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Considering Management Impacts on Carbon and Nitrogen Cycling in California Agricultural Systems

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Considering Management Impacts on Carbon and Nitrogen Cycling in California Agricultural Systems

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Acknowledgments

Undertaking a doctoral degree was never something that I thought I would have the opportunity to undertake, let alone finish. I knew I was a decent student from the time I was very young, but I would have never seen the potential in myself to accomplish this without the mentorship and support from so many.

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Chapter 1

Assessment of Soil Conservation Practices on Soil Carbon and Soil Health Indicators in California Annual Cropping Systems

1.1 - Introduction

Farming practices and the need for productive soils have intensified over the last 50 years (Horwath and Kuzyakov, 2018), yet losses of topsoil and soil function still amount to over $8 billion a year (Sartori et al., 2019). This disconnection between humanity's need to maintain agricultural productivity and our disregard for the limited and vulnerable nature of the soil resource is quite alarming. Soil degradation can be halted or reversed by adopting soil conservation and best management practices. Horwath and Kuzyakov (2018) determined that we can optimistically expect to maintain or even increase soil carbon in the future if we alter management practices by implementing practices like cover cropping and no-till. Methods used to quantify soil health practices' impacts will have a strong impact on the affects that are shown. Therefore, there is a need to have a baseline set of indicators that researchers agree are useful in determining the viability of practices.

Indicators of and practices that improve soil health, have been of interest to agricultural researchers for nearly as long as there has been agricultural research. However, the term soil health gained prominence in the academic discourse after the Soil Science Society of America (SSSA) formed an ad-hoc committee to discuss the subject and attempted to gain some clarity about what defines it, how to measure it, and which data was needed to understand it better (Karlen et al., 1997). The discourse continues today with discussions about which practices have the most potential to sequester carbon in soils, what defines a healthy soil, and what the most useful indicators are to measure changes in Soil Health(Chambers et al., 2016; Karlen et al., 1997; Kravchenko and Robertson, 2011; Minasny et al., 2017; Morgan et al., 2020). Practices including cover cropping, compost addition, manuring, residue/cover retention, and no or reduced tillage are known as conservation agriculture practices promoting soil health (Crystal-Ornelas et al., 2021; Kim et al., 2020; Nunes et al., 2020; USDA, 2016a). However, quantifying soil health management practices impacts on soil health indicators, especially on soil carbon cycling and storage in soils, has become an area of spirited debate in recent years because if the variability in the relationships between practices and indicators as well as by confounding factors present in different systems such as climate, soil texture and the cropping system in question (Amundson and Biardeau, 2018; Moinet et al., 2023; White et al., 2017). Soils have been considered as a potential sink for anthropogenic carbon emissions(Minasny et al., 2017; Mitchell et al., 2019a; Morgan et al., 2020) and so more data is necessary, especially in arid and semi-arid climates and within intensive cropping systems to determine which practices really have a significant effect that would justify increased costs associated with their adoption. Increased costs from adoption of cover crops include seed, labor, irrigation, time lost for growing cash crops, termination, incorporation, etc.

The metrics soil scientists use to assess changes in soil health are many and multiplying, and there is confusion about which indicators are the most accurate and useful; this only adds to the already difficult task of measuring soil health and determining which practices will be the most effective in any given system. The North American Project to Evaluate Soil Health Measurements *(NAPESHM),* led by the Soil Health Institute, aimed to define the minimum appropriate parameters necessary to measure soil health (Norris et al., 2020). This project evaluated more than 20 indicators using data from more than 120 different sites across the United States, Canada, and Mexico and determined which indicators respond to management and therefore are the most useful in measuring soil health. Resulting data and analysis revealed that total soil organic carbon (SOC) and permanganate oxidizable carbon (PoxC) were both found to be valuable indicators for assessing soil health (Liptzin et al., 2022). Both of these indicators responded positively to cover cropping and reduced/no-till management, which is of interest in this study (Liptzin et al., 2022). However, Liptzin et al. (2022) also determined that reduced or no-tillage management did not always have a positive relationship with soil carbon and that climate played an outsized role in the effectiveness of reduced/no-till on increasing soil carbon. In warmer climates with a long growing season, microbial activity continues to oxidize soil organic matter more of the year and the increased residue at the surface simply results in more microbial activity, especially when these soils are irrigated (Davidson and Janssens, 2006). This means that carbon inputs on the surface of soils will be more susceptible to loss than subsurface inputs, especially in the thermic mediterranean climate of California. Other meta-analyses agree that reduced-till management often has a positive impact on carbon, but not always, this variability calls into question whether reduced/no-till is as effective at sequestering carbon in all soils as some claim (Bai et al., 2019; Luo et al., 2010; Ogle et al., 2019). Considering that the positive impacts of reduced/no-till on carbon stocks can be variable and limited in more arid climates, and that systems in California are not only arid but intensively managed and irrigated, and the data on these practices impacts in the west are very limited (Davidson and Janssens, 2006; Liptzin et al., 2022; Luo et al., n.d.; Ogle et al., 2012).

Understanding the long-term effects of management on soil health, carbon dynamics, and yield, among other things, is essential if scientists are going to make claims about those practices. The Century experiment (https://asi.ucdavis.edu/programs/rr/century-experiment) in Davis, CA, (USA) as well as a long-term experiment in Five Points, CA, offer two unique opportunities for researchers to better understand how long-term management will impact soil health indicators in semi-arid Mediterranean regions (Mitchell et al., 2019a; Tautges et al., 2019). The data collected so far from these long-term (>10 years) sites indicated that no-till can increase carbon in the surface of soils (0-30cm), and cover cropping has the same effect, but data also shows that sub-surface dynamics (60-200 cm) carbon may decrease under cover cropping. The majority of research regarding tillage, cover cropping and soil carbon comes from east of the Rocky Mountains in climates that have warm wet summers and long frozen winters (Norris et al., 2020; Powlson et al., 2014). When we contrast that with the short cool and wet winters and long hot growing seasons (under irrigation no less) found here in California, we can start to understand how the climate and agriculture practices present in California are so conducive to microbial decomposition and respiration of carbon for much more of the year than other places. This lack of adequate, reproducible data for semi-arid regions and the question of whether these healthy soil practices will have the same positive effects as in other climates is the knowledge gap we hope to address with this study. Another knowledge gap in the majority of existing data on conservation agriculture practices is the fact that this data most often only considers the top 30 cm of soil profiles. In the last decade, several studies have established the importance of considering more of the soil profile before broad generalizations are made (Balesdent et al., 2018; Harrison et al., 2011; Tautges et al., 2019). Tautges et al. (2019) found that when only considering the first 30 cm of the soil profile, soil carbon concentrations appear to increase (Tautges et al., 2019). However, when considering the whole profile to 1-2 meters, this increase disappears, cover cropped treatments actually lose carbon in the subsurface, so no increase was observed. Previous research at the long-term research site in Five Points, Ca only considered the first 30 cm of the soil profile (Mitchell et al., 2019a, 2017, 2015a, 2007; Veenstra et al., 2007a). This study looked at soil C distribution with depth in sites with long- versus with short-term management to estimate the duration of practice implementation needed to see increases in soil C at depth.

Implementation of these practices is not only a scientific question though. Farmers and land managers are business owners and operate in a competitive market, so economic considerations must also be made. Implementing soil health practices such as cover cropping and no-till can provide an economic net-benefit when yields increase or are maintained, or cost is reduced or at least not increased (such as preventing erosion or reducing fuel or labor costs). For instance, implementation of cover cropping may require additional input costs including seed, planting, labor, fertilization termination of the cover crop; as well as the opportunity-cost of limited cash-crop choices because of timing (Bergtold et al., 2019)(Bergtold et al., 2019). On the other hand, the use of cover crops can result in economic benefits such as yield increases, forage for grazing animals soil protection, weed control, or reduced need for tillage passes. A cost benefit analysis (CBA) is commonly used by decision-makers for determining if an environmental practice should be implemented (Bergtold et al., 2019; Hanley et al., 2009)(Bergtold et al., 2019; Hanley et al., 2009). Cost Benefit analyses need to include all relevant costs for implementation as well as the expected benefits to be useful in decision making (Hanley et al., 2009)(Hanley et al., 2009). Costs and benefits from proposed practices are not usually realized immediately and so, often a discounted present value (PV) is often calculated over the whole lifetime of the project or practice implementation to make the CBA more accurate(Hanley et al., 2009) (Hanley et al., 2009). Then, once these values are calculated, a net present value test such as a benefit cost ratio (BCR) is calculated to determine if the project or practice should proceed. We believe that farmers, land managers and other stakeholders are unlikely to adopt practices that are economically “nonstarters,” and so we believe this perspective is important.

The main objective of this study was to characterize the effects of cover cropping and no-till on soil organic carbon stocks and other soil health indicators and compare long-term managed sites to sites that have been managed for only a few years. Additionally, we completed a simple cost benefit analysis by considering practice implementation costs and changes in yield. We intend for this economic analysis to give some insight into the economics that our farmers face and to indicate whether implementation would be economically efficient if the practices were not being subsidized by an outside entity, given the realized costs (range), and observed benefits. Our study provides additional data in determining whether no-till should be promoted for its carbon sequestration potential, or for other soil health benefits. This study will add significant value and provide decision makers, policymakers and even the public to continually strive to improve working landscapes. This more complete picture should help decision-makers and stakeholders better understand the costs and benefits of each management practice in the context of the arid to semi-arid mediterranean conditions found in much of California's annual production systems.

1.2 - Material and Methods

1.2.1- Field trials

In this study, we collected data from three field trials evaluating the effects of tillage and cover crops on soil carbon and soil health. The trials were located along a gradient of decreasing precipitation from Sutter County in the Sacramento Valley (CA, USA) to Fresno County in the southern San Joaquin Valley. A summary of the sites studied is provided in **Table 1.**

1.2.1.1 – Short-Term Effects of a Legume Winter Cover Crop

This trial was located in Sutter County (California USA). The trial, started in 2018 was a short-term experiment (3 years total, with practice implementation in the winter of 2018-2019 and 2019-2020) with vetch (*Vicia americana*) planted as a winter cover crop at two rates (32 and 64 kg ha^{-1} 2018-19; 16 and 32 kg ha^{-1} 2019-20) and a control with no cover crop. These treatments were applied to 8-bed plots replicated three times and distributed in the field following a randomized complete block design. Soils are mapped as Shanghai, silt loam, clay substratum - 90 % and similar soils (*fine-loamy, mixed, superactive, nonacid, thermic Aquic Xerofluvents*) Annual precipitation was reported to be 37 cm on average during the study years("NOAA NCEI U.S. Climate Normals Quick Access," n.d.). Previous crop history in this site was conventionally grown rice in 2015 and 2016, left fallow in 2017. Processing tomatoes were planted at the end of April 2019. Tomatoes were harvested in August 2019. Vetch cover crop was planted on November 13, 2019 (Year 2). Poultry manure compost was added to all the plots, including the control, on Fall 2018 at an approximate rate of 11.2 Mg ha^{-1} as part of the grower practices.

1.2.1.2 - Short-Term Effects of a Mixed Winter Cover Crop

The field trial was located in Mendota, Fresno County (California, USA) and has been in cotton (*Gossypium barbadense or hirsutum*) production for 11 years (2011-2017). The field site was established in 2018 and consisted of two treatments: (i) cover crop (CC) and (ii) no cover crop or control (NO). For the CC treatment, in 2019 a mix of 95% small grain (*often Triticum aestivum or Triticosecale Wittm. Ex A. Camus*) and 5% tillage radish (*Raphanus raphanistrum*) was drilled seeded. In 2019-2020 the seed was flown on by plane, and a majority legume mix was used. Treatments were applied in three plots with treatment (CC) and control (NO) on three adjacent fields (plots). Plots were distributed following a randomized split plot design. The soil in two of the experimental plots is classified as 85% *Elnido* series (*Coarse-loamy, mixed, superactive, thermic Typic Endoaquolls).* The soil in the third plot is classified as 85% *Palazzo series* (*Fine-loamy, mixed, superactive, thermic Fluvaquentic Endoaquolls)*. Preceding this experiment, the site was planted to continuous cotton (*Gossypium barbadense or hirsutum*) for ~11 years (2011-2017); preceding that, the field was planted to alfalfa (*Medicago sativa)* for four years. Recent farmer records report problems with infiltration, ponding, and water retention, causing yield reduction since ending the chicken manure applications.

All experimental plots received 4.48 t ha^{-1} green waste compost in 2018 and 2019, and gypsum was applied in 2019 to address poor infiltration. The cover crop treatment was not irrigated in any year. The cotton crop was irrigated by a furrow-flooding approximately once every 7 days during the growing season. Different strategies were utilized each year to plant the cover crop.

Cover crops were terminated each year by first applying herbicide (glyphosate), flail-mowed, and incorporated by discing. After this, normal practices of roto-tilling and bed-formation preceded planting of the cotton cash-crop.

1.2.1.3 – Long-Term Effects of Winter Cover Crops and Reduced Till

This long-term experimental site was established in 1999 to examine reduced tillage and cover cropping in intensively managed and irrigated annual production systems in the Mediterranean climate in California. The study site is located at the University of California's

West Side Research and Extension Center (WSREC) in Five Points, CA (USA). Soils are classified as Panoche clay loam (87% and similar soils) (*fine-loamy, mixed, superactive, thermic, Typic Haplocambids*). A tomato-cotton rotation was practiced from 1999 until 2013. The systems were changed to sorghum (*Sorghum bicolor*) and garbanzo beans (*Cicer arietinum*) in 2014. Processing tomatoes (*Solanum lycopersicum*) were planted in 2018, Cantaloupe melon (*Cucumis melo var. cantalupensis*) was planted in 2019 and butternut squash (*Cucurbita moschata 'Butternut'*) in 2020. Management treatments included tillage and cover cropping combined in a factorial design, including standard tillage without cover crop (STNO), standard tillage with cover crop (STCC), no-tillage without cover crop (NTNO), and no-tillage with cover crop (NTCC). The CC mix during this study consisted of Juan triticale (*Triticosecale* Wittm.), Merced rye (*Secale cereale* L.), and common vetch (*Vicia sativa* L.) (30% triticale, 30% rye, and 40% vetch by weight). From 2010 to 2014, the CC mixture was changed to pea (*Pisum sativum* L.), faba bean (*Vicia faba* l.), radish (*Raphanus sativus*), and Phacelia (*Phacelia tanacetifoli*). Each treatment was replicated four times in a randomized complete block design. Treatment plots consist of six beds, each measuring 9.1 × 82.3 m. Six-bed buffer areas separated tillage treatments to enable the different tractor operations that were used in each system. Standard tillage operations consisted of residue shredding, multiple disking's to incorporate residues to a depth of 20 cm, use of a subsoiling shank before planting, additional disking to 20 cm to break up soil clods, listing of beds, and power incorporation of the surface 10 cm of soil using a cultimulcher (BW Implement, Buttonwillow, CA).

The no-till (NT) systems were managed from the general principle of trying to reduce primary intercrop tillage to the greatest extent possible. Controlled traffic farming, or zone production practices that restrict tractor traffic to certain furrows, were used in the NT systems, and planting beds were not moved or destroyed in these systems during the entire study period. The only soil disturbance operations used in the NT systems were shallow cultivation during the first eight years for the tomato and cotton crops. As the project progressed, the NT treatments became true no-tillage systems in 2012, with the only soil disturbance occurring at the time of seeding or transplanting crops and cover crops. The tomato and cotton crops were furrow irrigated from 2000 to 2012. In keeping with trends in the region toward more efficient systems, the study site was converted to subsurface drip irrigation in 2013 with 34 mm diameter tape buried 30 cm in the centers of each 150 cm-wide planting bed.

Table 1 - Experimental sites - Summary of management period, conservation practices, treatments, cropping system and dates of soil sampling.

Site	Management period	Soil Conservation Practice	Treatments	Main Crop	Soil Sampling
Sutter	Short-term (3 years)	Winter Legume Cover crops	Fallow (Control)	Processing Tomatoes (2019) Rice (2020)	Fall 2018 Fall 2019 Spring 2020
			Low cover crop seed rate (Low CC)		
			High cover crop seed rate (High CC)		
Mendota	Short-term (3 years)	Winter Cover crops (Legume and non-legume mix)	No cover crop (NO)	Cotton (2018, 2019, 2020)	Spring 2018 Spring 2019 Fall 2020
			Cover crop (CC)		
WSREC	Long-term (21 years)	Winter cover crops (legume and non-legume mix) Reduced tillage	Standard Tillage/ No cover crop (STNO)	Processing tomatoes (2018) Cantaloupe(2019) Butternut squash (2020)	Spring 2018 Spring 2019 Fall 2020
			Standard Tillage/ Cover crop (STCC)		
			No-Tillage/ No cover crop (NTNO)		
			No-Tillage/ Cover crop (NTCC)		

1.2.2. – Soil and Cover Crop Biomass Sampling and Analysis

Soil sampling was carried out annually from 2018 to2020 at all sites in the periods as indicated in **Table 1**. To evaluate carbon dynamics, sampling was done to a depth of 90 cm in 2018 and 2020. In 2019, soil samples were collected to a depth of 30 cm. We collected 10 subsamples from each treatment plot from a straight transect down the middle bed on alternating side of the buried drip-tape. Sub-samples were composited and homogenized in the field and brought back to the lab for processing in sealable gallon plastic bags. Soils were then air-dried and sieved to <2mm removing any visible plant residues.

Cover crop biomass was assessed before the termination and incorporation of cover crops to the extent possible. Three one-meter squared (m^2) sections of cover crop was harvested by cutting by hand to the ground, drying, averaging dry biomass, and extrapolating results to the field level.

1.2.3. - Soil Analyses

Sieved and pulverized samples were analyzed for soil pH, EC, and total carbon and nitrogen using the methods outlined in **Table 2**. Un-sieved samples were analyzed for aggregate stability using the Slake test (NRCS, 2001).

Permanganate oxidizable carbon (PoxC) was used as a proxy for active carbon and determined according to the cited method (**Table 2**), but with the following improvements to help control variability. Briefly, samples were analyzed in batches of nine, and replicates were distributed in different batches. Black polypropylene centrifuge tubes were used to minimize light exposure, permanganate was added every 30 seconds to each sample, and samples were diluted every 30 seconds in the same consecutive order. Quality control consisting of 2.5g of cornstarch was included in each analyzed batch to account for variation among batches. Replicates were accepted when the coefficient of variation (CV) was ≤ 20%.

Total soil C was analyzed through dry combustion (Table 2). The change in soil carbon (C) stock was calculated as the difference between tons of organic C ha^{-1} in the soil at the beginning and end of each experiment for the short-term sites. For the long-term sites, only data collected in 2018 is reported. Values were calculated by experimental unit (plot), using bulk density and core length (15 cm – 30 cm) to convert from the percentage of total carbon to tons C ha^{-1}.

1.2.4 Economic analysis

A simple cost/benefit analysis (CBA) was performed based on information provided by farmer partners and scientific collaborators. Calculations are based on Hanley et. al (2009) methods for determining costs and benefits and calculations for cost/benefit ratio (CBR) as being simply the expected returns (in this case mean yield per acre) divided by the realized costs (range over the course of the three-year study). When the CBR is greater or equal to 1, the practice is considered economically viable according to this method. We did not apply discounts to costs or benefits as this economic analysis was not meant to be used to make economic decisions, but simply as a lens through with to view the measured benefits we observed.

1.2.3.3. Statistical analysis

Data were analyzed separately for each site, with data only being presented together for purposes of understanding the broader patterns and comparing long versus short-term implementation of practices. Data was analyzed using RStudio (R Development Core Team, 2020. Linear models using the “lmer” function were used to perform ANOVA comparing treatment groups within each soil depth section. For experimental sites containing blocks in their design, blocks were added as the random factor to account for random error associated with spatial variability between blocks. ANOVA results were analyzed for significant differences between treatment groups with $\boldsymbol{\alpha} = 0.05$ (95% confidence interval). If treatments were found to be significantly different according to these criteria, then a Post-hoc Tukey test was used to confirm the statistical significance between treatments.

Table 2 - Methods of physiochemical analysis used for all experimental sites.

Variable Measured	Method
pH	pH Probe, saturated paste extract (Richards, 1954)
EC	EC probe, saturated paste extract (Rhoades, 1982)
Total Organic Carbon (TC)	Combustion (International, 1997)
Total Nitrogen (TN)	Combustion (International, 1997)
PoxC (permanganate oxidizable carbon)	Improved, (Culman et al., 2012a; Culman et al., 2012b; Weil et al., 2003)
Soil Moisture	Mass lost after drying to 105^{O}C
NO_3-Nitrogen	Vanadium (III) Chloride Reaction, (Doane and Horwáth, 2003)
NH_4-Nitrogen	Berthelot Reaction, (Rhine et al., 1998)
Aggregate Stability	NRCS Soil Test Kit Guide, (NRCS, 2001)
Surface Condition (Water Infiltration)	Infiltration, Soil Quality Test Kit Guide, (NRCS, 2001)

1.3. Results and Discussion

1.3.1 Effects of the Treatments on Total Soil Carbon

As was expected, total soil carbon was highly correlated with depth at all sites. However, surprisingly, each study year was also significantly different, and overall, treatment did not have a significant effect in any of the sites. The change in soil carbon stocks can be seen in **Table 3**, all of the p-values obtained through the statistical analysis are summarized. The use of a cover crop and compost over three years (Sutter) as well as a cover crop managed with no-till for 21 years (WSREC site), did not increase soil C stocks compared to standard practice. We did notice a trend showing an increase in the average carbon stocks at the Sutter site in the 0-15 cm depth section over the three years of the experiment, but statistical analysis (ANOVA) determined that the differences between treatments and control were not significant. It is likely that the addition of compost to all treatments hid potential treatment effects from the cover crop. This can explain why all the treatments including the control at the short-term managed site (Sutter), we measured an increase in the carbon stocks after only 3 years of management. This increase in carbon as a result of compost addition has been observed in similar studies in California (Tautges et al., 2019).

Interestingly, at the Mendota site, which received only cover crop, we observed an increase not in the surface, but in the 30-60 cm depth section. This difference shows why it is so important to consider the whole profile to at least 1 meter depth as surface dynamics seem to be much more variable. A textural change at 30 cm (depositional clay increase) was also observed in all studied plots at this site, and this can further explain the carbon accumulation at 30 cm, as it is just below the layer that is regularly disturbed with tillage. An increase in clay at this depth is significant as the surface is notably course textured and higher clay content has been shown to have a positive effect on carbon accumulation in other studies (Schweizer et al., 2021). Furthermore, the decrease at 0-15 cm in all plots over the three-year study can be explained through the fact that the cover crop establishment was largely unsuccessful in the first and third years of the study period (~1.24 Mg ha^{-1} in 2018, ~7.41 Mg ha^{-1} in 2019 and ~0.25 Mg ha^{-1} in 2020) and the small addition of compost (~ 4.5 Mg ha^{-1}) was not adequate to increase or even maintain carbon stocks in this intensively managed cotton system. It is also notable that this site received flood irrigation and carbon that was observed at depth could have been deposited in the first year and translocated in subsequent years. Movement of dissolved organic carbon has been demonstrated through wet-dry cycles (including irrigation) in other California systems (Lundquist et al., 1999).

More surprising was the fact we did not detect a treatment effect at the long-term managed site in Five Points (WSREC) that received cover cropping and was managed as no-till for 20 years. Previous work at this site did indicate an increase in carbon in these treatments in the 0-30 cm depth section (Mitchell et al., 2017), but when accounting for the full 0–90 cm profile, there was no difference between treatments in this study in 2018. Subsequent years (2019 and 2020) did not indicate differences either, but this data is not included in this work. This new data is consistent with the findings of Tautges et al. (2019) who found that cover cropping alone did not increase carbon when the whole soil profile (0-2 m) is considered. Two recent meta-analyses show the importance of measuring carbon in the whole profile, as even deeper soil layers are affected by land use and management regimes (Balesdent et al., 2018; Harrison et al., 2011). Those analysis and our data indicate that tillage is resulting in a redistribution of carbon from the surface into the subsoil and that this redistribution might actually mean that tillage can have a positive effect on soil carbon if it is not excessive. If tillage is significantly increasing the contact between organic matter and soil mineral surfaces (Cotrufo et al., 2019) and is increasing microbial turnover (Cotrufo et al., 2013), and is and moving carbon into the subsurface where conditions are cooler (Davidson and Janssens, 2006; Kirschbaum, 1995; Trumbore et al., 1996), than the carbon in tilled systems in this warm mediterranean climate might be more stabilized than the particulate carbon accumulating in the surface would be in the same climate. This indicates that, unlike other regions, we should not necessarily expect cover cropping, even when combined with no-till to significantly increase carbon to one meter depth in the semi-arid to arid, thermic, Mediterranean climate of California's Central Valley. Many other experiments and meta-analysis indicate increases in carbon, but only report to a max depth of 30 cm (Mitchell et al., 2015b; Steenwerth and Belina, 2008; White et al., 2020; Wolff et al., 2018). However, data suggests that compost addition seems to have a stronger positive effect on increasing carbon to a depth of a meter or more, which others have also found (Tautges et al., 2019).

