EFFECTS OF NITROGEN MANAGEMENT AND CULTIVAR ON STRAWBERRY PRODUCTION UNDER DISEASE PRESSURE

A Thesis

Presented to

the Faculty of California Polytechnic State University,

San Luis Obispo

In Partial Fulfillment

Of the Requirements for the Degree

Master of Science in Agriculture

Specialization in Plant Protection Science

By

Kamille Garcia-Brucher

December 2021
COMMITTEE MEMBERSHIP

TITLE: Effects of nitrogen management and cultivar on strawberry production under disease pressure

AUTHOR: Kamille Garcia-Brucher

DATE SUBMITTED: December 2021

COMMITTEE CHAIR: Charlotte Decock, Ph.D.
Assistant Professor of Soil health and fertility
Department of Natural Resources Management & Environmental Sciences

COMMITTEE MEMBER: Gerald Holmes, Ph.D.
Director of the Cal Poly Strawberry Center
Department of Horticulture & Crop Science

COMMITTEE MEMBER: Christopher Appel, Ph.D.
Associate Professor of Environmental soil chemistry
Department of Natural Resources Management & Environmental Sciences
ABSTRACT

Effects of nitrogen management and cultivar on strawberry production under disease pressure

Kamille Garcia-Brucher

California strawberry growers face increasing regulatory pressures to manage nitrogen (N) applications in their production system. Standard practice in the California strawberry industry is to apply a synthetic pre-plant controlled release fertilizer (CRF) to ensure the crop has sufficient N during winter establishment. Some research from the UC Cooperative Extension suggests this practice is not efficient at delivering N to the crop since most of the N is released from CRF before strawberry crop N uptake is significant. Another concern for California strawberry growers is loss of their crop to a myriad of soilborne pathogens. Compost is commonly applied as a soil amendment in California strawberry fields as it offers both agronomic and environmental benefits including the potential for disease suppression. In light of legislation restricting N in some California cropping systems, Ag Order 4.0, and incentives programs established to promote soil conservation practices, compost may be a viable substitute for synthetic pre-plant CRF N. In this study, we investigated the effects of pre-plant fertilizer and strawberry cultivar on fruit yield, disease incidence, soil and plant N dynamics and soil carbon (C) at the Cal Poly Strawberry Center, San Luis Obispo, CA in a field infested with Macrophomina phaseolina. Pre-plant fertilizer treatments included 100 lb N/ac Cal Poly certified organic compost, 100 lb N/ac synthetic CRF and a control treatment (0 lb N/ac). Strawberry cultivars included three UC varieties, ‘Monterey,’ ‘Albion,’ and ‘San Andreas,’ and one Driscoll’s proprietary cultivar. Fruit yield and plant mortality data were collected throughout the growing season. Soil C was measured from soil samples collected in the root zone (6 in) while soil nitrate was measured from pore water samples collected in and below the root zone (6 and 12 in, respectively). Strawberry crop N uptake was determined using destructive plant samples while fruit N concentration was determined from subsamples of harvested fruit taken in April, May, June, and July each year. Although compost application did not significantly affect C sequestration and did not reduce disease incidence, there was no significant difference in total yield between compost and CRF treatments suggesting that compost can substitute for synthetic CRF without negatively affecting yield. There was significantly less plant mortality in control treatments compared with compost and CRF treatments suggesting excessive pre-plant N impacts disease incidence by M. phaseolina but more research is needed to better understand the mechanisms of infection by this soilborne pathogen. Total yield in this experiment was lower compared with statewide averages and crop N concentration was lower compared with the literature which is likely a result of disease pressure. Fruit N concentrations for the cultivars in this study were lower than the conversion coefficient defined by the Ag Order which means growers are removing less N through harvest allowing them more room in their N budget. Based on our results, compost may be substituted for synthetic CRF without negatively affecting yield and perhaps even make desirable soil improvements in this production system. And in fields with significant
levels of *M. phaseolina* in the soil, N applications should be considered as it was seen to impact disease incidence.

Keywords: compost, controlled release fertilizer, *M. phaseolina*, nitrogen uptake, Ag Order 4.0
ACKNOWLEDGEMENTS

I would like to thank the Agricultural Research Institute (ARI) of the California State University for funding this project and my coursework. I would like to thank my thesis committee, Dr. Charlotte Decock and Dr. Christopher (Chip) Appel of the Department of Natural Resources Management & Environmental Sciences at California Polytechnic State University, San Luis Obispo, and Dr. Gerald Holmes of the Cal Poly Strawberry Center and the Department of Horticulture & Crop Science. I would also like to thank Dr. Shashika Hewavitharana of the Cal Poly Strawberry Center and the Department of Horticulture & Crop Science and Mr. Drew Summerfield of the Cal Poly Strawberry Center.

I would also like to extend my gratitude to the many graduate students and undergraduate students who assisted me with this project, especially during harvest. Specifically, Ms. Connie Wong, Ms. Robyn Brooks, Ms. Janelle Rey and Ms. Morgan Morris who all made a huge impact on my work. I would also like to thank the staff of the Department of Natural Resources Management & Environmental Sciences at California Polytechnic State University, San Luis Obispo, especially Mr. Craig Stubler who always made time to assist with lab work and maintain the equipment essential to the project.

I would like to thank my parents and sisters who have all been incredibly supportive and encouraging. And finally, I have to extend my sincerest gratitude to my husband, Allen Brucher and my sweet daughter, Margot Olavi Brucher, for their continued love and support.
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CHAPTER 1

Introduction

California grows nearly 90% of the strawberries produced in the U.S. on less than 1% of the state’s total farmland (California Strawberry Commission (CSC), 2021). The high production of strawberries in California can be attributed to the yield potential of the cultivars grown, the mild coastal climate, the use of annual production systems that use pathogen- and pest-free planting stock each year, the intensive management of the crop, and the length of the growing season (USDA, 1999). In addition to meeting consumer demands, strawberry growers must battle issues relating to pests and disease, labor, water resources, nutrient management, and mounting legislation. Most strawberry production is centered in the coastal valleys of California with major production regions in Oxnard, Santa Maria, Salinas and Watsonville where groundwater monitoring has revealed nitrate levels that exceed the federal drinking water standard of 10 mg N/L (Bottoms et al., 2013; EPA, 2019). Strawberry growers are under increasing regulatory pressure to improve their nitrogen (N) management practices with regards to N application in their production system.

Agriculture has long been a source of anthropogenic N pollution. Nitrogen is a key limiting nutrient for most crops (Good and Beatty, 2011). Growers compensate for this limitation with the use of synthetic and organic N fertilizers. Like most crops, the yield and quality of strawberry fruit is strongly affected by plant N status (May and Pritts, 1990). Recent legislation from the State Water Resources Control Board (SWRCB) is meant to limit nitrate pollution in ground and surface waters by monitoring and, if
necessary, restricting N applications. The Central Coast Regional Water Quality Control Board (RWQCB) has prepared a new permit, Agricultural Order 4.0 (Ag Order 4.0) to regulate the discharge of agricultural pollutants from irrigated lands within the Central Coast region (RWQCB, 2021). This order became effective in early 2021 and outlines targets for discharge of agricultural wastes until 2050. California strawberry growers commonly apply N fertilizer in two forms: a pelletized, coated pre-plant fertilizer or control release fertilizer (CRF) and in-season liquid N fertilizer via drip irrigation, a method called fertigation. Synthetic pre-plant fertilizer offers short-term N availability to ensure the crop has sufficient N during winter establishment (Bolda et al., 2012). The decision to use pre-plant fertilizer may depend on several factors including anticipated rainfall, soil texture, previous crop, initial soil nitrate concentration, and additional N contribution from other sources such as high nitrate in irrigation water (Cahn, 2019). A 2012 survey of 21 Santa Maria strawberry growers revealed that growers typically apply more nitrogen in the pre-plant form compared with in-season fertigation (Bolda et al., 2012). This is changing, though, as some research has shown that routine use of high CRF rates is not an efficient practice and reducing CRF application rates could substantially improve N use efficiency (Bottoms et al., 2013). A more recent survey of 14 Salinas and Watsonville strawberry growers, conducted in 2017, placed less emphasis on pre-plant fertilizer application and a greater emphasis on in-season fertigation to meet the strawberry crop N demand (Cahn, 2019). Annual N uptake by strawberry crops has been reported to range from 59 kg/ha to 200 kg/ha (Albregts and Howard, 1980; Latet et al., 2002; Strik et al., 2004; Tagliavini et al., 2004, 2005). Strawberry crop N uptake curves have been shown to be cultivar-related and may become a useful tool for adjusting
fertilizer application rates (Agüero and Kirschbaum, 2013; Santos and Whidden, 2007; Simonne et al., 2001; Tagliavini et al., 2005). Research examining the effect of genotypic and phenotypic differences in strawberry cultivars on varying N application rates has shown that morphological differences such as canopy size and root density influence rate of crop N use efficiency (Agüero and Kirschbaum, 2013; Santos and Whidden, 2007; Santos and Chandler, 2009; Simonne et al., 2001; Tagliavini et al., 2005). More research is needed across cultivars to better understand early-season strawberry N requirements and the fate of the nitrogen in pre-plant fertilizers in California strawberry production.

In order to incentivize N use efficiency in California cropping systems, the Healthy Soils Initiative has been established by the California Department of Food and Agriculture (CDFA). This initiative provides financial incentive programs such as the Healthy Soils Program (HSP) for California farmers and ranchers to implement practices that improve soil health and reduce greenhouse gas (GHG) emissions (Gravuer, 2016). One agricultural practice with considerable soil health improvement and GHG reduction potential is the application of compost to croplands and rangelands. Compost is already a common soil amendment used in commercial strawberry production as it can provide both agronomic and environmental benefits (Lloyd et al., 2016). Some long-term studies have shown the repeated application of composted materials can enhance soil organic N content, storing it for mineralization in the following cropping seasons, while one study in particular demonstrated organic compost input to be superior to conventional synthetic fertilizer in building soil nutrient levels and reducing nutrient losses to ground and surface waters (Diacono and Montemurro, 2010; Hepperly et al., 2009). Other studies such as Hartz et al. (2002), report an N mineralization rate of 31 manures and composts
to be relatively low, which may pose limitations for enhancing short-term N availability but can be valuable in long-term soil building. Moreover, it is possible that compost can have different effects on soil N when used across different strawberry cultivars. Research in wheat showed root phenotypic differences across genotypes alter the soil rhizosphere community and impact nitrogen cycling through enhanced enzyme activity and microbial biomass in compost amended soils (Kallenbach et al., 2017). With its ability to cycle and retain nutrients and its beneficial effects on long-term soil organic N, compost may be a suitable alternative to synthetic pre-plant fertilizer in California strawberry production systems.

Another issue concerning California strawberry growers is the impact of soilborne pathogens on this high value crop. Managing the soil is important to California strawberry growers because changing fumigation practices have increased the incidence and severity of many soilborne diseases (Koike et al., 2012). Beginning in 2005, strawberry growers in southern California reported an increasing problem with collapsing strawberry plants (Koike et al., 2013). Initially documented in Orange and Ventura Counties, *Macrophomina phaseolina*, was found in Santa Barbara and San Luis Obispo Counties and then later in the Central Coast regions of Monterey, Santa Cruz, and Santa Clara Counties (Koike et al., 2013). *M. phaseolina* survives in both infected crop debris and soil as microsclerotia, survival structures that serve as the primary source of inoculum in the subsequent strawberry crop (Carter, 2016). Nearly all the initial outbreaks of this disease have been associated with strawberry fields that were no longer fumigated with methyl bromide (MeBr) used in combination with another fumigant, chloropicrin (Koike, 2008). The Montreal Protocol mandated the phaseout of methyl
bromide beginning in 1991 to be completed by 2005. Because no effective alternative to methyl bromide had been identified, California strawberry growers were granted critical use exemptions allowing them to continue using the fumigant, though their quantities were far more limited and approved amounts for use by strawberry growers declined until its total phaseout in 2016 (Guthman, 2017).

Soilborne pathogens have long plagued the strawberry industry and new diseases continue to emerge. There is ongoing research in the strawberry industry with a focus on alternatives to methyl bromide for control of diseases by various soil borne pathogens. The use of genetic resistance to control soilborne disease has become one of the most practical and successful initiatives in the strawberry industry. All major strawberry breeding programs have made soilborne disease resistance a top priority (Holmes et al., 2020). Other disease management strategies include biological methods such as anaerobic soil disinfestation, soil solarization, soil steaming, and the use of organic amendments like compost. Compost has been identified as a contributor to disease suppression based on qualitative and quantitative changes it induces in the soil microbial community (Lloyd et al., 2016; Noble and Coventry, 2005). Given the potential for cultivar and nutrient management interactions to influence soil N, it is possible that this interaction can also affect disease incidence by soilborne pathogens.

To study the effect pre-plant fertilizer management strategies and cultivar have on strawberry crop yield, soil and crop N dynamics, soil carbon and disease incidence, we set up a controlled field experiment at the Cal Poly Strawberry Center in October of 2018 in a field previously infested with M. phaseolina. In light of recent legislation impacting N application in strawberry production systems and increasing disease pressures, our
project aimed to assist growers with pre-plant N management strategies that may reduce
disease incidence while preserving yield. This study was undertaken to evaluate three
pre-plant fertilizer treatments: a green waste/manure compost, synthetic CRF, no pre-
plant N and four strawberry cultivars: Monterey, Albion, San Andreas, and a proprietary
cultivar. The objective was to determine whether pre-plant fertilizer treatments
differently affect (i) total yield, (ii) soil NO$_3^{-}$-N in the active root zone and below the
active root zone, (iii) soil C in the active root zone, (iv) crop and fruit N uptake, and (v)
disease incidence across the four strawberry cultivars. We also hoped to identify cultivar
by pre-plant nutrient management interactions that simultaneously reduce disease,
improve soil N dynamics and increase crop yield.

We hypothesize that pre-plant fertilizer treatment will have no significant effect
on total yield and that this is cultivar dependent. We hypothesize that plant mortality will
be lower in compost treatment blocks compared with control and CRF treatment blocks.
We also hypothesize that N uptake in vegetative tissue and fruit tissue is cultivar
dependent and that pre-plant fertilizer treatment will not be significant enough to affect
plant N sufficiency levels. And finally, we hypothesize that levels of active soil C will be
greatest in compost treatments and that some cultivars may even show greater C
allocation to plant roots under compost treatment.
CHAPTER 2

Literature Review

2.1 California Strawberry Production

Prior to World War II, California strawberries accounted for only 6-7% of the total U.S. strawberry production (Thomas, 1939). The Pacific Northwest led the nation in production due to their lower costs for land and labor (Thomas, 1939). After the war, the industry took a turn when population growth in California provided growers of fresh strawberries with two of the largest markets in the nation, namely the Los Angeles area and the San Francisco Bay area, within easy transportation distance (Geissler and Horwath, 2014). Between 1945 and 1957, the strawberry acreage in California increased from 1,100 to more than 20,000 acres (Geissler and Horwath, 2014). In 1957, over 550 million pounds of strawberries were harvested in the U.S. with California accounting for over 40% (Bain and Hoos, 1963). Today, the California strawberry crop represents a value of $2.8 billion, making this industry an integral component of the agricultural economy in the state (CSC, 2021). The following chapter briefly summarizes the cultural practices in conventional strawberry production systems and the current legislation affecting the California strawberry industry.

2.1.1 Cultural Practices

Although strawberries are naturally a perennial plant, in California they are primarily cultivated as an annual crop for higher yields and lower disease and pest pressures in the first year (Hancock, 1999; Strand, 2008). California strawberry production is centered in the coastal valleys with Oxnard, Santa Maria, and
Salinas/Watsonville comprising 19.4%, 36.8% and 42.9% of the state’s total fall-planted strawberry acreage, respectively (CSC, 2021). Because of the mild climate and fertile soil, strawberry production can be shared across these coastal valleys and this system can essentially operate year-round. It is widely accepted that the best land for strawberries is sandy loam to loam soil to allow drainage and still provide important organic matter levels (Bolda et al., 2015). These deep, well-drained sandy loam soils also make for easier field preparation, more effective fumigation, lower salt accumulation, and are better suited for frequent irrigation and field activity (Bolda et al., 2015).

Field preparation for commercial strawberry production in California usually begins with soil fumigation and bed listing with most of the planting for fruit production occurring in the fall for short-day varieties. Chemical soil fumigation typically occurs several weeks before planting and takes place under a sealed plastic tarp (USDA, 1999). With the phaseout of methyl bromide in 2016, agronomists and growers are collaborating to find alternatives that match the disease control seen with methyl bromide application but other chemical fumigants such as chloropicrin, meta sodium, and 1,3-dichloropropene + chloropicrin are applied in a similar way (USDA, 1999). Once the soil is fumigated, tractor implements shape the field into raised beds which promote soil drainage and boost yields (Wilhelm and Sagen, 1974). Standard practice in California strawberry production is to apply a pre-plant fertilizer in the form of a controlled release N-P-K (nitrogen-phosphorous-potassium), ‘strawberry mix,’ below the drip line often between plant rows (Hartz and Bolda, 2011) or below the plant line directly. The raised beds are then tightly covered in polyethylene mulch to increase bed temperature to benefit root development in the winter and reduce evaporative losses of irrigation during the warm seasons (Strand
In coastal production fields of California, bare-root strawberry transplants from high elevation nurseries are planted in holes punched in the plastic mulch at equal spacing. The transplants are typically irrigated with overhead sprinklers for the first five weeks to leach salts and maintain plant turgidity (Daugovish et al., 2016). During the remainder of the season, plants are irrigated and fertilized via drip lines placed 3-7 cm in the soil and between plant rows (Daugovish et al., 2016). Strawberry fields currently receive on average about 200 lb per acre fertilizer N over a production season, but N fertilization rates and timing vary widely among growers (Hartz et al., 2018). Production in southern regions (Oxnard and Ventura) begins first while production in Salinas and Watsonville begins last (Bolda et al., 2015). Strawberries are harvested in one or more growing regions every month of the year, with peak production occurring in late spring (USDA, 1999; Bolda et al., 2015). Fruit harvest times vary but it is harvested roughly every 3 to 4 days depending on temperature and market demand and by the end of the season, yields can be between 50,000 and 67,000 lb/ac (Bolda et al., 2000; Bottoms et al., 2014).

