Title

Changes in Soil Nitrous Oxide Emissions, and Nitrogen and Water Use Efficiencies, Associated With Soil Organic Carbon Storage

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Changes in Soil Nitrous Oxide Emissions, and Nitrogen and Water Use Efficiencies, Associated With Soil Organic Carbon Storage

By

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Abstract

Efforts to build soil organic carbon (SOC) in global croplands are rapidly expanding. Evidence suggests that long-term increases in SOC can lead to improved crop yield and reduced nitrogen (N) fertilizer and water requirements, two important sustainability challenges. However, increases in SOC may also trigger higher soil nitrous oxide (N_2O) emissions due to changes in labile soil carbon and N pools, among other soil functions. Using wheat as a case study in a controlled environment, we tested the hypothesis that increasing SOC will improve yields and the efficiency of water and N fertilizer use, but this will come at the cost of higher soil N_2O emissions. Mesocosms were constructed using intact soil cores (30 cm diameter by 25 cm depth) sampled from two treatments in a long-term experiment that differed in SOC following 20 years of conservation soil management, labeled as SOC_{low} and SOC_{high} for this study. Wheat was grown in each soil at three different N fertilizer levels (0, 100, and 200 kg N ha^{-1}) under drip irrigation in the greenhouse. Soil N_2O emissions were measured using the closed chamber methodology. Results indicate that SOC_{high} did not increase wheat grain yield, thus water and nitrogen use efficiencies were similar at the different N levels. Yet, soil N_2O emissions significantly increased by 25-112% under SOC_{high} conditions, which represents a tradeoff for climate change mitigation. While enhancing SOC storage in croplands is likely to bring well-documented crop and environmental benefits, these results suggest that changes in soil N_2O emissions should also be considered to determine the magnitude of net GHG emission reductions.

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1.0 Introduction

Efforts to mitigate the effect of greenhouse gas (GHG) emissions on the environment have led to an increased focus on the ability of soils to cycle carbon (C) from the atmosphere. The positive effect of improving soil organic carbon (SOC) on climate change mitigation is well documented (Blanco-Canqui et al., 2013; Horwath & Kuzyakov, 2018; Lal, 2004a; Paustian et al., 2019; Schjønning et al., 2007), and can be achieved through changes in management practices and land use such as agroforestry, no-till, cover cropping, biochar and organic amendments (OM), and crop rotation. Converting uncultivated land to agricultural ecosystems has been shown to deplete the SOC pool by 40-75% (Kucharik et al., 2001), but can be replenished at a rate of 50-1000 kg C ha^{-1} yr^{-1}, by changing management practices (Lal, 2004b; West & Post, 2002). However, what is less often considered is that changes in SOC will also influence soil nitrogen (N) dynamics and the potential for N losses due to the tightly coupled biogeochemical cycling of C and N in agricultural systems. Thus, concerns remain that the magnitude of increases in SOC needed to meet climate change mitigation goals may correspond with greater N input requirements and potentially negative impacts on N_2O emissions and N leaching losses. This potential tradeoff has received increasing scientific attention, in part because alterations to the N cycle, including higher N_2O emissions, can offset the climate change benefits from SOC sequestration by increasing net GHG emissions (C. Li et al., 2005; Trost et al., 2013).

The main pathways to N_2O production, nitrification and denitrification, are driven by the availability of soil carbon substrates, leading to speculation that increasing SOC can result in higher N_2O emissions (Guenet et al., 2021). Methods to increase SOC often involve carbon inputs in the form of root exudates or plant residue (Blanco-Canqui et al., 2013). The accumulation of these plant materials can increase nitrification and denitrification rates due to changes in soil properties, as well as greater concentrations of the carbon substrates needed to carry out the processes (Kallenbach et al., 2016). Recent studies have shown that when more carbon is present in the soil, microbial activity increases, which leads to anoxic conditions and greater N_2O production via denitrification (Chen et al., 2013; Y. Li et al., 2021; Senbayram et al., 2012). Additionally, root exudates or plant residue can lead to changes soil properties such as decreased porosity and increased water holding capacity, which contribute to the formation of anoxic conditions and denitrification-driven N_2O emissions (Blanco-Canqui & Lal, 2009; Z. Li et al., 2022). Despite multiple studies examining the effects of changing SOC on N_2O conditions, there are many contradictory results, likely the result of these highly interconnected and complex processes. Water inputs and soil temperature are key variables controlling emissions rates (Skiba et al., 1998), and the inability to control for these variables in field environment make possible strategies for mitigation unclear. Due to the high global warming potential of N_2O, and its ability to offset the benefits of carbon sequestration, understanding interactions between environmental drivers that control N_2O production is vital.

Changes in soil physical, chemical, and biological properties due to higher SOC, have effects beyond increasing N_2O emissions. Crop growth and yield have been shown to be positively impacted by these changes through direct and indirect pathways (Oldfield et al., 2019). Long-term studies have documented that management practices which increase SOC can increase yields due to changes in soil compaction, aeration, nutrient cycling, or aggregate stability (Blanco-Canqui et al., 2013; King et al., 2020). Similarly, evidence suggests that improved infiltration and water storage as a result of improved SOC management can support better plant growth and higher productivity per unit of irrigation input, thereby increasing water productivity (Shehzadi et al., 2017). However, these positive impacts on yield are not always observed, meaning it is necessary to account for potential tradeoffs with efforts to mitigate GHG emissions on crop productivity (Xia et al., 2018, Shang et al., 2021).

To limit the global environmental footprint of agriculture, the potential of higher yields also offers the possibility of increasing the efficiency of external of N fertilizer and water, both major drivers of crop productivity. Increased SOC storage is accompanied by increasing organic N stocks, leading to higher indigenous soil N supply and potentially lower N fertilizer demand. Moreover, improved soil structure and quality have been shown to increase yield potential and crop response to fertilizer, translating to gains in N use efficiency (Ernst et al., 2020). While these sustainability co-benefits are often acknowledged in the literature, few studies have simultaneously determined the degree to which long-term changes in SOC in a single field impact crop yield and associated resource use efficiencies.