Table 3 - Change in carbon stocks after 3 years of treatment at each site, sites, depths, stock change, standard error and p-values are shown. Mendota: cover crop (CC), no cover crop (NO), Sutter: no cover crop (Control), Low cover crop rate (Low CC), High cover crop rate (High CC).

Site	Depth (cm)	Treatment	Mean C Stock Change (tons/ha)		SE	p-value (α=0.05)
Mendota	0-15	CC	-3.24	±	1.01	0.068
		NO	-0.73	±	1.90	
	15-30	CC	8.93	±	0.51	0.445
		NO	10.46	±	1.21	
	30-60	CC	13.25	±	2.16	0.038
		NO	9.75	±	2.31	
	60-90	CC	11.93	±	0.62	0.200
		NO	7.43	±	1.86	
Sutter	0-15	Control	8.68	±	0.18	0.189
		Low CC	10.12	±	1.45	
		High CC	11.42	±	0.71	
	15-30	Control	6.18	±	1.83	0.984
		Low CC	6.53	±	2.94	
		High CC	6.81	±	1.55	

	30-60	Control	4.42	±	0.30	0.287
		Low CC	3.80	±	0.29	
		High CC	3.70	±	0.33	
	60-90	Control	4.47	±	0.79	0.278
		Low CC	3.38	±	1.33	
		High CC	2.94	±	1.45	

Table 4 - Soil organic carbon and nitrogen concentration (% of dry matter), standard errors and bulk densities (g cm^{-3}) are shown for all long and short-term sites. Mendota: cover crop (CC), no cover crop (NO), Sutter: no cover crop (Control), Low cover crop rate (Low CC), High cover crop rate (High CC), WSREC: : cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST).

Site	Depth	Treatment	%C (± SE)			%N (± SE)			Bulk Density
WSREC	0-15	CCNT	1.2	±	0.23	0.13	±	0.02	1.23
		CCST	1.14	±	0.09	0.13	±	0.01	1.09
		NONT	0.93	±	0.11	0.11	±	0.01	1.26
		NOST	0.9	±	0.07	0.11	±	0.01	1.31
	15-30	CCNT	1.11	±	0.18	0.12	±	0.02	1.6
		CCST	0.88	±	0.07	0.1	±	0.01	1.6
		NONT	0.98	±	0.12	0.11	±	0.01	1.6
		NOST	0.79	±	0.07	0.09	±	0.01	1.6
	30-60	CCNT	0.51	±	0.11	0.06	±	0.01	1.45
		CCST	0.43	±	0.03	0.05	±	0	1.45
		NONT	0.36	±	0.04	0.04	±	0	1.45
		NOST	0.38	±	0.02	0.05	±	0	1.45
	60-90	CCNT	0.42	±	0	0.04	±	0	1.45
		CCST	0.54	±	0.13	0.06	±	0.01	1.45
		NONT	0.45	±	0.04	0.04	±	0	1.45
		NOST	0.43	±	0.06	0.04	±	0	1.45
Mendota	0-15	CC	0.59	±	0.05	0.03	±	0	1.6
		NO	0.67	±	0.04	0.04	±	0	1.6
		CC	0.96	±	0.02	0.06	±	0.01	1.7
	15-30	NO	1.04	±	0.08	0.07	±	0	1.7
	30-60	CC	0.96	±	0.08	0.07	±	0.01	1.7
		NO	0.9	±	0.11	0.06	±	0.01	1.7
	60-90	CC	0.81	±	0.1	0.05	±	0.01	1.6
		NO	0.75	±	0.03	0.05	±	0	1.6
Sutter	0-15	Control	1.29	±	0.02	0.14	±	0.002	1.3
		High CC	1.4	±	0.03	0.15	±	0.011	1.3
		Low CC	1.38	±	0.09	0.15	±	0.003	1.3
	15-30	Control	1.02	±	0.09	0.11	±	0.009	1.4
		High CC	1.11	±	0.03	0.12	±	0.015	1.3

		Low CC	1.08	±	0.14	0.11	±	0.004	1.3
	30-60	Control	0.69	±	0.02	0.07	±	0.002	1.6
		High CC	0.67	±	0.01	0.07	±	0.001	1.6
		Low CC	0.65	±	0.01	0.07	±	0.002	1.5
	60-90	Control	0.8	±	0.03	0.09	±	0.006	1.6
		High CC	0.74	±	0.06	0.08	±	0.004	1.6
		Low CC	0.76	±	0.05	0.08	±	0.005	1.6

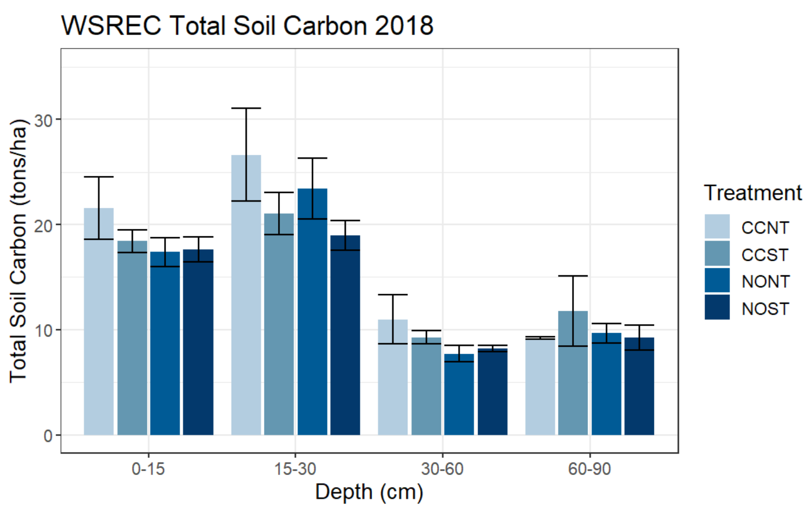

Figure 1 - Total Carbon stocks (tons ha^{-1}) at all depths for long-term experimental site at WSREC in 2018, ~20 years after practice implementation. *: cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST)*. Values are averages ± SE. (N=16).

1.3.2 Effects of the Treatments on Soil Nitrogen Dynamics

Nitrogen concentrations were also found to be overall not significantly different with respect to treatment. However, we did observe significant differences when comparing years. Nitrogen concentrations at the Sutter and Mendota sites were significantly different from year to year (increasing). We have determined that this year-over-year difference is most likely due to compost applications at both of these sites. As discussed previously, the application of compost at these two sites was applied to all treatments at the request of the farmer partners and was not a controlled treatment variable, but the additions had an observed impact. Comparatively, in the 60-90 cm depth section at the WSREC site we did notice a significant difference in nitrogen concentration, but this could likely be due to residual nitrogen leaching past the root-zone from the sub-surface drip fertigation, and differences in porosity from the tillage and cover cropping treatments. Recent work at Russel Ranch (Davis, CA) showed that cover cropping increased transport of carbon and nitrogen to the subsoil (Rath, 2022). We expect that no-till might exacerbate this effect, and the infiltration data presented below (Section 3.5, **Figure 7**) appears to agree with this.

Table 5 - ANOVA results for percent total nitrogen from soil samples at all sites. Treatment, Year and Block p-values are shown.

Site	Treatment	Depth	Year	Block
Mendota	0.78	0.037	0.0005	0.47624
Sutter	0.4974	< .001	< .001	0.6698
WSREC	0.024	< .001	NA	NA

Table 6 - P-values showing the significance of treatment effect on nitrogen content by depth at the WSREC site, calculated with only 2018 data which was used in this study.

Depth	Treatment p-value
0-15	0.401848
15-30	0.236943
30-60	0.160439
60-90	0.050088

Percent N changed over the course of the study in Mendota and Sutter as is shown in the significance of year (**Table 5**), likely due to compost applications, although we cannot be sure as this was not a controlled variable, no treatment effect was observed. There was a treatment effect on N concentrations at WSREC as discussed earlier. **Figure 2** (below) shows the differences of the nitrogen effect that was observed at the WSREC site. We do not expect that this difference has any substantial meaning as this field was managed with subsurface fertigation and residual nitrogen is expected to be found at depth in some treatments, potential mechanisms are discussed above. **Figure 3** (below) shows post-hoc Tukey analysis and indicates that the only two treatments with differences are the no cover crop no-till (NONT) and the cover crop standard till (CCST) plots. It is possible that the combination of a cover crop and standard tillage resulted in more nitrogen redistribution to this lower depth after 20 years, although complete mechanism are unclear.

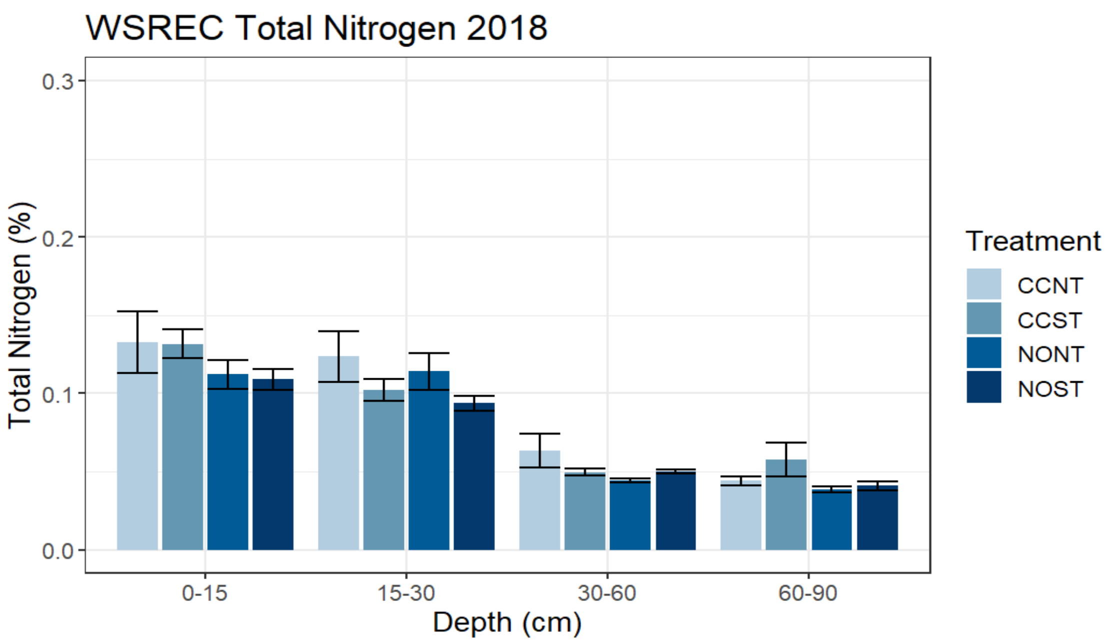

Figure 2 - Total Nitrogen (%) by depth section for long-term experiment at WSREC. Data Collected in 2018. cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST

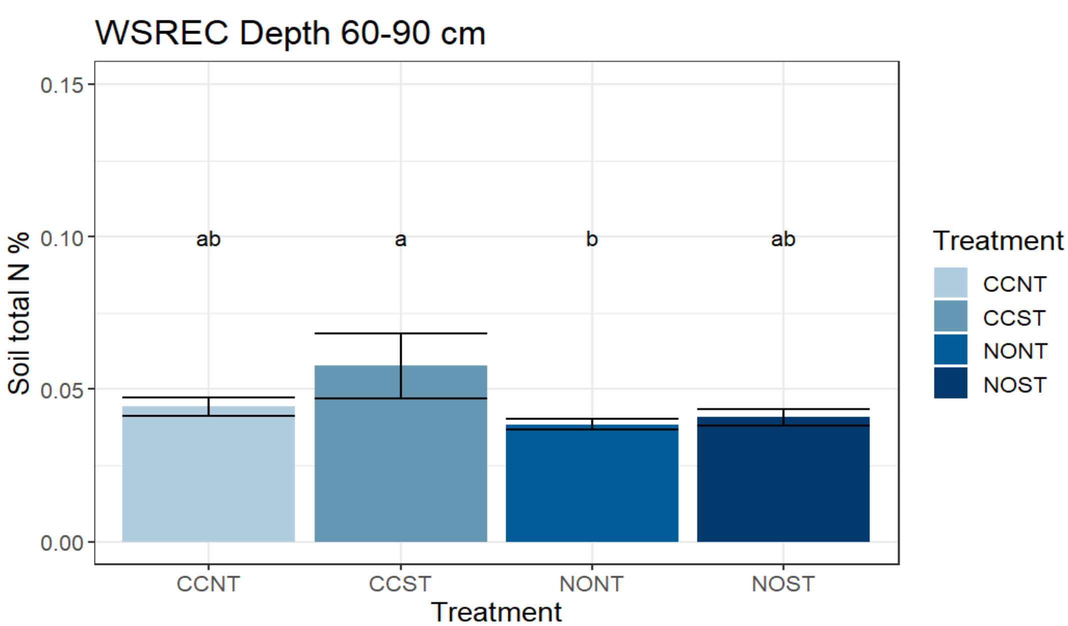

Figure 3 - Soil total nitrogen (%) with Tukey plot showing significance of treatment, from 2018 data. cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST).

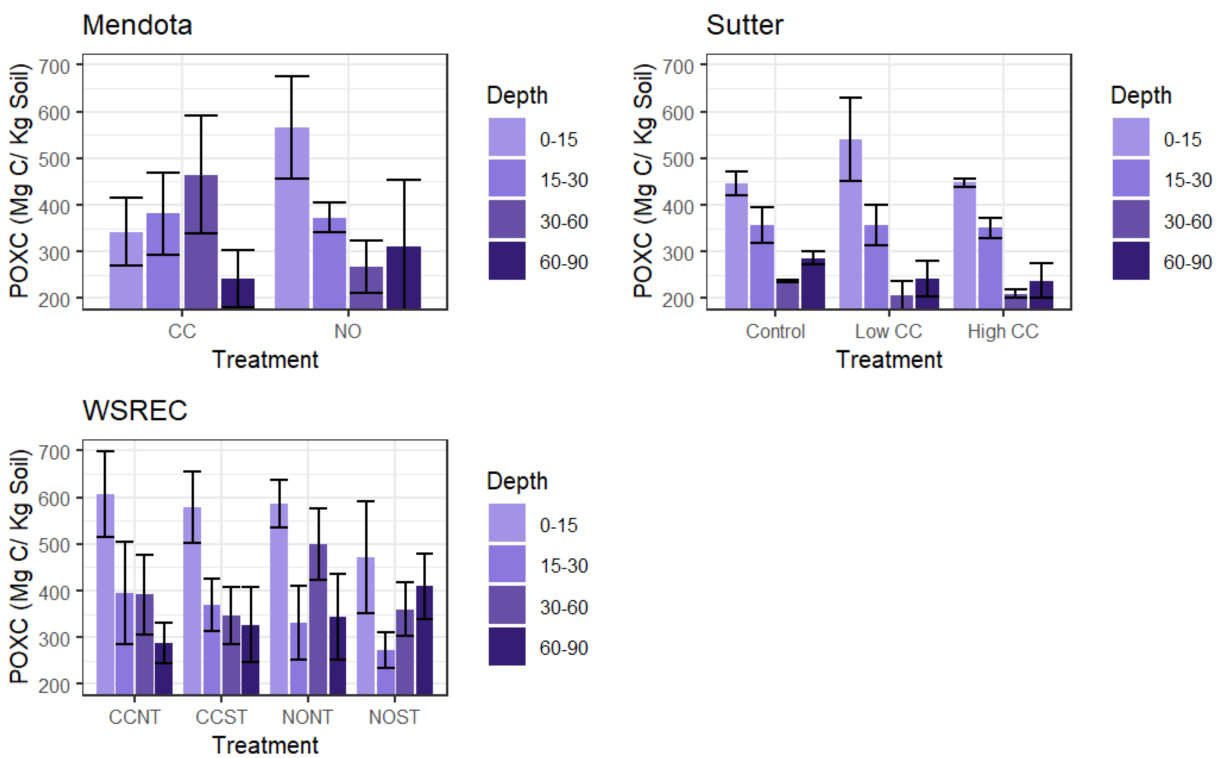

Figure 4 -POXC results for all sites at all depths for 2020. Mendota: Cover crop(CC) and No cover crop (NO). Sutter: control, Low-rate cover crop (Low CC), High-rate cover crop (High CC). WSREC: cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST).

1.3.3 - Permanganate Oxidizable Carbon (PoxC)

We did not observe a significant treatment effect in any site (ANOVA results summarized in **Table 7**), but we obtained significant differences with respect to depth. PoxC often decreases with depth, and this is true in some of our sites (**Figure 4: WSREC and Sutter**) and in other work (Culman et al., 2012; Wade et al., 2020), which usually correlates with the total carbon content of each depth. However, despite lack of significant treatment differences, the Mendota site had an interesting dynamic. PoxC is thought of as proxy for the labile fraction that is supposed to be responsive to short-term management (Culman et al., 2012; Hurisso Horwath et al., 2016; Wade et al., 2020). The increase in PoxC can predict carbon accrual (Culman et al., 2012). PoxC tends to increase in deeper layers for the CC treatment. This could potentially be explained by the presence of a subsurface layer with higher clay content at 30-60 cm. The accumulation of oxidizable carbon could have been produced by deposited root exudates from the cover crop roots. Root exudates contain sugars and organic acids that are labile (Panchal, 2022). The accumulation of carbon at deeper layers can be a promising route for stable carbon accumulation, in subsequent years of treatment (Wade et al., 2020). The trend is very distinct from the no cover crop treatment (NO), where labile C decreases at deeper layers. The different carbon allocation by depth between both treatments, shows what has been seen in other studies that cover crops can alter the formation and size distribution of soil pores allowing carbon to move down the soil profile with water (Panchal et al., 2022).

For the two sites with cover crop treatments (Mendota and Sutter), we observed varying trends. In Sutter PoxC decreased with depth in all treatments including the control. However, the effect of cover crops at Sutter could be hidden by the addition of compost to all plots. The significance of the year at the Sutter and WSREC sites could be attributed to difference cropping systems. Different root exudate dynamics, and therefore, different carbon inputs from year to year (Panchal et al., 2022).

Table 7 - ANOVA results for treatment and year, by depth, for PoxC in all sites in all years (top). Second table (bottom) shows only 2020 ANOVA results for all sites for PoxC showing the significance of depth.

Site	Depth	p-value Treatment	p-value Year
Mendota	0-15	0.453	0.911
	15-30	0.670	0.369
	30-60	0.469	0.017
	60-90	0.904	0.194
Sutter	0-15	0.507	5e-4
	15-30	0.630	0.896
	30-60	0.377	0.001
	60-90	0.401	0.004
WSREC	0-15	0.537	0.001
	15-30	0.619	0.322
	30-60	0.467	0.000

	60-90	0.388	0.002

Site	Treatment	Depth
Mendota	0.971903851	0.000243436
Sutter	0.987410799	1.51E-21
WSREC	0.619981536	1.62E-07

1.3.4 - Soil pH and EC

As presented in **Figure 5 and Table 8**, we did not observe a significant difference in soil pH after 3 years of treatment at each site. The p-values are summarized in **Table 8**. Most sites kept a similar pH between all the treatments. Electrical conductivity (EC) effects were insignificant from our observations. These results can be found in **Figure 6** below. Although we did not observe any significant effects at our study sites, compost quality and source are important factors to consider, especially in soils with existing salinity issues, as large additions of compost with high EC could result in elevated soil EC (Gondek et al., 2020) that would affect crop establishment and yield (Jamil et al., 2006) if not managed with adequate leaching irrigation to remove soluble salts.

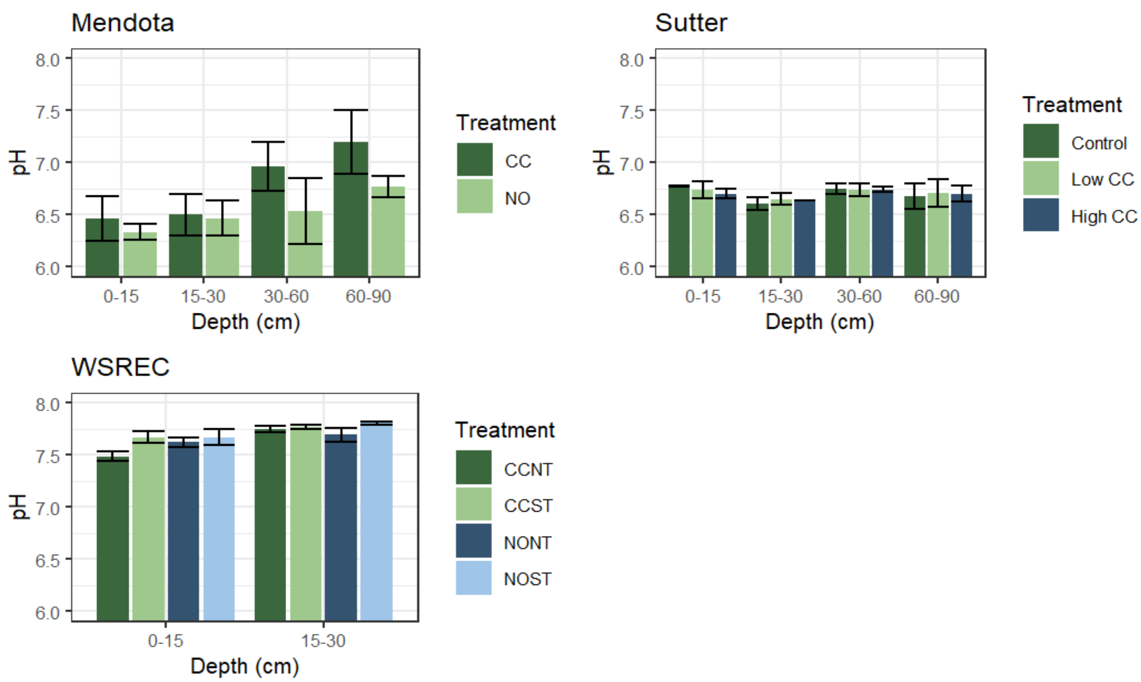

Figure 5 -Soil pH results for all sites at all depths for 2020. Mendota, Cover crop(CC) and No cover crop (NO). Sutter, control, Low-rate cover crop (Low CC), High-rate cover crop (High CC). WSREC, cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST).

Table 8 ANOVA results by treatment and block for soil pH in all sites in 2020.

Site	Depth	P-value Treatment	P-value Block
Mendota	0-15	0.3828	0.1857
	15-30	0.9175	0.7935
	30-60	0.1215	0.1202
	60-90	0.3061	0.6500
Sutter	0-15	0.3950	0.1095
	15-30	0.7589	0.4514
	30-60	0.9425	0.2026
	60-90	0.9013	0.0242
WSREC	0-15	0.2074	NA
	15-30	0.2496	NA

Table 9 - ANOVA results for bulk density for all sites and depths. P-values showing the significance of treatment and year are shown.