2.1.2 Legislation

Agriculture has long been a source of environmental contamination. Growers apply nutrients on their fields in the form of chemical fertilizers and animal manures. Excess nutrients can be washed from the fields and into waterways during rain events and when snow melts and can also leach through the soil and into groundwater over time. High levels of nitrogen and phosphorous can cause eutrophication of water bodies leading to hypoxia (“dead zones”) and harmful algal blooms and disrupt wildlife and
produce harmful toxins (EPA, 2020). In addition to excessive nutrient applications, chemical pesticides for managing unwanted insects, weeds, and pathogens can contaminate air, soil, water, turf, and other vegetation and become toxic to a host of non-target organisms including humans. California is one of the leading regions for agricultural production, producing half of the nation’s fruits, vegetables, and nuts (Guthman, 2018). Therefore, it is no surprise that it should be the premier example for regulation to reduce the negative effects agriculture can have on the environment.

2.1.2a Nitrate Pollution and the Irrigated Lands Regulatory Program

Nitrate pollution of drinking water supplies is a critical problem throughout the central coast of California. Studies indicate that fertilizer from irrigated agriculture is the largest primary source of nitrate pollution in drinking water wells and nitrate loading continues as a result of agricultural fertilizer practices (Carle et al., 2006). Hundreds of drinking water wells serving thousands of people throughout the region have nitrate levels exceeding the drinking water standard (CA Department of Public Health, 2017). Beginning in 2003, the State Water Resources Control Board implemented the Irrigated Lands Regulatory Program (ILRP) to regulate discharges from irrigated agricultural lands to protect surface water and groundwater throughout California. This included an order released in 2004 (2004 Agricultural Order) which found that discharge of waste from irrigated lands had impaired and polluted the waters of California and of the U.S. within the central coast region (California RWQCB Central Coast Region Order No. R3-2017-0002, 2017). Over the years, this order has developed and become increasingly definitive regarding N management in several cropping systems including strawberry production systems in the central coast region.
Although this legislation aims to protect public health and ensure safe drinking water by controlling nitrate loading to groundwater and public water systems, it has serious implications for strawberry growers throughout this region of California. Understanding the N dynamics in the strawberry production system can influence this legislation and its impact on N management decisions for strawberry growers.

2.1.2b Methyl bromide and the Montreal Protocol

Methyl bromide is recognized as an ozone-depleting substance and is known to be problematic to public health and, as a result, has been phased out of production and use as of 2016. Although soil treated with MeBr is covered with plastic tarps immediately after application, 50 to 95% of the MeBr eventually enters the atmosphere leading to increased depletion of ozone allowing increased ultraviolet radiation to reach the earth’s surface (EPA, 2020). The Montreal Protocol is an International treaty designed to reduce the use of products containing substances responsible for ozone depletion (EPA, 2020). It mandated the phaseout of methyl bromide beginning in 1991 to be completed by 2005 but because no effective alternative to methyl bromide had been identified, California strawberry growers were granted critical use exemptions allowing them to continue using the fumigant until its total phaseout in 2016.

2.2 Disease Management

Since the 1960’s, MeBr combined with chloropicrin was almost universally used to control soilborne diseases in conventional strawberry and fresh market vegetable production systems. It was unsurpassed in the ability to control a myriad of pathogens
and was cost effective over a range of soil conditions and production systems (Chellemi, 2002). In the wake of its phaseout, growers have tackled disease management through alternative chemicals, intensive cultural sanitation practices, and alternative methods including integrated pest management approaches to control for soilborne pathogens. The following chapter introduces the emergence of soilborne diseases in the strawberry production system and the common methods to control soilborne diseases including chemical control, such as fumigation with methyl bromide, plant breeding of specific cultivars for disease resistance, and alternative methods of disease management such as compost, investigations into the rhizosphere and how management of nitrogen applications can impact disease.

2.2.1 Emergence of soilborne diseases

Since the phaseout of MeBr, the California strawberry industry has suffered production losses caused by soilborne fungi not previously recognized as strawberry pathogens in California. Verticillium wilt was the primary threat to strawberry fruit production in California (Holmes et al., 2020). Changes in fumigant chemistries and application methods have been associated with the emergence of important soilborne diseases including Fusarium wilt (caused by *Fusarium oxysporum*) and *Macrophomina* charcoal rot (caused by *Macrophomina phaseolina*) (Holmes et al., 2020; Koike, 2008; Zveibil and Freeman, 2005). This section of the literature review will focus on *Macrophomina* charcoal rot.

Beginning in 2005 or before, strawberry growers reported an increasing problem with collapsing strawberry plants. Symptoms included wilting of foliage, plant stunting,
and drying and death of older leaves. Plants eventually collapsed and died, especially if such plants were subjected to environmental stresses or were bearing a heavy fruit load. When plant crowns were cut open, internal vascular and cortex tissues were dark brown to orange-brown. The fungus *Macrophomina phaseolina* was consistently associated with these problems (Koike et al., 2013). *M. phaseolina* is a well-known soilborne pathogen that infects a wide range of crops (Koike et al., 2013). Originally found in only two California counties (Orange and Ventura), the disease caused by *M. phaseolina* known as charcoal rot has been confirmed in all of the major strawberry production regions in the state (Koike et al., 2013). The fungus has a wide range of hosts and upon infection and plant death, *M. phaseolina* produces large numbers of microsclerotia, which are known to survive for many years in fallow fields (Carter, 2016). In other pathosystems, the survival of *M. phaseolina* in soil and on debris has been reported for up to 15 years (Baird et al., 2003). The plants are predisposed to charcoal rot when exposed to stress factors such as extreme heat, drought, or excessive fruit load (Bolda et al., 2015). Plants infected with *M. phaseolina* do not show symptoms initially, however, once the plant undergoes stress (e.g., starts to produce fruit), symptoms develop (Koike, 2012). Symptoms include discoloration of leaves, wilting, and overall plant decline and plant death (Gupta et al., 2012; Kaur et al., 2012; Koike, 2012; Koike and Bolda, 2013). Charcoal rot symptoms are absent while plants are small during fall and winter but increase rapidly during the spring and summer due to higher soil temperature and increased water stress (optimal range 25-30°C) (Mihail, 1992; Wyllie et al., 1984; Zveibil et al., 2012). Fruit production is not affected by onset of symptoms until the plant starts to collapse from infection (Mertely et al., 2010; Mertely et al., 2014).
2.2.2 Chemical Control

Soil fumigation has successfully controlled disease caused by soilborne pathogens in addition to promoting a positive plant growth and yield response even in the absence of soilborne pathogens (Chamorro et al., 2016). One of the most momentous shifts in cultural practices for strawberries was the introduction of preplant soil fumigants, beginning with chloropicrin in the 1950s and MeBr in the 1960s (Tourte et al., 2016). The use of these fumigants led to higher and more predictable yields and fruit quality and further enabled the development of more stable markets for strawberries (Wilhelm and Westerlund, 1994). Yields for strawberries statewide increased from a range of two to four tons per acre prior to the introduction of soil fumigants to 16 tons per acre by 1969 (Geisseler and Horwath, 2014). The combination of MeBr and chloropicrin has proven efficacious against diseases such as soilborne fungi that cause Verticillium wilt, Phytophthora root and crown rots, anthracnose, black root rot, charcoal rot, and, significantly, other soilborne pathogens of unknown etiology that impact strawberry plant yield and quality (USDA, 1999). Conventional strawberry growers typically inject soil fumigants approximately 30 days prior to planting at a rate of 300 to 400 lb ai combination/ac (USDA, 1999). Alternative fumigant chemistries are available but limitations in terms of efficacy relative to MeBr continue to exist (Duniway, 2002). And, despite advances in fumigation technology that have led to reduction in emissions, the long-term availability of these alternative fumigant chemistries is not certain (Mazzola et al., 2017).

Multiple MeBr alternatives exist and are registered including chloropicrin, 1,3-dichloropropene, and methyl isothiocyanate (Ajwa et al., 2003). Chloropicrin has strong
fungicidal activity (Shaw and Larson, 1999) and is the most widely used preplant fumigant. It was initially introduced to control Verticillium wilt (Martin, 2003). Initially marketed for its nematicide properties, 1,3-dichloropropene has also been recognized for its fungicidal properties (Winslow, 2019). However, there has been a history of regulatory concerns with this product due to its contamination of groundwater and air quality (Duniway, 2002). Methyl Isothiocyanate has strong broad-spectrum activity against plant pathogenic nematodes, weeds, oomycetes and fungi but has been deemed unreliable due to its variable efficacy and low strawberry yields following its use (Duniway, 2002; Winslow, 2019). As MeBr use has declined, the use of chloropicrin has increased proportionally and is currently the most widely used fumigant in the California strawberry industry (Holmes et al., 2020).

2.2.3 Genotypic Effects on Disease

Higher levels of genetic resistance to disease in strawberry cultivars can be achieved through breeding (Lloyd and Gordon, 2016). However, because MeBr was so effective at controlling soilborne diseases, breeding programs focused on developing other traits (Guthman, 2017). There is an absence of commercial strawberry cultivars that possess high levels of resistance to multiple pathogens (Nellist, 2018). Since the phaseout of MeBr, use of genetic resistance to control soilborne diseases became more attractive (Miles et al., 2018). Commercial strawberry cultivars vary in their susceptibility to pathogens. Resistance to pathogens has been provided by the use of dominant resistance genes (R-genes), which recognize pathogen avirulence genes (Avr) and are based on gene-for-gene resistance such as in the interaction of the cultivated strawberry with Phytophthora fragariae (van de Weg, 1997). For other diseases such as Verticillium wilt
caused by *Verticillium dahliae*, resistance is under complex control of multiple genes (Antanaviciute et al., 2015). Resistance in strawberry to Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *fragariae*, and to anthracnose, caused by *Colletotrichum acutatum*, is a result of single dominant resistance genes, *Fw1* and *Rca2*, respectively (Pincot et al., 2018; Denoyes-Rothan et al., 2005). Several studies have focused on resistance in strawberry to *M. phaseolina* which causes the disease charcoal rot. One such study included a screening of 90 strawberry cultivars planted for a field trial at the Cal Poly Strawberry Center in San Luis Obispo, California. Strawberry plants were inoculated shortly after transplanting during the 2017 growing season and were subjected to water stress in late summer. This produced a wide range of responses showing that host resistance can be used to partially control or delay this disease (Holmes et al., 2020). However, similar studies exploring the susceptibility of different cultivars to charcoal rot produce conflicting results and therefore further work is required to explore the tolerance and resistance mechanisms.

2.2.4 Alternative management strategies

In addition to chemical fumigation by MeBr and host resistance strategies, other methods of controlling soilborne pathogens are gaining popularity among commercial strawberry growers in the wake of the MeBr phaseout. These include the use of other chemical fumigants such as chloropicrin and dichloropropene, careful management of cultural practices such as preventative sanitary measures and crop rotation, and the addition of organic amendments through composts or a technique called anaerobic soil disinfestation (ASD).
2.2.4a Rhizosphere

Plant resistance is one of the effective strategies in plant protection and attempts have been made to study effects of resistant and susceptible cultivars on microbial communities (Azad et al., 1987; An et al., 2011; Yao and Wu, 2010; Lazcano et al., 2021). Plant genotype can have a significant impact on soil microbial community structure, and differences in the rhizosphere microbial community have been suggested to contribute to the difference in resistance to disease (Nallanchakravarthula et al., 2014; Lazcano et al., 2021). Hotspots of microbial diversity exist in the soil in microenvironments like the soil-root interface, otherwise termed the rhizosphere. The rhizosphere represents the thin layer of soil surrounding plant roots and the soil occupied by the roots and supports large active groups of microorganisms (Geetanjali and Jain, 2016). Rhizodeposits include sloughed-off root cap and border cells, mucilage, and root exudates (Boyd, 2019). The vast organic compounds such as amino acids and sugars secreted by plant roots in the rhizosphere provide a food source for microorganisms increasing microbial biomass and their activity in the rhizosphere (Geetanjali and Jain, 2016). The release of organic compounds by the plant root, or rhizodeposition, drives the formation of microbial communities near, on, or within the root. Plants benefit from increased nutrient availability and improved resistance to biotic and abiotic stressors provided through complex interactions with microbial associates.

Root exudates include a wide array of primary and secondary metabolites including, vitamins, amino acids, hormones, phenolic acids and enzymes (Lynch and Whipps, 1990). Exudation is most common at the root tip and is both an active and passive process in the plant with the function of sensing, manipulating, and
communicating with the soil environment (Canarini et al., 2019). These functions have the capacity to attract, deter or kill belowground insects, herbivores, nematodes, and microorganisms, inhibit the growth of competing plants, and sense resource availability (van Dam and Bouwmeester, 2016). In this way, root exudates can act as a first line of defense against invading pathogens or attract beneficial microorganisms to establish a niche in the rhizosphere (Philippot et al., 2013).

2.2.4b Compost

Reducing the amount of pesticide used in food production continues to be a major objective in agricultural policy to reduce environmental contamination and has become increasingly important among consumers. Compost is commonly applied as a soil amendment in conventional strawberry production systems at a rate between 4.4 and 6.6 tons/ha (3,500 to 5,400 lb/ac) (Lloyd et al., 2016). Compost can also be used to help suppress disease. Consistent and sustained biological control of diseases caused by several types of soilborne plant pathogens can be achieved in differing compost-amended growing media as long as variables such as consistency of the parent organic material, moisture content, salinity, carbon to nitrogen ratio, and process parameters are controlled in the compost (Cohen et al., 1998; Hoitink et al., 1977; Quarles and Grossmann, 1995). In a review article on the use of composts to suppress soilborne diseases, Noble and Coventry (2005), found that disease suppressive effects increased with the rate of application of organic amendments in the majority of the 92 studies reviewed. Although effects of compost on disease suppression are more consistently seen in container-based studies in greenhouses or growth rooms, composts have also been shown to suppress
several diseases in the field (Noble and Coventry, 2005). The greater efficacy of
composts in suppressing disease in greenhouses or growth rooms than in the field may be
due to enhanced activity of microbial antagonists at higher temperatures and/or the better
mixing of compost in containers than in field plots (Noble and Coventry, 2005). Some
disease suppressing effects of compost used in field settings have been demonstrated with
common soilborne strawberry pathogens. Lodha et al. (2002) found that amendment of
soil with compost applied at a rate of 4 lb per acre prepared from crop or weed residues
reduced the severity of dry root rot in clusterbeans caused by *M. phaseolina*. However,
there has been more success seen in container-based studies. Antoniou et al. (2017) and
Borrero et al. (2004) reported the suppressive effect of plant-based composts on wilts
caused by *Fusarium oxysporum* and *Verticillium dahliae* on tomato plants grown in
containers. Spring et al. (1980) found significantly lower rates of apple seedling kill
cau sed by *Phytophthora cactorum* in media containers with bark compost compared with
media containers with peat.

Careful management of soil microbial communities can reduce pathogen disease
incidence and increase plant and soil health (Beneduzi et al., 2012; Santoyo et al., 2012).
The use of organic soil amendments promotes soil microbial diversity, including specific
taxa known to suppress soil-borne pathogens (Lupatini et al., 2017). There are several
known mechanisms by which certain soil bacteria can achieve their disease suppressing
properties. Some soil bacteria have been seen to produce substances that directly suppress
pathogens including antibiotics and siderophores (Beneduzi et al., 2012; Inderbitzen et
al., 2018). Others include indirect mechanisms such as successful competition for
nutrients by beneficial microorganisms, activation of disease-resistance genes in plants...
by microorganisms, and improved plant nutrition and vigor leading to enhanced disease resistance (Hoitink and Boehm, 1999).

Compost prepared from a range of feedstocks is already widely used in the USA for suppressing diseases on organic and conventional crops (Goldstein, 1998). In their study comparing the application of four commercially available composts in strawberry fields, Lloyd et al. (2016) found that all composts evaluated (manure, mushroom, yard trimmings, and vermicompost) led to substantial stimulation of microbial activity for a minimum of seven months, irrespective of geologic location and native microbial populations indicating potential for disease suppression should pathogens be present. In this particular study, applications of manure, mushroom and yard trimming composts were made at a much higher rate (67.2 tons/ha or ~60,000 lb/ac) than commonly found in conventional strawberry production systems (Lloyd et al., 2016). One impediment to using compost in this production system is the large cost associated with transport and application (Holmes, G. pers. comm. 2021). Although compost is already used as a soil amendment in California strawberry production, it is not necessarily used as a tool to control for disease by soilborne pathogens.

2.2.4c Nitrogen Management and Plant Disease

Nutrients are important for growth and development of plants and microorganisms and they are also important in disease control (Agrios, 2005). Although plant disease resistance and tolerance are genetically controlled (Agrios, 2005), they are affected by the environment and especially by nutrient deficiencies and toxicities (Marschner, 1995; Krauss, 1999). The use of fertilizers produces a more direct means of
using nutrients to reduce the severity of many diseases and, along with cultural practices, can affect the control of diseases (Marschner, 1995; Atkinson and McKinlay, 1997; Oborn et al., 2003). However, nutrients can affect the development of a disease by affecting plant physiology or by affecting pathogens, or both (Dordas, 2008). Nitrogen is by far the most extensively reported element affecting plant disease and is also the nutrient element applied in the largest quantity and the element most frequently deficient in cultivated soils (Datnoff et al., 2007). There are several reports of the effect of N on disease development that are inconsistent and contradictory. The causes of this inconsistency are poorly understood and may be a result of the different forms of N nutrition of the host (Huber and Watson, 1974; Celar, 2003; Harrison and Shew, 2001), the type of pathogen (Büschbell and Hoffmann, 1992; Marschner, 1995) or the development stage of N application (Carballo et al., 1994). In a meta-analysis conducted on 57 articles to identify the way plant disease severity of fungal pathogen-induced infection is modified following fertilization, N fertilization increased disease severity in the vast majority of instances (Veresoglou et al., 2013). However, the authors noted they could not detect any significant contrasts and failed to obtain significant one-trial-per-study subsets and remain skeptical of the tests due to the low replication level of most treatments (Veresoglou et al., 2013).