The ability for SOC to promote crop yields is an important area of interest from both an economic and food insecurity perspective. Staple crops like wheat are main sources of nutrition for approximately 30% of the world's population and have an economic value of approaching $39 billion annually (Grote et al., 2021). Some studies have predicted a 5-7% decrease in yields as a result of climate change, therefore necessitating investigation into whether SOC can mitigate some of these losses (Shiferaw et al., 2013). Findings are mixed on whether SOC directly contributes to yield increases, partly because the correlation is difficult to quantify due to confounding effects from other crop production strategies like nitrogen (N) fertilization (Blanco-Canqui et al., 2012; Lorenz et al., 2019). Therefore, studies looking at the direct effects of building SOC on crop yields are minimal (Lal, 2006, 2010; Swanepoel et al., 2018; Xu et al., 2019). Some of these studies focused on crop yields in marginalized soils, so yield increases may be greater relative to those from more productive soils due to improvements in overall soil health. Additionally, other studies have shown that yields either decreased or stayed level under increased SOC conditions (Oelofse et al., 2015; Swanepoel et al., 2018). Improving yields has direct implications for global economic and food security development goals (FAO et al., 2020), and the lack of clarity on whether higher SOC stocks can improve yields may make it more difficult to develop successful initiatives to meet these targets.

Recent studies have highlighted the possibility that practices contributing to SOC gains can increase N_2O emissions partially offsetting the reduction in net GHG emissions (Guenet et al., 2021; Lugato et al., 2018; Wu et al., 2022). Studying soils where long-term management has resulted in increased SOC is critical for shedding light on whether efforts to build SOC will have unintended consequences for N_2O emissions. Therefore, the objectives of this study were: (a) to investigate the effect that increased SOC will have on N_2O emissions and wheat yield (*Triticum aestivum*), and (b) determine whether long-term SOC development will improve yield potential while reducing the need for nitrogen fertilizer. We hypothesized that at SOC_{high} levels yields will be higher due to greater water and nitrogen use efficiency. Additionally, we hypothesized that nitrous oxide emissions will also be elevated in SOC_{high} soils. Insights from this study will help determine the costs and benefits of long-term increases in SOC for ecosystem processes related to climate regulation and food production.

2. Methods and Materials

2.1 Site Description and Experimental Design

This study was conducted in a greenhouse located on the University of California, Davis campus in Davis, CA, USA (38.5382^{o}N, 121.7617^{o}W) during 2022. Soil mesocosms were constructed using intact soil cores (30 cm in diameter and 25 cm depth) using polyvinyl chloride (PVC) pipes. The soil used for the study was sampled from a long-term experiment at the University of California West Side Research and Extension Center (WSREC), which is located approximately 35 miles southwest of Fresno, CA USA (36.3419^{o}N, 120.1103^{o}W). Soils at the site are Panoche clay loam (fine- loamy, mixed superlative, thermic Typic Haplocambids) (Arroues, 2006). To address the study objectives, samples were obtained from treatments representing the largest difference in SOC that had resulted from 20 years of management. Standard practice for the region includes tillage without cover crops, which was designated as the control (SOC_{low}). Soils representing the SOC_{high} treatment were sampled from the treatment that had utilized no-till and cover crop practices. Both of these practices are promoted as fundamental opportunities to enhance SOC in croplands. Other aspects of management in the long-term experiment were similar and reflective of typical summer irrigated crop rotations in this region— with the field most recently planted with sorghum (*Sorghum bicolor)* and garbanzo beans (*Cicer arietinum)*. The most recent cover crop mixture consisted of triticale (*Triticosecale Wittm.*), cereal rye (*Secale cereale L.*), common vetch (*Vicia sativa*), radish (*Raphanus sativus*) and clover (*Trifolium incarnatum*), and had a C:N of 42:1 due to a higher ratio of tritcale and rye to legumes and vetch. In the mesocosms most cover crop residue was removed from the soil surface to establish similar conditions for SOC_{high} and SOC_{low}, but remaining root biomass and smaller pieces of above ground residues in SOC_{high} were left undisturbed. A detailed description of management history and crop yields is provided in (Mitchell et al., 2017, 2022). Key soil characteristics determined in these studies are reported in Table 1. Pre-study levels of soil nitrate were extracted and measured using 30mL of 0.5M potassium sulfate and 6g of soil that was oven-dried, homogenized, and sieved to 2mm (Mulvaney 1996).

The experiment was arranged in a randomized split-plot design where soil C levels (SOC_{high} and SOC_{low}) were considered main-plots and inorganic N fertilizer rates as subplots (0, 100, and 200 kg N ha^{-1}). “SOC_{high}” and “SOC_{low}” are subjective terms and therefore are used only to indicate the relative differences in SOC between the two soils used in this study, and not as comparisons to SOC levels in other soils. Each treatment was replicated four times. The N fertilizer was applied as urea, equally split between two stages of plant growth—initial emergence and tillering following recommended practices to increase plant uptake of applied N fertilizer for this region (Orloff et al., 2012). Foliar application of urea has been shown to cause leaf burn during early stages of crop growth (Clay et al., 2021) so urea granules were distributed across the soil surface during each fertilization event and then irrigated with 0.3L to ensure dissolution and subsoil incorporation.

2.2 Wheat Management

The wheat was planted 8 days after an initial watering event which consisted of an initial 2.0L of DI water applied over two days in order to saturate the top 5cm of the soil profile, followed by regularly scheduled drip irrigation events of approximately 0.5L of DI water. This was done to ensure that there was adequate water available for initial germination, since the soil had an average moisture content of 3.5% at time of sampling. Previous research on soil water dynamics at this site indicated that field capacity was around 18-21% volumetric water content, determined 3 days after a 4.8 cm irrigation event (Araya et al., 2022). Wheat was planted at a rate of 250-320 plants m^{-2} –equal to the recommended planting density for irrigated wheat in California. To accommodate the size of the pots used (0.019 m^3), the seeding rate was adjusted accordingly to 86.0 plants per m^2 (Fan et al., 2016; UCANR, 2022). AP Octane wheat treated with Dividend Extreme (AgriPro Wheat Inc., Kansas, USA) was planted 2.5 cm below the soil surface, and spaced 7.6 cm apart in a square within the pot in order to facilitate room for root growth. At the time of planting, four seeds were planted in each well, and then thinned after emergence.