Site	Depth	P-value Treatment	P-value Year
Mendota	0-15	0.334282	0.392696
	15-30	0.334282	0.392696
	30-60	0.343436	0.343436
	60-90	NA	NA
Sutter	0-15	0.273730	0.000000
	15-30	0.461577	0.000000
	30-60	0.077630	0.000000
	60-90	0.370783	0.000000
WSREC	0-15	0.247089	NA
	15-30	0.235597	NA
	30-60	0.235597	NA
	60-90	0.235597	NA

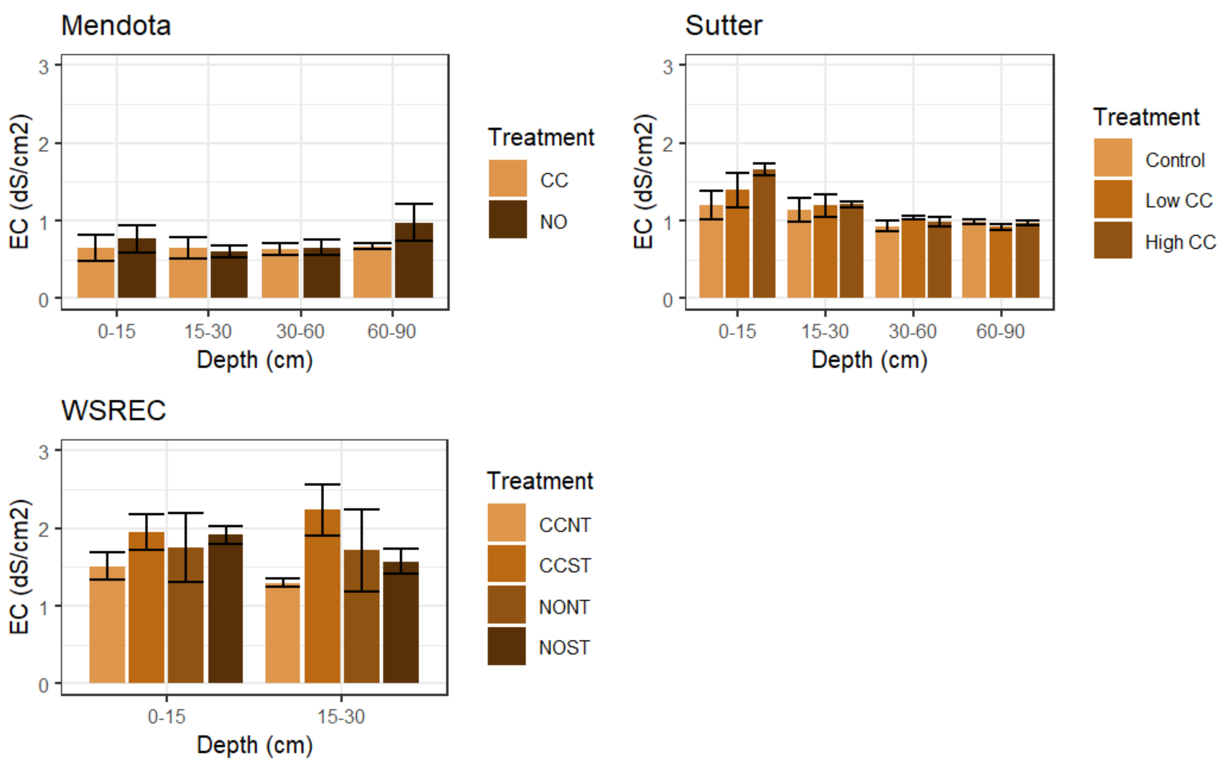

Figure 6 - Soil EC results for all sites at all depths for 2020. Mendota, Cover crop(CC) and No cover crop (NO). Sutter, control, Low-rate cover crop (Low CC), High-rate cover crop (High CC), WSREC, cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT), no cover crop standard till (NOST).

1.3.5 - Soil Bulk Density, Wet Aggregate Stability, and Infiltration

As is shown in **Table 10** (below), we did not observe a significant effect for bulk density for most of the sites at all depths. The primary reason bulk density data was collected in this this study was for the accurate calculation of carbon stocks, and no significant differences in bulk density were expected with respect to treatment. It has been noted in other work that bulk density can be negatively impacted by tillage (Li et al., 2019). However, many studies agree that impacts are often small and do not affect crop growth (Grant and Lafond, 2011; Lampurlanés and Cantero-Martínez, 2003; Li et al., 2019; Logsdon and Karlen, 2004).

Table 10 - ANOVA results for bulk density for all sites and depths. P-values showing the significance of treatment and year are shown.

Site	Depth	P-value Treatment	P-value Year
Mendota	0-15	0.334282	0.392696
	15-30	0.334282	0.392696
	30-60	0.343436	0.343436
	60-90	NA	NA
Sutter	0-15	0.273730	0.000000
	15-30	0.461577	0.000000
	30-60	0.077630	0.000000
	60-90	0.370783	0.000000
WSREC	0-15	0.247089	NA
	15-30	0.235597	NA
	30-60	0.235597	NA
	60-90	0.235597	NA

Table 11 – Wet aggregate stability scores for all sites, treatment, mean score, low and high confidence intervals are shown as well as whether the treatment effect was significant or not and the associated p-value. All data is from 2020 in the final year of the experiment.

Aggregate Stability				
Site	**Treatment**	**Mean Score**	**CI low**	**CI high**
WSREC				
	NOST	3.167	3.038	3.30
	CCNT	5.807	5.716	5.898
	CCST	5.178	5.048	5.306
	NONT	3.932	3.804	4.062
ANOVA		**Significant? Yes**	P Value = 2.2 e -16 ***	
Mendota				
	NO	2.118	2.055	2.032

	CC	2.118	2.052	2.160
ANOVA		**Significant? No**	P Value = .752	
Los Banos				
	0 tons/ac	2.76	2.59	2.93
	15 tons/ac	2.54	2.37	2.72
	30 tons/ac	2.95	2.77	3.12
ANOVA		**Significant? No**	P Value = 0.14	
Sutter				
	Control	2.38	2.87	1.89
	Low-Rate CC	2.84	3.46	2.22
	High-Rate CC	2.63	3.39	1.87
ANOVA		**Significant? No**	P Value = 0.14	

Wet Aggregate Stability

The short-term management sites (Sutter and Mendota) didn't show a significant difference in wet aggregate stability with respect to treatment (**Table 11**). The only differences were observed for the long-term management site (**Table 11**), but these differences were notable. As expected, the no-cover crop and standard tillage treatment (NOST) had the lowest aggregate stability, with a mean score of 3.2 This makes sense since tillage disturbs the soils and breaks-up aggregates and there was no additional biomass (roots, shoots, and residue) or carbon inputs (exudates) to increase aggregate stability. The cover crop standard till treatments (CCST) had much higher wet aggregate stability with a mean score of 5.2, which surprisingly higher than the no-till no cover crop treatment (NONT) with a means score of 3.9. This indicates that cover cropping seems to contribute more to aggregate stability than no-till. Unsurprisingly, the highest score was from the combination of no-tillage and cover crop (CCNT) with a score of 5.8. The high aggregate stability in the cover crop treatments can be explained by the effect of cover crop active roots. Root exudates work as a binding agent (Odesa, 1979) that holds soil particles together. In addition, root exudates promote microbial activity around the roots which in turn generates extracellular polymers that form aggregates. The uptake of water in the root zone causes localized wet-dry cycles that help stabilize the aggregates formed (Six et al., 2002). The greater contribution of cover cropping to increase aggregate stability is notable. Soil aggregates protect carbon (Plaza-Bonilla et al., 2013) in the soil and can be an indicator of carbon sequestration (Blanco-Canqui and Lal, 2010). However, the connection between tillage, cover cropping, quantity of aggregates, their size, clay content and minerology of soils and the stability of carbon held within aggregates is still a matter of significant and important research among scientists (Blanco-Canqui and Lal, 2010; Cotrufo et al., 2019; Jastrow et al., 2007; Plaza-Bonilla et al., 2013; Sundermeier et al., 2011). That debate is well outside the scope of this study but is no-doubt nuanced and interesting.

1.3.6. Cost Benefit Analysis

Table 12 – Simple cost benefit analysis using cost ranges from applied practices. Practices are listed as well as expected resulting biomass contributions from the practice, costs (low and high), resulting expected return (benefit), and cost benefit ratio (CBR).

Cost Analysis - Cover Cropping and Compost							
		Mg ha^{-1}	**Cost in \$ ha^{-1}**		**\$ ha^{-1}**	**Cost Benefit Ratio (CBR)**	
Site	**Practice**	**Biomass Contribution**	**Cost Low**	**Cost High**	**Yield Change (2018-20)**	**Low Estimate**	**High Estimate**
WSREC							
	Cover Crop Management (irrigated)	**5.60**	**237.00**	**367.00**	**123.50**	**0.52**	**0.34**
	(from UCANR Study 2003)	**(mean from 2018/2019)**					
Pikalok							
**Actual Costs*	Cover Crop Management (un-irrigated) Poor Rain	**0.56**	**125.00**	**175.00**	**123.50**	**0.99**	**0.71**
**Biomass Yield estimated*	Cover Crop Management (un-irrigated) Good Rain	**5.60**	**125.00**	**175.00**	**889.20**	**7.11**	**5.08**
***Farmer Estimated Costs*	Cover Crop Manamgement (irrigated)	**6.73**	**200.00**	**312.50**	**1333.80**	**6.67**	**4.27**
	Compost Application	**3.36**	**150.00**	**200.00**	**889.20**	**5.93**	**4.45**
	Gypsum Amendment	**0.00**	**150.00**	**200.00**	**444.60**	**2.96**	**2.22**
Sutter	Control (standard Practice)	**0.00**	**0.00**	**0.00**	**N/A**	**N/A**	**N/A**
	Cover Crop - Low Rate	**1.57**	**172.90**	**296.40**	**1694.70**	**9.80**	**5.72**
	Cover Crop - High Rate	**2.24**	**197.60**	**370.50**	**2541.70**	**12.86**	**6.86**
			*assuming \$360/bale of cotton				

Simple cost benefit analysis using cost ranges from applied practices, practices are listed, expected resulting additional biomass contribution expected, costs (low and high), resulting expected return (benefit) and cost benefit ratio (CBR).

1.3.6.1 - Economic Analysis – Short-Term Cover Crop (Sutter County)

The cover crop treatments had higher tomato yields compared to the control plots without cover crop (see **Table 12**), but these differences were not found to be statistically significant. The high cover crop treatment averaged 22.23 Mg ha^{-1} higher than the control, and the lower rate cover crop treatment averaged 14.82 Mg ha^{-1} higher than control. The value of organic processing tomatoes in 2019 was approximately \$126 Mg^{-1}, resulting in each cover crop treatment providing an extra \$2902.25 ha^{-1} (T2, high) and \$2035.28 ha^{-1} (T1, low) compared to the control (See **Table 12**). On the other hand, the cover crop treatments had expenses related to vetch seed costs, planting and termination costs from equipment passes, labor and fuel. The annual vetch seed cost was \$139 ha^{-1} in 2018 because of an error calibrating the planter and planting double the rate of vetch. In 2019, cover crop seed cost was \$75.95 ha^{-1}. Still, even with increased costs, the cover cropping increased yields adequately to economically justify the added costs (CBR >1, See **Table 12** above).

1.3.6.3 Economic Analysis – Short Term Cover Crop (Fresno County)

As can be seen in **Table 12** (above), the economic upside of cover cropping depends highly on market conditions (potential income from cash crop) and weather (to establish the cover crop without irrigation). The risk versus potential reward (cost benefit ratio - CBR) for each of the practices is dependent upon whether the cover crop can be irrigated or not. At this site winter irrigation was not possible during study years. Furthermore, early winter rainfall was not sufficient to establish a good stand of cover crop early enough in the season to be effective in the second and third year of the study. Therefore, the return from additional biomass was severely limited. But the cost of seed, planting and termination is still there. This means that there is a greater inherent risk to planting cover crops without irrigation. If rain is not adequate to cause any real benefit, the sunk cost still exists. However, in wet years, especially when the rain comes at the right time to establish a good stand of cover crop, the benefits might be substantial.

However, the benefit of other practices (compost addition and/or gypsum amendment are compared here) that improve soil and increase yield carry inherently less risk and do not depend on rainfall quantity and timing that is increasingly unpredictable with climate change. Therefore, it seems the most economically viable to apply compost, or other amendments directly as the benefits are much more certain.

1.3.6.4 Economic Analysis – Long-Term Experiment – Cover Crop and No-Till (WSREC)

In contrast, the economic situation is quite a bit different at the Five Points study site. Here, we did not see a significant-enough increase in yield to pay for the increased management costs and so the economic returns for the management practices do not even pay for the practices themselves (seen in CBR <1). It should be noted that this site was managed more intensively with more passes for cover crop termination rather than herbicide used to terminate the cover crop. This shows the differences in cost depending on how practices are implemented and how this can greatly affect the economics of implementation. Therefore, there are significant differences in the cost associated with these practices. Still, it is not expected that farmers or land managers would implement practices that are not expected to have a positive economic impact on their operations. This is arguably the most important factor that drives farmer decision making and so it should not be ignored.

1.4. Conclusions

Overall, we did not find significant evidence of changes in carbon stocks for these conservation agriculture practices at our study sites in the mediterranean climate of California's Central Valley. Even with long-term management we did not measure significant increases in carbon when accounting for more of the soil profile. Instead, we observed that in the standard tillage systems, carbon is redistributed to a deeper depth but does not increase with these practices. The only significant increases that were observed were in 30-60 cm depth section at the Mendota site, under short-term cover crop management.

Although we did not observe carbon sequestration at any site, we did observe other soil health indicators were positively impacted by these practices. We especially noticed benefits from long-term implementation of cover cropping and no-till at the WSREC site, with respect to wet aggregate stability and infiltration. In our study it seems that benefits from cover cropping and no-till are not apparent after only a few years of implementation.

Overall trends in economics seem to indicate that there is often a positive economic impact from cover cropping and compost addition. However, with cover cropping there is much more risk to the farmer. In years where there is adequate rain and irrigation is not necessary, cover cropping provides a significant positive impact. However, in years where there is drought, where irrigation is unavailable or comes at a high price, there is a risk of cover cropping not resulting in adequate improvement in yield to be worth the investment.

We did not see significant benefits in terms of soil health from cover cropping in the short term. With this in mind, we saw the most significant soil health benefits from cover cropping in the long term at WSREC. Overall, it seems that in the short-term legume cover crops can provide adequate economic benefits to justify implementation, but to see significant soil health benefits, it needs to be practiced in the long term.

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End of Chapter 1

Chapter 2

Nitrous Oxide Emissions Remain Low, But Are Influenced by Tillage and Cover Cropping in Sub-Surface Irrigated Annual Production Systems in California

Abstract

Greenhouse gas emissions form agriculture are primarily due to nitrous oxide emissions from soils and fossil fuels used in the production of fertilizer and in farming operations. Tillage and cover cropping affect the structure of soil movement of water, wet-dry cycles, and alter carbon and nitrogen cycles, and no-till has been proposed as a way to reduce fuel use and improve soil health. Implementation of no-till and cover cropping have been shown to impact nitrous oxide flux. However, the direction of the effect varies depending on fertilization, irrigation, and cover crop choice. Therefore, a factorial randomized complete block (RCBD) design with treatments having either the presence or absence of winter cover crops and tillage was undertaken, and greenhouse gas measurements were taken periodically over the course of a three-year study. Results indicate no significant treatment effect, and positive fluxes of nitrous oxide were found to be low in all treatments. Correlation analysis and stepwise linear regression indicate that factors controlling N_2O flux were more closely correlated to water dynamics, fertilization, and season. Our results indicate the value of employing sub-surface drip in these systems not only to decrease water use and improve yields as other researchers have shown, but also to reduce greenhouse gas emissions from these high-input, highly productive agricultural systems.

2.1 - Introduction

Agricultural greenhouse gas (GHG) emissions have been established to be one of the more intractable challenges that agriculturalists must address to prevent further climate warming (Shukla et al., 2019). Not only do GHG have the direct effect of exacerbating an increasingly unstable climate (IPCC, 2013), but they are also an indicator of environmental losses of applied fertilizer; as incomplete heterotrophic denitrification produces nitrous oxide (Bayer et al., 2015; Del Grosso et al., 2008; Horwath and Burger, 2012) a potent greenhouse gas (Lashof and Ahuja, 1990). Yet soils have been lauded as potential solutions to climate change through carbon sequestration (Horwath and Kuzyakov, 2018; Lal, 2011; Minasny et al., 2017; Poeplau and Don, 2015). So, which is it? Are soils a source or a sink for greenhouse gasses? Realistically, the answer is both. It is well understood that plants are a sink for carbon through photosynthetic carbon fixation. However, we also know that cropping systems are often a source of GHG emissions, primarily nitrous oxide (Bayer et al., 2015; Del Grosso et al., 2008; Horwath and Burger, 2012). Nitrous oxide flux from soils has been shown to be highly correlated to specific practices such as irrigation, fertilization, and additions of cover crops (Mosier et al., 1998). In the mediterranean climate of California's San Joaquin Valley (SJV), farming systems are intensively managed using irrigation during the entire growing season, fertilization, and significant tillage operations which has resulted in significant GHG emissions from these soils (De Gryze et al., 2011). However, in the past two decades, implementation of sub-surface drip irrigation in the SJV of California has reached or exceeded 90% for many crops such as processing tomatoes (J. P. Mitchell et al., 2012). Sub-surface irrigation has been shown to increase water and nitrogen use efficiency, reduce pest and disease losses, and improve yields per unit of input (Phene et al., 1992). Therefore, this practice is only being implemented more and more here in California.

In the context of GHG production and sub-surface drip, past research has indicated that GHG fluxes (especially N_2O) from SDI fields are lower than furrow irrigated fields, even when a winter legume cover crop is grown (Kallenbach et al., 2010). But questions remain about whether tillage and cover cropping will increase or decrease GHG fluxes under SDI. It is generally understood that no-till and cover cropping have a positive impact on soil health (Crystal-Ornelas et al., 2021; Kim et al., 2020; Nunes et al., 2020; USDA, 2016b). But do these practices have a positive, negative, or insignificant effect on the production of GHG that could potentially negate the positive impacts? Therefore, questions remain about whether no-till and cover cropping are providing an environmental net benefit in sub-surface irrigated annual production systems in California (Haddaway et al., 2017; Luo et al., 2010; Minasny et al., 2017; Mitchell et al., 2013; Moinet et al., 2023).

Long-term research in Five Points, CA at the UC Westside Research and Extension Center (UC-WSREC) has already offered us a unique opportunity for research to better understand how long-term management can impact soil health (Mitchell et al., 2019b, 2007; Veenstra et al., 2007b), but the associated greenhouse gas implications at this site are so far unknown. What we have learned so far from research at this site is extremely valuable, as it was one of only two long-term research sites in California that are considering conservation agriculture practices with respect to carbon sequestration potential, and associated soil health. Previous research at another long-term managed site in Davis, California, indicate that cover cropping can increase GHG emissions under flood irrigation as mentioned earlier (Kallenbach et al., 2010). However, Kallenbach et al also observed that in sub-surface irrigated systems, nitrous oxide fluxes were reduced significantly. Previous research at WSREC indicates that yields were not significantly affected by the presence of a winter cover crop (Mitchell et al., 2015b). However, maintaining yield may not justify the additional cost(s) of implementing cover-crops, especially if those practices result in greater GHG emissions. The effect of no-till alone or combined with cover cropping on GHG fluxes in this system are also unclear as there is a dearth of data for GHG flux from SDI systems under cover cropping and no-till. The primary goal of this study was to investigate whether changes in GHG flux accompany winter cover cropping alone or combined with no-till. This additional information should help decision makers and stakeholders better understand the full picture of costs and benefits of each management practice alone or in tandem. The main objective of this work was to observe a system that had been managed under cover cropping and no-till for approximately 20 years and SDI fully implemented and determine if these practices increase, reduce, or do not affect GHG fluxes. There is a dearth of information available about tillage and cover cropping's effects on GHG fluxes in this arid, sub-surface irrigated annual cropping system in the highly productive southern San Joaquin Valley of California. This locale is unique in its combination of soil texture, climate, sub-surface irrigation and this research should fill a significant knowledge gap that should be useful for improving practices in the region. So, our objectives were to 1) determine if there was a significant effect of cover cropping and tillage management practices on GHG fluxes, 2) explore correlations between GHG flux and predictive variables, 3) Determine which predictive variables were the most highly correlated to GHG flux and then to discuss findings in the context of past research and future goals to inform management in these highly productive annual production systems.

2.2 - Materials and Methods

2.2.1 - Site Description

This long-term experiment was established to examine reduced tillage and cover cropping and was started in 1999 to demonstrate the effectiveness of conservation agriculture practices in California's intensively managed and irrigated annual crop production systems in the mediterranean climate in California. The study site is located at the University of California's West Side Research and Extension Center (WSREC) in Five Points, CA (36°20′29″N, 120°7′14″W). Soils are mapped as *Panoche clay loam* (87% and similar soils) (*Panoche clay loam series: fine-loamy, mixed, superactive, thermic, Typic Haplocambids*). In 1998 before the study began, a uniform barley (*Hordeum vulgare* L.) crop was grown. At the start of the long-term management, a tomato-cotton rotation was practiced until 2013. To better achieve the conservation agriculture goal of crop rotation diversity, the systems were changed to sorghum (*Sorghum bicolor*) and garbanzo beans (*Cicer arietinum*) in 2014. Management treatments included a factorial arrangement of tillage and cover cropping, including standard tillage without cover crop (STNO), standard tillage with cover crop (STCC), no-tillage without cover crop (NTNO), and no-tillage with cover crop (NTCC). The CC mix consisted of Juan triticale (*Triticosecale* Wittm.), Merced rye (*Secale cereale* L.) and common vetch (*Vicia sativa* L.) (30% triticale, 30% rye and 40% vetch by weight). Between 2010 and 2014, the basic CC mixture was changed to include a greater diversity of species including pea (*Pisum sativum* L.), faba bean (*Vicia faba* l.), radish (*Raphanus sativus*), and Phacelia (*Phacelia tanacetifoli*). Each treatment was replicated four times in a randomized complete block design. Treatment plots consist of six beds, each measuring 9.1 × 82.3 m. Six-bed buffer areas separated tillage treatments to enable the different tractor operations that were used in each system.

The no-till systems were managed from the general principle of trying to reduce primary intercrop tillage to the greatest extent possible. Controlled traffic farming, or zone production practices that restrict tractor traffic to certain furrows were used in the no-till (NT) systems, and planting beds were not moved or destroyed in these systems during the entire study period. The only soil disturbance operations used in the NT systems were shallow cultivation during the first eight years for the tomato and cotton crops. As the project progressed, the NT treatments became true no-tillage systems in 2012 with the only soil disturbance occurring at the time of seeding or transplanting crops and cover crops.

The tomato and cotton crops were furrow irrigated from 2000 to 2012. In keeping with trends in the region toward more efficient systems, the study site was converted to subsurface drip irrigation in 2013 with 34 mm diameter tape buried 30 cm in the centers of each 150 cm-wide rows with 75cm beds. During this study (2018-2020), a different cash crop was grown each summer. Processing tomatoes (*Solanum lycopersicum*) were planted in 2018, Cantaloupe melon (*Cucumis melo var. cantalupensis*) was planted in 2019 and butternut squash (*Cucurbita moschata 'Butternut'*) in 2020.

The climate in this part of the SJV is ideal for production of many crops as it has a very long growing season with essentially no precipitation falling during the growing season. Average seasonal temperature and precipitation can be seen in **Table 1** below.

Table 13 - Average seasonal temperature and precipitation for the Southern San Joaquin Valley between 2006 and 2020, from NOAA Climate Normals Quick Access (accessed 05/14/2023).

Season	Temp (^oC)	Precip (mm)
Winter	9.61	106.68
Spring	17.33	56.38
Summer	26.77	3.3
Fall	18.83	25.4
Annual Avg	25.22	191.77

2.2.2. - Greenhouse Gas Collection and Analysis

2.2.2.1 - Greenhouse Gas Sample Collection

Greenhouse gas samples were collected every two weeks to the extent possible and specifically before and after the first significant rainfall events in the fall. Greenhouse gas measurement was accomplished using a closed static chamber method targeting CO_2, CH_4, and N_2O. Guidelines for the chamber design and sampling follow the protocols set forth in chapter 3 of the USDA-ARS GRACEnet protocol (Parkin and Venterea, 2010). Chambers measured 52.70 cm by 32.39 cm wide and 15.24 cm tall. Chambers were installed directly over the beds crosswise to capture the flux from as much of the bed as possible. A second chamber measuring 17.78 cm by 15.24 cm by 15.24 cm was also place in each experimental unit and gas samples were collected from chambers at the same time. Chamber bases were installed in experimental units and left in the field to the extent possible to minimize disturbance artifacts, and flux chamber tops were installed only for sampling. Sampling took place over one hour at three equi-distant time points, and all chambers are sampled in the same part of the day to reduce temperature effects. 25 mL gas samples were collected with a syringe and transferred to 12.5mL evacuated (<.05 ATM) glass exetainers (Exetainer, Labco Ltd., Buckinghamsire, UK), fitted with new chlorobutyl septa. Gas samples were kept at standard temperature and pressure until analysis was completed in our lab in Davis, CA. Ambient temperature (shaded) as well as soil temperature (15 cm below the surface) at the beginning and end of each sampling event were also recorded and used to calculate gas fluxes.

2.2.2.2. GHG Sample Analysis

The gas samples were analyzed on a Shimadzu gas chromatograph (Model GC-2014) with a 63Ni electron capture detector (ECD) and flame ionization detector (FID) for CH_4 using high purity hydrogen. The GC is linked to a Shimadzu auto sampler (Model AOC-5000). The autosampler uses a gas-tight syringe to remove 2 mL gas from the sample vials and injects it into the GC port. The GC uses as carrier gas a mixture of helium and P5 (mixture of 95% argon and 5% methane. The carbon dioxide (CO2) and N_2O are separated by a Haysep Q column at 80°C. The ECD is set at 320°C and the pressure of the carrier gas flowing into the ECD is 60 kPa. The minimum quantity of N_2O detected by this GC system is 0.1 pg s-1. After the acquisition of the sample, the autosampler's syringe and the GC's sample loop are purged with helium to back flush water and other slow chromatically resolved analytes. Gas standards were prepared before analysis from a minimum of three gas standards in the expected range of the analyte gasses.