Nitrogen is intimately involved in most of the plant’s physiological processes for growth and disease resistance (Datnoff et al., 2007). It can induce various anatomical and biochemical changes such as reduced activity in key enzymes of phenol metabolism and lower lignin content, both of which are part of the defense system of plants against infection (Dordas, 2008). This along with the increase in the content of the low-
molecular-weight organic nitrogen compounds which are used as substrates for some parasites, is believed to be the reason for increased susceptibility to obligate parasites at high N rates (Dordas, 2008). Alternatively, nitrogen influences plant resistance by reducing the frequency of successful penetration by pathogens or slowing parthenogenesis after penetration (Datnoff et al., 2007). The situation is even more complex for soilborne pathogens as on the root surface there are many more microorganisms than in the bulk soil (Dordas, 2008). Also, there is competition between and repression of different microorganisms, and there are chemical barriers such as high concentration of polyphenols in the rhizodermis and physical barriers such as silicone depositions on the endodermis (Huber, 1980).

According to Datnoff et al. (2007) strategies for reducing disease with nitrogen nutrition include maintaining a balanced fertilizer program with full sufficiency of N for optimizing plant growth and yield, making timely applications of N to avoid periods of excessive N and predisposing environmental conditions for pathogens, using different forms of N to enhance disease control, and modifying environmental conditions to influence the predominant form of N that is optimum for plant resistance or less conducive to the pathogen itself.

Disease resistance will never be complete, and it will always be subject to compromise as resident pathogen populations evolve and new strains are introduced (Lloyd and Gordon, 2016). There may be no single solution to control the detrimental effects of soilborne pathogens in the strawberry industry and reducing inoculum levels of strawberry pathogens in the soil will require the implementation of multiple strategies.
2.3 Nitrogen Dynamics

The massive increase in anthropogenic N introduced into the environment, largely through N fertilizers, has had significant negative environmental consequences (Good and Beatty, 2011; Vitousek et al., 1997). Research in California indicates that irrigated agriculture contributes approximately 78% of the nitrate loading to groundwater in agricultural areas (Viers et al., 2012). Though crop fertilization is not the only contribution to nitrate contamination in groundwater, like other contributors, it is one that is ongoing and essential to the industry and the commerce of the State of California. Consequences associated with nitrate contamination of drinking water include human health hazards such as methemoglobinemia in infants and environmental hazards such as the eutrophication of fresh water and marine ecosystems. Although rare in industrialized countries, the use of nitrate-contaminated drinking water to prepare infant formula is a well-known risk factor for infant methemoglobinemia also called blue baby syndrome (Knobeloch et al., 2000). The body will convert these nitrates into nitrites which bind to hemoglobin forming methemoglobin which is unable to carry oxygen turning oxygen-deprived areas of the body blue (Knobeloch et al., 2000). Eutrophication of marine ecosystems can result in harmful algal blooms and create ‘dead zones’ depriving aquatic organisms of oxygen. Any negative human or environmental effect is made more concerning when the long-term impacts are not well understood.

Strawberry is a nitrogen-sensitive crop; the yield and quality of strawberry fruit is strongly affected by plant N status (May and Pritts, 1990). The following chapter outlines the extensive research that has focused on the N demand of the strawberry crop, including N uptake by specific strawberry cultivars, as well as N inputs, synthetic and
organic N sources and N dynamics in the soil once applied in this production system. This chapter also covers specific research that has been conducted to explore the effect of preplant control release fertilizer on N dynamics in the strawberry production system.

2.3.1 Nitrogen in conventional strawberry production systems in California

More recently, the strawberry industry has focused on ‘fine-tuning’ fertility and water management for more efficient resource use, along with additional yield and fruit quality improvements (Bottoms, Bolda, et al. 2013; Bottoms, Hartz, et al. 2013). The most effective N management strategy for a cropping system is one that provides adequate levels of soil N throughout the growing season by recognizing the pattern of N demand by the crop and the N release characteristics of significant N sources (Doerge et al., 1991). Cultural practices include pre-plant fertilizer application to fall-planted strawberries in California production systems. This pre-plant fertilizer is typically a coated, pelletized, control release fertilizer (CRF) designed to slowly release plant available N and other nutrients during the course of winter establishment. According to Shaviv (2005), the term controlled-release fertilizer applies to fertilizers in which factors dominating the rate, pattern and duration of release are well known and controllable during CRF preparation. Controlled-release fertilizers are typically coated or encapsulated with inorganic or organic materials that control the rate, pattern, and duration of plant nutrient release (Liu et al., 2014) such as polymer-coated urea CRFs (Du et al., 2006; Loper and Shober, 2012).

Extensive research has been performed to investigate fall-applied (pre-plant) N applications in strawberry plastic mulch systems throughout the United States. Excessive
pre-plant N rates have been reported to reduce yields, delay ripening, and produce soft fruit (Albregts et al., 1991; May and Pritts, 1990; Voth et al., 1967). Additionally, a high rate of N often results in production of excessive foliage (Voth et al., 1967) leading to increased disease susceptibility (Stadelbacher, 1963) and interference with harvest. A 2013 survey of 45 coastal strawberry fields revealed that about 50% of seasonal N is in the form of CRF and that, on average, growers apply more N in pre-plant form (~110 lb N/ac) compared with in-season N application through the drip system (~92 lb N/ac) (Hartz et al., 2018). This is changing, though, as research by Thomas Bottoms and Michael Cahn of the UC Cooperative Extension suggests that such high rates of CRF N may not be the most efficient N delivery system to the crop (Bottoms et al., 2013; Cahn, 2019). However, a moderate amount of CRF N provides insurance in case of nitrate loss during crown establishment from winter rains (Bolda et al., 2010). In Florida, the other major U.S. strawberry production region, the sandy soils throughout the state provided little organic matter to the crop and pre-plant N is often applied to supplement crop N demand. However, research by Agehara et al. (2007) found that applying starter N fertilizer did not improve monthly or total strawberry yield and other Florida studies have found little to no impact of pre-plant N rates on strawberry early and total yields (Albregts et al., 1991; Santos and Whidden, 2007).

Determining an N management strategy is essential and should consider any residual soil N and N applied through the irrigation water. Soil monitoring of 45 commercial fields in major California strawberry growing regions has shown that strawberry fields of heavier soil texture and particularly those in rotation with vegetable crops, tend to have high soil NO$_3$-N at planting (Hartz et al., 2018). Bolda et al. (2010)
conducted pre-fertilization sampling of eight strawberry fields coming out of vegetable production and found these fields tend to have an average of 115 lb/ac soil NO$_3^-$-N in the top 12 inches of soil. With good irrigation management during transplant establishment, the soil can retain significant residual NO$_3^-$-N into the winter (Hartz et al., 2018). Using a CRF product with an N release rate better matched to the crop N uptake pattern should also improve efficiency (Hartz et al., 2018). Following pre-plant N application, conventional strawberry growers fertigate using the drip irrigation system to deliver N fertilizer to the plant.

2.3.1a Crop N uptake

Annual N uptake by strawberry crops has been reported to range from 53 to 178 lb/ac (Albregts and Howard, 1980; Latet et al., 2002; Strik et al., 2004; Tagliavini et al., 2004, 2005). This wide range of N uptake is largely driven by crop productivity. Less research in California strawberry plant N uptake has been conducted but a study by Bottoms et al. (2013) of the UC Cooperative Extension showed total plant N uptake to be between 180 lb/ac and 220 lb/ac in plants throughout the coastal California production regions (Bottoms et al., 2013a and b; Hartz et al., 2018). Following transplanting, there is a period of slow growth where N uptake is low as transplants become established. A prolonged period of relatively constant growth and N uptake begins at the early flowering stage and continues through harvest period during which N uptake can average 1 lb/ac/day (Hartz et al., 2018). The California Department of Food and Agriculture has compiled optimal leaf nutrient concentrations for strawberries determined by a study in 53 commercial fields with ‘Albion’ strawberries located in the Watsonville-Salinas and
Santa Maria areas over two production seasons (CDFA, 2020). Leaf N concentrations between 3.1% to 3.8% at early flowering stage indicate optimal plant N status with a slow decline in leaf N of 2.7% to 3.2% by early harvest and from 2.4% to 3.0% by late harvest (Hartz et al., 2018). Plant N uptake is high during the fruit ripening stage when plants partition a high fraction of absorbed N to the fruit (Tagliavini et al., 2005). About 50% of total crop N is removed by fruit harvest (Hartz et al., 2018).

Several studies have focused on the uptake, assimilation, and partitioning of N in plant organs. Tagliavini et al. (2005) conducted a field study to compare the uptake and partitioning of nutrients in field grown strawberry plants under fertilized and unfertilized conditions and found that the yield was significantly greater in the fertilized plants compared with the unfertilized control plants and that most of the N in the fertilized trial was partitioned to the fruit compared with vegetative organs. The same authors also conducted a container-based study to compare N uptake and remobilization under high N application rates and low N application rates and found that under high N application rates, the amount of N remobilized to developing organs (new growth) was significantly greater than under low N application rates and that plant N uptake was especially high during fruit ripening when plants partitioned a high fraction of absorbed N to the fruit (Tagliavini et al., 2005). In a greenhouse study comparing different external NO$_3^-$-N concentrations on NO$_3^-$ uptake and nitrate reductase activity on NO$_3^-$-N uptake and assimilation in a short-day cultivar, Darnell and Stutte (2001) found increased NO$_3^-$ uptake with increasing external NO$_3^-$-N concentration was reflected in tissue NO$_3^-$ concentrations of all organs except the fruit and that strawberry growth and fruit yield were not affected by differing external NO$_3^-$-N concentrations. The authors suggest that
growth and fruit yield of strawberry is limited not by its ability to take up NO₃⁻-N but by its ability to reduce and assimilate NO₃⁻ into the tissue (Darnell and Stutte, 2001). This suggests that although excess fertilization may result in luxury consumption, it will not necessarily affect fruit N concentrations and yield. It is also likely that phenotypic differences across cultivars will influence the fruit N concentrations rather than fertilization strategies based on a cultivar’s ability to assimilate NO₃⁻ into the tissue.

2.3.2 Effect of Cultivar on N Dynamics

Strawberry breeding plays a major role on the response of specific genotypes to N fertilization practices. Fruit earliness and rain-damage tolerance, total fruit yield, disease and insect resistance, flavor, and postharvest quality are among the most important traits for breeding cultivars. However, when breeding for specific traits, variations in nutritional requirements may occur, thus leading to modified fertilization practices (Santos and Chandler, 2009).

In winter production regions such as Florida, California, and southern Spain, nitrogen fertilizer application rates fall into a broad range of between 150 to 300 lb N/ac (Agüero and Kirschbaum, 2013). Annual N uptake by strawberry crops has been reported to range from 53 to 178 lb N/ac (Albregts and Howard, 1980; Latet et al., 2002; Strik et al., 2004; Tagliavini et al., 2004, 2005) which could be an acceptable range for plant nutrition studies since excessive fertilizer rates could cause damage to the crop, increase production costs, and contaminate the environment (Giuimerà et al., 1995). Several studies have examined the effect of genotypic differences in strawberry cultivars on varying N application rates and vice versa. Most of these studies for U.S. grown
strawberries have taken place in Florida, the second largest production region for fresh market strawberries after California and a region whose waterbodies also show increased nitrate levels as a result of agricultural fertilizers. Much of this research focuses on strawberry yield response to differing N rates. Simonne et al. (2001) found that strawberry response to N rate is cultivar dependent and the lowest N rate tested yielded significantly less marketable fruit compared with the other two N rates used. Santos and Chandler (2009) performed a similar study comparing plant canopy diameter, marketable fruit weight and marketable fruit number for two cultivars commonly grown in Florida, ‘Strawberry Festival’ and ‘Winter Dawn. In two different growing seasons, the authors found that the response in these measurements of the two strawberry cultivars depends on the range of N rates used for fertilization attributing this difference in performance to morphological differences in the two cultivars. ‘Strawberry Festival’ produced a vigorous wide plant with a deep and profuse rooting system whereas ‘Winter Dawn’ is considerably smaller suggesting the former cultivar may require higher N rates than ‘Winter Dawn’ to satisfy its nutritional requirements for growth and development. This supports prior research that N rates need to be adjusted based on the size of vegetative biomass of a particular cultivar (Simonne et al., 2001; Tworkoski et al., 2001). In their study comparing the amount of nitrogen removed in a strawberry crop among four strawberry genotypes, Black et al. (2005) found that fruit N concentration, total yield and harvest removal of N differed significantly across the genotypes. The basis for genotypic differences in harvest removal of N was attributed to both fruit N concentration and yield; for example, the variety with the lowest yield, ‘B51,’ was among the highest in fruit N concentration but still had the lowest harvest removal of N (Black et al., 2005). By
contrast, two other varieties, ‘Jewel’ and ‘Allstar’ had similar yields but the former had higher fruit N concentration resulting in the highest harvest removal of N while the latter had a similar harvest removal of N to ‘B51’ (Black et al., 2005). However, in their container-based study comparing N uptake and remobilization under high and low N rates in two strawberry cultivars, Tagliavini et al., (2005) only report significant differences in N uptake or N partitioned to plant organs between fertilizer treatments and did not find differences between cultivars. More research on the effect of cultivar on N uptake and partitioning in commonly grown California cultivars can help support best management practice efforts of growers.

2.3.3 Effect of Compost on N Dynamics

Long-term management of soils with organic amendments increases soil organic matter and microbial biomass populations and activity compared with fertilized or unamended soils (Martyniuk and Wagner, 1978; McGill et al., 1986; Fraser et al., 1988; Dick et al., 1988; Johnston, 1991; Dick, 1994; Fauci and Dick, 1994). Compost contains micro- and macronutrients, enhances water-holding capacity, and improves soil structure (Lloyd et al., 2016). Although compost application occurs more frequently and at a higher rate in organic strawberry production systems, some conventional strawberry growers in California’s central coast are known to apply compost every one to two years in various forms including manure compost, spent mushroom compost, vermicompost, and yard trimming compost.
2.3.3a Soil N Dynamics

Composts differ in their effects on soil nitrate levels. Increasing rates of vermicompost application can increase soil NO$_3^-$ concentrations due to enhanced NO$_3^-$/NO$_4^-$ ratios typical in “mature” vermicompost (Arancon et al., 2006; Atiyeh et al., 2001; Mullane et al., 2015). Lloyd et al. (2016) found increasing soil NO$_3^-$-N levels in field applications of manure compost compared with non-amended control fields two weeks after incorporation. In this study, manure compost was applied at a rate of ~600 lb total N per acre and soil NO$_3^-$-N was determined in lab analyses using dry soil and sodium acetate: acetic acid extraction by a horticultural advising and testing lab in Watsonville, CA. With these results, the authors concluded that vermicompost and mushroom compost are good sources of early season nitrate, and mushroom, manure, and vermicompost were good sources of slow-release nitrogen throughout the season and that all forms of compost used in the study could provide sufficient nitrogen for optimal strawberry production based on typical N application rates in conventional production systems in coastal California (Lloyd et al., 2016). Another study conducted in a nursery setting found total soil N increased with increasing amounts of vermicompost (Broz et al., 2017) while Beck et al. (2016) found a combination of vermicompost addition and cover crop increased soil N compared to synthetic fertilizer.

2.3.3b Crop N dynamics and fruit yield

Broz et al. (2017) reported only slightly increased total N in strawberry tissue when compared to synthetic fertilizer while Hargreaves et al. (2009) reported no significant differences in strawberry leaf N when fertilized with ruminant compost or
synthetic fertilizer. However, when using compost as a fertilizer, it was reported that strawberry tissue had higher amounts of N than plants fertilized with synthetic fertilizer (Hammad et al., 2014; Mahadeen, 2009; Wang and Lin, 2002). Some studies comparing yield and quality of strawberries using different fertilizer and organic amendment combinations also show that the soil texture may influence strawberry leaf N concentrations. Results from a greenhouse study showed greater strawberry leaf N in plants grown in a silty soil amended with composted and fresh poultry litter compared with synthetic fertilizer, but there were no differences in leaf N in plants grown in clay and sandy soils (Preusch et al. 2004). This increase in leaf nutrient concentration in plants amended with poultry manure can be attributed to the increase in quantity and activity of soil microorganisms which result in considerable accumulation of N in plant leaves (Tejada et al., 2006; Garcia et al., 1997).

Although their field study evaluating four commercially available composts did not include a leaf nutrient analysis, Lloyd et al. (2016) found a notable trend in plant canopy growth that included a larger canopy diameter in the early part of the growing season in vermicompost amended soils compared with the non-amended control plots and plots with other compost-amended soils. However, in their greenhouse experiment, Broz et al. (2017) reported no differences in strawberry biomass across their vermicompost and synthetic fertilizer treatments with the exception of their 25% vermicompost + synthetic fertilizer treatment which had significantly greater mean above ground vegetative biomass relative to plants treated with synthetic fertilizer only.

Although compost additions can be an important sustainable practice that enhances soil organic matter, soil microbial communities, and available N in
conventional strawberry production, it does not always enhance strawberry yields. Lloyd et al. (2016) saw no significant increase in cumulative marketable yields in compost amended trials compared with non-amended trials while Beck et al. (2016) found that small vermicompost additions increased total and marketable strawberry yields but only in the second year, possibly a result of the impact of vermicompost on the soil microbial community and functioning that may have built up in the second year. In one study evaluating the efficacy of a recycled food waste-based liquid compost on strawberry yield on a conventional strawberry field in Santa Maria, California, researchers found significantly greater marketable yield in food waste-treated plots compared with grower standard proprietary fertilizer regimen (Dara 2016). Hammad et al. (2014) reported significantly higher total and marketable yields in strawberry field plots treated with a manure and green waste compost compared with untreated plots. The impact of compost on strawberry yields is inconsistent and seem to vary based on feedstock, N application rate, and specific soil properties (texture, pH, etc.) therefore, more research is needed across different soil types and for different composts and application rates.