All pots received equal amounts of water via 8, 1L hr^{-1} flow irrigation emitters controlled by a timer. Watering schedules were adjusted based on environmental factors and plant growth needs with the goal of reducing water as a limiting factor (Mathesius et al., 2021). Two EM50 data loggers (Meter Group, Pullman, WA) equipped with three ECH_2O 5TM Volumetric Water Content (VWC) and Temperature sensors (Meter Group, Pullman, WA) were placed in the 0-15cm horizon of three representative pots—two SOC_{high} pots and one SOC_{low} pot. Over the course of the growth period, the sensors recorded soil temperature and VWC every hour. VWC content was expressed as an average of all three sensors.

Wheat was harvested when 90% of plants in each pot reached the ripening stages (Feekes 11.3-11.4). When this occurred, all aboveground biomass was harvested by hand and separated by heads and stem/leaf biomass. Heads were harvested at the topmost node on the plant stem.

2.3 Soil N_2O Emissions

Soil N_2O fluxes were measured following the closed-static chamber method outlined in (Parkin & Venterea, 2010). Adjustments were made to the methodology to account for the greenhouse environment and study objectives. Anchors were placed in the middle of the pot to a depth of 5cm, with wheat planted around the outside. Anchors remained in the soil for the duration of the study to minimize disturbance from repeated insertion and removal.

Gas sampling started two days after the initial irrigation event. Subsequent measurements were taken weekly, except for the periods directly after fertilizer application, during which additional gas samples were taken 1, 3, and 5 days post-application. The chambers used for N_2O sampling were constructed following the steps outlined in Pitton et al., (2021). Briefly, the chambers were constructed out of insulated, vented, and round PVC chambers. Each cylindrical chamber was 10 cm in diameter and 15 cm in height, and fitted with a PVC lid.

Each sampling event occurred between 8:30-10:00am, during which all 24 pots were sampled. Samples were collected by block, with 12 pots being sampled at once. Ambient samples were taken at the start of the sampling event and served as the Time 0 sample points. Subsequent samples were then taken at 10, 20, and 30 minute intervals.

Samples were taken at each of the time intervals by inserting a 30mL syringe fitted with a needle into a rubber butyl septa (Labco Ltd., Lampeter, U.K.) on the chamber lid. A 25mL gas sample was removed, of which 5mL of the sample was then ejected. The remaining 20mL of gas was immediately transferred into a previously evacuated 10mL glass vial. The exetainers were sealed with butyl rubber stoppers (Labco Ltd., Lampeter, U.K.) and a clear silicone adhesive sealant. The samples were stored in the glass vials until analysis using gas chromatography (GC) to determine N_2O concentration. The GC analysis was conducted using a Shimadzu 2014 (Shimadzu Co., Kyoto, Japan). The GC used helium as a carrier gas, and was calibrated for analysis using N_2O standards between 0-9.95 ppm.

A restricted quadratic regression (RQR) procedure was used to calculate daily fluxes (dN_2O) as a function of the rate of change in N_2O concentration in the chamber headspace over time. RQR was chosen in order to minimize the effects of measurement errors compared to standard nonlinear methods. Unlike linear regression models, RQR procedures account for suppression of the vertical gas concentration gradient at the soil-atmosphere interface during chamber deployment (Venterea et al., 2020). A trapezoidal integration of flux versus time was used to estimate the cumulative area-scaled N_2O emissions (cN_2O, kg N_2O-N ha^{-1}).

2.4 Data Analysis

The effect of soil C and N rates and their interaction were analyzed for each variable using linear mixed models in R software (lme function, nlme package; Pinheiro et al., 2017). Soil C level, N rates, and their interaction were considered fixed effects, whereas random effects included C level nested within blocks. Plot residuals were inspected to assess normality assumptions and constant error variance. Analysis of variance was used to test the significance of effects at $p < 0.05$. There were no significant interactions between soil C level and N rate for all variables analyzed except vegetative, grain, and total N uptake values. Thus, the interaction term between C and N rates was removed from models and only main effects reported, except for vegetative, grain, and total N uptake results where analysis of variance was used to assess the effect of soil C at each N level, with linear models including soil C level as fixed effect and blocks as random effects.

After harvesting straw and grain, the biomass samples were sent to the University of California, Davis Analytical Lab for analysis of total nitrogen and carbon. These concentrations were obtained via combustion on a Leco TruSpec CN Analyzer (St. Joseph, MI, USA; AOAC International, 1997).

3. Results

3.1 Environmental conditions

Daily irrigation volumes were consistent during the crop establishment up to the tillering phase of wheat growth, with cumulative water inputs reaching 168.3 mm 23 days after seeding (DAS). Irrigation volumes were further increased to meet crop water demand during reproductive growth, including two surface irrigation events of 1.5 L of DI H_2O at 42 and 63 DAS, which together represented 24% of cumulative irrigation supplied during the second half of the season. Average soil VWC was 16.7% over the entire study, but varied between a low of 12.0% and a high of 21.1%. Pore volume was approximately 8.7L and 9.5L in the SOC_{high} and SOC_{low} soils, respectively. The highest VWC was recorded early in the growth period following germination, in the days following the initial application of urea. Soil VWC steadily declined between 20-35 DAS, and reached a low when the wheat in the tillering and booting growth stages (34 and 54 DAS, respectively). Notable increases in VWC corresponded with the two N fertilizer application events and two surface irrigation events, particularly in the week following the second N application (35-41 DAS).

Although daily average air temperature remained mostly consistent throughout the course of the study, there was an approximately $1^{o}C$ decrease in overall average temperatures after whitewash meant to decrease solar radiation was applied to the greenhouse walls and ceilings in early April (Figure 1). Soil temperatures fluctuated slightly more, ranging from 21.4-26.7 ^{o}C. The highest daily average soil temperatures occurred during the mid- to late-tillering growth stage, while the lowest occurred in mid-April during the booting growth stage.