2.2.2.3 - Calculating Gas Fluxes

Linear gas fluxes were calculated according to USDA-ARS GRACEnet protocol (Parkin and Venterea, 2010). Linear flux measurements were accepted as linear and used in the model according to the following criteria: $R^2 > 0.9$ for CO_2, $R^2 > 0.9$ for N_2O, and $R^2 > 0.7$ for CH_4. All linear fluxes were converted into mass of gas (g) day^{-1} ha^{-1} using the measured environmental conditions of each sampling date and specific chamber sizes. Then, gas fluxes were calculated using the "GasFluxes" package in RStudio (Version 2023.03.0+386). Outputs of gas flux data are in µmol target gas m^{-2} hr^{-1}. After fluxes were calculated metadata including gravimetric water content (GWC), nitrate content (NO_3-N mg kg^{-1} dry soil) and season were added back to the data set for the creation of linear models. Flux data was not transformed after being calculated and all statistical analysis and modeling was performed on untransformed data.

2.2.2.4 Soil Analysis

In addition to the gas samples, a composite (3 sub-samples) soil sample from the top 15 cm surrounding each chamber was collected at each chamber during every sampling event. Soil samples were analyzed for mineral nitrogen (NH_4-N, NO_3-N) according to (Rhine et al., 1998) and (Doane and Horwáth, 2003) as well as gravimetric moisture content calculated as mass loss after drying at 105°C for 24 hours). These mineral nitrogen and water content data were used to help explain gas flux based on physiochemical conditions present in each sampling event (see GHG Analysis above, Section 2.2.3).

2.2.2.5 - Flux Data Analysis

All data analysis, modeling and statistics was performed in RStudio (R Development Core Team, Version 2023.03.1+446). Analysis of data followed a multi-step process. First, a likelihood ratio test (LRT) was performed to look for treatment effects on response variable (GHG flux) data modeled using the "lmer" function of the "lme4" package with treatment treated as a fixed effect and block as random effect. These models were compared to null models created using the same response variable and block as random effects but excluding the fixed treatment effects. Then the models were compared using the "anova" function resulting in p-values that test the null hypothesis that the more complex model is true over the less complex model (see **Table 1**). Then correlation analysis was completed for continuous variables and their correlation to GHG flux (Soil Nitrate-N, Water Content, and Ammonium-N) using the "cor.test" function comparing the response variable to the predictive variable. Correlation for categorical variables (chamber placement, season, and year) was completed by first converting the variables into factors, then by preforming chi-squared tests on comparison, between the categorical variables and response variables. Finally, multiple linear regressions were completed using the "lm" and "lmer" function to further explore variable connections between response and predictive variables. Significance of treatment effect was determined to be accurate when $\alpha \leq 0.05$ or p-values were less than (0.05), other p-values are shown for interpretation, but make not that many (Nitrate, Ammonium, Water Content, Season, Chamber Placement, etc.) variables were not manipulated and p-values are used to aid in understanding the relationships between the variables and GHG flux.

Table 14 - Likelihood Ratio Test Results (LRT) – Model Type, factors included – F-Fixed Effects, R-Random Effects, Akaike Information Criterion (AIC) scores, Bayesian Information Criterion (BIC) and P-values included. This analysis was used to determine if treatment effect was significant for each of the response variables. Treatment effect was determined to be significant if the p-values were < .05 indicating the model with treatment included fit the data better than the null model with only random effects from treatment block.

Model	Factors (F=Fixed, R=Random)	AIC	BIC	P-Value	Significant? (Y/N)
Null_CO2	Block-R	-3775	-3761.3	N/A	-
Treat_CO2	**Treatment-F, Block-R**	**6044.1**	**6071.5**	**1**	**NO**
Null_N2O	Block-R	-2309.3	-2296.8	N/A	-
Treat_N2O	**Treatment-F, Block-R**	**-2308.8**	**-2283.9**	**0.1338**	**NO**
Null CH4	Block-R	-2775.5	-2763.2	-	-
Treat_CH4	**Treatment-F, Block-R**	**-3759.6**	**-3734.9**	**$<2.2\ e^{-16}$**	**YES**

2.3.0 - Results and Discussion

2.3.1 - Treatment Effects on GHG fluxes

As is indicated in **Table 2** above, there was no significant treatment effect with either N_2O or CO_2 as response variables. These results are rather surprising, as cover cropping and tillage have been shown to have a significant effect on aggregation and surface condition (Haruna et al., 2020) as well as soil moisture dynamics (J. Mitchell et al., 2012) and carbon and nitrogen cycling (Crystal-Ornelas et al., 2021; Veenstra et al., 2007a). However, mixed results from meta-analysis show only weak connection between the presence of cover crop and nitrous oxide flux (Basche et al., 2014; Muhammad et al., 2019) and instead indicate that soil edaphic conditions (clay content, precipitation, nitrogen fertilization, cover crop type) have a greater influence than whether a cover crop is present or not. It is interesting to note that the no cover crop no till treatment has the highest flux, this is difficult to explain with available data. However, results do indicate that fluxes were very low in all treatments, aligning with the previous research showing the mitigating effect SDI has on nitrous oxide emissions (Kallenbach et al., 2010). In this study, the cover crop was primarily non-legume (see site description, **section 2.1**), the soil surface conditions were dry for much of the year (xeric soil moisture regime, sub-surface drip irrigation), and clay content is above average (clay loam). The table below (**Table 3**) shows the mean daily and yearly fluxes by treatment. It is important to note that the average fluxes for all treatments are very low. The largest yearly average flux was calculated to be only 34.6 g ha^{-1} for the no-till, no cover crop treatment (**Table 3**).

Table 15 - Mean fluxes of Nitrous Oxide by treatment. Treatments are cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT) and no cover crop standard till (NOST). SE indicates standard error of mean daily flux. Bolded numbers indicate the treatment with the highest average fluxes over the study period.

Treatment Means for Nitrous Oxide Flux					
Treatment	**Mean Yearly Flux (g ha^{-1})**	**Mean Daily Flux (g ha^{-1} day^{-1})**	**+/-**	**SE of daily flux**	**Observations (n)**
CCNT	13.200	0.036	+/-	0.011	109
CCST	17.300	0.047	+/-	0.013	132
NONT	**34.600**	**0.095**	**+/-**	**0.037**	**107**
NOST	13.000	0.036	+/-	0.010	120

Fluxes of carbon dioxide were also not significantly altered by treatment as can be seen in **Table 2**, but methane flux was. This is interesting as it is well understood that global soils are a sink for methane, which our data agrees with (Curry, 2007; Kirschke et al., 2013), but not in all treatments. As can be seen in **Table 2** there was a treatment effect when considering methane as the response variable. Mean fluxes can be seen in **Table 4** below and weekly average fluxes can be seen in **Figure 2**. Although the fluxes are very small, it is quite interesting to note that all of the no-till and all of the cover crop treatments had negative values. We caution the reader to notice that flux values were only used in the data set when the R^2 values were greater than (0.7). This resulted in differing amounts of data for each plot and could explain the results, as non-linear fluxes are not included here.

Table 16 -Mean fluxes of Methane by treatment. Treatments are cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT) and no cover crop standard till (NOST). SE indicates standard error of mean daily flux. Bolded numbers indicate the treatment with the highest average fluxes over the study period.

Treatment means for Methane Flux					
Treatment	**Mean Yearly Flux ($g\ ha^{-1}$)**	**Mean Daily Flux ($g\ ha^{-1}\ day^{-1}$)**	**+/-**	**SE of daily flux**	**Observations (n)**
CCNT	-0.794	-0.002	+/-	0.002	59
CCST	-0.296	-0.001	+/-	0.003	53
NONT	-0.173	0.000	+/-	0.002	54
NOST	**0.759**	**0.002**	**+/-**	**0.001**	**63**

Table 17 - Mean fluxes of Carbon Dioxide by treatment. Treatments are cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT) and no cover crop standard till (NOST). SE indicates standard

error of mean daily flux. Bolded numbers indicate the treatment with the highest average fluxes over the study period.

Treatment Means for Carbon Dioxide Flux					
Treatment	Mean Yearly Flux ($g\ ha^{-1}$)	Mean Daily Flux ($kg\ ha^{-1}\ day^{-1}$)	+/-	SE of daily flux	Observations (n)
CCNT	**158.300**	**57.820**	**+/-**	**19.978**	**171**
CCST	121.736	44.464	+/-	13.823	183
NONT	85.934	31.388	+/-	11.069	175
NOST	62.853	22.957	+/-	6.845	179

Carbon dioxide fluxes followed an expected pattern of being highest in the cover crop + no-till treatment (**Table 5**). It is impossible to separate carbon dioxide produced from heterotrophic versus autotrophic respiration from soil and so plant root respiration shows up here. Still, no significant treatment effect was observed (**Table 2**).

When viewed graphically, it is clear that nitrous oxide emissions are very low no matter which treatment or season, as can be seen in **Figure 1** (below). In the first few months of observation, a noticeable flux was observed that coincided with the first wetting of the soil in the fall by sprinkler irrigation to establish cover crops. This pattern of a large flux of nitrous oxide occurring after a long dry period has been observed in other studies but was only observed in the first year here. Subsequent sprinkler irrigation in 2019 to establish cover crops did not have the same effect, but a noticeable increase did occur in fall of 2019, but it was not as large of a flux. As has been show in other research, these increases in flux occurred when overhead irrigation increased water filled pore space in the surface of the soil, driving denitrification (Linn and Doran, 1984; Ruser et al., 2006, 2001).

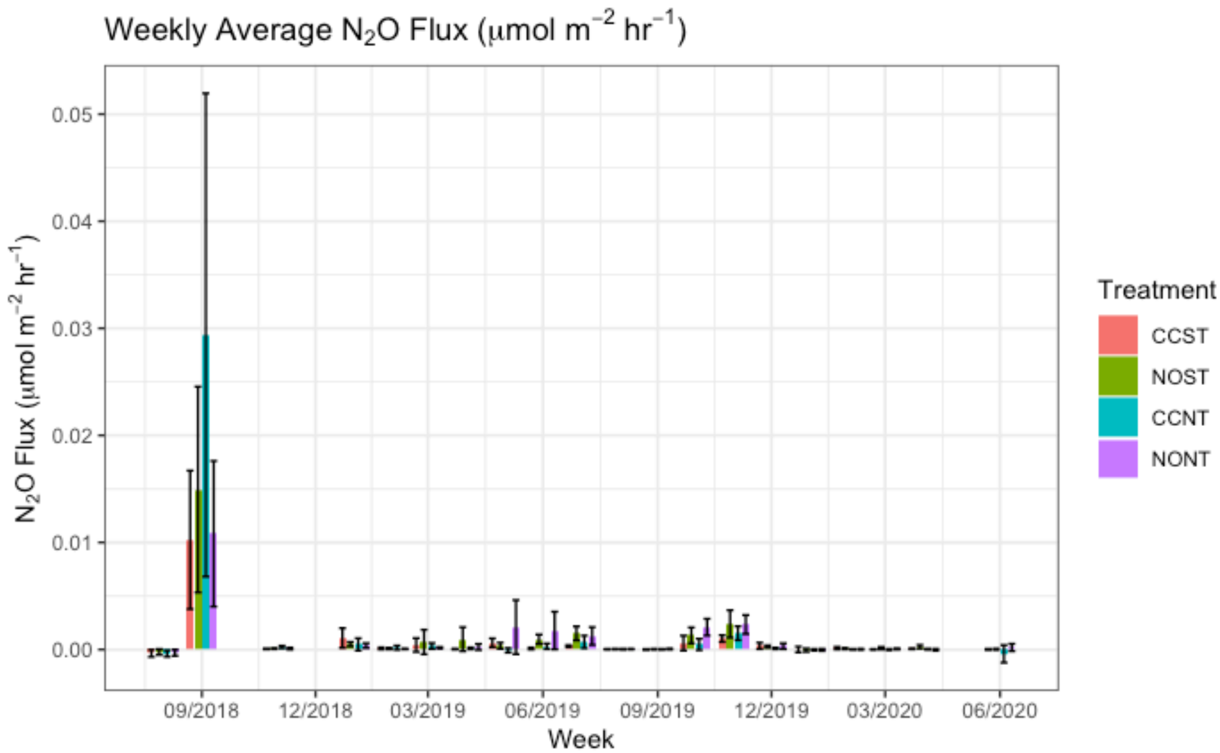

Figure 7 - Weekly average nitrous oxide flux by treatment during the duration of the study period. Gaps indicate times when accessing chambers was impossible due to weather conditions or due to covid-19 lockdowns. Treatments are cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT) and no cover crop standard till (NOST).

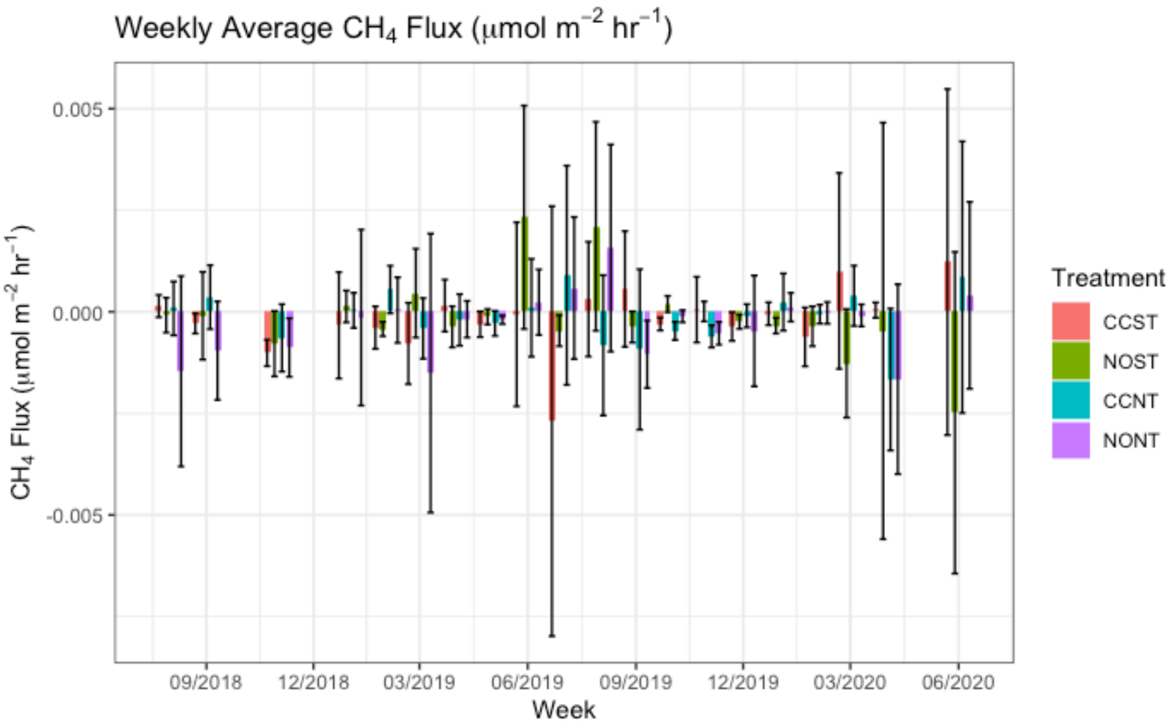

Figure 8 - Weekly average methane flux by treatment during the duration of the study period. Gaps indicate times when accessing chambers was impossible due to weather conditions or due to covid-19 lockdowns. Treatments are cover crop no-till (CCNT), cover crop standard till (CCST), no cover crop no-till (NONT) and no cover crop standard till (NOST).

2.3.2 – Correlation Analysis

As is discussed in Section 2.2.4 above, after determining that treatment effects were not significant, a correlation analysis was completed using correlation matrixes for continuous variables and then Chi-Squared analysis for categorical (factor) variables. These analyses were completed to better understand the factors controlling flux of GHG in this study. Results can be seen in **Tables 6-9** below. As can be seen in Table 6, water content was the most highly correlated with nitrous oxide flux having a positive value of 0.366. This agrees with previous findings (Linn and Doran, 1984; Mosier et al., 1998; Ruser et al., 2006), and indicates that (in this experiment) water content tightly coupled with nitrous oxide flux, as denitrification is an anaerobic process(Groffman et al., 1999) and when diffusion of oxygen is restricted, denitrification proceeds. As can be seen in **Table 9**, none of the categorical variables (chamber placement, season, year) showed a significant correlation when considered with N_2O flux alone. This data again suggests that in sub-surface irrigated systems like this one, that the placement of water and fertilizer below the soil surface is either 1) reducing denitrification to a much lower level or 2) allowing denitrification to proceed completely by the time gasses flux from the soil surface only undetectable N_2 gas is produced. A weaker but notable correlation can also be seen between nitrous oxide flux and concentration of inorganic nitrogen sources (NO_3-N and NH_4-N), which can also be seen in the tables below. This is expected as nitrate and ammonium are both substrates that can be converted into nitrous oxide through denitrification via heterotrophic denitrification (Nitrate) (Groffman et al., 1999; Korom, 1992) and nitrifier denitrification (Wrage et al., 2001).

Table 18 - Nitrous Oxide flux Correlation Matrix - showing the correlation of continuous variables measured at the same time as flux. nitrous oxide (N_2O), gravimetric water content (GWC), nitrate-nitrogen (NO_3), and ammonium nitrogen (NH_4). Values are all between -1 -> 1 and positive or negative numbers indicate the direction of the correlation.

N_2O Correlation Matrix - Continuous Explanatory Variables				
	N_2O	GWC	NO_3	NH_4
N_2O	1.000	0.366	0.091	0.100
GWC	0.365	1.000	-0.092	0.214
NO_3	0.091	-0.092	1.000	-0.006
NH_4	0.100	0.214	-0.006	1.000

Table 19 Carbon Dioxide flux Correlation Matrix - showing the correlation of continuous variables measured at the same time as flux. nitrous oxide (N_2O), gravimetric water content (GWC), nitrate-nitrogen (NO_3), and Ammonium Nitrogen (NH_4). Values are all between -1 -> 1 and positive or negative numbers indicate the direction of the correlation.

CO_2 Correlation Matrix - Continuous Explanatory Variables				
	CO_2	GWC	NO_3	NH_4
CO_2	1.000	0.360	0.079	0.052
GWC	0.360	1.000	-0.092	0.214
NO_3	0.079	-0.092	1.000	-0.006
NH_4	0.052	0.214	-0.006	1.000

Table 20- Methane flux Correlation Matrix - showing the correlation of continuous variables measured at the same time as flux. nitrous oxide (N_2O), gravimetric water content (GWC), nitrate-nitrogen (NO_3), and Ammonium

Nitrogen (NH_4). Values are all between -1 -> 1 and positive or negative numbers indicate the direction of the correlation.

CH_4 Correlation Matrix - Continuous Explanatory Variables				
	CH_4	GWC	NO_3	NH_4
CH_4	1.000	0.021	0.008	0.037
GWC	0.021	1.000	-0.092	0.214
NO_3	0.008	-0.092	1.000	-0.006
NH_4	0.037	0.214	-0.006	1.000

Table 21 - Chi-Squared correlation matrix - showing response variable in the left column, categorical variable tested, chi-squared value (X-Squared), degrees of freedom (df) and probability that this factor is influencing flux (P-Value). These were pairwise tests so only one variable was tested at a time.

Response Variable	**Categorical Variable**	**X-Squared**	**df**	**P-Value**
N_2O flux	Chamber Placement	515	514	0.4793
N_2O flux	Season	2859	2856	0.4807
N_2O flux	Year	1906	1904	0.4828
CO_2 Flux	Chamber Placement	515	514	0.4793
CO_2 Flux	Season	2859	2856	0.4807
CO_2 Flux	Year	1906	1904	0.4828
CH_4 Flux	Chamber Placement	515	513	0.4669
CH_4 Flux	Season	2859	2856	0.4649
CH_4 Flux	Year	1906	1904	0.4699

Results of correlation analysis for carbon dioxide follow an expected pattern (**Table 7**). When soil is dry, less respiration is expected and so when dry soil is moistened, then microbial respiration (as well as plant root respiration), proceeds. A weak positive correlation between CO_2 flux and inorganic nitrogen sources can be explained by the fact that all respiring organisms require nitrogen as well and so when nitrogen is available, then respiration increases.

The correlation between methane flux and treatment was shown to be significant, as discussed earlier (**Table 2**). However, only weak correlations were found in this analysis and suggest that our approach, using only linear fluxes ($R^2 > 0.7$), might be inadequate to capture the relationships between the measured variables and fluxes of methane. Methane fluxes were always small (**Figure 2**) and seemed to have greater magnitude in the warmer seasons. This could be due to faster rates of growth from warmer temperatures, resulting in increased methanotrophy as well as methane production happening simultaneously. Methane fluxes from upland soils are mentioned in the literature (Boeckx and Van Cleemput, 1996), but mechanisms and edaphic conditions that control flux are less known.

2.3.3 - Soil Moisture and Nitrate

As can be seen in **Table 10** (below), soil moisture varied significantly with minimums showing almost no water in the surface soils (~ 1%) and maximums at or slightly above ~30%. This variability in soil moisture was highly correlated to nitrous oxide flux. But as can be seen, moisture content was not highly variable between treatments. Also, as previously mentioned, the nitrous oxide fluxes were extremely low in all treatments.

Table 22 - Moisture content, calculated as a percent of oven dry mass of soil. Maximum, minimum, and average values are shown along with standard errors for all treatments. Treatments are the same as the above tables and figures.

Treatment	Min (%)	Max (%)	Average (%)	+/-	SE
CCCT	1.38	33.33	15.79	+/-	0.52
CCST	1.36	29.66	15.33	+/-	0.52
NOCT	1.43	32.87	13.23	+/-	0.46
NOST	1.59	29.71	14.44	+/-	0.47

Table 23 - Nitrate-nitrogen as measured in the surface 0-15 cm of soil surrounding chambers. Samples were taken at the same time as nitrous oxide flux samples. All values are reported in mg of NO_3-N kg^{-1} of dry soil.

Treatment	Min	Max	Average	+/-	SE
CCNT	0.00	639.14	33.02	+/-	5.21
CCST	0.00	225.53	37.98	+/-	2.94
NONT	0.09	246.81	43.13	+/-	2.62
NOST	0.02	875.60	75.63	+/-	7.09

Soil nitrate showed similar variability from very high to very low concentrations observed. Differences in average nitrate nitrogen observed between treatments could be least partially due to presence and lack of tillage and cover crops. For instance, both of the cover cropped treatments had lower average nitrate-nitrogen than the non-cover cropped treatments. Differences between treatments were shown to be statistically significant with respect to treatment according to simple linear regression using treatment and block compared to a null model where only block (random effects) were used to explain flux (as employed above in section 2.2.4). Subsequent ANOVA comparing these two models indicated that treatment was a significant explanatory variable compared to the null model (p-value = <.001). This can be explained by the fact that the cover crop mixed used here was primarily non-legume and simply utilized more nitrogen to support growth. Conversely, the non-cover cropped treatments had higher average values. With respect to tillage, since fertilization occurred primarily in the sub-surface through the buried drip it makes sense that mixing of the soil through tillage would bring more nitrogen to the surface as can be seen in both of the standard tillage treatments.

Similar analysis with respect to water content, with treatment used as the explanatory variable and compared to a null model, indicate that there is a significant effect of treatment on water content (p-value = 0.038). It is important to remember, the water content and nitrate - nitrogen data we analyzed here is only in the top 15cm of soil surrounding GHG collection chambers and was only collected when GHG samples were collected so may not be the best indication of overall, field level dynamics.

However, even though this data is limited, it still indicates that there are differences with respect to cover cropping and no-till when it comes to water and nitrogen cycling. It has been demonstrated elsewhere that nitrogen fertilization and water filled pore space are strongly correlated to nitrous oxide production (Basche et al., 2014; Del Grosso et al., 2008; Grandy et al., 2006; Horwath and Burger, 2012; Mitchell et al., 2013; Wrage et al., 2001; Zhu-Barker et al., 2019), and so this information can be useful when considering nutrient management, residual nitrogen left after harvest, and the potential for soils to emit nitrous oxide.

2.3.4. - Stepwise Regression – Considering All Variables Together

Our final analysis of the gathered data took the form of multiple, stepwise linear regression to explore collinearity, and correlation in the data that might be useful. In this analysis we built two linear models then compared the p-values for explanatory variables to what we learned earlier. This is similar to the approach of Cynthia Kallenbach and her co-authors (2010). Although we understand that we cannot use this data to address our central question as we did not manipulate these data in our treatments. This data will be useful to other researchers intending to undertake research on nitrous oxide flux from agricultural soils and wish to better control experiments to reduce colinear effects between variables.