2.3.4 Effect of CRF on crop and soil N dynamics and fruit yield

Controlled-release nitrogen fertilizers are commonly used in some vegetable production systems, rice systems and of course, strawberry production systems. Controlled-release nitrogen fertilizers are often associated with positive characteristics such as reduced burn, consistent release of N over a long period, and possible reductions in nitrate leaching (Shaviv and Mikkelson, 1993; Simonne and Hutchinson, 2005). Polymer-coated urea exemplifies CRFs (Du et al., 2006; Loper and Shober, 2012). These
fertilizers control the release of nutrients with semi-permeable coatings, occlusion, protein materials, or other chemical forms by slow hydrolysis of water soluble, low-molecular-weight compounds (Trenkel, 2010). The category of CRF most often used in strawberry production systems are those with a physical coating around the urea fertilizer. Typical coating materials are sulfur, wax, or a plastic resin, or some combination of these materials (Geurtal, 2009). Nitrogen release from coated products may be dependent on soil moisture, soil temperature, microbial activity, coating thickness, orifice size in the coating, or some mixture of these variables (Geurtal, 2009). In their review of coating materials and mechanisms of release for different CRFs, Lawerencia et al. (2021) reported a broad range of nutrient release rates. Some sulfur and mineral-based coated CRFs release 75% of their nutrient content in as little as a few minutes while some synthetic polymer-based and natural polymer-based coated CRFs can take up to 77 days to release the same amount of nutrients (Lawrence et al., 2021). They can have unpredictable release patterns especially if environmental variables such as soil or climatic conditions affect the N release rate. As with all fertilizers, the best and most efficient use of CRF should recognize crop N needs and time this with the N release rate. Research has shown diverse effects of preplant CRF N practices and sources on strawberry production (Santos, 2010).

In their 2011 field trial, Bottoms et al. (2013) monitored the N release of fall-applied 18N-3.5P-10.8K CRF in two different strawberry fields that followed vegetable plantings and found that nearly 80% of the initial N content had been released by the end of April (roughly six months after burial in the field) and did not properly time with crop N uptake which was limited between fall transplanting through March. Because this CRF
was applied post-planting, this result likely understated the N release in commercial fields in which CRF application is done preplant. This poorly timed release of N from preplant CRF resulted in substantial winter NO$_3^-$-N leaching of roughly 30 kg/ha N below 30 cm depth (estimated from 70% of 101 kg/ha CRF N and determining an average biomass N accumulation of about 40 kg-ha$^{-1}$ by strawberry plants). The authors concluded that current N fertilization practices did not efficiently match the crop N uptake pattern observed particularly in strawberry production systems that follow vegetable crop plantings which result in higher residual soil mineral nitrogen. In addition to inefficient N uptake, of their three field trials, the authors found only one showed a significant effect of CRF application rate on cumulative fruit yield where the higher rate of 121 kg/ha had significantly greater cumulative fruit yield compared with the rate of 61 kg/ha (Bottoms et al., 2013). Several other studies have shown no benefit to preplant N fertilization. Albregts et al. (1991) showed that the use of preplant fertilizer on strawberry may not enhance fruit yields and quality, or plant size, if sufficient fertilizer is supplied by drip irrigation as soon as the plant roots are able to absorb it. Santos (2010) performed a study comparing preplant N and sulfur sources on strawberry growth and yield and found that preplant N application had no effect on total strawberry yields regardless of the N source used supporting the findings of Albregts et al. (1991) and an earlier study by Santos and Whidden (2007) which found that application of preplant starter N fertilizer did not improve monthly or total strawberry yield.

However, the value of pre-plant N has been shown in some other studies. Miner et al. (1997) designed a study to evaluate rates of fall- and spring-applied N in strawberry plasticulture management systems in North Carolina and found that marketable yield
increased with increasing rate of fall-applied N. In a greenhouse study comparing preplant fertilization with traditional fast release NPK fertilizer, slow-release fertilizer applied at a depth of 10 cm and slow-release fertilizer mixed with soil, Cadahía et al. (1993) found less NO$_3$ losses by leaching in the slow-release fertilizer and slow-release fertilizer mixed with soil treatments compared with traditional fast release NPK fertilizer and reported greater root development in slow-release fertilizer mixed with soil treatments. The authors also found that the use of a slow-release fertilizer mixed in with soil was best to establish absorption increase of N by the plant and resulted in significantly higher yields compared with the other treatments (Cadahía et al., 1993). In a three-year field study comparing preplant CRF N application rates, Benedixen et al. (1998) found that in two of the three years, treatments with higher application rate of preplant CRF N of 160 lb N/ac had significantly higher total strawberry yield compared with treatments with a lower application rate of preplant CRF N of 80 lb N/ac and that in all three years, treatments with CRF N had significantly higher total strawberry yield compared with control treatments. These conflicting findings suggest a need for more research on the effects of CRF N on crop and soil N dynamics and strawberry yield.

2.4 Soil Health Indicators

Use of agro-chemicals, deep tillage and irrigation have degraded soils, polluted surface and groundwaters, and contaminated the air (Lal, 2008). Soil is an essential non-renewable resource with potentially rapid degradation rates and extremely slow formation and regenerations processes (Van-Camp et al., 2004). Soil fertility is the capacity of soil to provide physical, chemical and biological needs for the growth of plants for
productivity, reproduction and quality, relevant to plant and soil type, land use and climatic conditions (Abbott and Murphy, 2007). In recent years, growers have become more aware of sustainable management practices to ensure soil resource conservation. For example, the Healthy Soil Initiative, launched in 2015 by former California Governor Gov. Jerry Brown, is a collaborative measure that brings state agencies and departments together to promote the stewardship of healthy soils. The main objective of the Healthy Soils Initiative is to combine innovative farm and land management practices that contribute to building adequate soil organic matter to increase carbon sequestration and reduce overall greenhouse gas emissions (CDFA, 2020).

While disturbance and soil modification during modern crop production are inevitable, there are ways to manage these disturbances to mimic natural systems, thereby reducing the adverse impact of agriculture on the environment (Magdoff, 2001). Conventional strawberry production systems are intensively managed with a high input of agrochemicals such as fertilizers and pesticides which, despite contributing to the success of the crop, may have an adverse impact on the environment (Lovaisa et al., 2017). It is also well known that many crops experience a decline in productivity when replanted in the same site (Bennett et al., 2012). This could be related, in part, to some deterioration of the soil quality which could also be reflected in its microbial community, generally related to those functional groups that contribute to its fertility as well as the total microorganisms and their enzymatic activities (Lovaisa et al., 2017). It is believed that higher soil quality may have greater microbial functional capability favoring the resilience to stressing conditions in strawberry production (Reganold et al., 2010).
Properties of a healthy soil have been linked to important agronomic benefits such as disease and weed suppression, resilience to environmental stress and increased plant productivity (Berendsen et al., 2012). Therefore, implementing practices that sustain biological productivity, maintain environmental quality and promote plant performance in conventional strawberry production will help sustain this industry in California. The following section provides a brief overview of the importance of soil organisms and soil carbon, two indicators of healthy soil, and the extensive research that has focused on the benefits of organic amendment additions, such as compost, to improve soil health. This chapter also covers the contribution by the plant itself to soil health factors, particularly through the root-soil interface, also termed the rhizosphere. Finally, this chapter briefly covers how a healthy soil can have a positive effect on the plant by improving plant fertility, suppressing pests and disease and influencing crop N uptake.

2.4.1 Soil health indicators: soil organisms and soil carbon (C)

Soil organisms regulate many belowground functions that benefit plants, including organic matter decomposition, nutrient transformations, maintenance and formation of soil structure, and biocontrol of soilborne plant pathogens (Powlson et al., 2011). These benefits play a vital role in agriculture since the occurrence of beneficial soil organisms has been shown to suppress pathogens and diseases, improve nutrient availability, promote plant growth and thus increase crop yield (Yuan et al., 2014). When it comes to the effect of soil health on disease suppression, the overarching principle is that a healthy biological community outcompetes pathogens (Stirling et al., 2016). Microbial communities also supply plant-available N through biological N fixation and
mineralization of organic forms, and limit N losses by immobilizing it in soil organic matter (Scmidt et al., 2019). Organic fertility inputs such as compost and cover crop residues alter the abundance, diversity, and activity of various nitrogen-cycling microorganisms (Li et al., 2017; Tatti et al., 2013; Kong et al., 2010; Gu et al., 2017).

In addition to its capacity to sequester C and potentially mitigate climate change (Lal, 2004), soil organic carbon plays an important role in long-term soil conservation and restoration by sustaining its fertility due to the improvement of physical, chemical, and biological properties of soils (Sequi, 1989). Soil organic C is a heterogenous mixture of organic compounds in the soil that has an array of turnover times (McLauchlan, 2006). There is extensive research that supports the positive effects of compost application on soil organic matter (SOM) and C (Pinamonti, 1998; Morlat and Chaussod, 2008; Brown and Cotton, 2011; Mugnai et al., 2012; Peregrina et al., 2012; Gaiotti et al., 2017; Mondini et al., 2018). Furthermore, the addition of C-rich materials like compost can trigger an increase in microbial activity which increases microbial growth and promotes aggregate formation (Six et al., 2004), thereby facilitating stabilization and sequestration of C as biomass or within soil aggregates.

2.4.2 Effect of organic amendments on soil health

The use of organic soil amendments has been associated with desirable soil properties including higher plant available water holding capacity and CEC and lower bulk density, and can foster beneficial soil microorganisms (Doran, 1995; Drinkwater et al., 1995). Organic wastes such as animal manures, by-products of several kinds and composted residues can be used as amendments to increase soil fertility, since they are
important sources of nutrients for growing crops and a means for enhancing overall soil quality (Davies and Lennartsson, 2005). In addition to building soil carbon levels, organic amendments have been shown to alter specific components of the soil microbial community. Microbial communities supply plant-available N through biological N fixation and mineralization of organic forms, and limit N losses by immobilizing it in soil organic matter (Schmidt et al., 2019). In a study comparing the addition of synthetic versus organic amendments in both organic and conventional agricultural systems, Bulluck et al. (2002) found that, regardless of production history, the addition of organic soil amendments led to increased propagule densities of Trichoderma species (known biological control agents of plant pathogenic fungi), thermophilic microorganisms, enteric bacteria, and had decreased numbers of plant pathogenic microorganisms in the soil. Crop residues can also be a valuable source of organic matter for agricultural soils; they can improve the soil quality and productivity through favorable effects on soil properties such as plant available water holding capacity, cation exchange capacity, and the stimulation of beneficial microorganisms (Saroa and Lal, 2003).

Not only are organic amendment additions seen to improve fertility and microbial diversity, but it has also been observed that additions of some synthetic fertilizers can actually reduce microbial diversity and reverse the stable nutrient cycle. As more soluble N inputs enhance the breakdown of soil organic matter, the native soil fertility processes that are invested in soil organic material become depleted (Hepperly et al., 2009). Urea, one of the most common chemical forms of N fertilizer used worldwide can decrease microbial diversity, specifically nitrifier diversity, and selects for specific nitrifying strains (Staley et al. 2018). Addition of N fertilizers to soil, in various chemical forms,
has also been shown to reduce heterotrophic respiration due to a variety of factors that may include soil acidification and impacts on carbon cycling (Janssens et al., 2010; Ramirez et al., 2010). In a nine-year study comparing the effects of compost, manure, and synthetic fertilizer additions on crop yield and soil properties, Hepperly et al. (2009) found that soil N and carbon (C) levels either did not improve or were reduced in treatments with synthetic chemical fertilizers compared with compost treatments.

There are also several downsides to compost applications in commercial fields. The main factors that directly affect disease suppressive effects differ depending on the type of organic amendments added to soil (Litterick et al., 2004). The nature, degree of decomposition, C:N ratio, time of application, and quantity of fresh organic residues are crucial in determining their effects on both pathogen and beneficial soil microorganisms (Chung et al., 1990; van Bruggen and Termorshuizen, 2003). Although there is a myriad of research to support the disease suppressive effects of composts on soilborne diseases, some growers have reported poor disease control or increased disease following application of compost (Litterick et al., 2004). For example, composts prepared from heterogenous wastes that vary in salinity, N availability, and degree of decomposition may lead to marked increases in disease incidences and severity (Litterick et al., 2004). The composting process is complex and careful attention must be paid to ensure elimination of pathogens is achieved. Heat generated during the thermophilic high-temperature phase of aerobic composting appears to be the most important factor for the elimination of plant pathogens (Bollen and Volker, 1996) but if the composting process is not properly managed, harmful pathogens that pose health risks to both crops and humans may persist. Composting via thermal treatment aims to reduce pathogen levels without
eliminating beneficial microorganisms (Fuchs, 2010), but low levels of pathogens may survive in the soil for prolonged periods following incorporation (Jian et al., 2003; Islam et al., 2004).

Growers are sometimes discouraged from using compost due to food safety concerns (Olimpi et al., 2019). Research in the Central Coast has documented aversion to using organic soil amendments due to concern over the potential for cross-contamination of human pathogens from soils to crops, in particular, composted animal manure (Baur et al., 2016; Karp et al., 2015). On the other hand, if growers substitute synthetic fertilizers for compost and manures, changes in soil fertility and organic matter will likely result in soil microbial communities that are less diverse and less effective at suppressing pathogens (Lowell et al., 2010; Jones et al., 2019). This undermines the ability of natural processes to reduce food safety risks (Olimipi et al., 2019). The cost of transporting compost to the field and distributing it in the soil can be a deterrent to growers who already have great costs associated with labor and management and food safety regulations make it onerous and costly for growers to produce their own compost on site (Olimipi et al., 2019). A grower must take careful consideration including compost source, feedstock, and the ultimate goals they wish to achieve with their compost application.

Organic amendments have been a feasible option to improve the health of the soil biological community in strawberry production. Many agricultural and chemical companies have begun adding biological components, such as plant growth promoting rhizobacteria, to their products and other biofertilizers are widely available but expensive. More widely available organic amendments include rice bran, brassica seed meal, and
compost, each of which have received attention regarding their potential to suppress soilborne disease in strawberry cultivation (Decock (proposal), 2018). Rice bran and molasses have been used as soil amendments in the context of anaerobic soil disinfection (ASD) and when using rice bran as the carbon source, ASD has been shown to be a powerful disease suppressing agent against *Verticillium dahliae* (Muramoto et al., 2014; Muramoto and Shennan 2019). Mazzola et al. (2017) reported that Brassicaceae seed meal amendments suppressed the abundance of *M. phaseolina* detected in soil systems, but that optimal seed meal-induced pathogen suppression required a functional soil biology. And Lloyd et al. (2016) reported a significant reduction in *V. dahliae* root infections in some compost amended soils.

2.4.2a Effects of compost on soil health

As a general rule, the quantity and quality of organic material added to soils are the major factors in controlling the abundance of different microbial groups and the activity of microorganisms involved in nutrient cycling (Dianoco and Montemurro, 2010). Several long-term experiments have demonstrated that soil biological properties, such as microbial biomass C, basal respiration and some enzymatic activities, are significantly improved by compost treatments (Diacono and Montemurro, 2010). This is particularly evident in the upper layers of the soil because of the added labile fraction of organic matter (Zaman et al., 2004; Ros et al., 2006; Tejada et al., 2006, 2009). Benefits of compost amendments to soil also include pH stabilization and faster water infiltration rate due to enhanced soil aggregation (Stamatiadis et al., 1999). Since generally the composts are slowly decomposed in the soil, the continuous release of nutrients can
sustain the microbial biomass population for longer periods of time, compared with mineral fertilizers (Murphy et al., 2007). Hepperly et al. (2009) reported increased soil C and N levels and no required liming in compost treatments compared with synthetic chemical fertilizer treatments over their nine-year experiment in a maize-vegetable-wheat rotation at an experimental farm in Pennsylvania. Residual effects have also been observed from compost application. In their study evaluating the effects of manure, compost, and ammonium nitrate N application in corn production in Mead, Nebraska, Ginting et al. (2003) found four years after the last application of compost and manure resulted in 20 to 40% higher soil microbial biomass C compared with the N fertilizer treatment and residual effects of the compost and manure applications resulted in 42 to 74% higher potentially mineralizable N compared with synthetic N fertilizer treatment. Hepperly et al. (2009) also reported long term advantages through compost amendments in wheat crops which rely on residual N in the soil for fertilization establishing that compost amendments can support crop yields over time. Throughout a history of compost application and other organic farming practices, organic tomato plots at Russel Ranch in Davis, CA reported higher C and N levels compared with conventionally managed plots (Mukome et al., 2014).

Because soil carbon improves biological, physical and chemical qualities of soil, it is crucial to understand how compost application affects soil C levels. Permanganate oxidizable carbon (POXC) is a measurement of the fraction of the active C pool that is likely to be stabilized in soil. Mineralizable C (Min C) is a measurement of the fraction of active C pool that tends to be mineralized by soil microorganisms. POXC and Min C are known to be more sensitive to soil management and compost additions have been shown
to have a positive effect on both POXC and Min C (Culman et al., 2012; Hannam et al., 2016; Hurisso et al., 2016).

2.4.3 Effect of plant on soil health

Conventional cropping systems have their own effects on soil properties, indirectly through management and directly through the crops themselves via the microenvironment of the rhizosphere at the root-soil interface. Plants provide primary substrates for supporting an active and diverse soil food web via organic inputs (e.g., roots, aboveground residues, and root exudates) (Bais et al., 2006; Grayston et al., 1998; Marschner et al., 2001). Plant roots create additional complexity establishing resource-rich hotspots with distinct properties from the bulk soil and selectively recruiting microbial communities in the rhizosphere (Hinsinger et al., 2005; Fan et al., 2017). In their study exploring the interaction between agricultural management and plant selection on rhizosphere microbial communities, Schmidt et al. (2019) found that this interaction can result in plant recruitment of management-system-specific taxa and shifts in microbial networks and even N-cycling pathways in the rhizosphere. The authors suggest that since agricultural management practices impact rhizosphere microbial communities differently from the bulk soil, they can be used to guide research priorities in management decisions.