3.2 N_2O emissions

Nitrous oxide fluxes in the SOC_{high} treatment were typically higher than fluxes in the SOC_{low} treatment across all three N fertilizer treatments, with a few exceptions. As expected, N_2O fluxes were highest after irrigation and fertilization events across all treatments. After the initial application of water, daily N_2O fluxes peaked 1-2 days later—a trend that was seen in subsequent surface irrigation and fertilization events (Figure 2). Regardless of SOC and N rate treatment, there were positive N_2O fluxes subsequent to fertilizer and irrigation events, although the degree of response varied by treatment. The highest fluxes were recorded after the initial watering event and after the urea applications (Figure 2).

Daily soil N_2O emissions were consistently greater for SOC_{high} compared to SOC_{low} across the wheat growing season, and particularly during the three peak events. Accordingly, cN_2O emissions were significantly different for the two soils ($p=0.025$). The SOC_{high} soils had a mean seasonal flux of 941.48 g ha^{-1}, compared to the SOC_{low} soils which had a mean seasonal flux of 624.41 g ha^{-1}. This represents a 51% increase in N_2O emissions between the two soil types. Moreover, individual contrasts within each N rate show that N_2O emissions were 112%, 43%, and 25% higher for SOC_{high} compared to SOC_{low} at 0, 100, and 200 kg N ha^{-1}, respectively (Fig. 3). The effect of N rate was marginally significant ($p=0.062$), with 36% higher N_2O emissions for 200 kg N ha^{-1} inputs compared to 0 kg N ha^{-1} inputs (910 vs 671 g N ha^{-1} , respectively).

3.3 Yield and Water Productivity

Vegetative biomass and total biomass were 16% and 12% higher, respectively, for SOC_{low} compared to SOC_{high} (p=0.05 and 0.06, respectively) (Table 2). However, grain yield was not different for the two soils. In contrast, higher N inputs significantly increased vegetative biomass, total biomass, and grain yields (p=0.04, 0.03, and 0.04 respectively), with an N rate of 200 kg ha^{-1} increasing yield by 23% compared to 0 kg N ha^{-1}. Results for water productivity were similar because irrigation inputs were the same for all treatments, meaning differences in water productivity were only a function of grain yield.

In-season measurements of chlorophyll content taken 57 days after seeding (DAS) were significantly affected by the nitrogen rate (p=0.0479), but not the soil (p=0.153) (data not shown). Although the 100 kg N ha^{-1} nitrogen rate was not significantly different than the 200 kg N ha^{-1} and 0 kg N ha^{-1} rates, there was an average of 6% more chlorophyll in the 200 kg N ha^{-1} nitrogen treatments than the 0 kg N ha^{-1} treatments.

3.5 Plant nitrogen content, and nitrogen use efficiency.

Crop N uptake in the vegetative and grain portions of the wheat were similarly affected by the treatments (Table 3), with N rate having a significant impact on all N uptake variables (p= <0.001). Although differences were not observed between SOC_{low} and SOC_{high}, there was a significant soil by N rate interaction. At both 0 and 100 kg N ha^{-1}, there was no significant effect of soil on crop N uptake variables. However, SOC_{low} had significantly greater vegetative, grain, and total N uptake than SOC_{high} at the 200 kg N ha^{-1} treatment. The positive response to N fertilizer addition showed that as mineral N increased, so did the N content in the grain and vegetative biomass across both soils. Between the 0 kg N ha^{-1} and 200 kg N ha^{-1}, there was an approximately 40% increase in N across all biomass portions of the wheat.

Owing to the different response of crop N uptake to fertilizer N addition in each soil, nitrogen use efficiency was significantly impacted by the soil (p=0.014). Between the SOC$_{low}$ and SOC$_{high}$ treatments, there was a 3.5-fold increase in NUE (Table 3). The greatest different in NUE was observed between the two soils at the 100 kg N ha^{-1} (p=0.029), with more than a 5-fold increase in NUE, while a 2.6-fold increase in NUE was observed between the SOC$_{low}$ and SOC$_{high}$ soils at the 200 kg N ha^{-1} rate.

4.0 Discussion

4.1 N_2O response to treatments

Cumulative N_2O emissions from the SOC_{high} soils were 51% higher than the SOC_{low} soils, confirming the hypothesis that increasing SOC can lead to higher emissions.

Regardless of N rate, emissions were higher in the SOC_{high} soils by 25-112% (Figure 3)—which indicated that unique characteristics of the SOC_{high} soils likely contributed to higher nitrous oxide fluxes. Other studies have found that depending on the SOC storage method used, changes in soil characteristics such as: OM, microbial abundance, water holding capacity, compaction, and pore structure were observed to cause increased N_2O emissions (Cayuela et al., 2014; Charles et al., 2017; C. Li et al., 2005; Steinbach & Alvarez, 2006). However, all of these studies found that the positive relationship between N_2O emissions and SOC were variable, and typically site specific. Previous studies at the original sampling site indicated that the SOC_{high} soils had better pore structure, as well as higher OM, microbial abundance, infiltration rate, aggregation, and SHC—all of which likely contributed to higher emissions in the SOC_{high} soils (Araya et al., 2022; Mitchell et al., 2022; Schmidt et al., 2018).

In addition to the changes in soil physical properties, the higher emissions observed in the SOC_{high} soils were likely due to the overall increase in total C (Table 1). Availability of organic C in the soil is a primary control on denitrification rates (Z. Li et al., 2022) so the 52% increase in C in the SOC_{high} soils likely contributed to the higher N_2O emissions, and is not unexpected. High flux peaks observed in the SOC_{high} soils after fertilizer application corroborate this finding.

In all but one instance, the SOC_{high} soils saw higher fluxes after fertilizer application (Figure 2). The exception to this trend was in the 200 kg N ha^{-1} treatment, and was likely the result of rapid mineralization of N due to a drop in C:N resulting from the combined effects of high C content and high N rate (Senbayram et al., 2012). It is possible that N_2O fluxes in this study were inadvertently mitigated by the initial removal of large pieces of cover crops from the soil surface. While this was done to prevent known issues with crop growth in the SOC_{high} soils, the removal of surface residue has also been shown to decrease N_2O emissions by 11% (Essich et al., 2020). This further highlights the tradeoffs associated with improved SOC and heightened N_2O emissions and possible

The effect nitrogen on N_2O emissions was seen across all three N treatments, although the relationship was only marginally significant (p=0.06). Based on the results of other studies (Bouwman et al., 2002; Stehfest & Bouwman, 2006), we expected to see a significant and proportional increase in cN_2O with increasing nitrogen rates due to a greater concentration of available N in the soil. However, there was no significant change in N_2O emissions among the N treatments for the SOC_{high} soils. The SOC_{high} soils had greater organic N stocks (Table 1), so it is likely that microbial N mineralization was high in the 0 kg N ha^{-1} for the SOC_{high} treatment, leading to high baseline emissions. In turn, this large pool of initial organic N may have lessened the effect of fertilizer N addition on emissions from the SOC_{high} soils. Most literature backs the idea of linear or exponential increases in cN_2O emissions with increasing fertilizer application rates regardless of SOC (Bouwman et al., 2002; Hoben et al., 2011; McSwiney & Robertson, 2005). As such, the cN_2O trends seen in the SOC_{high} soils appear to be atypical.