The first model included treatment as well as all other measured variables mentioned previously. Including: inorganic nitrogen, water content, season, chamber placement, and year. The second model included all of the above except treatment. We compared these models as we wished to consider treatment effect from another perspective and determine how strong of an effect treatment had when combined with or excluded from other variables. Essentially exploring the colinear relationships in these data. Results are below in **Table 12 & 13** below. Modeling the data in this way essentially tells us how the model changes when different variables are added and removed. Of special interest here is treatment effect. **Table 12** show the ANOVA results for the model when treatment is included as an explanatory variable, and interestingly, when combined with the other data, treatment is shown to be significant (p-value = 0.009). However, as our previous analysis indicated, the p-value for water content was higher, indicating a potentially stronger effect. However, when treatment and year were removed from the second model, subsequent ANOVA (**Table 13**) shows that water content remains as having the strongest connection to explain results, nitrate becomes significant, and the p-values for chamber placement and season both decreases. It should also be noted that the relative proportion of sums of squares these variables, The relative proportion that GWC accounts for in the first table (Table 12) show that, like the p-value, GWC is explaining a large part of the variation in the data. Also notable in the second table (Table 13) this pattern repeats, and nitrate explains large part of the variation in addition to GWC. This analysis agrees with previous findings showing a strong correlation between water content and nitrogen concentration of surface soils and nitrous oxide flux (Horwath et al., 2015; Horwath and Burger, 2012; Kallenbach et al., 2010; Linn and Doran, 1984) and explain the lack of treatment effect in this studied system. The effect of the sub-surface drip on water content in the surface of soils and also the placement of nitrogen below the soil surface had a strong mitigating effect on nitrous oxide emissions.

Table 24 - ANOVA results for stepwise regression with N_2O flux as the response variable and all other measured variables as potential explanatory variables. gravimetric water content, nitrate-nitrogen (NO_3), ammonium-nitrogen (NH_4), chamber placement (Bed/Furrow), Season, and Year. P-values are shown, and significance of effect is also shown (P-Value and Significance). Significance codes are provided in the bottom of the figure.

Analysis of Variance Table - Stepwise Regression W/Treatment					
	DF	**Mean Sq**	**F Value**	**P-Value**	**Significance**
Treatment		8.225 e-06	3.925	0.009	**
GWC	1	1.029 e-04	49.143	3.264 e-11	***
NO_3	1	2.416 e-06	1.153	0.284	
NH_4	1	2.520 e-06	1.202	0.274	
Bed/Furrow	1	1.142 e-05	5.449	0.021	*
Season	3	9.047 e-06	4.317	0.006	**
Year	1	1.130 e-07	0.054	0.816	
Residuals	208	2.096 e-06	-	-	
Significance Codes: "*" 0.001, "**" 0.01, "*" 0.05, "." 0.1 " " 1**					

Table 25 - ANOVA results for stepwise regression with N_2O flux as the response variable and all other measured variables as potential explanatory variables excluding treatment. gravimetric water content (GWC), nitrate-nitrogen (NO_3), ammonium-nitrogen (NH_4), chamber placement (Bed/Furrow), Season, and Year. P-values are shown, and significance of effect is also shown (P-Value and Significance). Significance codes are provided in the bottom of the figure.

Analysis of Variance Table - Stepwise Regression W/Out Treatment					
	DF	**Mean Sq**	**F Value**	**P-Value**	**Significance**
GWC	1	8.879 e-05	40.742	1.07 e-09	***
NO_3	1	8.495 e-06	3.897	0.050	*
NH_4	1	1.800 e-06	0.825	0.364	
Bed/Furrow	1	1.431 e-05	6.567	0.011	*
Season	3	1.058 e-05	4.857	0.002	**
Residuals	212	2.179 e-06	-	-	
Significance Codes: "*" 0.001, "**" 0.01, "*" 0.05, "." 0.1 " " 1**					

2.5. - Conclusions

Overall, the results of this work indicate that cover cropping and tillage did not have a significant effect on nitrous oxide emissions. Water content was shown to strongly correlated to nitrous oxide flux, agreeing with previous work. Overall, nitrous oxide emissions from this system were found to be very low in all treatments, which supports the use of sub-surface irrigation in this region. This data should be useful in the design of future experiments examining nitrous oxide flux from soils in this region under sub-surface drip. We expect that more could be gleaned from experiments that explore the edaphic and climate conditions controlling nitrous oxide flux that we have discussed here.

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End of Chapter 2

Chapter 3

Nitrate Leaching Potential from Composting – Unexpected Fate of Nitrogen in the Vadose Zone

Abstract

The dairy industry in California is the largest of any in the US, producing 20% of the nation's dairy products . Manure from dairy operations results in 195 - 462 g of N $head^{-1}$ day^{-1} of manure nitrogen being produced by 1.7 million lactating cows. These dairies manage manure in a variety of ways, and some producers wish to compost the dairy manure solids. However, the existing research on composting does not compare static piling to composting of dairy manure. A further gap exists with regard to the timing and quantity of nitrate produced from composting of dairy manure and this is important to better assess leaching risk. We conducted field-based research with a commercial composting operation to compare nitrate production in static piles vs aerobic windrow composting at commercial scale. We determined that composting resulted in significantly more nitrate production than static piling of manure; at the end of our 15 weeks study the compost contained 55.74 g NO_3-N kg^{-1} dry matter compared to 3.76 g NO_3-N kg^{-1} dry matter for the static piled manure. However, ammonium and dissolved organic nitrogen levels were higher in the static manure pile through the entirety of the experiment. These constituents can be converted to nitrate in the soil after being lost from the static pile. We then sampled to nine meters below the composting operation to better understand the fate of nitrate produced. Subsequent $\boldsymbol{\delta}^{15}$N and $\boldsymbol{\delta}^{18}$O enrichment in the remaining nitrate indicated that leached nitrate was being attenuated by heterotrophic denitrification in sub-surface soil layers; no appreciable nitrate was observed to leach beyond the sampled depth in this system. Enrichment values ($\boldsymbol{\delta}$15N and $\boldsymbol{\delta}$18O) of nitrate are used as a proxy showing denitrification as the most likely mechanism for attenuation of nitrate. We then confirmed denitrification potential using soil incubations to ensure robustness of results.

3.1 - Introduction

The dairy industry in California is the largest of any in the US, producing 20% of the nation's dairy products (Chang et al., 2005a). Milk sales comprise greater than $6.4 Billion to the California Agriculture market, and California is the largest dairy producer in the United States (USDA, 2017). Manure from dairy operations results in the production of 195 - 462 g of N head^{-1} day^{-1} of manure nitrogen being produced by 1.7 million lactating cows (Chang et al., 2005a). Dairy manure and its management in California are of great interest to producers as dairy manure is a potential source of crop nutrients and organic matter for agricultural and urban soils, but also a concern. The concern is due to the potential of excess nitrogen to negatively impact water and air quality (Cativiela et al., 2019) and the excess of nitrate already present in many California groundwater aquifers (Esser et al., 2011)Click or tap here to enter text..

(Cativiela et al., 2019; Chang et al., 2005a; Meyer et al., 2019a, 2019b, 1997a). Dairy manure is currently managed in a variety of ways, compounding the complexity of the problem of how best to find solutions (Meyer et al., 1997b). With the exception of dry-scrape manure handling, most manure handling involves the use of water to flush manure from pens and collect it, then the solid and liquid fractions are separated using screens or other mechanical means (Meyer et al., 1997a). Then, the liquid manure is used as a nutrient input for dairy cropland on-farm to grow feed/forage; and some is exported off site (with varying degrees of treatment). Currently only a small portion of the manure is composted either on or off farm (Cativiela et al., 2019; Meyer et al., 1997b; Quality and Board, 2015).

Recent state commissioned reports (Cativiela et al., 2019; Quality and Board, 2015) have confirmed that the bulk of mineral nitrogen at-risk for leaching results from the liquid fraction of manure that is usually held in retention ponds (commonly called lagoons). This effluent is mixed with water and used to fertilize fields on or adjacent to the dairy where feed/forage crops are grown. Separated manure solids are commonly dried and reused for bedding for indoor barns known as (dry-packing) or exported for alternative uses off-farm (Cativiela et al., 2019; Chang et al., 2005b; Meyer et al., 1997a; Quality and Board, 2015). Even though it is not the primary source of leached nitrogen from dairies, manure solids still account for a large amount of material from dairy production that has the potential to provide benefit as a soil amendment (Chia et al., 2020; Giusquiani et al., 1988; Huang et al., 2016; Hubbe et al., 2010). However, manure solids may also represent a source of N pollutants to ground and surface water (Cativiela et al., 2019; Lockhart et al., 2013; Maynard, 1993) depending on how it is managed. On commercial dairies, manure solids are typically stored in large piles which can either be managed without disturbance (static) or actively turned to support aerobic microbial respiration (composted). It is important to consider the nitrogen present in the solids fraction and to understand the transformations of manure-nitrogen that occur during composting under these different conditions. Mineralization of organic matter releases ammonium (NH_4-N) which can quickly be converted to nitrate via nitrification under aerobic conditions. Yet, it remains unclear whether composting is more or less of a source of nitrate-nitrogen during and after composting compared to the current practice of static piles on California dairies, indicating a need for research.

Many compost studies only refer to volatilization or denitrification losses of nitrogen during the composting process (Li et al., 2013; Witter and Lopez-Real, 1988; Wong et al., 2017) or only refer to the final concentrations of nitrate in compost with respect to differing compost inputs or methodologies (Bargougui et al., 2019; Bernal et al., 2009; Finstein et al., 1985; Gale et al., 2006; Giusquiani et al., 1988; Hadas and Portnoy, 1994; Maharjan and Hergert, 2019; Mark Buchanan et al., 2001). Gao et al. (2010) have shown the evolution of nitrate as a result of the transformation from organic and ammonium forms to nitrate over the course of composting. Ammonium concentrations decrease and nitrate increases as composting progresses, peaking in the maturity phase (Gao et al., 2010). However, these N transformations have not been studied in California, leading to a gap in knowledge about whether there is significant difference between static piling (standard practice) of dairy manure and composting it with respect to nitrate production (both timing and quantity). Other factors influencing the total amount of nitrogen present in finished compost include the inputs used (Bernai et al., 1998; Bernal et al., 2009)as well as the method of composting that is employed (Maeda, 2020; Meng et al., 2016; Sánchez-Monedero et al., 2001). As little or no information is available about dairy manure composting in California, which requires additional management and inputs compared to static piling, it is critical to understand how it influences the risk of N leaching to ensure that composting dairy waste solids streams can be effectively managed by producers to reduce losses of nutrients.

Furthermore, the fate and transport mechanisms of nitrate leached into soils underlying composting operations in California are unknown as these systems do not appear in the published literature. Existing studies that discuss nitrate leaching in California and elsewhere commonly refer to soil texture and land use as major factors controlling the risk of leaching (Karimi and

Akinremi, 2018; Kurunc et al., 2011) but fail to explain the connections between shallow groundwater (<20 m) and deeper groundwater (50+ m) where agricultural and groundwater wells are actually located. It is generally known that soil texture is often highly variable in alluvial soils especially along alluvial fans and in basins where flooding is the primary source of new material and surface water courses vary significantly over geologic time. Alluvial soils dominate the agriculturally productive land in California, and this textural heterogeneity is expected both across space and with depth. The textural variability with depth can cause significant differences in how water percolates through soils, and as a result the residence time of that water at any given depth. If an aquitard –a less pervious layer of soil—is slowing the movement of water, then this will in-tern effect the quantity of substrates (nitrate is if interest here) that have time to be transformed before the water reaches deeper layers.

Regarding different sources of agricultural N pollution, an important factor is that when denitrification occurs, nitrate is consumed and no longer poses a leaching risk. Denitrification has been found to be a significant loss mechanism of nitrogen from the terrestrial environment, estimated by some researcher to account for 1/3 of all N lost from the unmanaged terrestrial biosphere (Houlton and Bai, 2009). This N transformation is the result of denitrifying bacteria reducing NO_3^- to N_2O, NO_x, and N_2 respectively (Delwiche and Bryan, 2003; Groffman et al., 1999). This process is favored under saturated and anaerobic conditions and is increased when there is an available carbon source (Delwiche and Bryan, 2003; Kozub and Liehr, 1999). It is well understood that denitrification is favored under anaerobic conditions where oxygen, the more favorable electron acceptor, is not available (Delwiche and Bryan, 2003), and is therefore an anaerobic process. Anaerobic conditions are commonly found in wetlands (Huang et al., 2020), bodies of water (Lehmann et al., 2003), groundwater (Böttcher et al., 1990; Chen and MacQuarrie, 2005a, 2005b) and in soils where anaerobic conditions occur (Aravena and Robertson, 1998; Delwiche and Bryan, 2003; Horwath et al., 1998; Qin et al., 2012a).

Stable isotope biogeochemical methods (SIBMs) involve measuring the relative abundance of the stable isotope(s) of a given element and how those ratios compare to standard abundances found in various systems (used as standards) (Chen and MacQuarrie, 2005a; Jones and Dalal, 2017; UC Davis SIF, 2020). When denitrification occurs over long time periods, there is an isotopic signature left behind due to the microbial preference for 14N over 15N (fractionation), and this signature can be used by researchers to evaluate denitrification in a given system relative to the natural abundance of these isotopes (Chen and MacQuarrie, 2005b; "GAMA - Domestic Well Project Site | California State Water Resources Control Board," n.d.; Houlton and Bai, 2009). SIBMs are increasing in popularity as analytical tools and methods are developed and as mechanisms that change the ratio of one isotope to another (fractionation) are identified. Isotope fractionation where the lighter isotope is favored, (i.e. more ^{14}N is used in the process compared to ^{15}N) results in an "enrichment" of the relative abundance of the heavier isotope. Denitrification is one such process that results in and enriched ^{15}N abundance that has been identified and is well studied (Aravena and Robertson, 1998; Böttcher et al., 1990; Lehmann et al., 2003; Waterhouse and Horwath, 2020). This study builds on the work of many of the researchers listed above, using ^{15}N enrichment ($\delta^{15}N$) as an identifying characteristic of denitrification (when coupled with loss of nitrate) that can be observed with the proper isotopic analysis.

3.2 - Materials and Methods

3.2.1 - Experimental Design

This research was carried out at a composting facility in the Hollister/Gilroy area of California (USA) (36.930246 °N,-121.443654 °W). Hollister has a thermic soil temperature regime, and a xeric soil moisture regime. The average high temperature between 2006 and 2020 was 21.78 °C and the average low temperature during the same period was 7.94 °C . The average annual precipitation is 354.08 mm, which mostly comes between the months of November and March ("NOAA NCEI U.S. Climate Normals Quick Access," n.d.). Soils at this site are mapped as 90% *Pacheco* series and similar soils (*fine-loamy, mixed, superactive, thermic, Fluvaquentic Haploxerolls)* (see **Figure 16)**. Two primary treatments were imposed to evaluate the impact of different manure management practices on carbon and nitrogen transformations: static piling (SP) and manure composting (MC). Approximately 12 truckloads (32.9 Mg per load) containing mechanically separated solid dairy manure from a partner dairy in the San Joaquin Valley (California, USA) were dropped at the composting site in early October 2020. The SP treatment was simply piled up in a conical shape and left static and comprised approximately 33 Mg of material (one double tractor trailer's worth).

The MC treatment was placed into a 250 m windrow and wood mulch (made from chipped wood waste) was added as a bulking agent to assist in maintaining aerobic conditions. The mulch was added in approximately the same volume as the dairy manure, following the composting partner's regular protocols. Both treatments were monitored side by side from the beginning of October 2020 (week 1) to the beginning of January 2021 (week 15).

During the course of the experiment, the composting facility implemented standard management in the MC treatment, including watering, turning, temperature monitoring once a week for 15 weeks. Microbial pathogen and heavy metal testing were completed at the end of composting, as required by federal and state guidelines. In late January 2021, a third treatment was begun. This treatment contained a mix of four manure types (horse, poultry, rats/mice, herbivore zoo animals) vegetable culls, grape pomace, and wood mulch and is hereafter referred to as mixed manure compost (MMC). The MMC treatment was managed in the same fashion as the MC treatment for ~15 weeks and finished in late April.

3.2.2 - Compost Sampling and Analyses

Compost samples were collected from the SP and MC weekly between weeks 1-4 and biweekly from weeks 5-15 to monitor the changes of carbon and nitrogen pools. For the MMC treatment, compost samples were only collected at week one and fifteen. For the MC and MMC treatments, compost windrows were divided into three equally sized lengthwise sections, and in each section, ten sub-samples were collected from randomized locations along that section and mixed together as one composite sample, sampling enough to fill a one-gallon sealable plastic bag. Samples were collected using a 0.5 m core at each sampling location. The SP treatment was a conical pile, so these sub-samples were collected in a differently than the windrows, with the pile divided into three sections like sections of a circle. Samples were taken following each compost turning event so compost materials were expected to be well mixed (in the composted treatments), nevertheless, sub-samples were still collected from the outside and into the pile to a depth of 50 cm into the pile. In the SP treatment a composite sample was taken in the same manner. However, the SP treatment had a conical shape and so sub-samples were taken in a circular pattern in evenly-spaced replicates around the circumference, but to a deeper dept of at least 1 m (this pile was much larger and became more anaerobic from the outside into the pile).

MMC samples were only collected in the first week and the final stage (week 15) of the composting process to characterize the input and outputs from the composting process and therefore do not appear in most figures.

During much of the composting process the samples from all treatments were hot or warm to the touch after collection (thermophilic) so no refrigeration was used until samples reached the lab. Samples were then stored in a cold room at 4^{o}C until further processing. Extractions of compost samples occurred within 5 days after collection and subsequent analysis occurred within 10 days after extraction. Samples were extracted with DI water and then analyzed for mineral nitrogen (NO_3^--N, NH_4^+-N), total dissolved nitrogen (TDN) by persulfate digest, total organic carbon (TOC), and total carbon and nitrogen by combustion. See Table 1 below for methods of analysis. Samples were also dried in a 105^{o}C oven and mass loss was measured to determine moisture content. All reported values are adjusted for moisture content and reported on a dry mass basis.

Compost samples were collected from the composite sub-samples and dried to 105^{o}C for gravimetric water content analysis (mass lost upon drying). Un-dried samples were filled into specimen cups and the cups were dropped from a height of 10 cm to pack the material slightly, more material was added to fill the container and the process was repeated 3 times for each sample. After packing, the material that exceeded the known volume of the specimen cups was removed and the sample and container were weighed. The bulk density is a calculation of mass over volume, and so the moisture adjusted dry mass of the compost was divided by the volume of the cup to give the bulk density of the material. This is an adapted method from the bucket-drop method (TMECC, 2011) cited in (Breitenbeck and Schellinger, 2004).

3.2.4 - Soil Sampling

To better understand the mechanisms occurring with respect to soil nitrogen leaching in this composting facility, deep (9 meter) soil core sampling was undertaken. A Geoprobe® push-drill system (Geoprobe Systems, Salina, KS) was used to collect a total of fifteen (five per transect) equally spaced soil cores split into three transects to nine meters depth. Transects passed through the center of the composting facility, a hay field adjacent to the facility directly to the east, and an unmanaged fallow field directly to the west across the road. See Figure10b for locations. All transects are mapped as the same map unit which is comprised of 90% Pacheco series (Fine-loamy, mixed, superactive, thermic Fluvaquentic Haploxerolls). These two additional fields (Hay Field and Control) were used as controls as they were outside the composting yard footprint. The Hay Field did have compost applied at a rate of ~8t yr^{-1} for the preceding 15 years. Sampling was completed on March 16^{th}-17^{th}, 2021, and the remaining on September 20^{th}-23^{rd}, 2021. Compost is regularly applied to this site, but a hay crop is grown and removed for sale each year. No mineral nitrogen fertilizer was ever applied to this field according to the land manager. However, potassium was applied on a semi-annual basis. The fallow field had not been managed for >20 years according to the property owner, but >20 years passed had been managed for alfalfa hay and annual row crops including processing tomatoes.

Soil cores were collected and kept cool during the transportation back to the lab where they were immediately removed from plastic tubes and sub-sampled, separating samples by depth sections as follows: 0-30 cm, 30-60 cm, 60-90 cm, 90-120 cm, 120-240 cm, 240-360 cm, 360-480 cm, 480 -600 cm, 600-720 cm, 720-840 cm, and 840-910 cm. This made for a total of five replicate cores for each of the three transects, all to a depth of nine meters. Cores were taken from the field and stored at ambient temperature for transport back to the lab where they were stored at 4˚C until sub sampling. Subsamples were collected from cores and extracted with 2M KCL and analyzed for mineral nitrogen (NO_3-N and NH_4-N), dissolved organic carbon (DOC) and total dissolved nitrogen (TDN). Total dissolved organic nitrogen was calculated as the difference between total dissolved nitrogen and inorganic nitrogen (Table 1). After sub sampling and extracting, the first set of cores (March 2021) were frozen. The second set of cores (September 2021) were not frozen but kept at 4˚C until incubations were complete.

3.2.5 - Denitrification

Potential denitrification incubations were performed using soils from September 2021 according to the protocol developed by Groffman et al (1999). In brief, incubations included samples from the center and end of each transect to show the variability across space, separated into four depths (0-30cm, ~ 3 m [at the first aquitard], 6 m, and 9 m). The 15 g samples were added to sealable glass 30 ml vials which were evacuated and purged with high purity nitrogen gas three times with three volumes of gas to remove oxygen. Then, three treatments: 1) Pure DI water, 2) 100 mg L^{-1} glucose, and 3) 100 mg L^{-1} glucose and 40 mg L^{-1} KNO_3 were made into aqueous solutions. Treatments were added (9mL) to each vial and the incubation timing began. Incubation was undertaken at ambient lab temperature (~23^{O}C) for six hours on a reciprocal shaker at 125 RPM. Headspace gas samples were collected at 30 min, 60 min, 90 min and 360 minutes after treatments were added. This method was used to identify if the soil in question had the adequate microbial life and substrates to allow denitrification to proceed. This is used as additional evidence to support our hypothesis of denitrification. I.e., if there is isotopic evidence as well as evidence that denitrification is possible through the incubations, then denitrification is the most likely explanation for the disappearance of nitrate. The gas samples were then analyzed using gas chromatography (Shimadzu GC 2000) to detect concentrations of nitrous oxide, within two days of the incubation being completed. To better understand the mechanisms of nitrate-N dynamics seen in the system, extractions were also analyzed for $\boldsymbol{\delta}^{15}$N and $\boldsymbol{\delta}^{18}$O of nitrate using the bacterial denitrification method at the UC Davis Stable Isotope Facility (UC Davis SIF, 2020). Briefly, after being analyzed for nitrate, the samples were filtered to 0.2 μm using Nalgene® Syringe Filters (surfactant free, acetate and cellulose acetate, 25mm). Isotope enrichment (δ) values were calculated for soil core sample extractions by the UC Davis SIF using appropriate standards and validated methods (UC Davis SIF, 2020). This data is presented without any further analysis, only calculating the standard error between samples for clarity and presentation in figures.

3.2.6 - Data analysis – Composting Experiment

Considering the nature of the data we collected in this experiment; we did not believe it would be appropriate to limit our approach by only use parametric statistical methods. Working on commercial compost operations dictated an experimental design where large compost piles were not replicated. Instead, replications were obtained from different sections of each pile, consistent with previous literature. However, this sampling strategy potentially violates the assumption of independence between replications when using parametric statistics. Therefore, non-parametric statistical methods were employed in addition to parametric methods. Parametric tests were completed first using the "anova" function used on a linear model created with the "lm" function of the data with Nitrate as the response variable and treatment as the factor potentially explaining the results. Following this the Kruskal-Wallis one-way ANOVA by ranks was used as a non-parametric alternative to add to the statistical evidence.

3.2.7 - Data Analysis – Fate of Nitrate

Statistical modeling and analysis are not used on the soil core extracts as our objectives were not necessarily to test differences between treatments but only to identify evidence of denitrification or lack thereof in our studied system to attempt to uncover a mechanistic explanation for our data. Error bars in the graphs represent the standard error for each set of replicates, calculated using the equation $SE = \frac{SD}{\sqrt{n}}$. Isotopic data are expected to have significant variability due to heterogeneous leaching patterns and therefore, uneven denitrification patterns across space in the field. If these patterns were to be modeled, we recommended that many more sites would be included with varying precipitation patterns, soil textures, and nitrate loading quantities. Future research could use isotopic tracers or other methods that would be more possible to parametrize and make further assumptions about, justifying additional statistics and more specific hypothesis-driven objectives.

3.3 - Results and Discussion

3.3.1 - Carbon and Nitrogen Transformations—Composting vs Static Piling of Dairy Manure

The mass and volume of composted materials decreased with time (**Table 2**). We determined that the MC and MMC treatments lost 15.79% and 26.67% of their mass respectively. Meanwhile, the SP (uncomposted) treatment lost only 8.79% of the initial mass. The much higher amount of mass lost in the compost treatments is consistent with what other researchers have observed (Breitenbeck and Schellinger, 2004). This likely due to higher microbial activity in the compost piles under aerobic conditions as well as the mechanical turning itself which provides oxygen and increases diffusion losses of volatile compounds.