Crop species and even genotypes have distinct rooting systems, affecting the ability of crops to facilitate plant-soil interactions (de Graaff et al., 2013; de Vries et al., 2017). The rhizosphere can harbor tens of thousands of individual species per gram of soil and is likely the most diverse microbial community on the planet (Garbeva et al.,
Roots release large amounts of inorganic C which directly affects the biogeochemistry of the soil (Cheng et al., 1993; Hinsinger 2001; Hinsinger et al., 2009) while the release of organic C produces dramatic changes in the physical, biological, and chemical nature of the soil (Jones et al., 2004). Despite this understanding of the impact plant roots can have on soil biological communities, there is little effort by plant breeding programs to target below-ground phenotypic traits that support plant-soil interactions (Van Bueren et al., 2011). Intensive soil management can alter soil biological communities and potentially lead to a decoupling of plant and soil communities (Postma-Blaauw et al., 2010). Targeting phenotypic traits for greater belowground C allocation can have multiple impacts on promoting beneficial plant-soil interactions as well as overall soil health (Junaidi et al., 2018). In their study comparing root traits and root biomass of wheat genotypes to organic amendments and earthworms, Junaidi et al. (2018) found that the magnitude of positive compost effects on soil N-cycling, N uptake, and rooting systems follows increases in genotypic belowground investment suggesting that root phenotypic plasticity, especially in terms of biomass allocation, can be an important breeding strategy for developing genotypes with performance advantages within organic and low input agroecosystems. Given the environmental concerns associated with conventional strawberry production, using more organic practices can help mitigate some of these concerns, it would be helpful to identify cultivars that exhibit traits such as greater root biomass that may help the plant to thrive if compost were to replace pre-plant synthetic CRF N.
CHAPTER 3

Materials & Methods

3.1 Field Site Description

The field trial was conducted in Field 35B at the Cal Poly Strawberry Center in San Luis Obispo, CA (35.305670, -120.673311) for both the 2018-2019 and 2019-2020 growing seasons. The average annual precipitation was 17.9 inches, 18.7 inches and 11.1 inches for 2018, 2019 and 2020, respectively and the average annual temperature was 62.4°F, 58.5°F, and 59.7°F, for 2018, 2019 and 2020, respectively (California Irrigation Management Information System, 2021). Although this trial took place in the same field for both growing seasons, the experimental plots were in different parts of the field but not more than 40 m apart. We were unable to relist the beds in exactly the same location in the field due to its small size and the other research projects present in the field. This field was infested with *Macrophomina phaseolina* in 2017 and has not been fumigated since in order for other experiments to determine strawberry cultivar tolerance to this soilborne pathogen. The field is mapped as 89% Salinas silty clay and 11% Los Osos-Diablo complex (Soil Survey Staff, 2021). According to a soil test report conducted by A&L Western Agricultural Laboratories (San Luis Obispo, CA) in August 2018 and September 2019 the soil texture is a clay loam soil. The soil test report indicated a residual soil NO$_3^-$-N of 75 ppm in August 2018 and 21 ppm in September 2019. Soil organic matter content was reported to be 2.9% in August 2018 and 3.4% in September 2019.

Standard grower practices from the southern regions (Oxnard and Santa Maria) were used. Transplants were planted into beds 162 cm between centers with four plant
rows spaced 25 cm apart. Plants were spaced 41 cm apart. Strawberry beds were 30 cm high and 2 rows of Tri-Cal low-flow drip irrigation tape (1.2 liter/30.4 m, 20 cm between emitters) were laid 2 to 3 cm below the soil surface. Beds were covered with a totally impermeable film, polyethylene mulch (TIF), which was 1 mm thick, black on top and white on the reverse side. Our experimental setup consisted of eight plastic mulch-covered, raised beds that were approximately 48 m long and 1.6 m wide in 2019 and 43 m long and 1.6 m wide in 2020. The experiment was established using a randomized complete block design with four replications and two factors: pre-plant fertilizer treatment and cultivar (Figure 1). One-third of each bed were ‘control’ plots with no pre-plant fertilizer, 1/3 were treated with 100 lb N/acre AgRx brand 18-8-13 urea coated custom blended strawberry pre-plant control release fertilizer (CRF), and 1/3 were treated with 100 lb total N/acre Cal Poly Certified Organic Compost (5.3 tons compost/acre) incorporated via hand tilling. This application rate was in line with the CDFA Healthy Soils Program incentive for compost application in California production systems. Cal Poly compost was made of processed livestock manure and green waste generated from campus operations (Table 1, Wong, 2021). A ten-foot buffer zone was established between the first and second and the second and third blocks in each bed to separate pre-plant treatments. For year one, the last block in each bed was cut shorter than the proposed design leaving a block length of only 10.5 m or 5.3 m for each individual plot.

Four cultivars were selected for this trial based on anecdotal evidence from Pest Control Advisors of San Luis Obispo and Santa Barbara Counties and included three day-neutral UC varieties (Monterey, San Andreas, and Albion) and one Driscoll’s proprietary cultivar which is kept confidential at the request of Driscoll’s. Cultivars were randomly
assigned to a single plot in each block. In 2019, each bed contained between 347 and 405 plants planted by a commercial planting crew on October 23, 2018. In 2020, each bed contained between 364 and 384 plants planted by Cal Poly staff and students on November 5, 2019.

**Table 1.** Comprehensive overview of properties of Cal Poly certified organic compost applied in October 2018 (Year 1) and November 2019 (Year 2). Values represent the dry weight of each nutrient contained in the compost. Moisture content was measured as a percentage of fresh weight of the compost (Wong, 2021).

<table>
<thead>
<tr>
<th>Compost content</th>
<th>Year 1</th>
<th>Year 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>C:N ratio</td>
<td>10</td>
<td>9.1</td>
</tr>
<tr>
<td>Organic matter</td>
<td>32.6%</td>
<td>34.9%</td>
</tr>
<tr>
<td>Organic C</td>
<td>15.0%</td>
<td>14.0%</td>
</tr>
<tr>
<td>Total Nitrogen (N)</td>
<td>0.95%</td>
<td>0.95%</td>
</tr>
<tr>
<td>Phosphorous (P as P₂O₅)</td>
<td>1.2%</td>
<td>1.4%</td>
</tr>
<tr>
<td>Potassium (Kas K₂O)</td>
<td>1.8%</td>
<td>1.8%</td>
</tr>
<tr>
<td>Calcium (Ca)</td>
<td>2.6%</td>
<td>3.0%</td>
</tr>
<tr>
<td>Magnesium (Mg)</td>
<td>1.6%</td>
<td>1.6%</td>
</tr>
<tr>
<td>Sodium (Na)</td>
<td>0.38%</td>
<td>0.31%</td>
</tr>
<tr>
<td>Chloride (Cl)</td>
<td>0.42%</td>
<td>0.41%</td>
</tr>
<tr>
<td>Sulfur (SO₄²⁻-S)</td>
<td>550 mg/kg</td>
<td>420 mg/kg</td>
</tr>
<tr>
<td>Total Boron (B)</td>
<td>30 mg/kg</td>
<td>30 mg/kg</td>
</tr>
<tr>
<td>Copper (Cu)</td>
<td>52 mg/kg</td>
<td>62 mg/kg</td>
</tr>
<tr>
<td>Iron (Fe)</td>
<td>16000 mg/kg</td>
<td>19000 mg/kg</td>
</tr>
<tr>
<td>Lead (Pb)</td>
<td>5.0 mg/kg</td>
<td>4.4 mg/kg</td>
</tr>
<tr>
<td>Manganese (Mn)</td>
<td>390 mg/kg</td>
<td>430 mg/kg</td>
</tr>
</tbody>
</table>
**Figure 1.** Field experiment design. Adapted from Decock et al., 2018.

For the 2018-2019 growing season pre-plant soil amendments included AgriMend (AgriFarm Group Inc., CO), a highly soluble form of calcium in all treatments. In-season fertilizer applications consisted of CAN-17 applied at a rate of approximately 7.5 gallons per acre every 2-3 weeks depending on weather and plant size with alternating use of 20-20-20 mix at a rate of 12.5 lb per acre tapering off in the later part of the season. Mid- to late-season fertilizer applications of 6-31-31 were made to facilitate increase in berry size and improved quality. All in-season fertilizer applications were made using the drip irrigation system in 20-minute time slots alternating between fertigation and no fertigation until all of the fertilizer was siphoned into the system. This method was employed to allow for the best chance of adsorption of all nutrients to the clayey soil (pers. comm. D. Summerfield, 2019). Irrigations followed industry standards using tensiometers to indicate timing of irrigation events. All in-season fertigation and irrigation events were the same across all experimental treatments in both years.
To measure soil moisture, 96 Watermark Soil Moisture sensors with 1.5 m of wire (Irrometer, Riverside, CA) were assembled using 0.75-inch plastic tubing cut to 8 and 14 inches to be installed at depths of 6 and 12 inches, respectively, sealed to the sensor with PVC glue. The extra 2 inches gave room for closing the tube by folding at the top and tying with a zip tie to prevent water from entering the tubing during sprinkler irrigation and rain events. Sensors were installed in each of the 48 individual experiment plots using a soil probe and metal insertion tool. To collect soil pore water, 48 Rhizon SMS 10 cm porous (15 µm) pore water samplers (Rhizosphere Research Products (RRP), Netherlands) were installed enclosed in PVC at depths of 6-8 inches and 48 MacroRhizon 10 cm porous (15 µm) pore water samplers with 60 cm extension PVC/PE tubing were installed at depths of 12-14 inches in each plot. To install the Rhizon samplers, a small hole to a depth of 6 inches was augured in moist soil at a 45-degree angle next to a plant in an outer row of the bed. To install the MacroRhizon samplers, a hole was augured in moist soil at a 45-degree angle next to a plant in an inner row of the bed using a 12 in soil probe and an RRP insertion tool.

3.2 Soil and plant sampling

Soil samples were collected twice per growing season (January and July) for Permanganate Oxidizable Carbon (POXC) and Mineralizable C (Min C) analyses. Six to eight samples from each experimental plot were taken at 0-15 cm depth and combined to create a mixed composite sample. Soils were stored in quart-sized Ziploc® bags until they were sieved using an 8 mm sieve and air dried in paper bags. Undisturbed soil samples were collected using a Giddings manual bulk soil core sampler with a diameter
of 5 cm (Windsor, CO) at approximately 30 cm (12 inches) deep and were separated into subsamples by depths of 0-15 and 15-30 cm by measuring length from the top of the soil cores.

Soil pore water samples were collected from each experimental plot using Rhizon and MacroRhizon pore water samplers installed at a depth of approximately 15.2 to 20.3 cm and 30.5 to 35.6 cm, respectively. Samples were taken approximately every two weeks and were used to analyze soil NO$_3$-N. To collect soil pore water, 20 mL syringes were attached to the samplers with plungers held in place with wooden retainers to create a vacuum. Syringes were left on samplers overnight to allow for proper water accumulation and recovered the next day. To collect water, syringes were carefully removed from samplers and water was ejected into labelled 20 mL scintillation vials and stored in a refrigerator ($4^\circ$C) until lab processing. Soil moisture data was collected using a WaterMark handheld meter with a reading in centibars (cb) (Irrometer, Riverside, CA) approximately every two weeks (at every pore water sampling event). Soil moisture data was used to determine soil NO$_3$-N exposure.

During the 2018-2019 growing season, plant samples were taken via destructive plant sampling on four occasions to measure vegetative N accumulation. Samples were taken April 4, May 16, June 13, and July 19. In April 2019, a random number was assigned to each plant in the plots and plants assigned the two smallest numbers were selected for removal by trowel. Plants were stored in a refrigerator ($4^\circ$C) in labelled paper bags until processing. This method did not give an equal volume of soil for root sampling, so improvements to the belowground biomass sample collection were made for the May sampling. In May and June 2019, a random number was assigned to each plant
in the experimental plots and plants assigned the two smallest numbers were selected and removed.

To remove aboveground plant parts, the plant was cut at the base of the crown at the soil level using pruning shears. To sample for plant roots, a 10 cm bulb planter (Edwards Tools) was inserted into soil where the crown was removed, and a soil core was extracted using a trowel to ensure an equal volume was removed with the roots. Plant parts were stored in labelled paper bags and soil cores with roots were kept in a labelled plastic bag in a refrigerator until processing. In July 2019 sampling, a random number was assigned to each plant in the experimental plots and plants assigned the four smallest numbers were selected for removal. In July 2019, four plant samples were removed instead of two to provide sufficient plant material for analysis under a different project. Sampling of above and below ground plant parts followed the same method as May and June samplings. For the 2019-2020 growing season, five destructive plant sampling events occurred (April 2, April 30, May 25, June 28, and July 23). The sampling protocol followed the same methods as in April and May 2019 with two plants removed from each plot for each sampling event.

To collect yield data, marketable and unmarketable fruit were picked twice per week from April 1 to July 19 for the 2019 season and from April 13 to July 23 for the 2020 growing season. Harvested fruit were weighed to determine fruit fresh weight per plot. Five fruit per plot were set aside once per month from April to July in each growing season and were weighed, cut into quarters, weighed again and stored in a labelled 50 mL falcon tube in the freezer until processing for N content.
3.3 Analysis of soil samples

3.3.1 POXC and Min C

Prior to soil analysis all soil samples were sieved to 8 mm. We determined bulk density (BD, g cm\(^{-3}\)) for each depth by dividing the oven-dried mass of the undisturbed soil core by the volume of the core. The composite samples collected for POXC and Min C were air-dried and stored in paper bags. Permanganate oxidizable carbon (POXC), which represents the portion of active C pool that tends to be stabilized and sequestered in soil (Hurisso et al., 2016), was colorimetrically determined using the revised protocol of Weil et al. (2003), where 2.5 g of sieved and air-dried soil samples reacted with 2 M potassium permanganate solution, and the absorbance of the reacted solution was measured using a spectrophotometer (Milton Roy, Houston, Texas, USA) at 550 nm. The concentration of POXC (mg kg\(^{-1}\) soil) was then calculated by the equation in Weil et al. (2003):

\[
POXC = \left[ \frac{0.02 \text{ mol}}{L} - (a + b \times Abs) \right] \times \left( \frac{9000 \text{ mg C}}{\text{mol}} \right) \times \left( \frac{0.02 \text{ L solution}}{m} \right)
\]

where \(a\) = intercept of the standard curve; \(b\) = slope of the standard curve; \(Abs\) = absorbance of the sample’s POXC measurement; 9000 = milligrams of carbon oxidized by 1 mole of MnO\(_4^-\) changing from Mn\(^{7+}\) to Mn\(^{4+}\); and \(m\) = mass of air-dried soil sample in kg. Mineralizable C (Min C), which represents the soil active C pool that is likely to be mineralized and consumed (Hurisso et al., 2016), was determined by rewetting 10 g of air-dried soil samples to 50% water-holding capacity with deionized water and measuring CO\(_2\) concentration (mg CO\(_2\)-C kg\(^{-1}\) soil hr\(^{-1}\)) with a Li-COR Li-850 CO\(_2\)/H\(_2\)O gas analyzer (Lincoln, NE, USA) after a 48-hour incubation.
3.3.2 Nitrate

Nitrate concentrations in soil pore water samples were determined by colorimetry. Soil pore water samples were diluted by extracting 10 µL of soil pore water and placing it in a 0.5 mL Eppendorf conical tube containing 90 µL of DI water. From this solution, 15 µL of sample was placed into a 2 mL cuvette with 1 mL of NO$_3^-$ reagent. Samples were mixed and allowed to sit for 8 to 48 hours before measuring absorbance at 540 nm using an Evolution 201/220 UV-Vis spectrophotometer (Thermo Fisher Scientific, Madison, WI) to determine plant available nitrogen concentrations (Doane and Horwath, 2003; Forster, 1995). Pore water NO$_3^-$-N concentration was converted to soil NO$_3^-$-N concentration based on soil moisture content. To determine soil NO$_3^-$-N exposure, a method described by Alexander et al. (2018) was used where the summed concentrations were plotted against time and trapezoidally-integrated to represent cumulative soil NO$_3^-$-N availability across the entire analysis period termed nitrate exposure.

3.3.3 Soil moisture

Soil moisture tension measurements collected from Watermark sensors were converted to volumetric moisture content based on a soil retention curve. The soil moisture retention curve was determined using the method described in Dexter and Bird (2001) on soil collected in 2019 to illustrate the relationship between the soil water content and soil water potential in the field. Batches of 8 mm sieved, air-dried soils were packed and wetted slowly from below by capillarity to saturation while placed in 1-cm high brass rings. The saturated soils were then drained to water suctions of 0.01 and 0.05 bars on a sandbox apparatus, and to pressure levels of 0.1, 0.33, 1, and 4 on ceramic
pressure plate extractors and 15 bars on a membrane extractor. The water contents remaining in drained samples were measured gravimetrically by oven drying at 105 °C for 24 hours (ThermoFisher Scientific, Pittsburgh, PA, USA) and were fitted to the van Genuchten (1980) equation using Gnuplot, an optimization and graphing software (Version 5.2, Williams and Kelley, 2019).

3.4 Analysis of plant samples

3.4.1 Biomass N

To determine vegetative biomass N, fruit was removed and the two (April, May, June 2019 and April, May, June, and July 2020) or four (July 2019) plant samples were combined and divided into two parts: leaves versus crown and stems. For April 2019 plants, roots were separated from the rest of the plant. Fresh plant parts were weighed, put in paper bags and oven dried for 24 hours at 65°C. Dry plants were weighed again to determine moisture content. Once dried and weighed, stems/crowns and leaves were ground into powder using a grinder (SP Bel-art Micro-mill Grinder 115VAC, 60 Hz; Wayne, NJ) and analyzed for C (%) and N (%) using the Vario Max CNS elemental analyzer (Elementar Americas, Ronkonkoma, NY) at 900 °C.

Soil cores with roots were weighed before soil was removed from roots using dry sieving technique. Large soil aggregates were removed by hand and then smaller aggregates were separated from the roots by placing root+soil clusters into a 500 µm sieve with a lid and catch pan and shaken until roots were mostly clean of soil. Once roots were extracted from soil cores the freed soil was weighed and a sub sample was taken, placed in a tin, oven dried overnight at 105°C, and weighed after 24 hours to
determine soil moisture. Clean roots were weighed, placed in a labelled paper bag and oven dried at 65 °C overnight. Once dry, roots were weighed again to determine dry mass. Root density was calculated as the dry mass of roots per dry mass of soil. Root biomass was ground using the Bel Art micro-mill grinder, and 300 mg of ground root was used to determine CN concentration on the Vario Max CNS elemental analyzer.

