ABSTRACT

LAY, AMANDA M. Impact of Pre-Plant Fertilizer Rates in combination with Polysulphate® on Yield, Soil Nitrogen Distribution, and Physiology of Strawberry (*Fragaria* x *ananassa* 'Camarosa' & 'Chandler'). (Under the direction of Dr. Mark Hoffmann and Dr. Michelle Schroeder-Moreno).

Annual hill plasticulture systems are the primary production method for strawberry (*Fragaria x ananassa*) in North Carolina (NC). Approximately 1,200 acres of strawberries are grown in NC in plasticulture systems, mostly for direct-to-consumer markets. Young strawberry plants are transplanted every year in September/October in order to produce fruit the next Spring. Fertilizers for this system are usually applied in two steps: as fall applied pre-plant fertilizer and in the following spring as liquid fertilizer. It is common practice for smaller growers to apply full-spectrum fertilizer as pre-plant, often at rates that match currently recommended 67 kg/ha (60 lbs/ac) Nitrogen (N). However, questions remain whether or not such practice leads to optimal plant nutrition during the first months of establishment. Polysulphate is a certified organic fertilizer which consists of 48% sulphate, 14% potassium, 6% magnesium, and 17% calcium. We hypothesized that Polysulphate co-applied with a standard full spectrum fertilizer will lead to higher yield and fruit quality, and when applied with lower full spectrum fertilizer will reduce N concentrations in strawberry beds. To investigate this, two field trials were conducted using *F.* x *ananassa* cv. Camarosa. Studies were conducted between September 2019 - May 2020 and September 2020 – June 2021. Six pre-plant fertilizer treatments were established at two research stations in North Carolina: Piedmont Research Station (clay loam soil, non-fumigated) and Central Crops Research Station (sandy loam soil, fumigated). Each trial was set up as demonstration plot in a split plot design, with one plot per treatment, and four replicates per plot. Following treatments were established: High N (1334.93 kg/ha 6-6-18); Grower Standard (1120.85 kg/ha of 6-6-18); Low Polysulphate (902.29 kg/ha 6-6-18 + 280.21

Polysulphate); Medium Polysulphate (684.84 kg/ha 6-6-18 + 560.43 Polysulphate); Half-Grower Standard (560.43 kg/ha 6-6-18); High Polysulphate (446.27 kg/ha 6-6-18 + 840.64 Polysulphate); and a non-treated control (NTC). To assess N concentration in soil, samples were taken at 0-12.7 cm and 12.7-25.4 cm depth for the first 5 months after transplanting. To assess nutrient content in plants, tissue samples were collected during the first 5 month after transplanting. All field trials were subject to spring fertigation at 17 kg N /ha/week. Marketable and total strawberry yields were assessed over 6 weeks in the spring of 2020 and 2021. The co-application of Polysuphate did not lead to a significant increase in yield or fruit quality. However, especially in sandy soil, N rapidly declined under plastic within the first 8 weeks after pre-plant fertilizer application. Treatments that contained Polysulphate and lower amounts of full spectrum fertilizer showed significantly lower N concentrations in the soil, while maintaining similar yields compared to grower standard per-plant fertilizer treatments.

To evaluate the impact of different N dosages on plant growth, a greenhouse study was conducted at the Horticulture Field Lab. In the greenhouse a complete randomized plot design was set up in a soilless container system (n=12 per treatment). Four N rates: 1.19 mmol/L of N; 2.36 mmol/L of N; 4.76 mmol/L of N; or 9.50 mmol/L of N were applied before planting and mixed into Coconut Coir: Perlite (50:50) substrate. Treatments were assessed in a soilless potted system. *F.* x *ananassa* cv. Chandler were planted as plug plants and grown over 14 weeks under greenhouse conditions. The following traits were assessed over the growing period: the number of flowers, crowns, and leaves, crown diameter, and chlorophyll. At the end of experiment, nutrient content of plant tissue, substrate, and dry weights of above and below ground plant organs were assessed. In this study we observed that N is left over in the substrate of the highest N treatment, while nitrogen content was generally similar in petioles and crowns across all

treatments. Our greenhouse study shows that the highest N rate did not result in the complete use of N by the plant in the first 14 weeks after planting.

Based on our studies, it is likely that the current recommendations of pre-plant N rates for NC plasticulture strawberry are too high, especially for highly aerated soils. We successfully reduced the amount of full-spectrum fertilizer applied as pre-plant and used Polysulphate to serve as source for sulfur, calcium, magnesium and phosphorous for plant establishment for the strawberry cultivar Camarosa. However, while our study was focused on the investigation of Nitrogen distribution in soil, future research on the impact of fertilizer on the overall nutrition requirements of strawberry plants is necessary for comprehensive fertilizer recommendations.