Losses of carbon and nitrogen in both treatments resulted in lowering of the C/N ratio of both treatments (**Figure 6**). The compost treatment maintained a higher C/N ratio throughout the experiment due to the added wood chips. Visual inspection and subsequent analysis of the static pile treatment showed a consistently higher moisture content through the composting process. This is important when considering the potential of constituents to become mobilized or dissolved in rainwater and the overall risk of leaching to the soil underneath. As it would take significantly more water to wet-up a drier compost pile enough to result in leaching (Webber et al., 2011).

The change in C and N pools over time was analyzed in water extracts since they most closely resembled leaching rainwater, the major transport pathway from compost piles to soil. Results indicate that the primary form of nitrogen found in the treatments was dissolved organic-N, with concentrations about an order of magnitude greater than that of NO_3^--N and about twice as high as NH_4^+-N at the beginning of the composting period (**See Figures 2-5**). This is not very surprising as it is well understood that manure nitrogen largely comes from urea and ammonified urea which are byproducts of digestion and metabolism (Nennich et al., 2005) but does offer some insight into the transformations that we expected and the potential risk of leaching nitrogen. Nitrate is more readily leachable and therefore the primary concern, but organic nitrogen and ammonium can also leach and nitrify upon entering the soil depending upon edaphic conditions. Future work should continue to monitor for high dissolved organic-N concentrations in manure piles.

We expected that organic nitrogen (DON) and ammonium (NH_4^+) forms of nitrogen would be the most prevalent in both the compost and static pile manure at the beginning as they were made from the same starting materials (**Figures 3-5**). This was found to be true with DON levels at 806 mg kg^{-1} for the SP treatment and 577 mg kg^{-1} for the MC treatment respectively at the beginning of the experiment (**Figure 4**). The difference is expected to be from some of the dissolved constituents sorbing to the added wood chips in the MC treatment. Ammonium levels were high compared to nitrate, which is expected considering the manure source of nitrogen, as discussed above (**Figure 3**). The MC treatment lost much of the ammonium pool of nitrogen by week six of composting with values ranging from 70.26 – 276.27 mg kg^{-1} NH_4^+-N at the beginning, to .88 – 2.03 mg kg^{-1} NH_4-N at the week 7 sampling (**Figure 3**). This fraction was likely lost during compost turning due to volatilization which is the primary loss pathway of nitrogen from composting systems (Witter and Lopez-Real, 1988; Wong et al., 2017; Zhang et al., 2016) (see **Figure 3**). However, the SP treatment maintained higher ammonium levels until around week seven when they also dropped. This indicates that volatization losses may exist even if materials are not turned or could also mean that ammonium forms of N are being converted into organic forms of nitrogen by microbial action. Volatization of ammonia from the static piling as well as volatile organic compounds (VOCs) appeared to be emitted from the SP treatment throughout the experiment based on the noticeable smell during each sampling event (anecdotal). The compost treatment did not have a significant smell in contrast (also anecdotal). Presence of VOCs in manure leachate are of concern in addition to nitrate as many of these compounds have a very unpleasant smell and can pose a health and environmental risk (Domingo et al., 2015; Wania, 2003). Although the presence of VOCs and other airborne losses were not quantified, this is another reason why further research about the differences between these two management regimes might prove useful. If aerobic composting results in little to no smell, then placement of composting facilities might help alleviate the significant issues with odor that dairy producers face (Brinkley and Vitiello, 2014) especially when these dairies are located near populated areas.

Nitrate-nitrogen followed a more regular pattern and was only found in low concentrations (<20 mg kg^{-1}) in both treatments for much of the duration of the experiment (**Figure 2**). Nitrate levels did not change significantly until the final sampling at week fifteen where the levels of nitrate in the compost treatment rose from <10 mg kg^{-1} to > 50 mg kg^{-1}, showing more than a five-fold increase. Differences were detected over time (week) and between treatments with both parametric and non-parametric ANOVAs (**Table 3**). This data supports our hypothesis that nitrate is primarily produced in the final "maturation" phase of composting. In contrast to the CP, less than 5 mg kg^{-1} of nitrate was detected in the SP treatment throughout the experiment. This is consistent with the static piling not resulting in significant alteration to the forms of nitrogen due to lower microbial activity, even after significant time. The presence of such high levels of DOC, DON, and the anaerobic nature of much of the pile indicate that the SP manure is still relatively unprocessed (**Figures 4 & 5**). Since SP manure is not "mature compost", it would potentially pose a significant risk to young crops (Bernai et al., 1998) and also a potential source of human pathogens (Li et al., 2020)

The dissolved organic carbon (DOC) found in extracts followed an expected pattern given the water content and nature of treatments (**Figure 4**). Throughout the experiment the values were consistently higher in the SP treatment (mean during 15 weeks of composting= 6745.9 mg kg^{-1}), compared to (mean during composting = 3903.8 mg kg^{-1}) for the MC treatment. This is expected as the DOC values and the DON values both followed a similar pattern, and the variability of both can be attributed to pile heterogeneity (inherent variability) in the windrow and pile we sampled from.

The results for DOC and DON highlight that both the windrow composting as well as the static piling resulted in leachable (extracted with water only) carbon and nitrogen, although the forms and quantity of nitrogen differ between management regimes. Considering that dissolved organic nitrogen and ammonium forms of nitrogen are both expected to mineralize and then nitrify relatively quickly upon entering and unsaturated soil system (Silver et al., 2018), the total amount of leachable nitrogen (both organic and mineral forms) is of greatest interest to understand the environmental risk of N pollution in this study. The availability of adequate dissolved carbon could also play a significant role in these systems, as the liable carbon (DOC) might result in increased immobilization of nitrogen by way of larger microbial biomass. We determined that the quantity and forms of nitrogen present in the static piled treatment pose a greater environmental risk. Even though this treatment did not produce as much actual nitrate, the total amount of dissolved organic nitrogen plus inorganic nitrogen was much greater. Since organic forms of nitrogen are expected to mineralize into organic forms and then nitrify in the surface soils where oxygen is not limiting, this organic nitrogen is going to be converted into nitrate that will then become an environmental concern. Therefore, composting, although it produces some nitrate in the maturation phase is in fact an improvement on the static piling practice.

3.3.2 - Fate of Leached Nitrate from Composting – Where is the Nitrate Going?

Soil nitrate concentrations are presented separately from the two different coring events in March of 2021 and September of 2021. The initial (March 2021) soil coring uncovered a surprising pattern with respect to nitrate concentrations below the compost facility. As can be seen in **Figure 7**, nitrate concentrations were highest at the surface of the compost yard area (190.8 mg kg^{-1}) but decreased with depth exponentially and by ~4 meters depth the concentration dropped to <1 mg kg^{-1}. Compared to the adjacent hay field which had only 5.7 mg kg^{-1} of nitrate at the surface, increasing to 85.9 mg kg $^{-1}$ in the depth section from 0.5 to 1 meter, then decreasing exponentially to just 3.4 mg kg^{-1} by 4 meters depth (**Figure 7**). Ammonium nitrogen concentrations were low through the whole soil profile, ranging from ~2 to ~5 mg kg $^{-1}$ in both the compost yard area as well as the adjacent hay field (**Figure 8**). Dissolved organic carbon (DOC) followed a similar pattern to the nitrate (**Figure 10**). In the compost area we expected carbon to have leached from the composting operation into the underlying soil, this is what we observed. As can be seen in **Figure 10**, DOC concentrations were higher in the compost area at the surface (0-1 m depth) at 97.8 mg kg^{-1} but decreased by a factor of 2 by 2 meters depth to ~ 40 mg kg^{-1} and remained at approximately that level through 9 meters depth. DOC concentrations in the adjacent hay field followed the same pattern as the compost yard samples from 1-9 meters depth. But were not elevated at the surface (39.7 mg kg^{-1} from 0-1 m depth) which is consistent with the management regime, as there were much smaller and less frequent inputs of carbon into this system.

The combination of nitrate and dissolved organic carbon leaching into the surface of the compost yard area indicated that (1) nitrate was being produced in adequate quantity from the compost operation to leach and create nitrate loading to surface soil layers that might continue to leach deeper in the profile, (2) considering the un-saturated nature of this soil (observed at the surface), we predicted that anaerobic conditions would not be present, even in the sub-soil, the would have favored denitrifying conditions. However, after studying the pattern of nitrate concentrations decreasing to background levels with increasing depth, this prediction was challenged. When coring was taking place, we did observe obvious stratigraphic differences in texture with depth. These soils are alluvial, and so patterns of flooding and erosion result in deposition of different soil textures was expected.

Upon dissecting the deep soil cores for analysis, it was clear that the surface texture of the soil did not continue to be consistent with depth and strata of clay dominant layers, strata of coarse sand, and more clay with increasing depth were observed. After closer examination of the mapped soil textures from this area, it appeared that the proximity to Tequisquita Slough had resulted in significant variation in the texture of these alluvially deposited soils and that these sub-surface clay strata were having an outsized effect on the movement of water. Therefore, we posit that the dissolved nitrogen and carbon contained therein could be affected by this 'perched' water table. Thus, water was in fact leaching, but only to a few meters' depth. After reaching the clay-rich aquitard, water movement appeared to have slowed and allowed microbial processes to remove oxygen, resulting in anoxic conditions that then would favor denitrification. Denitrification is expected under anoxic conditions found in wetlands (Horwath et al., 1998; Huang et al., 2020; Kozub and Liehr, 1999) and in deep groundwater (Aravena and Robertson, 1998; Chen and MacQuarrie, 2005a) and even deep in lakes (Lehmann et al., 2003) but was not expected here due to the proximity to the surface and apparent lack of extended aquic conditions. However, other researchers have found evidence of denitrification in the vadose zone and attribute it to soil texture and resulting residence time (Lenhart et al., 2021). Although our data shows even more complete denitrification, we expect the same mechanisms are at play. This is consistent with and could explain the variability of nitrate concentrations found in deep groundwater elsewhere in California (Esser et al., 2011; Miller et al., 2020). If this phenomenon of nitrate attenuation in the vadose zone could be accurately predicted using existing soil texture data it could be very helpful in protecting groundwater from nitrate pollution.

Soil core samples were extracted and analyzed for nitrate, then the extracted nitrate was analyzed using the SIBMs described above to determine if an enrichment signature had been left behind that would indicate denitrification. As can be seen in **Figures 11-15**, both of the coring events uncovered the same pattern. Nitrate was being attenuated as it percolated down through these soils (evidenced by decreasing concentration). We deduced that soil nitrate was being attenuated as it leached from the surface downward, and this was occurring not only in the compost yard where there was an excess of nitrate and dissolved carbon leaching, but also in the adjacent hay field and control fields where nitrate loading was much less. The level of enrichment that was observed exceeded any we could find in the literature under similar conditions and was observed not only in the cores below the compost operation, but also in the neighboring hay field and control field (see Figures 11-15). The high levels of enrichment observed at this site do not necessarily mean that this level of denitrification is not occurring elsewhere, as the soil conditions were not obviously abnormal. But rather indicates that this mechanism is not well documented in the literature and that this research should be duplicated. Soil texture for all three sample sites were mapped as the same map unit (Pc, 90% Pacheco Loam, see Figure 16). Delta values for the enrichment of 15N were observed to be low at the surface of all soils (20-30 ‰). But then between 3- and 5-meters depth (this varied across replicates and treatments slightly due to wavy abrupt boundary present between strata) the values increased by a factor of 2-6 to a maximum mean value of 127.5 ‰ at 6- meters in the compost yard and a maximum mean value of 307.2 ‰ in the hay field treatment (**Figures 11-12**). These values are all from the initial core sampling in March of 2021. The subsequent core sampling that occurred in September was undertaken to add validity the result we had previously observed and add additional data points to potentially address the variation in the delta values we had previously observed (**Figures 13-15**). We had significant standard error between cores in the same treatment as can be seen in **Figures 11-15**, with standard error of ~ 1-2 at the surface, but increasing to as much as ~178 (6-7 m depth) in the hay field treatment (numbers from March Cores **Figures 11-12**). The second coring event confirmed previous findings, but the standard error between cores was only found to be more variable. We observed standard error in the second set of cores to be as high as 300-600 (**Figures 13-15**). This may initially seem concerning due to the inability of the data to predict a specific enrichment value for any one core in this dataset. However, the variation makes sense when preferential flow patterns that are expected in alluvial soils especially under Mediterranean (conditions with periods of dry followed by saturated conditions) are considered (Fuentes et al., 2015). Considering the spatial variability of nitrate transport into the subsurface, it is reasonable to expect that if leaching nitrate concentrations are variable across replicates, then the subsequent denitrification that occurred would also be variable across replicates. Therefore, we believe that the data, although variable, does still establish a clear and consistent pattern in this soil system. This pattern is one that shows that nitrate does in fact leach through the loam textured surface soils. But when leachate reaches these aquitard layers in the subsurface, denitrification is favored, and the nitrate is attenuated to normal (near zero) background levels.

To validate these findings, a subset of the core samples from the second collection (September 2021) were used to complete a potential denitrification incubation **See Figure 17**. Incubations were completed in early 2022 on soils that had been exposed to oxygen during storage. Even with this significant disturbance to edaphic conditions that were present in situ, and after removing and storing these cores for several months, the soils still contained the microbes (or at least the enzymes) necessary to denitrify nitrate. Determining denitrification potential using the acetylene inhibition method is often criticized for underestimating denitrification (Qin et al., 2012b; Rudolph et al., 1991). We measured denitrification by the production of N_2O and observed N_2O production in almost all incubation treatments and from all field samples, even the treatments where only water was added. Furthermore, this method was used only used to support our results observed from our other data, and we determined that this is robust enough to serve that function. We identified denitrification potential in all of the studied soils and expect, given the potential underestimation discussed above, that denitrifications rates could be much higher than we observed.

3.4 - Conclusions and. Implications and Future Research

Compost operations do potentially pose a risk to underlying groundwater, as nitrate-nitrogen and other forms of leachable nitrogen are produced during the composting process. However, we also established that there are circumstances where edaphic conditions can result in almost complete attenuation of nitrate through denitrification.

There are significant implications stemming from these results. We consider this as an early study identifying denitrification in the subsurface, and the denitrification resulting in not just the reduction of nitrate concentrations, but the reduction of nitrate levels to almost undetectable levels. The mechanism we have identified could potentially be used to determine areas of greatest risk of leaching of nitrate, and therefore, the areas of lesser risk; making placement of manure handling operations or composting facilities less likely to result in nitrate leaching into groundwater resources.

Our study adds to the existing body of research regarding C and N changes during manure composting and specifically addresses the differences between static piling and composting of dairy manure. We established that composting resulted in significantly more nitrate being produced, and the nitrate was produced primarily after the thermophilic phase and increased as compost matured. However, considering both dissolved organic and inorganic N forms, the static pile posed a bigger risk for N pollution. Furthermore, we identified that dissolved carbon and nitrogen (both mineral and organic forms) could be leached into soils underlying the studies compost operation. We then demonstrated the connection between the attenuation of nitrate (show by decreasing concentration with depth) and denitrification (show with stable isotopic methods) happening in the vadose zone. We then confirmed our result further by establishing that soils underlying this compost facility contained the microbial potential to denitrify nitrate. We believe that the latter is a topic worthy of further study, especially in California where we have an abundance of not only sources of potential nitrate pollution, but also, extremely texturally variable soils both across space and depth. This textural heterogeneity can be a detriment (if nitrate sources are placed atop course textured soils). But, as we show, can help purify waters if conditions are suitable at depth. This means that researchers should consider not just the surface of soils present in any given system, but also what conditions exist in the sub-soil layers.

3.5 - Figures and Tables

Table 26 - Methods of physiochemical analysis. Analyte, method citation and units are shown.

Parameter	**Protocol Source/Citation**	**Resulting Unit**
Nitrate- Nitrogen (NO_3^-)	(Doane and Horwáth, 2003)	mg kg^{-1}
Dissolved Ammonium- Nitrogen (NH_4-N)	(Rhine et al., 1998)	mg kg^{-1}
Total Dissolved Carbon (DOC) in compost extracts	Horwath Lab Developed Method using (analytik jena multi-N/C 3100)	mg kg^{-1}
Total Dissolved Carbon in Soil Core Samples (DOC)	Horwath Lab Developed Method using (analytik jena multi-N/C 3100)	mg kg^{-1}
Total Dissolved Organic Nitrogen by Persulfate Digest (DON)	(Cabrera and Beare, 1993)	mg kg^{-1}
Total Carbon and Nitrogen in solid samples by combustion (TC/TN)	Horwath Lab Developed Method	mg kg^{-1}
Bulk Density of Compost Samples (BD)	(Thompson, 2001) cited in (Breitenbeck and Schellinger, 2004)	g cm^{-2} kg m^{-3}

Temperature of Compost during composting (T °C)	(Natural Resources Division - Cal ReCycle, 2021)	°C

Table 27 - Changes in physical properties (volume, bulk density, and total mass reported in standard SI units) of the dairy manure subjected to the different composting treatments. Windrow (WR). Manure Compost (MC), MMC (Mixed Manure Compost), Static Piled Manure(SP).

Stage	Treatment	WR Length (m)	WR Height (m)	WR Width (m)	WR Volume (m^3)	Bulk Density ($g\ cm^{-3}$)	Bulk Density ($kg\ m^{-3}$)	Mass of Pile (Mg)	% Mass Lost
Start	MC	173.13	1.90	2.00	328.94	0.14	140.00	46.05	
End	MC	173.13	0.80	2.00	138.50	0.28	280.00	38.78	15.79
Start	MMC	179.83	2.00	3.50	629.41	0.18	180.00	113.29	
End	MMC	179.83	0.80	3.50	251.76	0.33	330.00	83.08	26.67
Start	SP	9.11	3.20	4.56	69.68	0.14	140.00	9.76	
End	SP	9.11	2.28	4.56	49.47	0.18	180.00	8.90	8.72

Table 28 -Statistical Results for Composting vs. Static Piling of Manure. Predictive and response variable(s) are shown. Parametric and non-parametric approaches with accompanying P-Values are shown.

Models and Statistical Results - Compost vs Static Pile				
	Linear Model	**Predictive Variable**	**Response Variable**	**P-Value**
Parametric ANOVA	Model 1	Treatment	Nitrate	0.005142**
	Model 2		Dissolved Organic Nitrogen	1.96 e-05 ***
	Model 3		Dissolved Organic Carbon	3.93 e-07***
	Model 4		Ammonium	0.003245**
Kruskal Wallace ANOVA	Model 1	Treatment	Nitrate	0.02864*
	Model 2		Dissolved Organic Nitrogen	9.57 e-05***
	Model 3		Dissolved Organic Carbon	4.743 e-06***
	Model 4		Ammonium	0.002843**
Significance Codes: "*"=.001, "**"=.01, "*"= .05, "."=.1**				

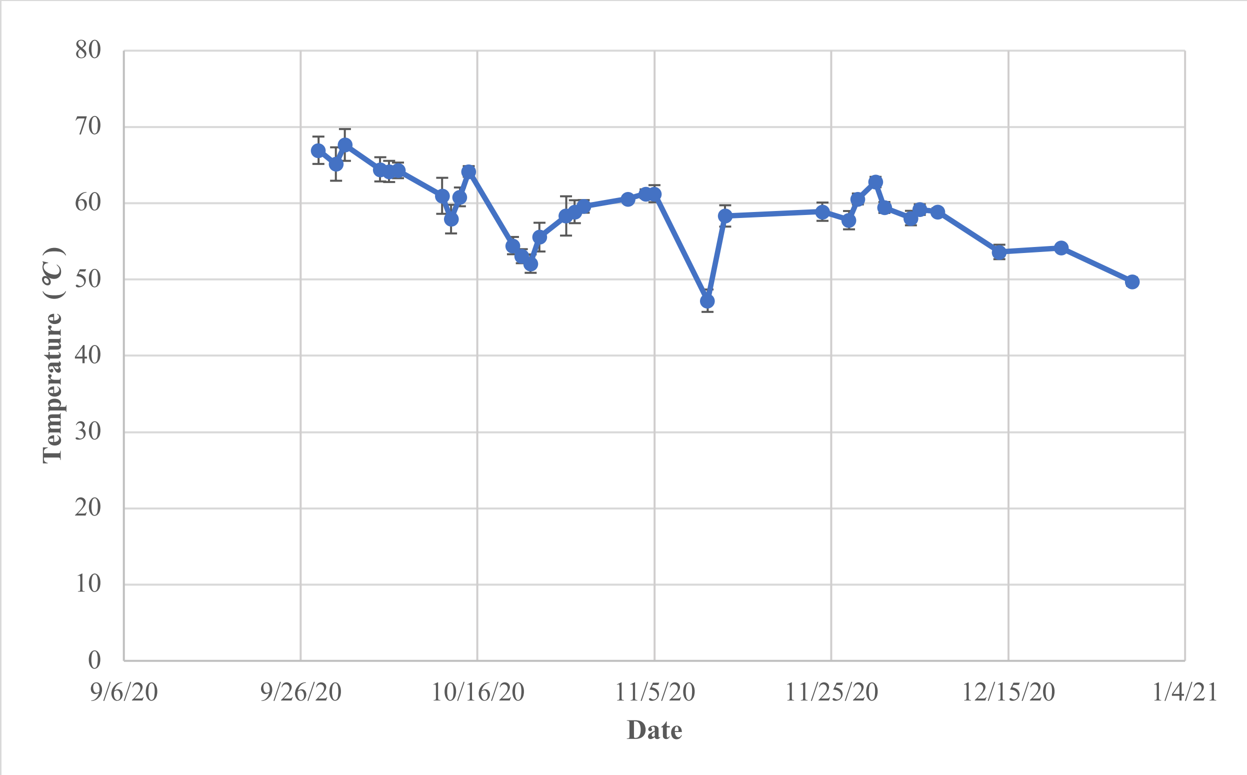

Figure 9 -Evolution of compost temperature (ºC) during the first 10 weeks of composting. Multiple temperature readings were taken each week to fulfill the state and federal reporting requirements governing minimum temperature requirements for composting, error bars represent standard error.

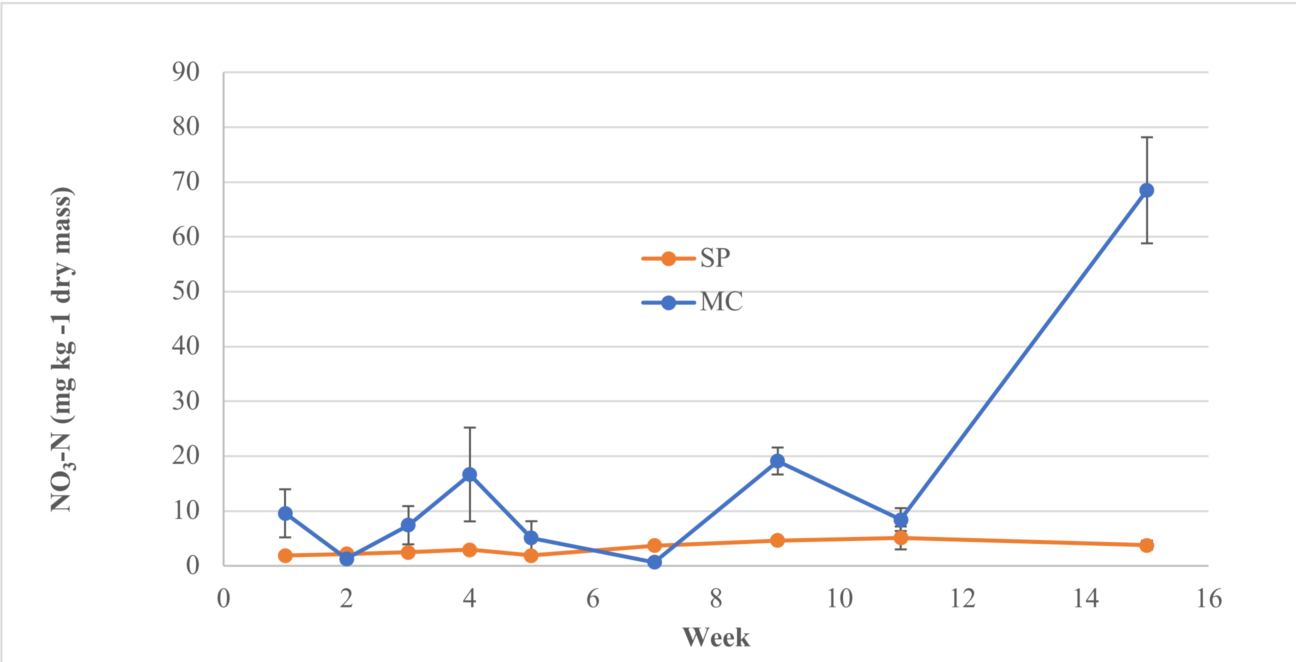

Figure 10 - Nitrate - N in Static Pile (SP) and Manure Compost (MC) Extracts (mg kg^{-1} dry mass), , error bars represent standard error for each sampling event (n=3).