Fruit samples that were set aside for N analysis were weighed, placed in 50 mL Falcon tubes with four small holes drilled in the cap, cut into quarters, weighed again, and stored in a -80 °C freezer until they could be freeze dried. Samples were lyophilized at -50°C, 0.039 mBar for 48 hours (Freezone® 4.5, Labconco® corporation, Kansas City, MO). Once freeze dried, fruit was weighed to determine moisture content and then ground using the Bel Art micro-mill grinder. Once pulverized, a 300 mg subsample of the fruit was measured to use for CN measurements using the Vario Max CNS elemental analyzer.

3.4.2 Plant Mortality and AUDPC

Visual plant mortality assessments were conducted weekly beginning May 13 and May 5 for the 2019 and 2020 growing seasons, respectively, and continued until the end of each season. Mortality assessment data was used to determine the area under disease progress curve (AUDPC). AUDPC is a useful quantitative summary of disease intensity over time (Jeger and Viljanen-Rollinson, 2001; Madden et al., 2007). The most common method used for AUDPC is the trapezoidal method, which can discretize the time variable (hours, days, weeks, months, or years) and calculate the average disease intensity.
between each pair of adjacent time points (Madden et al., 2007). AUDPC is calculated using the following formula:

$$AUDPC = \sum_{i=1}^{N-1} \frac{(y_i + y_{i+1})}{2} \times (t_{i-1} - t_i)$$

Where $y_i$ is the percent mortality for the observation number $i$, $t_i$ is the number of days from the initial observation, and $N$ is the total number of observations. AUDPC was expressed in %-days because severity ($y$) is expressed in percent and time ($t$) in days (Hagos et al., 2020). A subsample of 10 dead plants per plot were collected throughout the 2019 growing season beginning in May 2019. For plots that did not have ten dead plants, healthy plants were collected for a total of ten plants. These samples were used to determine % mortality by *M. phaseolina*. Plants were cut to the crown and each crown was cut into small parts for plating on Sorenson’s NP-10 medium. Sorenson’s NP-10 is a selective medium including agar and antibiotics developed to grow cultures of *V. dahliae* (Kabir et al., 2004) but has also been used as media to grow cultures of *M. phaseolina* because the two pathogens can be differentiated by morphological characteristics of the microsclerotia (pers. comm. S. Hewavitharana, 2021). Once plated, crowns were incubated at room temperature and allowed to sit for 3-4 days after which plates were analyzed for the presence of *Macrophomina* growth.
3.5 Statistical analyses

We performed linear mixed effects analysis using the package lme4 (Bates et al., 2014) in R (Team, 2013) to assess the effects of various factors for each response variable. Two-way split-plot-factorial ANOVA was used with the factors of pre-plant fertilizer treatment (main factor) and cultivar (subplot factor) on total yield, AUDPC, biomass N of vegetative tissue, harvest removal N in fruit, total N uptake, POXC, and Min C. Three-way split-plot-factorial ANOVA was used with factors of pre-plant fertilizer treatment (main factor), cultivar and depth (subplot factors) on soil NO$_3^-$-N exposure. Three-way split-plot factorial ANOVA was used with factors of pre-plant fertilizer treatment (main factor), cultivar and sampling date (subplot factors) on fruit N concentration and leaf %N. The assumptions of normality and homogeneity of variance were tested using the Shapiro-Wilk test and Levene’s test, respectively. Some variables required data transformation, mainly logarithm (AUDPC 2020, fruit N concentration 2020, soil NO$_3^-$-N exposure 2019 and 2020, POXC July 2019, leaf %N 2020, total N uptake 2020, and root density 2020), logarithm + constant (AUDPC 2019) and reciprocal (fruit N concentration May 2020), to meet normality and homogeneity of variance assumptions.
CHAPTER 4

Results

4.1 Yield

Statistical analyses to assess the effects of pre-plant fertilizer treatment and strawberry cultivar on all measurements was conducted on data from 2019 and 2020 separately. There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar on total yield in 2019 or 2020 (Table 2). In 2019, There was no significant effect of pre-plant fertilizer treatment on total strawberry yield but there was a significant effect of cultivar (p<0.001) (Figure 2). Total yield of cultivar Monterey (35,855.1 lb/ac) was significantly greater than all other cultivars. Total yield of the Proprietary (30,065.4 lb/ac) and San Andreas (27,958.3 lb/ac) cultivars were significantly greater than cultivar Albion (22,257.2 lb/ac). In 2020, there was a significant effect of cultivar on total yield (p<0.001) as well as a significant effect of the pre-plant fertilizer treatment (p=0.041). Total yield of ‘Monterey’ (34,358.0 lb/ac) was significantly greater than all other cultivars. Total yield of the Proprietary cultivar (28,409.3 lb/ac) was significantly greater than both ‘San Andreas’ (22,050.2 lb/ac) and ‘Albion’ (22,068.0 lb/ac). Total yield in the control treatment (28,472.2 lb/ac) was significantly greater than the CRF treatment (24,105.9 lb/ac).
Figure 2. Total yield (lb/ac) from strawberry harvests collected from 1 April 2019 to 19 July 2019 (year 1) and 4 April 2020 to 24 July 2020 (year 2). Error bars represent standard errors of the mean for each treatment (n=4). Uppercase letters indicate significant differences in total yield between cultivars in 2019 and 2020 measurements at p<0.05. Lowercase letters indicate significant differences in total yield between pre-plant fertilizer treatments in 2020 measurements at p<0.05.

Table 2. Two-way ANOVA on total yield by pre-plant fertilizer treatment and cultivar collected between 2019 and 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
<th>2019 F</th>
<th>p</th>
<th>2020 F</th>
<th>p</th>
</tr>
</thead>
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<td>pre-plant fertilizer</td>
<td>1.10</td>
<td>0.35</td>
<td>3.53</td>
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<td>cultivar</td>
<td>15.2</td>
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<td>17.3</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>1.33</td>
<td>0.27</td>
<td>1.39</td>
<td>0.25</td>
</tr>
</tbody>
</table>

4.2 Disease Incidence

4.2.1 AUDPC

There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar on disease incidence in 2019 or 2020 (Table 3). There was a significant effect of pre-plant fertilizer treatment on disease incidence (p=0.017) as well as a significant effect of cultivar in 2019 (p<0.001) (Figure 3). In 2019, disease incidence in the control treatment (169.8 %-days) was significantly less than in the compost and
CRF treatments (249.9 %-days and 256.3 %-days, respectively). In 2019, disease incidence of the Proprietary cultivar (107.3 %-days) was significantly less than ‘Monterey’ and ‘Albion’ (257.2 %-days and 367.5 %-days, respectively). Disease incidence of ‘San Andreas’ (169.3 %-days) was significantly less than ‘Albion’ cultivar. In 2020, there was no significant effect of cultivar on disease incidence but there was a significant effect of pre-plant fertilizer treatment (p<0.001). In 2020, disease incidence in the control and compost treatments (343.7 %-days and 451.2 %-days, respectively) were significantly less than in the CRF treatment (986.3 %-days).

**Figure 3.** Disease incidence determined using the area under the disease progress curve (AUDPC) from observation data collected from 10 May 2019 to 19 July 2019 (year 1) and 1 May 2020 to 24 July 2020 (year 2). Error bars represent standard errors of the mean for each treatment (n=4). Uppercase letters indicate significant differences in disease incidence between cultivar in 2019 measurements at p<0.05. Lowercase letters indicate significant differences in disease incidence between pre-plant fertilizer treatments in 2019 and 2020 measurements at p<0.05.
Table 3. Two-way ANOVA on disease incidence by pre-plant fertilizer treatment and cultivar collected between 2019 and 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
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<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>4.59</td>
<td>0.017</td>
<td>9.95</td>
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</tr>
<tr>
<td>cultivar</td>
<td>17.93</td>
<td>&lt;0.001</td>
<td>0.807</td>
<td>0.499</td>
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<tr>
<td>fertilizer:cultivar</td>
<td>0.48</td>
<td>0.82</td>
<td>0.672</td>
<td>0.673</td>
</tr>
</tbody>
</table>

4.2.2 Plating for Macrophomina phaseolina

A subsample of dead plants from each plot were collected to plate on nutrient media to check for infection by *M. phaseolina* in 2019 only. There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar and no main effects of pre-plant fertilizer treatment or cultivar on infection by *M. phaseolina* (%) (Figure 4; Table 4).

**Figure 4.** Results for plating to detect *M. phaseolina* in dead plants sampled from May to July 2019. Error bars represent standard errors of the mean for each treatment (n=4).
Table 4. Two-way ANOVA on M. phaseolina presence in dead plants (%) by pre-plant fertilizer treatment and cultivar collected in 2019. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
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<tr>
<td>pre-plant fertilizer</td>
<td>0.502</td>
<td>0.61</td>
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<tr>
<td>cultivar</td>
<td>1.13</td>
<td>0.35</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>0.928</td>
<td>0.49</td>
</tr>
</tbody>
</table>

4.3 Plant N Dynamics

4.3.1 Biomass N Vegetative Tissue

There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar on biomass N in vegetative plant tissue in 2019 (Table 5). There was a significant effect of cultivar type in 2019 (p<0.001) (Figure 5). Biomass N of the Proprietary cultivar (30.8 lb N/ac) was significantly greater than ‘San Andreas’, ‘Monterey’ and ‘Albion’ (23.1 lb N/ac, 21.7 lb N/ac, and 16.6 lb N/ac, respectively). Biomass N of ‘San Andreas’ was significantly greater than ‘Albion’. Similar to 2019, there was no significant interaction between pre-plant fertilizer treatment and cultivar on biomass N in aboveground vegetative tissue in 2020, but there was a significant effect of pre-plant fertilizer treatment (p<0.001) (Figure 5). In 2020, biomass N in plant tissue in the CRF pre-plant treatment (25.3 lb N/ac) was significantly greater than in the compost and control treatments (16.8 lb N/ac and 15.1 lb N/ac, respectively).
**Figure 5.** Biomass N accumulation in aboveground vegetative tissue from destructive plant samples collected in July of 2019 and 2020. Error bars represent standard errors of the mean for each treatment (n=4). Uppercase letters indicate significant differences in biomass N between cultivar in 2019 at p<0.05. Lowercase letters indicate significant differences in biomass N between pre-plant fertilizer treatment in 2020 at p<0.05.

**Table 5.** Two-way ANOVA on biomass N by pre-plant fertilizer treatment and cultivar collected in 2019 and 2020. F and p represent F-value and p value, respectively.

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<th>2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>2.27</td>
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</tr>
<tr>
<td>cultivar</td>
<td>17.4</td>
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</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>0.920</td>
<td>0.49</td>
</tr>
</tbody>
</table>

4.3.2 Harvest Removal of N (HRN)

There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar in HRN in 2019 or 2020 (Table 6). In both years, there was no significant effect of pre-plant fertilizer treatment on HRN, but there was a significant effect of cultivar type in 2019 (p<0.001) (Figure 6). In 2019, HRN for ‘Monterey’ (32.7 lb N/ac) was significantly greater than ‘San Andreas’ and ‘Albion’ (26.3 lb N/ac and 22.3 lb N/ac, respectively). In 2020, HRN for ‘Monterey’ (37.3 lb N/ac) was significantly
greater than all other cultivars and the Proprietary cultivar (30.8 lb N/ac) was significantly greater than ‘San Andreas’ (24.7 lb N/ac).

![Graph](image)

**Figure 6.** Harvest removal of N from data collected in April, May, June and July of 2019 and 2020. Error bars represent standard errors of the mean for each treatment (n=4). Uppercase letters indicate significant differences in HRN between cultivar in 2019 and 2020 at p<0.05.

**Table 6.** Two-way ANOVA on N accumulation in fruit by pre-plant fertilizer treatment and cultivar collected between 2019 and 2020. F and p represent F-value and p value, respectively.

<table>
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<tr>
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<th>2019</th>
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<th>2020</th>
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<td></td>
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<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
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<td>cultivar</td>
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<td>1.46</td>
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<td>0.32</td>
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</table>

**4.3.3 Total N Uptake (End of season)**

Total N uptake refers to the amount of N (lb/ac) removed by harvest and the amount of N (lb/ac) in healthy plants at the end of the growing season. There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar in 2019 or 2020 (Table 7). There was no significant effect of pre-plant fertilizer treatment on total N uptake but there was a significant effect of cultivar in 2019 (p<0.001) (Figure
7). In 2019, total N uptake for the Proprietary cultivar (58.6 lb N/ac) was significantly greater than ‘San Andreas’ and ‘Albion’ (49.4 lb N/ac and 38.7 lb N/ac, respectively). Total N uptake for ‘Monterey’ (54.4 lb N/ac) and for ‘San Andreas’ were significantly greater than for ‘Albion’. In 2020, there was a significant effect of both pre-plant fertilizer treatment and cultivar (p< 0.001 and p<0.001, respectively). In 2020, total N uptake in the CRF treatment (54.2 lb N/ac) was significantly greater than in the compost and control treatments (47.4 lb N/ac and 45.3 lb N/ac, respectively) across all cultivars. Total N uptake in ‘Monterey’ and the Proprietary cultivar (58.4 lb N/ac and 53.6 lb N/ac, respectively) were significantly greater than in ‘Albion’ and ‘San Andreas’ (42.7 lb N/ac and 41.2 lb N/ac, respectively).
Figure 7. Total N uptake by July of 2019 and 2020. Green bars represent total N uptake by aboveground vegetative tissue and red bars represent total N uptake by fruit. Uppercase letters indicate significant differences in total N uptake between cultivar in 2019 and 2020 at p<0.05. Lowercase letters indicate significant differences in total N uptake in pre-plant fertilizer treatments in 2020 at p<0.05.
Table 7. Two-way ANOVA on total N uptake in fruit and aboveground vegetative tissue by pre-plant fertilizer treatment and cultivar in 2019 and 2020. F and p represent F-value and p value, respectively.

<table>
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<td></td>
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<tr>
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<td>&lt;0.001</td>
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<td>fertilizer:cultivar</td>
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<td>0.7560689</td>
</tr>
</tbody>
</table>

4.3.4 Fruit N Concentration

For the purposes of this project, fruit N concentration refers to the amount of N per fresh fruit weight, expressed as lb N per lb fresh fruit. There was no significant interaction between pre-plant fertilizer treatment, cultivar, and month in the 2019 (Table 8; Figure 8), but there was a significant interaction between pre-plant fertilizer treatment and cultivar and a significant interaction between pre-plant fertilizer treatment and sampling month. In CRF treatment plots, ‘Albion’ (0.0012 lb N/lb fresh fruit) had significantly greater N concentration compared with all other cultivars (Figure 9) and in July, the Proprietary cultivar (0.00075 lb N/lb fresh fruit) had significantly lower fruit N concentration than all other cultivars (Figure 10). For 2020 fruit N concentration data, three-way ANOVA did not meet normal distribution of residuals assumptions. Instead, two-way ANOVA was used to detect treatment and cultivar effects for each sampling period (April, May, June, and July of 2020) (Table 9). Two-way ANOVA for each month determined there were no interactions between pre-plant fertilizer treatment and cultivar or main effects of pre-plant fertilizer treatment or cultivar in April, May, or June. However, in July, two-way ANOVA showed a significant interaction between pre-plant fertilizer treatment and cultivar, but post hoc Tukey HSD analysis revealed no significant differences in cultivar fruit N concentration across pre-plant fertilizer treatments and no
significant differences in fruit N concentration in pre-plant fertilizer treatment within cultivar. There was a significant effect of pre-plant fertilizer treatment and cultivar as main effects. Fruit N concentration in the CRF treatments (0.0012 lb N/lb fresh fruit) was significantly greater than in the control treatments (0.0010 lb N/lb fresh fruit) and fruit N concentration in ‘Monterey’ (0.0012 lb N/lb fresh fruit) was significantly greater than the Proprietary cultivar (0.0010 lb N/lb fresh fruit).

**Figure 8.** Fruit N concentration from data collected in April, May, June and July of 2019 and 2020. Error bars represent standard errors of the mean for each cultivar (n=4). Red lines represent the conversion coefficient determined by the State Water Resources Control Board. The conversion coefficient is a crop-specific coefficient used to convert from units of crop biomass, typically yield, removed per acre to units of nitrogen removed per acre. For strawberries, AgOrder 4.0 states the conversion coefficient to be 0.0013 lb N/lb fresh fruit.
Figure 9. Fruit N concentration across cultivars within compost (A), control (B) and CRF (C) pre-plant treatment blocks from data collected in April, May, June and July of 2019. Error bars represent standard errors of the mean for each cultivar (n=4). Uppercase letters indicate significant differences in cultivar within the CRF pre-plant treatment blocks at p<0.05.

Figure 10. Fruit N concentration across cultivars in April (A), May (B), June (C), and July (D) samples collected in 2019. Error bars represent standard errors of the mean for each cultivar (n=4). Uppercase letters represent significant differences between cultivar within the July sampling period at p<0.05.
Table 8. Three-way ANOVA on Fruit N (lb N/lb fresh fruit) in April, May, June, and July 2019 and 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
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<td>6.18</td>
</tr>
<tr>
<td>cultivar</td>
<td>3.76</td>
</tr>
<tr>
<td>month</td>
<td>76.6</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>5.02</td>
</tr>
<tr>
<td>fertilizer:month</td>
<td>0.903</td>
</tr>
<tr>
<td>cultivar:month</td>
<td>4.27</td>
</tr>
<tr>
<td>fertilizer:cultivar:month</td>
<td>0.948</td>
</tr>
</tbody>
</table>

Table 9. Two-way ANOVA on Fruit N (lb N/lb fresh fruit) in April, May, June and July 2020. F and p represent F-value and p value, respectively. Asterisk * indicates ANOVA showed significant effect but Tukey HSD determined there was no significant effect of cultivar within each pre-plant treatment or of pre-plant treatment within cultivars.