4.2 N_2O response to soil moisture

Other studies have found that N_2O emissions decreased when water filled pore space (WFPS) dropped below 70% (Butterbach-Bahl et al., 2013; Guo et al., 2014), so it was unsurprising that nitrous oxide emissions decreased during the period of soil moisture (Figures 1 and 2). Responses to changes in VWC were most apparent when soil moisture increased as a result of either the initial watering, or surface irrigation events (Figure 2). The initial watering period, which included all fluxes recorded before the initial urea application, accounted for 41 and 43% of cN_2O emissions in the SOC_{high} and SOC_{low} soils, respectively. Previous research has attributed these large pulses after a rewetting event primarily to the release of substrates for use by microbes in denitrification and nitrification (Fierer & Schimel, 2002; Guo et al., 2014). Unlike the initial watering event, the surface irrigation events occurred post-fertilizer application, so residual N in the soil contributed to the emissions fluxes observed in the 100 kg N ha^{-1} and 200 kg N ha^{-1} treatments (Figure 2). This provided an opportunity to examine residual effects of N fertilizer on emissions even after the final application. The fluxes seen in the 100 kg N ha^{-1} and 200 kg N ha^{-1} treatments indicated that large irrigation events can stimulate high fluxes in fertilized soils days to weeks after the last fertilization event. Effective net GHG accounting requires quantification of emissions in both the short- and long-term, so understanding the interactions between irrigation, residual fertilizer, and N_2O emissions is important.

4.3 Yield response to treatments

4.3.1 SOC and Nitrogen responses

Grain yields were not significantly different for the two soils, disproving our hypothesis that increases in SOC would significantly increase yields (Table 2). This is in contrast to the results of other studies. A recent global meta-analysis found that yields were 1.2 times greater in soils with 1.0% SOC compared to soils with 0.5% SOC (Oldfield et al., 2019). Previous field research at the long-term experimental site for this study reported both mostly positive effects of SOC on yields, but found that the results were crop-dependent (Mitchell et al., 2015, 2022). Pronounced improvements in soil health (e.g. aggregation, water infiltration rate, biological activity, total N stocks) for treatments with SOC_{high} supported higher yields, while lower yields were attributed to high levels of surface residue and compaction that impeded crop establishment in some years. These findings are similar to other studies showing that surface residues can breakdown slowly, leading to N immobilization and consequently decreased yields (Alijani et al., 2012). This highlights that rather than being only influenced by long-term changes in SOC itself, crop yields in SOC_{high} soils are also a function of short-term processes in the soil environment.

4.3.2 NUE

Expected NUE in wheat typically falls within the range of 40-50% (Ladha et al., 2005), indicating that N fertilizer utilization for the wheat grown in the SOC_{high} soils was significantly inhibited. This was surprising, as many studies have shown significant positive correlations between N retention and methods of increasing SOC stocks such as no-till, cover cropping, biochar amendments, and agroforestry (Dalal et al., 2011; Rosenstock et al., 2014; Steiner et al., 2008). In these studies, improvements were primarily attributed to changes in soil properties as a result of the presence of plant residues that facilitated greater N availability. Dalal et al. (2011) found that the removal of plant residue reduced NUE because it reduced both the long-term organic N supply and the amount of fertilizer N available for loss via leaching. Therefore, the initial removal of large pieces of plant residue from the soil surface in the SOC_{high} pots may have contributed to the non-significant results observed. When plant residue is removed, there is greater plant reliance on fertilizer for N uptake, which likely accounts for the higher crop response in the 100 kg N ha^{-1} and 200 kg N ha^{-1} treatments. Additionally, previous studies at the original sampling site found that the SOC_{high} soils had faster infiltration rates but no significant difference in water storage capacity, suggesting that low N uptake in those soils may have also been the result of N losses via leaching (Araya et al., 2022; Mitchell et al., 2015).

The low NUE but high N_2O emissions in the SOC_{high} treatment suggested that the wheat was not taking up as much of the mineral N available, and instead it was being lost via N_2O—which would account for the higher emissions observed in the SOC_{high} treatment.

4.5 Net GHG accounting & Broader Implications

Assessments of net GHG mitigation strategies must account for both SOC storage and emissions of non-CO_2 GHGs including N_2O emissions, recognizing the potential for tradeoffs identified above (Xia et al., 2018; Shang et al., 2021). In the scenario examined in this study, the increase in N_2O emissions from the SOC_{high} soils may not negate the benefit of carbon sequestration, if the gains are larger than the losses in terms of CO_2 equivalents (CO_2e). Across the three N rates, there was an increase of 0.317 kg N_2O-N/ha in the SOC_{high} soil—representing a 0.140 Mg CO_2e ha^{-1} yr^{-1}. Compared to the 1.65 Mg CO_2e ha^{-1} yr^{-1} storage rate, this represented an annual offset of roughly 8.5% yr^{-1}. Another way to estimate emissions in this scenario is to use IPCC emissions factors to examine N fertilizer-induced emissions. Assuming N inputs of 100 kg N ha^{-1} yr^{-1} for wheat, the SOC_{low} soils would produce 0.465 Mg CO_2e ha^{-1} yr^{-1}. The 50% increase in emissions observed in the SOC_{high} soils would produce 0.698 Mg CO_2e ha^{-1} yr^{-1}. This increase of 0.233 Mg CO_2e ha^{-1} yr^{-1} would offset annual SOC storage by 14%.