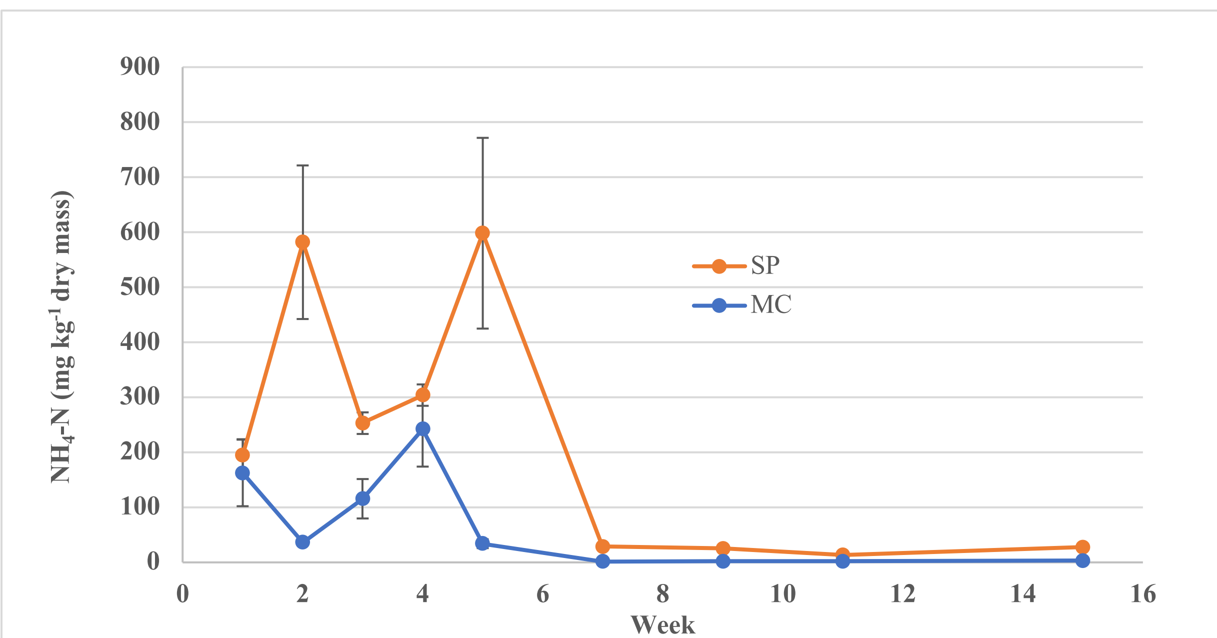

Figure 11 – Ammonium -N in Treatment Extracts, Static Pile (SP) vs Manure Compost (MC) (mg kg^{-1}dry mass), , error bars represent standard error for each sampling event (n=3).

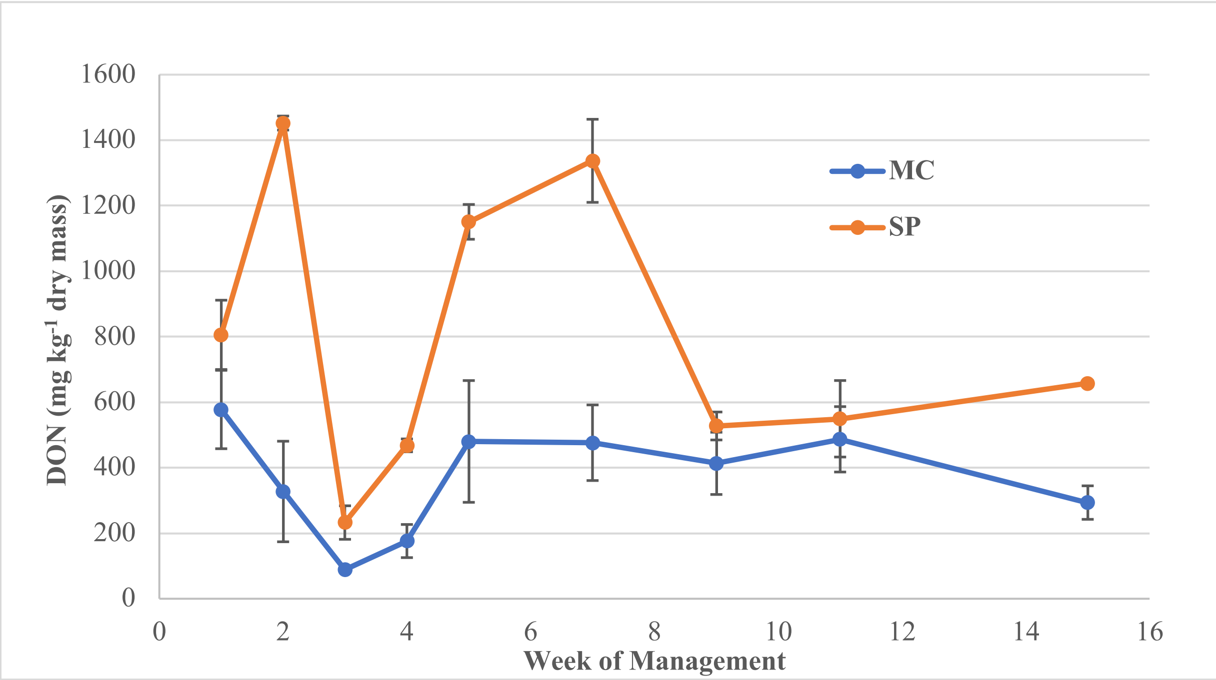

Figure 12 - Dissolved Organic Nitrogen (DON) in Treatment Extracts, error bars represent standard error for each sampling event (n=3).

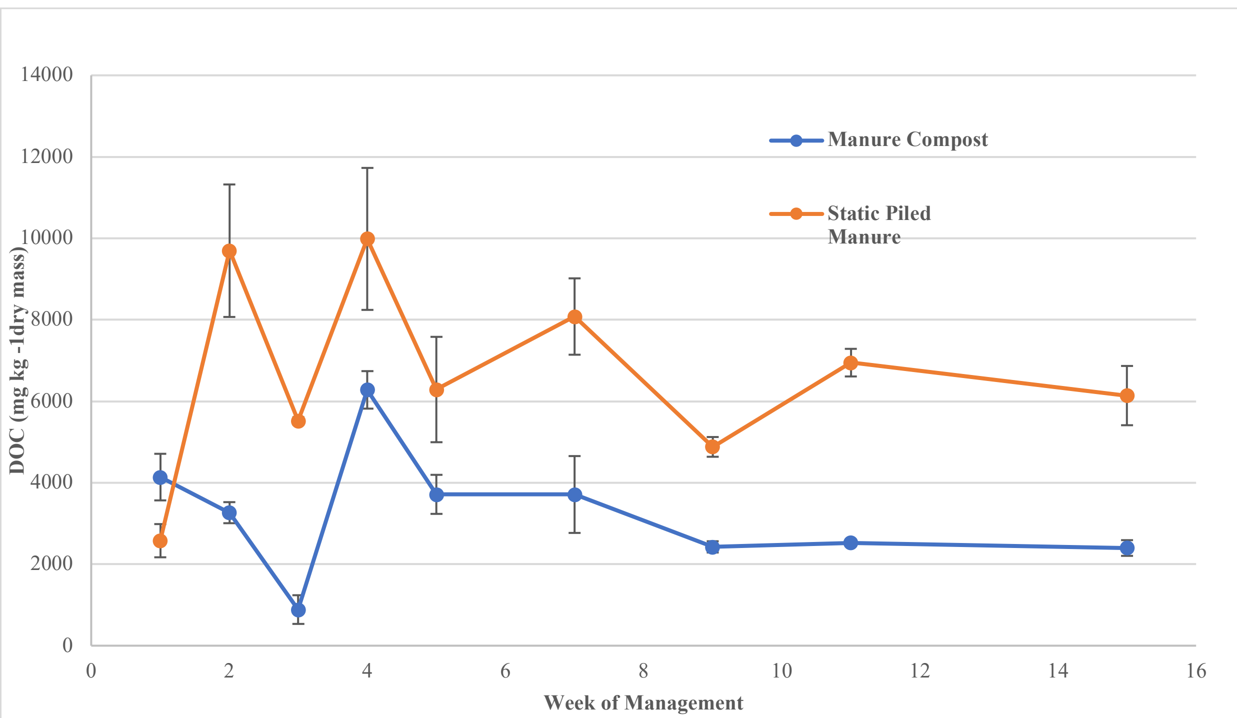

Figure 13 -Dissolved Organic Carbon (DOC) in Treatment Extracts During 15 weeks of composting, error bars represent standard error for each sampling event (n=3).

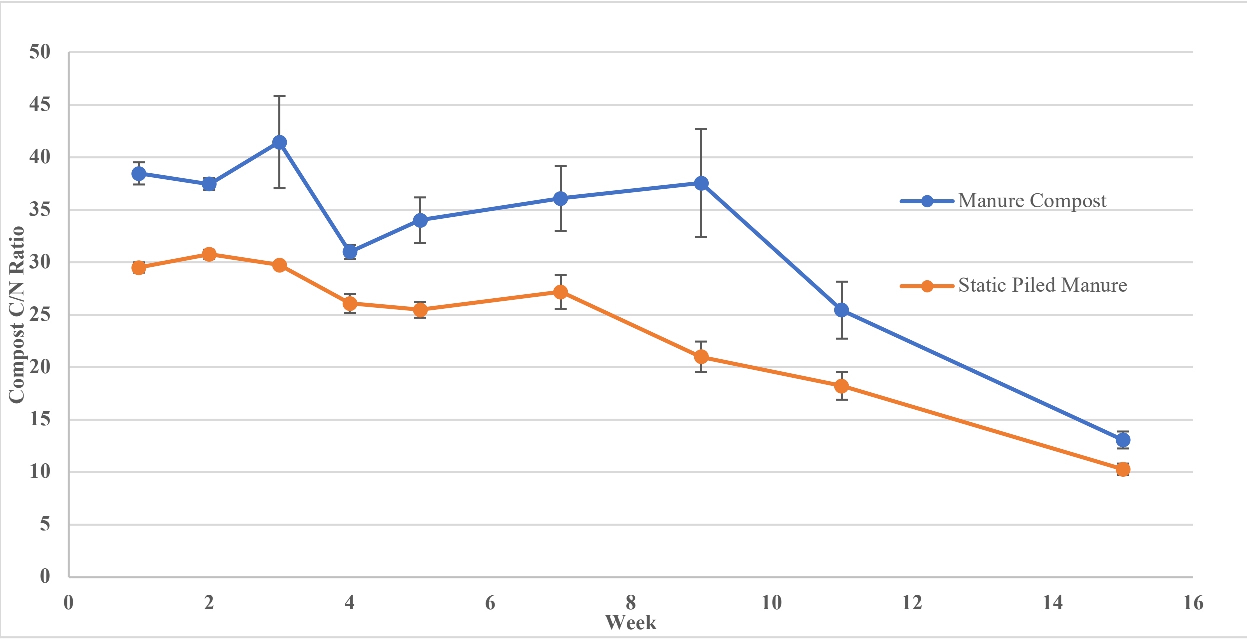

Figure 14 -Carbon to Nitrogen (C:N) Ratio in Solid fraction of Manure Compost and Static Piled Manure over 15 weeks of treatment.

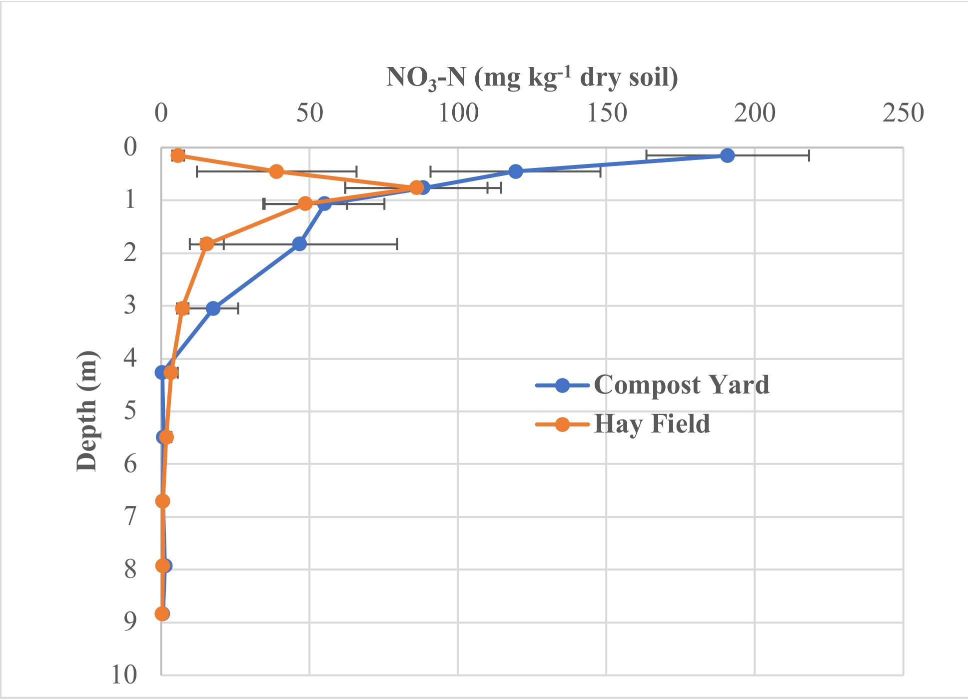

Figure 15 - Nitrate-N in Soil Core Extracts, error bars represent standard error for each depth section and extraction (n=5). March 2021 Sampling.

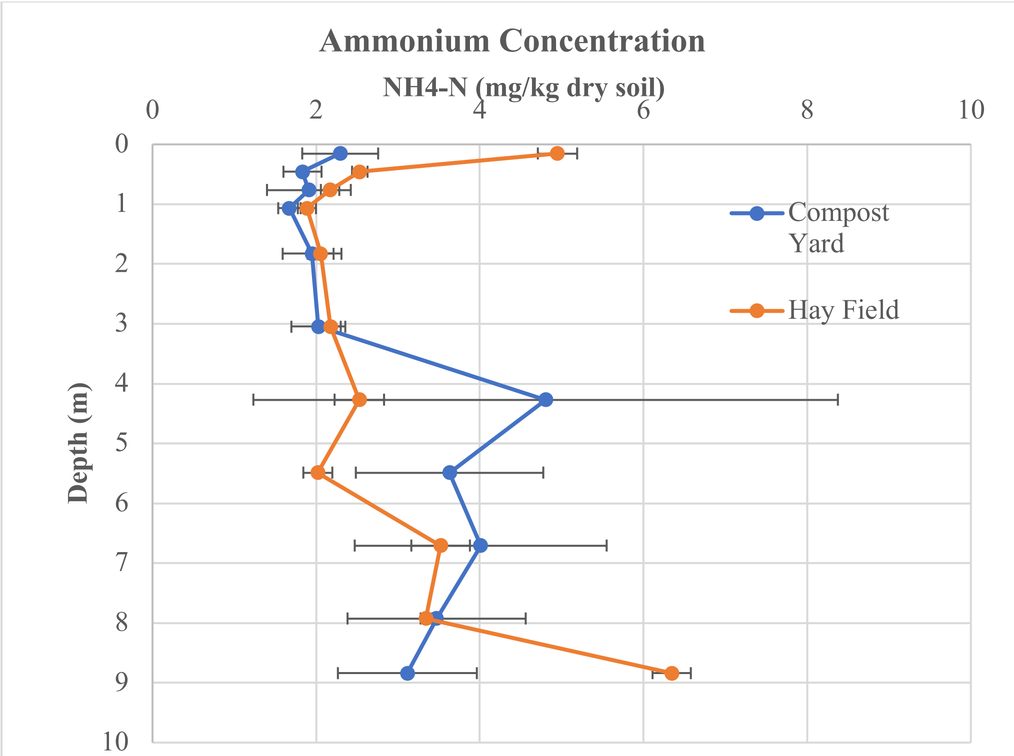

Figure 16 - Ammonium-N in Soil Core Extracts, error bars represent standard error for each depth section and extraction (n=5). March 2021 Sampling.

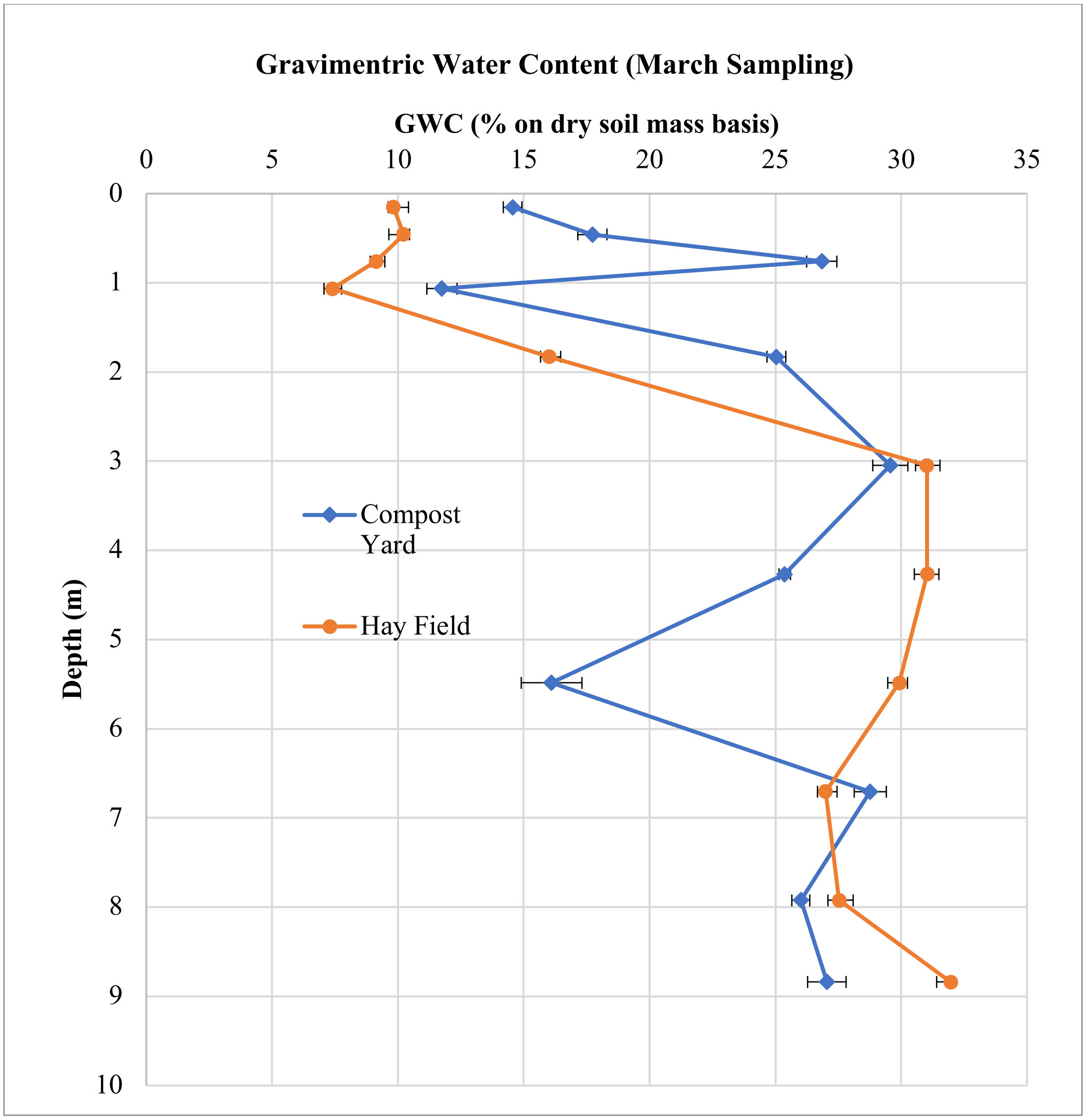

Figure 17 - Water Content , measured as mass lost after drying soil to 105 °C for 24 hours.

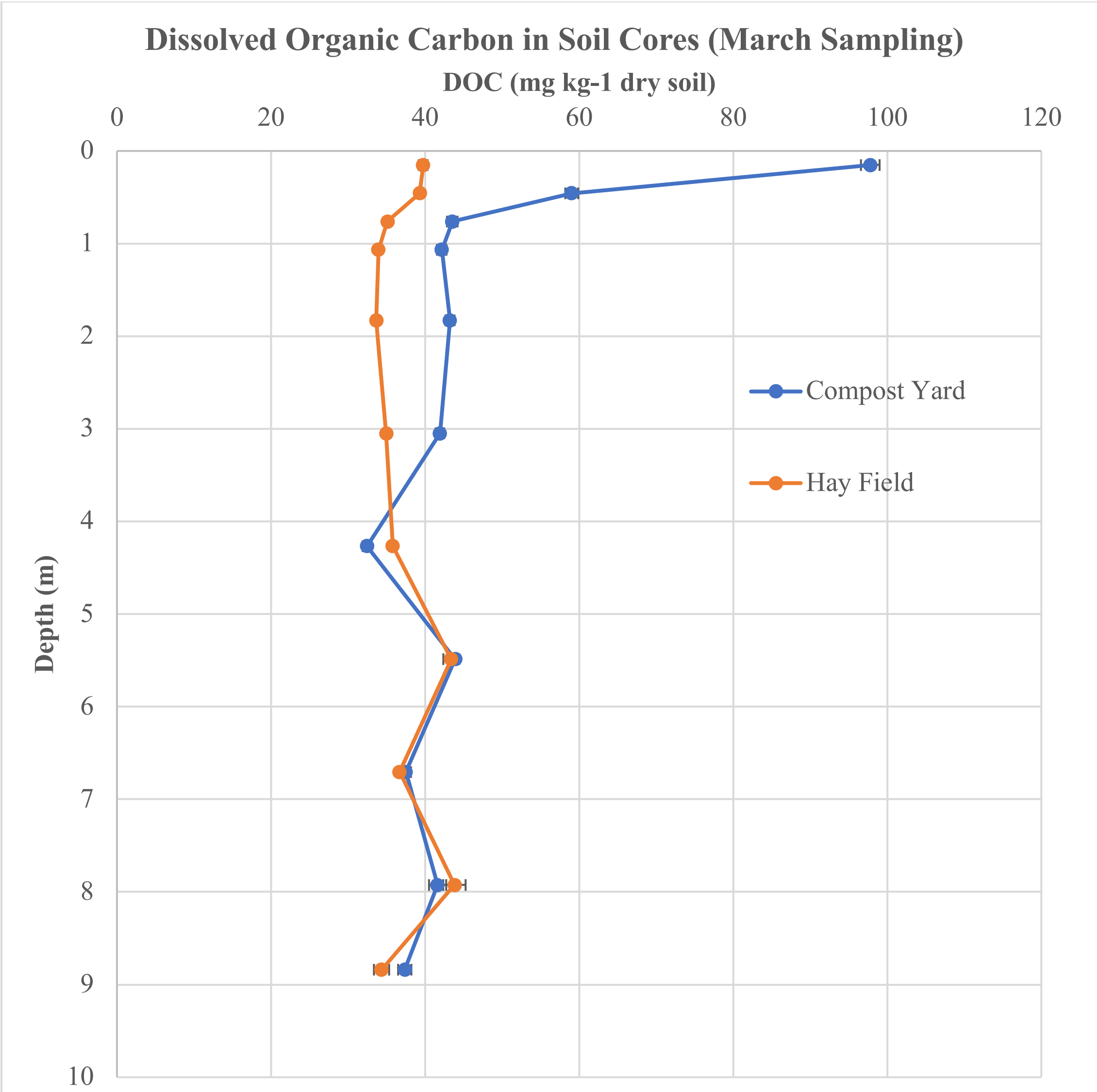

Figure 18 - Dissolved Organic Carbon (DOC) in Soil core Extracts, from March 2021 sampling event, error bars represent standard error calculated between depth section replicates (n=5).