<table>
<thead>
<tr>
<th></th>
<th>April</th>
<th>May</th>
<th>June</th>
<th>July</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>1.01</td>
<td>0.32</td>
<td>0.55</td>
<td>0.58</td>
</tr>
<tr>
<td>cultivar</td>
<td>0.43</td>
<td>0.73</td>
<td>0.21</td>
<td>0.89</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>1.85</td>
<td>0.12</td>
<td>1.15</td>
<td>0.36</td>
</tr>
</tbody>
</table>

4.3.5 Leaf Tissue N

In 2019, three-way ANOVA with pre-plant fertilizer treatment, cultivar, and sampling month did not meet normal distribution of residuals assumptions. Two-way ANOVA was used to detect effects of pre-plant treatment and cultivar on leaf tissue N in each sampling month (Figure 11). For the 2019 growing season, two-way ANOVA showed no significant interaction between pre-plant fertilizer treatment and cultivar in April, May, June or July (Table 10). In April and June of 2019 there was no effect of pre-plant fertilizer treatment or cultivar on leaf tissue N. In May 2019, the Proprietary cultivar (2.34% N) had significantly greater leaf tissue N compared with ‘Albion’ and ‘Monterey’ (2.20% N and 2.05% N, respectively) and ‘San Andreas’ (2.26% N) had
significantly greater leaf tissue N compared with ‘Monterey’ (Figure 12A). In July of 2019, the Proprietary cultivar (1.84% N) had significantly greater leaf tissue N compared with ‘Albion’ (1.71% N) (Figure 12B). For 2020, three-way ANOVA determined there was no significant interaction between pre-plant fertilizer treatment, cultivar, and sampling date (Table 11). However, there was a significant main effect of treatment, cultivar, and month (p<0.001, p=0.0011, and p<0.001, respectively) (Figure 12). The CRF and compost treatments (1.91% N and 1.92% N, respectively) had significantly greater leaf tissue N compared with the control treatment (1.84% N) across all cultivars and sampling dates. The Proprietary cultivar and ‘San Andreas’ (1.96% N and 1.93% N, respectively) had significantly greater leaf tissue N compared with ‘Monterey’ and ‘Albion’ (1.84% N and 1.82% N, respectively) across all treatments and sampling dates. Across all treatments and cultivars, leaf tissue N was significantly greater in samples taken on the earliest sampling date on April 2 (2.45% N) compared with all later sampling dates. Leaf tissue N was significantly greater in samples taken on April 30 (2.16% N) compared with samples taken in May, June, and July (1.75% N, 1.54% N, and 1.53% N, respectively) across all treatments and cultivars. Leaf tissue N was significantly greater in samples taken in May compared with samples taken in June and July across all treatments and cultivars.
Figure 11. Leaf tissue N from data collected in April, May, June and July of 2019 and 2020. Error bars represent standard errors of the mean for each treatment (n=4). Green bar represents the leaf %N sufficiency levels during main harvest (2.4 to 3.0%) according to the CDFA fertilizer guidelines 2021. Uppercase letters indicate significant differences in leaf tissue N between pre-plant fertilizer treatments in 2020 at p<0.05. Lowercase letters on legend indicate significant differences in leaf tissue N between cultivars in 2020 at p<0.05 and lowercase letters on graphs in 2020 indicate significant differences in leaf tissue N between sampling dates at p<0.05.
**Figure 12.** Leaf tissue N from data collected in May (A) and July (B) of 2019. Error bars represent standard errors of the mean for each treatment (n=4). Uppercase letters indicate significant differences in leaf tissue N between cultivar in May 2019 (A) and July 2019 (B) at p<0.05.

**Table 10.** Two-way ANOVA on leaf tissue N in April, May, June, and July 2019. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
<th>April F</th>
<th>April p</th>
<th>May F</th>
<th>May p</th>
<th>June F</th>
<th>June p</th>
<th>July F</th>
<th>July p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-plant fertilizer</td>
<td>1.02</td>
<td>0.37</td>
<td>2.10</td>
<td>0.14</td>
<td>2.19</td>
<td>0.13</td>
<td>0.596</td>
<td>0.56</td>
</tr>
<tr>
<td>cultivar</td>
<td>1.39</td>
<td>0.26</td>
<td>13.4</td>
<td>&lt;0.001</td>
<td>2.29</td>
<td>0.079</td>
<td>3.19</td>
<td>0.035</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>1.21</td>
<td>0.32</td>
<td>0.67</td>
<td>0.67</td>
<td>0.47</td>
<td>0.83</td>
<td>1.40</td>
<td>0.24</td>
</tr>
</tbody>
</table>

**Table 11.** Three-way ANOVA on leaf tissue N (%N) in April, May, June, and July 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
<th>2020 F</th>
<th>2020 p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-plant fertilizer</td>
<td>10.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>cultivar</td>
<td>10.5</td>
<td>0.0011</td>
</tr>
<tr>
<td>month</td>
<td>481.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>1.37</td>
<td>0.23</td>
</tr>
<tr>
<td>fertilizer:month</td>
<td>1.29</td>
<td>0.25</td>
</tr>
<tr>
<td>cultivar:month</td>
<td>1.16</td>
<td>0.32</td>
</tr>
<tr>
<td>fertilizer:cultivar:month</td>
<td>1.18</td>
<td>0.26</td>
</tr>
</tbody>
</table>
4.4 Soil NO$_3$-N

The effects of pre-plant fertilizer treatment, strawberry cultivar and depth on soil NO$_3$-N exposure were assessed during the period from January to July 2019 for the 2019 growing season and November 2019 to April 2020 for the 2020 growing season. Three-way ANOVA determined there were no significant interactions between pre-plant fertilizer treatment, strawberry cultivar, and sampling depth in 2019 or 2020 (Table 12). There was no significant effect of pre-plant fertilizer treatment or cultivar type on soil NO$_3$-N concentration but there was a significant effect of sampling depth in both 2019 and 2020 (Figure 13). In 2019 and 2020, the soil soil NO$_3$-N exposure was significantly greater below the root zone (10.59 mg N/kg soil/day and 22.9 mg/kg soil/day, respectively) compared to the root zone (1.6 mg N/kg soil/day and 5.9 mg N/kg soil/day, respectively).

![Figure 13](image-url)

**Figure 13.** Soil nitrate exposure from January to July 2019 and November 2019 to April 2020 for the 2019 and 2020 growing seasons, respectively. Error bars represent standard errors of the mean for each treatment (n=4). Uppercase letters indicate there was a significant effect of depth in each year with a greater nitrate concentration below the root zone compared with in the root zone (p<0.05).
Table 12. Three-way ANOVA on soil nitrate exposure from January to July of 2019 and November 2019 to April 2020. F and p represent F-value and p value, respectively. Asterisk * indicates a significant interaction but Tukey HSD showed no significant pairwise comparisons.

<table>
<thead>
<tr>
<th></th>
<th>2019</th>
<th></th>
<th>2020</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>0.727</td>
<td>0.49</td>
<td>2.54</td>
<td>0.089</td>
</tr>
<tr>
<td>cultivar</td>
<td>0.551</td>
<td>0.66</td>
<td>0.999</td>
<td>0.43</td>
</tr>
<tr>
<td>depth</td>
<td>167.4</td>
<td>&lt;0.001</td>
<td>71.9</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>3.10</td>
<td>0.010*</td>
<td>0.867</td>
<td>0.53</td>
</tr>
<tr>
<td>fertilizer:depth</td>
<td>0.164</td>
<td>0.85</td>
<td>0.779</td>
<td>0.46</td>
</tr>
<tr>
<td>cultivar:depth</td>
<td>0.210</td>
<td>0.89</td>
<td>2.43</td>
<td>0.075</td>
</tr>
<tr>
<td>fertilizer:cultivar:depth</td>
<td>0.452</td>
<td>0.84</td>
<td>1.13</td>
<td>0.36</td>
</tr>
</tbody>
</table>

4.5 Soil Carbon

4.5.1 Permanganate Oxidizable Carbon (POXC)

Two-way ANOVA determined there was no significant interaction of POXC between pre-plant fertilizer treatment and strawberry cultivar in January 2019 and 2020 or July 2020 (Table 13 and Table 14, respectively). Two-way ANOVA determined there was a significant interaction between pre-plant fertilizer treatment and strawberry cultivar on POXC in July 2019 but Tukey HSD showed no significant differences between treatments or cultivars (Table 14). In January and July of both years, there were no significant main effects of pre-plant fertilizer treatment of cultivar on POXC (Figure 14).
**Figure 14.** POXC in soils sampled in January 2019 and 2020 and July 2019 and 2020. Error bars represent standard errors of the mean for each treatment (n=4).

**Table 13.** Two-way ANOVA on POXC in January 2019 and January 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
<th>January 2019</th>
<th>January 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>2.06</td>
<td>0.14</td>
</tr>
<tr>
<td>cultivar</td>
<td>0.526</td>
<td>0.67</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>2.12</td>
<td>0.078</td>
</tr>
</tbody>
</table>

**Table 14.** Two-way ANOVA on POXC in July 2019 and July 2020. F and p represent F-value and p value, respectively. Asterisk * indicates a significant interaction but Tukey HSD showed no significant pairwise comparisons.

<table>
<thead>
<tr>
<th></th>
<th>July 2019</th>
<th>July 2020</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>0.295</td>
<td>0.75</td>
</tr>
<tr>
<td>cultivar</td>
<td>1.09</td>
<td>0.37</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>3.33</td>
<td>0.011*</td>
</tr>
</tbody>
</table>
4.5.2 Mineralizable Carbon (Min C)

There was no significant interaction between pre-plant fertilizer treatment and strawberry cultivar on Min C in January and July 2019 or January and July 2020 (Table 15 and Table 16, respectively). There were also no significant main effects of pre-plant fertilizer treatment or cultivar on Min C (Figure 15).

![Bar graphs showing Min C in soils sampled in January and July 2019 and 2020. Error bars represent standard errors of the mean for each treatment (n=4).](image)

**Figure 15.** Min C in soils sampled in January 2019 and 2020 and July 2019 and 2020. Error bars represent standard errors of the mean for each treatment (n=4).

**Table 15.** Two-way ANOVA on Min C in January 2019 and January 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>0.280</td>
<td>0.75</td>
</tr>
<tr>
<td>cultivar</td>
<td>0.237</td>
<td>0.87</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>1.14</td>
<td>0.36</td>
</tr>
</tbody>
</table>
Table 16. Two-way ANOVA on Min C in July 2019 and July 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
<th>July 2019</th>
<th></th>
<th>July 2020</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>1.91</td>
<td>0.17</td>
<td>0.507</td>
<td>0.61</td>
</tr>
<tr>
<td>cultivar</td>
<td>1.93</td>
<td>0.14</td>
<td>0.722</td>
<td>0.55</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>0.544</td>
<td>0.77</td>
<td>0.564</td>
<td>0.76</td>
</tr>
</tbody>
</table>

4.5.3 Root Density

In 2019, three-way ANOVA of root density measurements with pre-plant fertilizer treatment, cultivar, and sampling month did not meet normal distribution of residuals or homogeneity of variance assumptions. Two-way ANOVA was used to detect effects of pre-plant treatment and cultivar on root density in each sampling month (Figure 16; Table 17). Due to sampling error, root density in April 2019 could not be determined. Two-way ANOVA showed no significant interaction or any significant main effects of pre-plant fertilizer treatment and cultivar on root density in May 2019. In June of 2019, there was a significant interaction between pre-plant fertilizer treatment and cultivar. Cultivar Monterey in CRF treatment plots (0.045 g dry roots/g dry soil) had significantly greater root density compared with ‘Albion’ in the control and CRF treatment plots (0.019 g dry roots/g dry soil). Two-way ANOVA determined there was a significant interaction between pre-plant fertilizer treatment and strawberry cultivar on root density in July 2019 but Tukey HSD showed no significant differences between treatments or cultivars and there were no significant main effects.

Three-way ANOVA was used to determine significant effects of pre-plant fertilizer treatment, cultivar and sampling month in 2020 (Figure 17; Table 18). There were no significant interactions but there was a significant effect of each main effect.
Plants in the CRF treatment blocks (0.027 g dry roots/g dry soil) had significantly greater root density compared with plants in the control and compost blocks (0.022 g dry roots/g dry soil). Cultivar ‘Albion’ had significantly greater root density (0.028 g dry roots/g dry soil) compared with ‘Monterey’ cultivar (0.021 g dry roots/g dry soil). The plants sampled on April 2 (0.032 g dry roots/g dry soil) had significantly greater root density compared with the plants sampled in May, June and July (0.021 g dry roots/g dry soil, 0.021 g dry roots/g dry soil, and 0.017 g dry roots/g dry soil, respectively). Plants sampled on April 30 (0.028 g dry roots/g dry soil) had significantly greater root density compared with plants sampled in July.

Figure 16. Root density for plants sampled in May, June and July 2019. Error bars represent standard error of the mean for each treatment (n=4). Lowercase letters indicate significant differences in pre-plant fertilizer treatment x strawberry cultivar in June 2019 at p<0.05.
Figure 17. Root density for destructive plant sampling in each plot taken on April 2, April 30, May, June and July 2020. Error bars represent standard error of the mean for each treatment (n=4). Lowercase letters indicate significant differences in pre-plant fertilizer treatments at p<0.05. Uppercase letters next to strawberry cultivar names indicate significant differences across cultivar and uppercase letters on graphs represent significant differences between sampling dates at p<0.05.

Table 17. Two-way ANOVA on root density in May, June, and July 2019. F and p represent F-value and p value, respectively. Asterisk * indicates a significant interaction but Tukey HSD showed no significant pairwise comparisons.

<table>
<thead>
<tr>
<th></th>
<th>May</th>
<th></th>
<th>June</th>
<th></th>
<th>July</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
<td>F</td>
<td>p</td>
</tr>
<tr>
<td>pre-plant fertilizer</td>
<td>0.314</td>
<td>0.73</td>
<td>0.788</td>
<td>0.46</td>
<td>0.180</td>
<td>0.84</td>
</tr>
<tr>
<td>cultivar</td>
<td>1.82</td>
<td>0.16</td>
<td>2.69</td>
<td>0.063</td>
<td>2.06</td>
<td>0.13</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>0.628</td>
<td>0.92</td>
<td>2.63</td>
<td>0.04</td>
<td>3.03</td>
<td>0.02*</td>
</tr>
</tbody>
</table>
Table 18. Three-way ANOVA on root density for April 2, April 30, May, June and July 2020. F and p represent F-value and p value, respectively.

<table>
<thead>
<tr>
<th></th>
<th>2020 F</th>
<th>2020 p</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-plant fertilizer</td>
<td>7.08</td>
<td>0.0011</td>
</tr>
<tr>
<td>cultivar</td>
<td>6.97</td>
<td>0.010</td>
</tr>
<tr>
<td>depth</td>
<td>7.71</td>
<td>0.003</td>
</tr>
<tr>
<td>fertilizer:cultivar</td>
<td>1.83</td>
<td>0.097</td>
</tr>
<tr>
<td>fertilizer:month</td>
<td>1.62</td>
<td>0.12</td>
</tr>
<tr>
<td>cultivar:month</td>
<td>1.16</td>
<td>0.32</td>
</tr>
<tr>
<td>fertilizer:cultivar:month</td>
<td>0.936</td>
<td>0.55</td>
</tr>
</tbody>
</table>
5.1 Effects of pre-plant fertilizer and cultivar on strawberry yield

The profitability of strawberry production depends on many factors, including cultivar and environmental conditions, so the right cultivar selection is dependent in part on the interaction of abiotic factors with the environment. We hypothesized the pre-plant fertilizer treatment would have no significant effect on total yield and that any differences would be the result of cultivar. We saw different results in the 2019 and 2020 growing seasons regarding the effect of pre-plant fertilizer treatment on strawberry yield. In 2019, strawberry plants in plots treated with synthetic CRF as the source for pre-plant N did not have statistically different yield compared with strawberry plants in plots treated with compost as a source of pre-plant N or plots that did not receive pre-plant fertilizer treatment (Figure 2). This finding supports Florida research trials by Agehara et al. (2007), Albregts et al. (1991) and Santos and Whidden (2007) who found little to no impact of pre-plant N rates on total strawberry yield as well as research on CRF efficiency in California strawberry fields by Bottoms et al. (2013). A rule of thumb is to have 10 to 15 ppm mineral N (nitrate + ammonium) in the upper foot during the early seasons, which is equivalent to 35 to 60 lb N/ac depending on the soil texture (Cahn, 2019). Prior to planting in 2018, soil analyses concluded there was a high concentration of soil residual NO$_3^-$-N at 75 ppm. It is possible that this surplus of plant available N masked any effect of pre-plant fertilizer treatment on yield. However, in 2020, strawberry plants in plots that did not receive a pre-plant N application (control treatment) had significantly higher yield compared with strawberry plants in plots treated with synthetic...
CRF N as a pre-plant fertilizer treatment (Figure 2). Prior to planting, soil analyses concluded there was a concentration of 21 ppm residual soil NO$_3$-N. This result may suggest that this residual soil NO$_3$-N concentration provided sufficient N supply to support winter plant establishment and plant N needs prior to any in-season fertigation applications and fruit production.

Cultivar type did have a significant effect on yield in both the 2019 and 2020 growing seasons as ‘Monterey’ significantly out performed all other cultivars in both years, despite some severe plant mortality (Figure 2; Figure 3). This outcome is consistent with the objectives of strawberry breeding programs that focus on disease tolerance and/or resistance and yield. ‘Monterey’ also outperformed other UC cultivars including Albion and San Andreas in trials conducted at the Watsonville Research Facility in research trials that determined yield potential and fruit quality for UC bred varieties (UC Davis, 2007).

Our findings conclude that cultivar influences yield more than pre-plant N management and that growers should consider this when developing nutrient management plans for their production system. Overall, total yield in this experiment was much lower compared with yield in conventionally managed California strawberry production systems. Statewide fresh strawberry production averages 50,000 to 70,000 lb/ac each season (CSC, 2021; Hartz et al., 2018). It is important to note that our trial was conducted on a field that was infested with the soilborne pathogen, *Macrophomina phaseolina*, in 2017 and remained unfumigated since. It is common practice in conventional production systems to apply a chemical fumigant to strawberry beds prior to
planting to reduce disease during the growing season resulting in high yields (Bolda et al., 2015).