These calculations involve many assumptions and determining the net GHG budget should be a priority for future work. For example, the amount that N_2O emissions offset SOC storage would increase with higher N rates and especially if indirect N_2O emissions were also considered. Conversely, if SOC_{high} corresponded with a reduction in N fertilizer requirements, this would likely decrease the tradeoff for N_2O emissions because N inputs are a key factor controlling N losses to the environment. SOC storage will reach a limit at some point due saturation, after which the negative impacts of higher N_2O emissions will hold increased importance. Long-term efforts to improve SOC in agronomic settings typically provide clear benefits such as improved water holding retention, soil structure, and nutrient availability—although these benefits are site specific. This can lead to higher yields and lower N fertilizer and water requirements; therefore reducing the cumulative environmental impact of agriculture (Lal, 2010; West & Marland, 2002). However, as seen in this study and others, nitrous oxide emissions may be higher under certain high SOC conditions, offsetting some of the benefit (Guenet et al., 2021; C. Li et al., 2005). While this offset is generally not enough to negate the benefits of carbon sequestration, it must be accounted for in order to accurately determine best practices for improving the health of agroecosystems and mitigating climate change.

The global emphasis on promoting SOC cycling to mitigate climate change has led to new agricultural initiatives and market incentives. Once example is the “4 per mil” (4p1000) initiative launched in 2017 by France (Minasny et al., 2017), attributing its name to the target of increasing soil C stocks by 0.4% annually. Although the 4p1000 Initiative states that practice recommendations must account for non-CO_2 emissions “to ensure that net greenhouse emissions do not exceed the offset benefit from increased SOC sequestration” (Rumpel et al., 2020), studies quantifying this relationship are scarce for different regions. Management practices aiming to increase SOC stocks have been extensively studied (Paustian et al., 2016), however, the potential synergies or tradeoffs between SOC storage and N_2O losses remain unclear. A recent global meta-analysis reported that practices aiming to increase SOC storage successfully reduced net GHG emissions without impacting yields but were highly dependent on N rate, temperature, and crop residue management (Shang et al., 2021). Another global analysis focusing on SOC accumulation under straw return found that net changes in reactive N increased resulting in higher N_2O emissions (Xia et al., 2018), while Abdalla et al. (2019) found that cover crops were successful at increasing SOC without having significant effects on N_2O emissions, thus providing net GHG mitigation of 2 Mg CO_2-eq ha−1 yr−1 (Abdalla et al., 2019). Our results contribute to a growing body of evidence that practices for increasing SOC can trigger higher N_2O emissions, partially or fully offsetting the climate benefits over time (Guenet et al., 2021; Lugato et al., 2018).

4.6 Limitations

Nitrous oxide emissions are a function of the environment being measured, thus the data and conclusions are reflective of the management strategies used to increase SOC in these soils. The SOC_{high} soils had been managed using cover cropping and no-till practices for the last twenty years (Mitchell, unpublished data). A meta-analysis on the effects of SOC and N_2O emissions indicated that different management practices meant to increase SOC affect pathways for N_2O production differently (Guenet et al., 2021). Therefore, the results of this study are representative of a single part of this larger network of N_2O production pathways. GHG mitigation strategies should incorporate results from other methods of increasing SOC in order to more fully understand the effects of SOC on emissions.

By controlling water, temperature, and other inputs, it was possible to examine the effects of SOC on N_2O in the relative absence of other confounding factors on N_2O production. Although on-farm trials better replicate real-world environmental conditions that greenhouse studies may be unable to simulate (FAO, 2003), this greenhouse study allowed for greater focus on SOC. However, one noted benefit of improved SOC is the ability to retain water under drought conditions, and by controlling water inputs to ensure they were not limiting, it is possible that this benefit and its associated positive effect on yields (Lal, 2004b) may have been obscured.

Although field trials are preferred for simulating greenhouse gas emissions, other studies have shown that intact soil cores are the most effective way to replicate field soil conditions in greenhouse environments, and are therefore a preferred alternative if field trials are not feasible (Ogunkunle & Beckett, 1988; Schaufler et al., 2010). The intact soil cores helped mitigate aeration and soil-water dynamics issues by minimizing disturbance of the overall soil profile. However, we believe that field studies are necessary to fully understand the effects of high carbon soils on nitrous oxide emissions within the broader context of global agriculture. This study will serve to better understand what is driving emissions under certain conditions, and what strategies should be utilized to minimize N_2O emissions.

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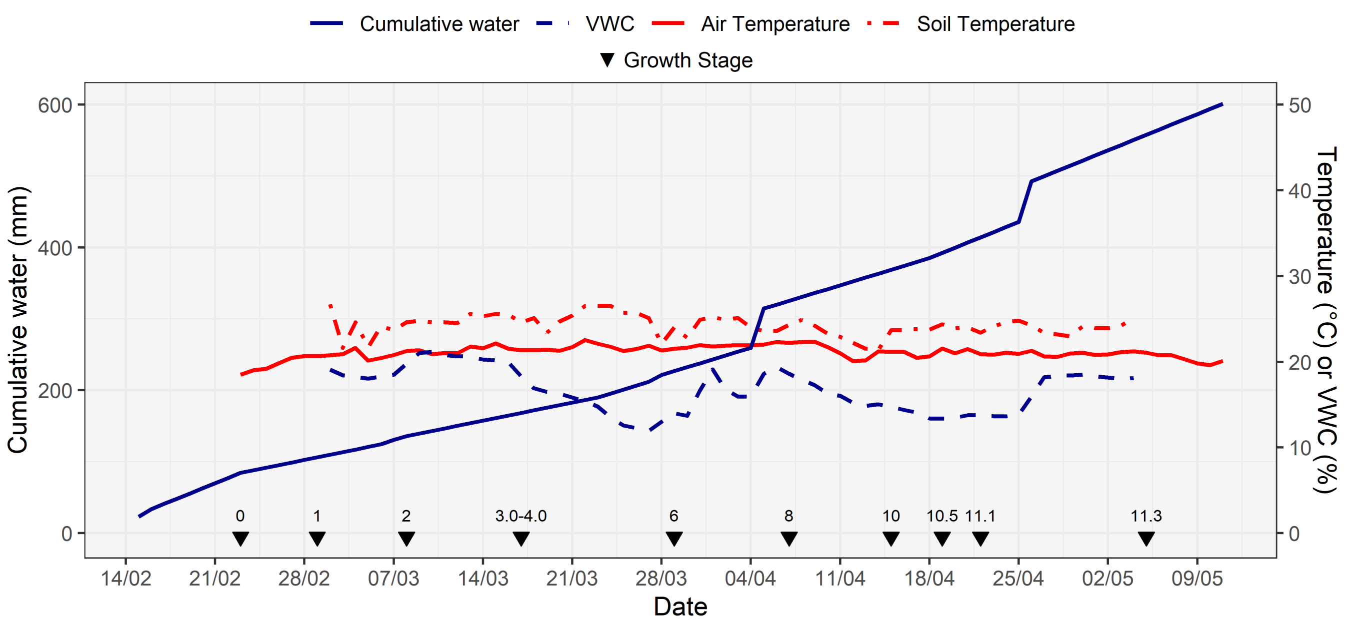
Tables and Figures