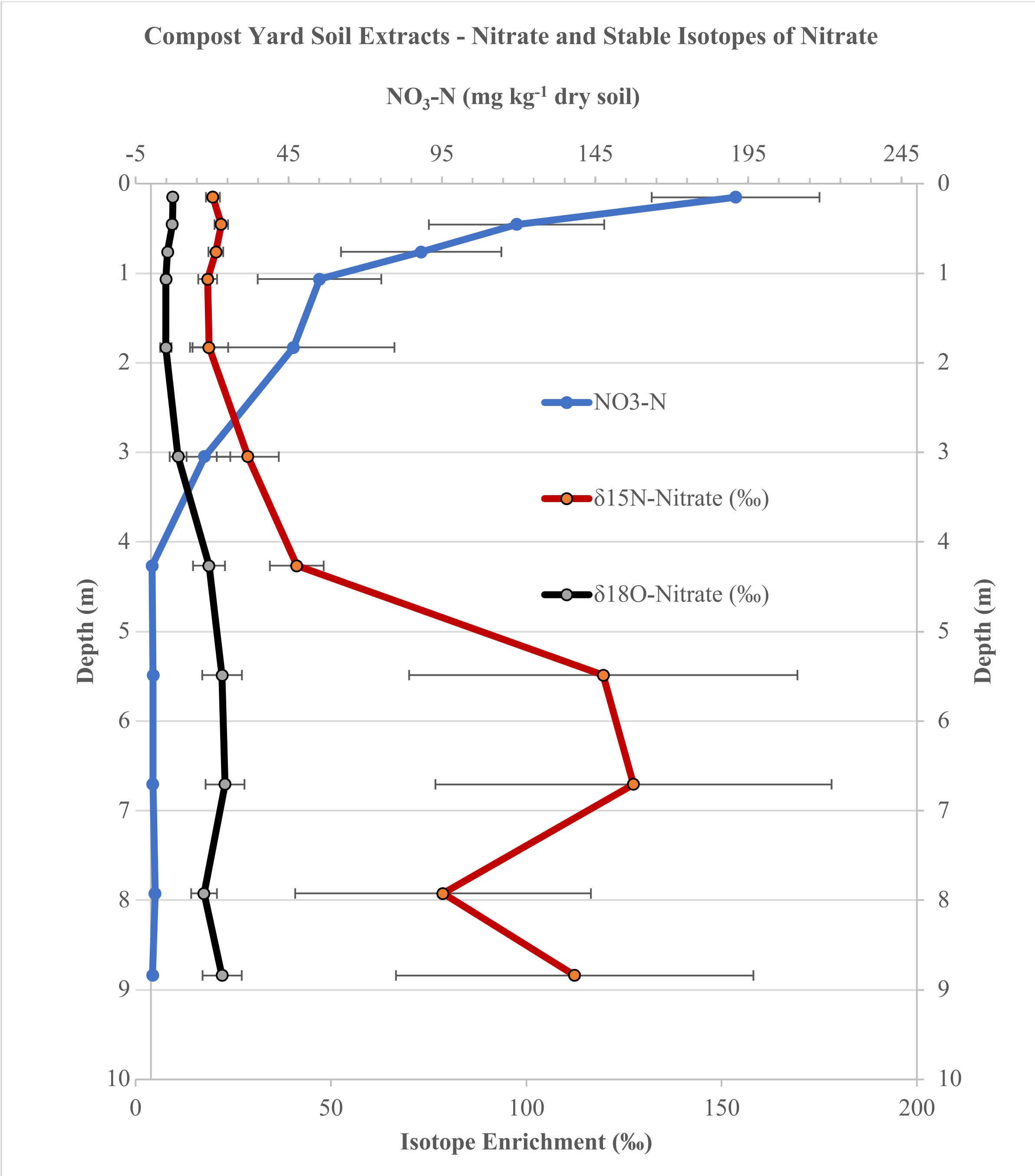

Figure 19 -Nitrate Concentration and Stable Isotope Enrichment (delta15N) and (delta18O) values of Soil Core Extracted Nitrate, with NO_3-N – (March 2021 Soil Cores), error bars represent standard error between depth section replicates (n =5).

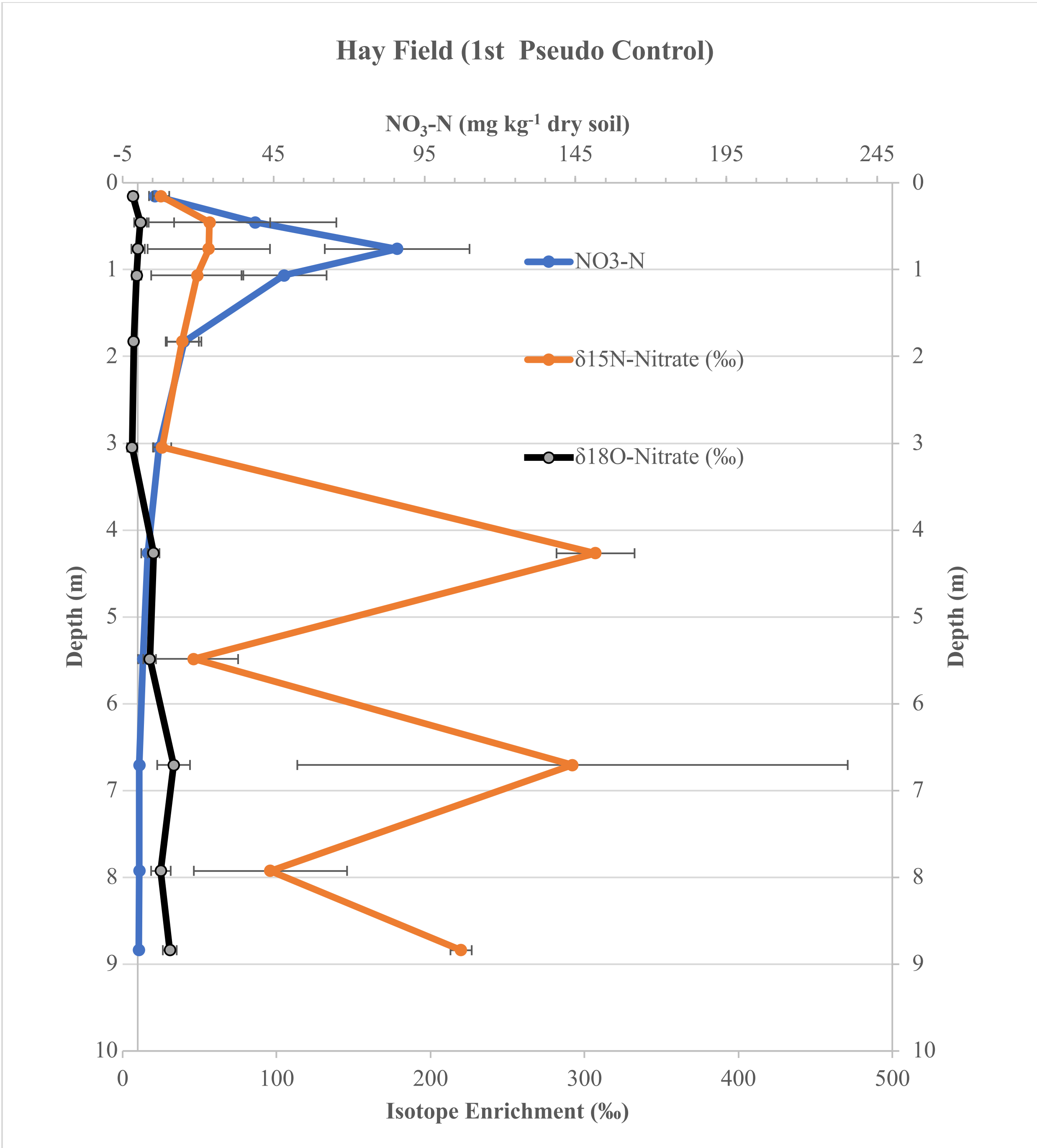

Figure 20 -Nitrate Concentration and Stable Isotope Enrichment (delta15N) and (delta18O) values of Soil Core Extracted Nitrate, with NO3-N – (March 2021 Soil Cores), error bars represent standard error between depth section replicates (n =5).

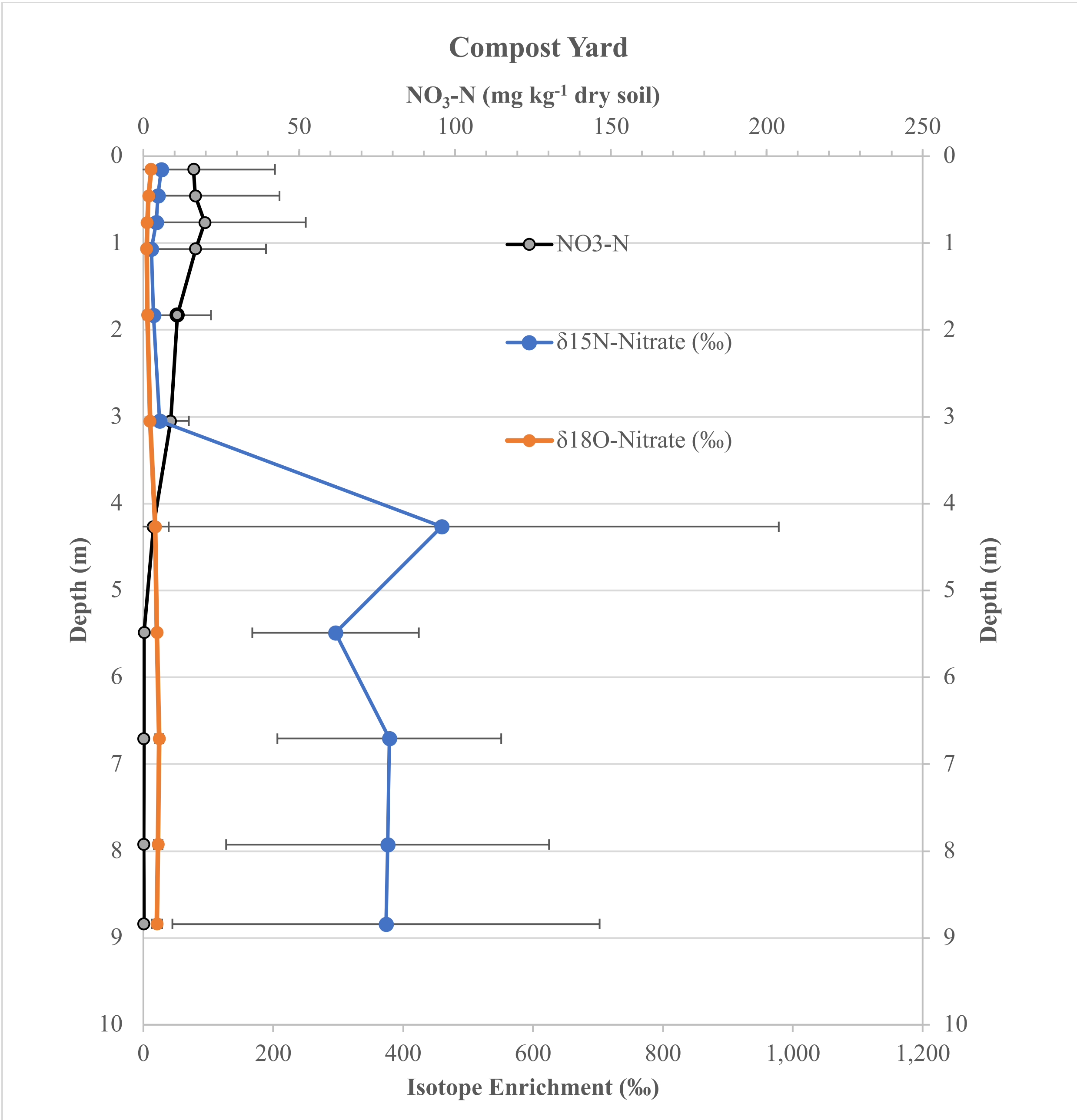

Figure 21 - Nitrate Concentration and Stable Isotope Enrichment (delta15N) and (delta18O) values of Soil Core Extracted Nitrate, with NO3-N – (Sept 2021 Soil Cores), error bars represent standard error between depth section replicates (n =5).

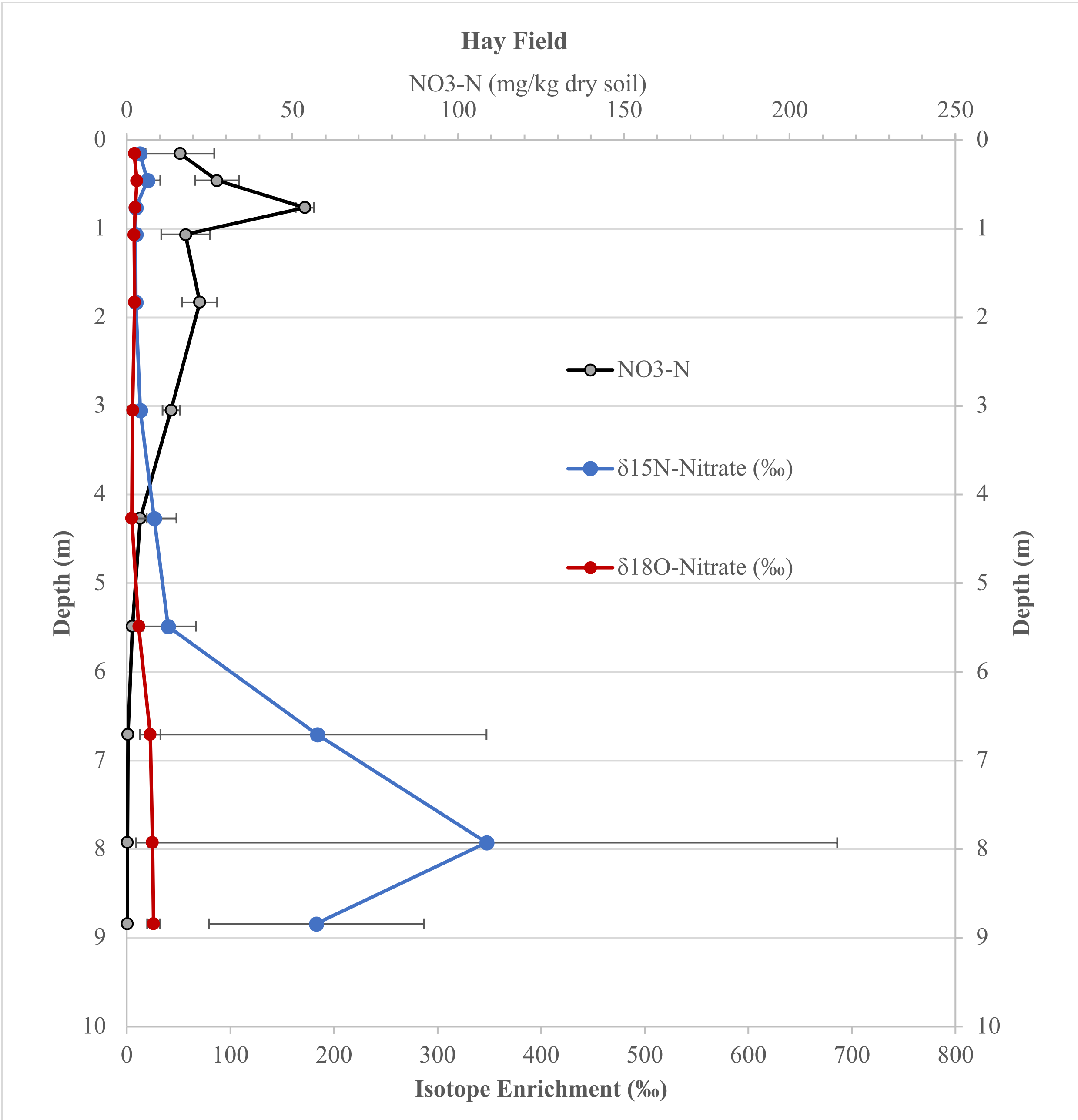

Figure 22 -Nitrate Concentration and Stable Isotope Enrichment (delta15N) and (delta18O) values of Soil Core Extracted Nitrate, with NO3-N – (September 2021 Soil Cores), error bars represent standard error between depth section replicates (n =5).

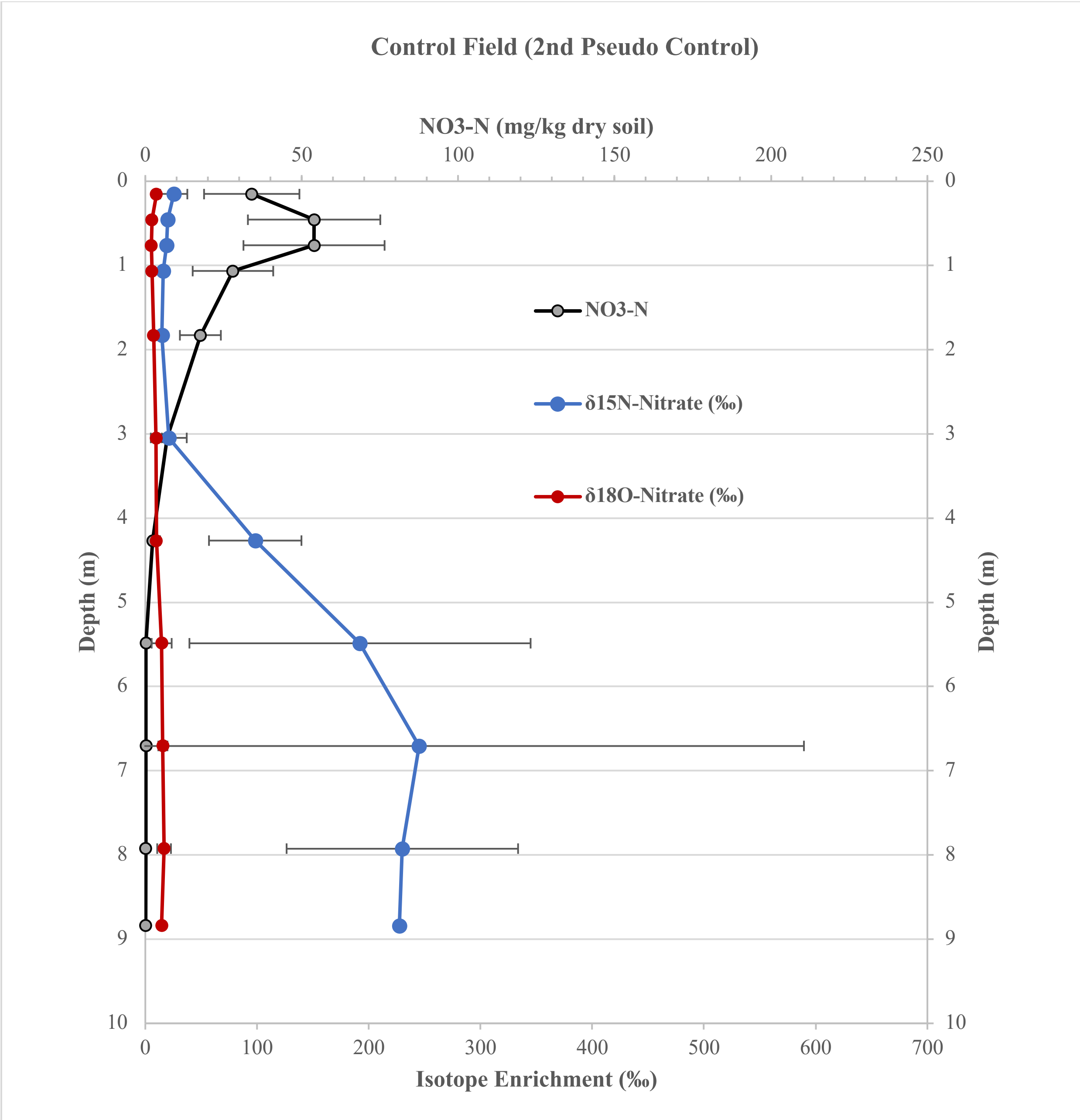

Figure 23 - Nitrate Concentration and Stable Isotope Enrichment (delta15N) and (delta18O) values of Soil Core Extracted Nitrate, with NO3-N – (September 2021 Soil Cores), error bars represent standard error between depth section replicates (n =5).

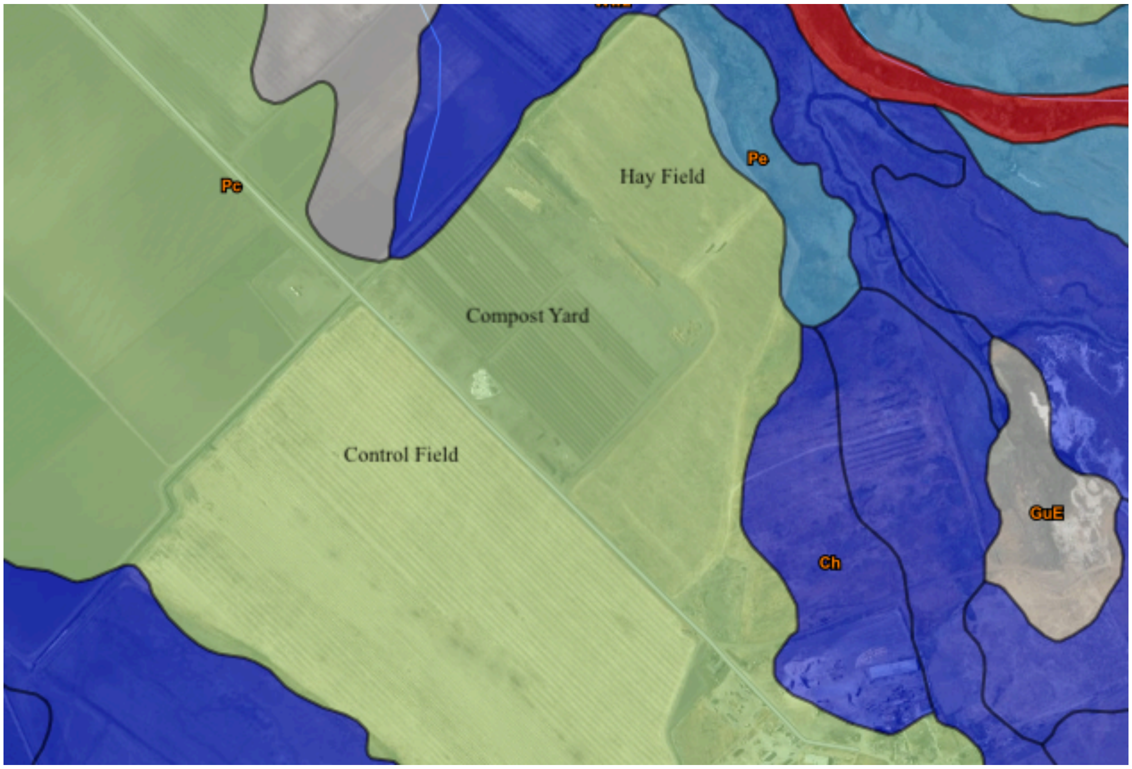

Figure 24 – Annotated Soil Map of Experimental Fields and Surrounding Landscape—Compost Yard (CY), Hay Field (HF) and Control Field (Con) are shown. (USDA NRCS Soil Survey, 2022).

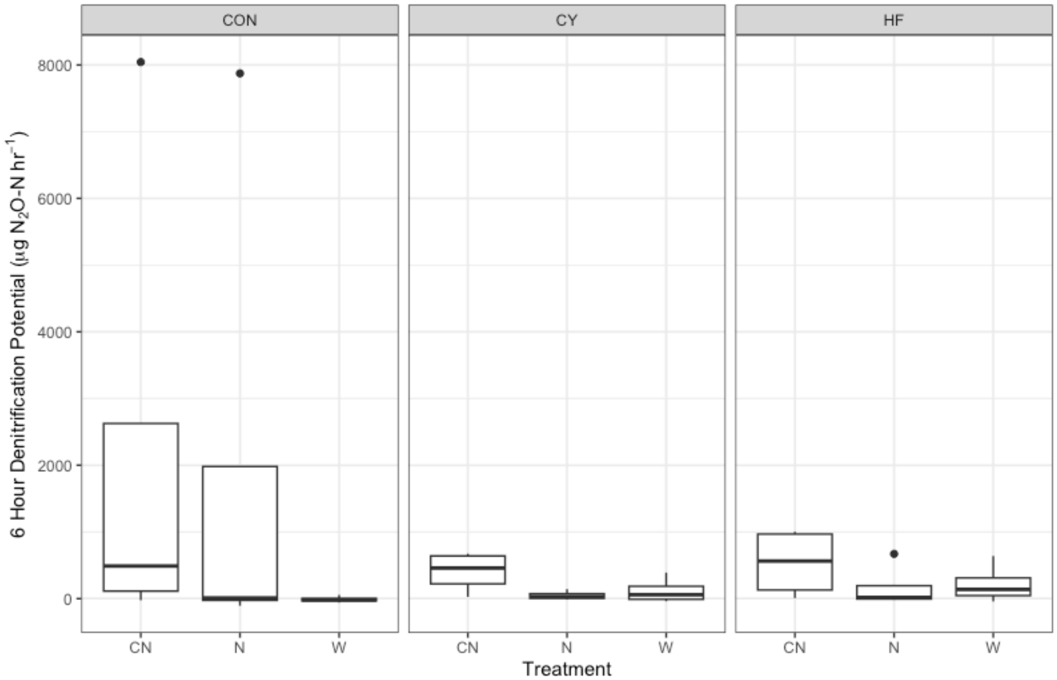

Figure 25 - Denitrification Incubation Results- Boxplots from Left to Right are for the control field (CON), the compost yard (CY), and the Hay Field (HF), treatments are indicated at the bottom of the plots: glucose and nitrogen added (CN), nitrogen only (N) and water only (W). No statistical difference between field or treatment was observed.

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