5.2 Effects of pre-plant fertilizer and cultivar on disease incidence

As previously mentioned, the experimental field was infested with *M. phaseolina* in 2017 and is also known to contain *Phytophthora cactorum* (S.M. Mansouripour, pers. comm. 2019). Although we cannot definitively say plant death was the result of *M. phaseolina*, physical characteristics of the dead and dying plants in the experiment were consistent with known symptoms of *M. phaseolina* including wilting foliage, plant stunting, and drying and death of older leaves, with the central youngest leaves remaining green and alive after disease onset (Koike, 2012). Strawberry plants are predisposed to the disease when exposed to stress factors such as extreme heat, drought or high fruit load (Bolda et al., 2015).

We hypothesized that plants in compost treatments would have lower plant mortality compared with plants in the control and CRF pre-plant fertilizer treatment blocks as a result of indirect effects (through improved soil health) and direct effects on plant pathogens and beneficial microorganisms in the compost (Litterick et al., 2004). In both years (2019 and 2020), there was significantly greater plant mortality in CRF pre-plant treatment blocks compared with the control blocks and in the first year of the experiment (2019), there was also significantly greater plant mortality in the compost treatment compared with the control treatment (Figure 3). This contradicted our hypothesis that compost treated plots would have a lower incidence of disease resulting in lower plant mortality rates. In certain instances, composts have been seen to confer
disease control and improve plant growth due to an enhanced overall level of biological activity (Chen et al., 1998; Lloyd et al., 2016). It is acknowledged that the addition of organic amendments to field soils often leads to improved soil structure, water penetrations and drainage, enhanced soil health, and greater complexity for microorganisms and soil food webs (Bulluck et al., 2002; van Bruggen, 1995). Field biological control of plant pathogens by composts can occur through a variety of mechanisms including competition for nutrients, antibiosis, hyperparasitism, and induced protection (Hornby, 1990) but most reports of disease suppression suggest that competition and/or antibiosis and hyperparasitism are the principal mechanisms (Litterick et al., 2014). The main factors that directly affect disease suppressive effects differ depending on several factors including the compost feedstock, C:N ratio, and the time of application (Chung et al., 1990; van Bruggen and Termorshuizen, 2003; Litterick et al., 2004). Olive and grape waste-based composts have been shown to suppress a variety of soilborne pathogens including some that can have significant impacts on strawberry cultivation such as *V. dahliae* and *F. oxysporum* f.sp. *fragariae* (Curlango-Rivera et al., 2013; Ntougias et al., 2007; Yangui et al., 2010). Organic amendments such as green manures, stable manures, and composts have been recognized to facilitate biocontrol of soilborne pathogens such as *Phytophthora* and *Fusarium* spp. (Hoitink and Boehm, 1999; St. Martin, 2014). There is also evidence of significant variability in compost capacity to suppress disease when multiple isolates of the same fungal pathogen species were evaluated (Termorshuizen et al., 2007).

We expected to see a significant effect of cultivar on disease incidence and plant mortality in both years since prior experiments with UC cultivars on susceptibility to
*Macrophomina* and *Fusarium* under different fumigation treatments resulted in significantly greater mortality in ‘Albion’ compared with ‘San Andreas’ and ‘Monterey’ (Albion > San Andreas > Monterey) across all treatments (Koike et al., 2013; Mansouripour et al., 2018). However, we only saw a significant effect of cultivar on plant mortality in 2019. This was perhaps due to the differences in disease pressure on cultivars as a result of complex interactions between pathogens, plants, and abiotic factors.

It is thought that a healthy plant may be less susceptible to diseases. Part of what makes a healthy plant includes meeting its nutritional needs. Adequate nutrition can make the plant more tolerant or resistant to diseases (Sullivan, 2001) as mineral nutrients regulate plant metabolic activity, which is related to plant resistance and pathogen virulence (Huber and Graham, 1999). Datnoff et al. (2007) concluded that nitrogen influences plant resistance by reducing the frequency of successful penetration of pathogens by slowing parthenogenesis after penetration. However, nitrogen is also known to induce various anatomical and biochemical changes such as reduced activity in key enzymes of phenol metabolism and lower lignin content, both of which are part of the defense system of plants against infection (Dordas, 2008). This can often increase susceptibility to obligate parasites at high N rates (Dordas, 2008). High N rates have been shown to produce succulent growth that can lead to exacerbation of such foliar diseases as powdery mildew in various plants (Powel and Lindquist, 1997). Nam et al. (2006) found elevated nitrogen and potassium concentrations in fertilizer solution increased disease severity by anthracnose in strawberry plants grown under hydroponic conditions.

It is difficult to explain the higher rates of plant mortality in plots treated with synthetic CRF but in the soil, particularly in the rhizosphere, competition among
microorganisms both beneficial and antagonistic, can influence inoculation by soilborne pathogens. Soil fertility and chemistry including soil pH, calcium, phosphorus and zinc levels and nitrogen form can all play a major role in the management of soilborne diseases (Panth et al., 2020). Nitrogen uptake in the form of nitrate makes the root zone less acidic, though, the beneficial effect of higher pH is lost by using the acidifying ammonium form of nitrogen. Woltz and Jones (1973) found the use of nitrate nitrogen produced higher soil pH and resulted in better control of Fusarium wilt in tomato production. We did not see significantly greater concentrations of NO$_3$-N in the root zone of experiment plots treated with synthetic CRF compared with compost or control plots. However, different N compounds have been shown to influence susceptibility to some diseases (Smith, 2009) and therefore different N fertilizers may affect infections by different soilborne diseases resulting in plant death. The source and level of N in fertilizers has been shown to affect the severity of anthracnose crown rot in strawberry (Smith, 2009) so it is possible this may be true for other soilborne pathogens. Little is known about the mechanisms involved in infection of the strawberry plant by *M. phaseolina* but based on our results, N levels may play a role in susceptibility of strawberry to charcoal rot. This is because in both years, the plants in the control treatment had significantly less plant mortality compared with the CRF treatment. More research on how N availability can affect mechanisms used by soilborne pathogens like *M. phaseolina* is needed.
5.3 Effect of pre-plant fertilizer and cultivar on plant N and soil N dynamics

Reports on seasonal strawberry crop N uptake indicate it is between 180 and 220 lb/ac in plants throughout the coastal California production regions (Bottoms et al., 2013a and b; Hartz et al., 2018), but have been reported to be as low as 70 lb/ac (Lieten and Misotten, 1993; Tagliavini et al., 2005) while N uptake in organic production systems has been reported to be ~107 lb/ac. In other production regions like Florida, N uptake has been reported to be even lower at only 53 lb/ac (Albregts and Howard, 1978, 1980). The majority of these reports are from conventionally managed strawberries in winter production regions such as Florida and California where the field is fumigated prior to planting and N fertilizers are applied in the mineral form. Total N uptake by strawberry plants in this experiment more closely matched the reports of lower N uptake with averages between 38 and 62 lb N/ac (Figure 4). Plant uptake would likely be higher in a field with better yield or higher plant population such as in a commercial managed production system. Leaf tissue analyses determined %N in leaf tissue across pre-plant treatment and cultivar were somewhat lower (avg. 2.1%) (Figure 11) than optimal leaf tissue %N (2.4 to 3.0%) during the main harvest period but based on CDFA fertilizer recommendations, %N in leaf tissue in pre-harvest samples indicated there were likely no N deficiencies in any of the pre-plant fertilizer treatments or cultivars (CDFA, 2021).

There was a trend across pre-plant treatment and cultivar in harvest removal N (lb of N in fruit) with a greater proportion of N acquisitioned to the fruit compared with crown, stems and leaves (Figure 6). This contradicted research from the UC Cooperative Extension from 13 commercial fields across Ventura, Santa Maria and Salinas growing regions who reported that approximately 100 lb N/ac would be removed from a field at
the state average yield of 35 T/ac representing about 50% of total crop N uptake (Hartz et al., 2018). It is possible this was due to the disease pressures in our experimental field caused by soilborne pathogens that infected aboveground vegetative tissue. Infection by *M. phaseolina* initially begins with discoloration of leaves, wilting and eventually overall plant decline and plant death after plants undergo stress from fruit production (Gupta et al., 2012; Kaur et al., 2012; Koike, 2012; Koike and Bolda, 2013).

Our results in both years were consistent with the literature in that plant nutrient concentrations and partitioning were cultivar dependent. This suggested that growers can plan their nutrient management program based on cultivar selection and adjust nutrient applications accordingly. Fruit N concentration (lb N per lb fresh fruit) in cultivars used in this field experiment reported lower N concentration than is referenced for strawberry N removal rates in Ag Order 4.0. Because actual N removal rates were less than the reference value, strawberry growers have more room in their N budget in terms of applied N vs removed N. Anecdotal evidence supports our finding that cultivars have different N needs and our research now provides data to support this. Although it is common practice to apply N fertilizer in a synthetic slow-release form prior to planting, research has indicated this N is not necessarily available during periods of more significant crop N uptake (Bottoms et al., 2013). Eliminating this application from the overall nutrient management plan will help reduce total N applications and focus on in-season N applications when crop N uptake is significant. This can also have economic benefits to the grower as pre-plant fertilizer applications can cost over $400/ac (Bolda et al., 2016).
A surprising result from this study was the effect on soil NO$_3^-$-N exposure. We did not see any significant difference in soil NO$_3^-$-N exposure in or below the root zone across pre-plant fertilizer treatments or cultivar (Figure 13). This was unexpected considering CRF and compost treatment plots received an additional 100 lb of N prior to planting compared with control plots. Because the soil in the field is of a fine clay loam texture compared with the sandy loam soils prevalent in coastal California strawberry fields, this may have influenced the soil organic N pool and affected the concentrations of mineral N in the soil solution. Even under high synthetic N inputs, more than half of plant N uptake can be derived from mineralization of soil organic matter, demonstrating the complexity of processes affecting the fate of fertilizer N and its interactions with soil organic matter (Yan et al., 2020).

In recent studies adjusting the framework for plant, microbe and mineral influences on bioavailable N, Daly et al., (2021) suggested that the proportion of bioavailable N derived from particulate organic matter (POM) or mineral associated organic matter (MAOM) depended on the ratio between N mobilized from POM, via depolymerization and solubilization, versus the potential for mineral sorption. The latter arises from the properties of soil colloids, soil texture, and the overall chemistry and quantity of MAOM and N in the soil solution (Rillig et al., 2007; Dippold et al., 2014). Their framework emphasizes the role of minerals in intercepting, immobilizing, and releasing bioavailable N via sorption and desorption processes (Daly et al., 2021). Possibly, immobilization of N into MAOM in the clay loam soil (89% Salinas silty clay) prevented buildup of fertilizer N in the soil solution in our study.
In addition to the complexity of N cycling in soil, the release rate of N in CRF and compost may have been too slow to pick up any differences in the soil NO$_3^-$-N in our pore water samples across pre-plant fertilizer treatments. According to the company who manufactures the CRF product used in this study, the nutrient release rate is approximately three to four months for complete dissolution of their product (pers. comm. AgRx, April 2020). At an application rate of 100 lb N/ac, this means that only ~1 lb N would be released per day in a three-month period. Because composts are known to have slower nutrient release patterns (Hartz et al., 2002), this too could make it difficult to detect any differences in soil NO$_3^-$-N exposure across pre-plant fertilizer treatments.

5.4 Effect of pre-plant fertilizer treatment and cultivar on soil C and root density

Permanganate oxidizable carbon and mineralizable C, two C pools known to be more sensitive to soil management compared to total soil organic C (Hurisso et al., 2016), were measured to provide insights of how C pools might change after receiving compost. We hypothesized that compost treatment plots would have greater POXC compared with CRF and control treatment plots. We did not see any significant differences across pre-plant fertilizer treatments in POXC levels (Figure 14), which contradicts literature that reports organically managed surface soils contain greater total C (Hepperly et al., 2009; Mukome et al., 2014; Reganold et al., 2010). Because strawberries are grown in an annual production system and due to some limitations we experienced in field design, our experiment was set up in two different parts of the field for the first and second year. This
could have impacted any differences in soil C we may have seen if compost application had taken place in the same plots each year.

Min C is a measure of the fraction of active C that tends to be mineralized by soil microorganisms and can, therefore, be used to indicate soil microbial activity. We hypothesized the compost treatment blocks would have higher microbial activity in soils sampled within the root zone compared with CRF and control treatment blocks, but this finding was not supported (Figure 15). We suspect the reason was similar to results for POXC levels and that this short-term compost application may not have been sufficient to affect this carbon pool.

Crop species and even genotypes have distinct rooting systems, affecting the ability of crops to facilitate plant-soil interactions (de Graaff et al., 2013; de Vries et al., 2017). Plant roots also establish resource-rich hotspots with distinct properties from the bulk soil and selectively recruit microbial communities in the rhizosphere (Hinsinger et al., 2005; Fan et al., 2017) and there is recent evidence that the rhizosphere microbiome is cultivar dependent and can play a role in resistance to soilborne pathogens and nutrient uptake of strawberry cultivars (Lazcano et al., 2021).

Junaidi et al. (2018) found greater C allocation in some wheat genotypes under compost inputs. We hypothesized there may be a significant effect of cultivar or perhaps even an interaction between cultivar and pre-plant fertilizer treatment on root density and that some cultivars may enhance C allocation to the root zone in the compost treatment blocks. Enhanced root allocation is thought to support processes such as biologically mediated nutrient availability (Junaidi et al., 2018).
Our hypothesis was not supported and in fact, our results were opposite of what we expected to see and did not support the research by Junaidi et al. (2018). In 2020, plants in the CRF treatment blocks had significantly greater root density compared with control and compost treatments. Junaidi et al.’s (2018) study with wheat took place in a greenhouse and so there was greater control over biotic and abiotic factors relative to our study. Again, our short-term compost application may not have been sufficient to identify cultivars that can allocate more C to root systems.
CHAPTER 6

Conclusions

In light of recent legislation impacting N application in strawberry production systems and increasing disease pressures, our project aims to assist growers with pre-plant N management strategies that may reduce disease incidence while preserving yield. This study was undertaken to evaluate the effect of pre-plant N fertilizer and cultivar on total yield, soil NO$_3^-$-N in and below the root zone, soil C in the root zone, crop and fruit N uptake, and disease incidence. We also hoped to identify cultivar by pre-plant nutrient management strategies that simultaneously reduce disease, improve soil N dynamics and increase crop yield.

Our results support our hypothesis that total yield is cultivar dependent since, in both years, we saw a significant effect of cultivar and a significant effect of pre-plant fertilizer treatment only in year one. In year two, there was a significant effect of pre-plant fertilizer treatment, but it was the control treatment that had the highest mean yield compared with compost and CRF treatments. This suggests that synthetic pre-plant CRF does not necessarily produce higher yields and compost can be substituted for synthetic CRF without negatively affecting yield. Overall, our total yield was lower compared with the statewide average of 50,000 to 70,0000 lb/ac which is likely a result of disease pressure.

Our results did not confirm the findings from other studies that compost can have disease suppressive effects on soilborne pathogens. In both years, our control treatments had significantly lower plant mortality compared with both compost and CRF plots. This could perhaps be a result of the low application rate of compost as other studies suggest
application rates of compost as a soil amendment are much higher. It could also be that the compost was not applied in the same location in the field each year and so these were short term applications. The field was infested with *M. phaseolina* and our study shows that pre-plant N levels affect disease incidence by this soilborne pathogen. More research is needed to better understand the mechanism of infection of the strawberry crop by *M. phaseolina* but these preliminary results can guide other research to investigate the influence of N in infection by *M. phaseolina*.

We hypothesized that compost and control treatment plots would have lower soil NO$_3^-$-N concentration both in and below the root zone compared with CRF treatment plots and therefore, reduce NO$_3^-$-N leaching. We did not detect any differences across pre-plant treatments from pre-plant fertilizer source. This was likely a result of the residual soil NO$_3^-$-N levels in the field prior to planting (75 and 21 ppm in year 1 and year 2, respectively). And because the soil in the field is of a finer texture compared with the sandy loam soils prevalent in coastal California strawberry fields, this may also influence the soil organic N pool and affect the concentrations of mineral N in the soil solution. Growers should continue to soil sample prior to planting to determine if pre-plant N is necessary.

Our results support our hypothesis that N uptake in vegetative and fruit tissue is cultivar dependent and pre-plant N treatment did not affect N sufficiency levels based on %N in leaf tissue. Ag Order 4.0 requires growers to limit N discharge from their fields from 500 lb N/ac/ranch/year in 2023 to 50 lb N/ac/ranch/year by 2050. Although this will be difficult to do, the fruit N concentration for cultivars used in this study were lower compared with the conversion coefficient defined in Ag Order 4.0. This means that
growers remove less N through harvest and therefore have more room in their N budget when it comes to N applications through fertilizer or other sources like irrigation water. Overall, total crop N uptake was much lower compared with the literature which, again, is likely a result of disease pressure and lower biomass production in general.

California strawberry growers will face extreme challenges as a result of continued N monitoring and looming N discharge limits. Our results add to the mounting evidence that shows the insignificant impact of synthetic pre-plant CRF N on strawberry yield. Although the decision to use pre-plant N depends on several factors including anticipated rainfall, soil texture, previous crop and initial soil nitrate concentration, eliminating this application would allow for more room in the N budget for in-season applications that can more closely match crop N uptake. More research should be done to further investigate the mechanisms of infection by *M. phaseolina* as this could have implications for N management in fields with *M. phaseolina* infestation. Additional research is needed to determine how compost applications as a substitute for synthetic pre-plant N in the long term can impact disease suppression, soil NO₃⁻-N, NO₃⁻-N leaching, and soil C.
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APPENDIX

Field Information

A.1 Soil Test Reports

Figure 18. Soil test report in August 2018 for Field 35B at the Cal Poly Strawberry Center.
Figure 19. Soil test report in September 2019 for Field 25 and 35B at the Cal Poly Strawberry Center.

Methods

A.2 Soil Moisture Retention Curve

Figure 20. Soil moisture retention curve for Field 35B at the Cal Poly Strawberry center. Created with Gnuplot in 2019.