TABLE 1: Soil Properties

Source of Variation	% Sand	% Silt	% Clay	SHC (cm/hr)	BD (g cm^{-3})	Total C (%)	Active C (mg /kg soil)	Total N (%)	NO_3^--N (mg/kg soil)	PMG (mg/kg soil)	OM (%)
SOC_{high}	40	32	29	77.3	1.17	1.32	575	0.13	10.99	82.8	3.1
SOC_{low}	39	32	29	25.9	1.13	0.87	398	0.09	47.83	56.7	2.3

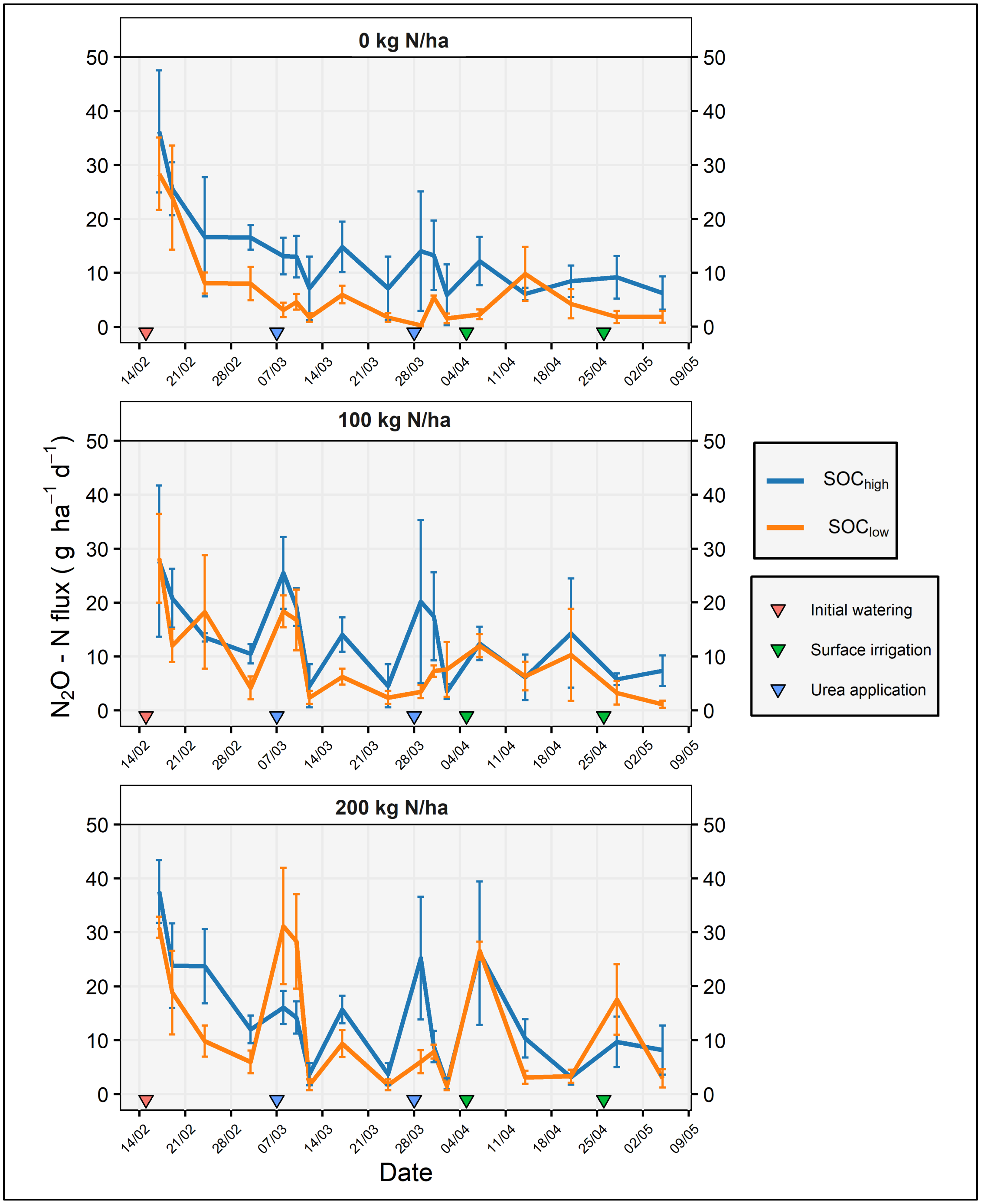
Note: Soil properties from (Mitchell et al., 2017, 2022)
SHC= Saturated Hydraulic Conductivity, BD= Bulk Density, PMN= Potentially Mineralizable Nitrogen, OM= Organic Matter

FIGURE 1: Ambient and Soil Environmental Data



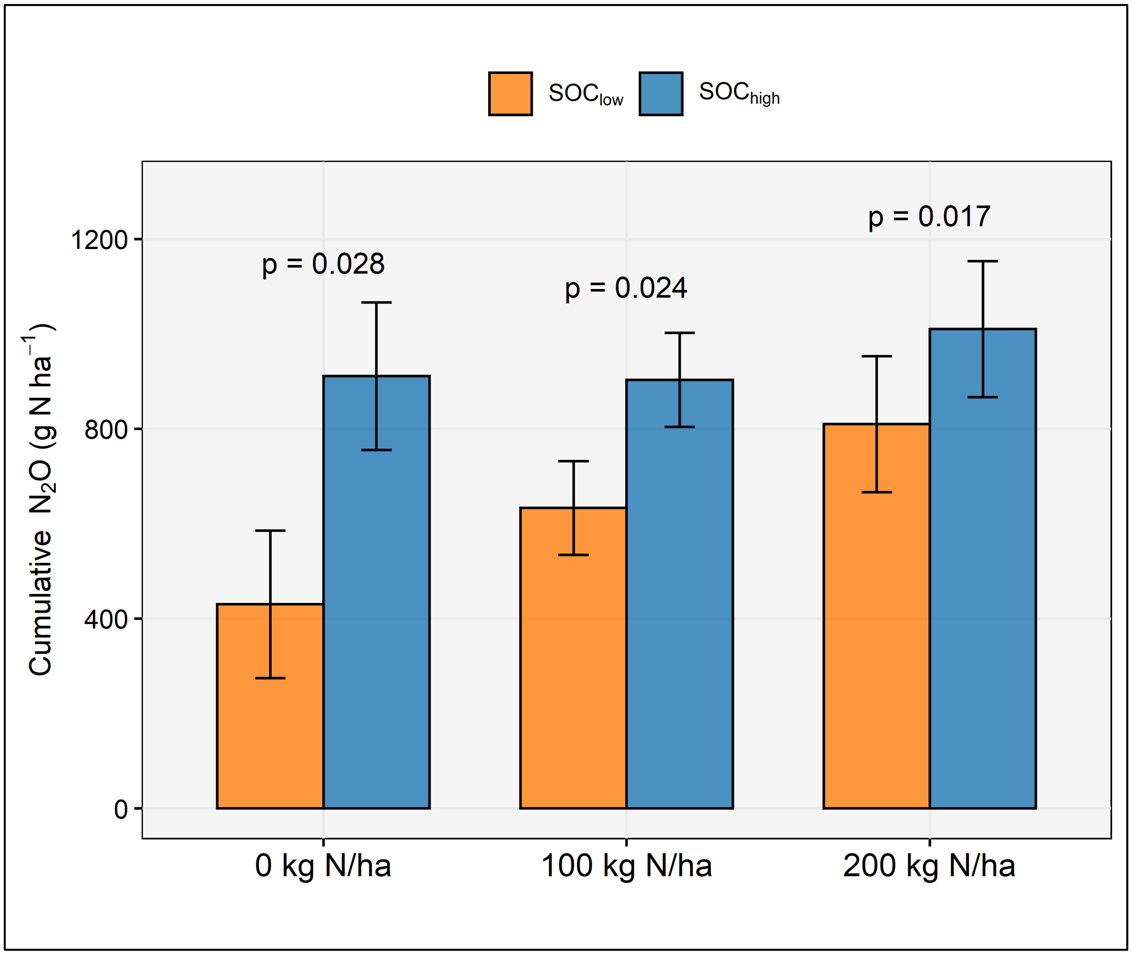
Note: Growth stages measured using Feekes Scale.
0=planting, 1.0=germination, 2.0=tillering, 3.0-4.0= tiller formation, 6.0= first node developed, 8.0= flag leaf developed, 10.0= booting, 10.5=booting, 11.1=milk, 11.3=hard kernel

FIGURE 2:Effects of soil carbon and nitrogen fertilizer on mean nitrous oxide fluxes.



Note: Error bars represent error of the mean. n=4

FIGURE 3: Mean cumulative nitrous oxide emissions across treatments



Note: p values represent differences in soils within each separate N rate

TABLE 2: Vegetative (non-grain), grain yield, total biomass, and water productivity for wheat grown on two soils (elevated and baseline SOC) under three N levels.

Soil	Nitrogen	Vegetative Biomass(g)	Grain (g)	Total Biomass (g)	Water productivity (g mm^{-1})
Elevated SOC		39.5	40.6	80.1	0.068
Baseline SOC		45.8	44.6	90.4	0.074
	Low	39.4 b	38.5b	77.8b	0.064b
	Medium	41.2 ab	42ab	83.2ab	0.0699ab
	High	47.3 a	47.4a	94.7a	0.0789a
P-VALUES					
Soil		0.05137	0.1473	0.05819	0.147
Nitrogen		0.04666	0.0309	0.03506	0.031

Note: Values followed by same letter within a column are not significantly different at p<0.05

TABLE 3: Nitrogen uptake in grain, dry matter, total crop N uptake, and N use efficiency (NUE) for wheat grown on two soils (elevated and baseline SOC) under three N levels.

Soil	Nitrogen	Vegetative N (g)	Grain N (g)	Total N uptake (g)	NUE (%)
$\mathrm{SOC}_{\mathrm{high}}$		0.39	0.78	1.17	13.8 b
$\mathrm{SOC}_{\mathrm{low}}$		0.46	0.92	1.38	48.7 a
	0	0.36 b	0.71	1.07 b	-
	100	0.40 b	0.82	1.23 b	30
	200	0.51 a	1.02	1.53 a	32.5
P-VALUES					
Soil		0.2203	0.2288	0.2222	0.01431
Nitrogen		p < 0.001	p < 0.001	p < 0.001	0.720
$\mathrm{SOC}_{\mathrm{high}}$	0	0.367	0.712	1.08	-
$\mathrm{SOC}_{\mathrm{low}}$		0.354	0.699	1.05	-
$\mathrm{SOC}_{\mathrm{high}}$	100	0.354	0.736	1.09	9.41
$\mathrm{SOC}_{\mathrm{low}}$		0.45	0.912	1.36	50.5
$\mathrm{SOC}_{\mathrm{high}}$	200	0.44	0.895	1.34	18.1
$\mathrm{SOC}_{\mathrm{low}}$		0.576	1.14	1.72	46.9
P-VALUES					
	0	0.857	0.935	0.91	-
	100	0.122	0.202	0.17	0.029
	200	0.045	0.005	0.015	0.009

Values followed by same letter within a column are not significantly different at p<0.05