Impact of Pre-Plant Fertilizer Rates in combination with Polysulphate® on Yield, Soil Nitrogen Distribution, and Physiology of Strawberry (*Fragaria* x *ananassa* 'Camarosa' & 'Chandler')

by
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DEDICATION

To Sawyer Walters, you always kept me going.

To my parents, Mr. Stephen Lay and Dr. Anna Scheyett, you got me to where I am.

Thank you.

BIOGRAPHY

Amanda Lay-Walters grew up in Chapel Hill, North Carolina where she learned about the food system and produce production at Spence's Farm (now known as Sunrise Community Farm Center). Amanda continued her education at North Carolina State University with a BS in Horticultural Science. At NC State she met many amazing mentors both inside and outside of the department. During her undergraduate program she studied abroad in Oaxaca, Mexico, spent a summer working at the University of Georgia UGArden, and worked for the Department of Horticultural Science for two years. Under the direct supervision of Dominic Gillooly and Chris Heim, Amanda helped to maintain ornamental sweet potato germplasm populations and cross pollinate table stock sweet potatoes, leading her to realize she wanted to focus on horticultural crop production.

She began her M.S. in horticulture at NC State in the Fall of 2019. Thanks to the encouragement of Dr. Mark Hoffmann, she applied and was accepted to be a graduate fellow of the Center for Environmental Farming Systems (CEFS) and met a wide array of wonderful faculty and graduate students. During her M.S. she collected 900 soil samples, experienced working from home during a global pandemic, got married, and conducted two field experiments and one and a half greenhouse experiments. She has been accepted as a PhD student at the University of Arkansas in the Horticulture Department and will start in Spring 2022.

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Chapter 1: Literature Review

Strawberry Production and Industry in North Carolina

Globally, the United States leads in strawberry production per unit of area and is second in production by volume after China (Samtani et al., 2019). In in 2007, strawberry production in the southeastern United States was estimated to cost $13,556 per acre and could have gross returns ranging from $21,000 to $30,000 per acre (Leandro et al., 2007). This cost has increased significantly since then; however, we do not have updated costs currently. In the United States the majority of strawberries produced is located in California (>80%) and Florida (19-8%) (Samtani et al., 2019). North Carolina (NC) and Oregon produce approximately 1% each of all strawberries produced every year (Samtani et al., 2019). NC is the second largest strawberry producer on the East Coast, after Florida (Samtani et al., 2019).

Most growers in NC are producing strawberries on less than five acres (Poling, 2012). There are an approximately 15-20 NC farms frequently producing strawberries on 10 or more acres. Of those farms, 5-6 farms produce frequently on 30 acres or more (Poling, 2015). The largest NC grower in the 2019-20 season had approximately 150 acres of strawberry production (personal communication with Hoffmann). NC growers often sell their strawberries directly to consumers via Pick your own, roadside stands, and farmers markets. Larger growers in NC ship to whole-sellers and grocery stores (McWhirt et al., 2020). NC has approximately 1,200 acres of strawberries each year; the vast majority of that acreage is produced in annual hill plasticulture systems (McWhirt et al., 2020).

Annual hill plasticulture is a system that uses plastic mulch to cover raised beds before transplanting (Poling, 2016). In plasticulture systems, plastic mulch is used to prevent weed competition and optimize soil drainage and soil temperature. Annual hill plasticulture

strawberries in NC are drip irrigated through a single drip line buried two inches underground in the center of the bed. Strawberries grown in plasticulture systems can be up to two and a half times more productive than bare-ground matted-row production (Poling, 2015). However, annual hill plasticulture is also more management intensive than matted-row strawberry production, which leaves a considerably smaller margin of error in regard to management and marketing decisions (Poling, 2016).

In mid-summer soils are tested for nutrient content and pH to predict how much dolomitic lime and potash (K_2O) will be needed before bedding (Hicks et al., 2015). In NC, pre-plant fertilizers are broadcasted across fields in August or September as a one-time granular application. Preplant fertilizers regularly include N, phosphorous, and potassium in large quantities (Hicks et al., 2015). Raised beds are then shaped and drip tape and plastic mulch are laid down. It is common practice to disinfest the soil using soil fumigants shortly before or after plastic mulch is laid down. Fumigation minimizes the risks associated with soil borne pathogens (Leandro et al., 2007; Melanson, 2021). Common chemicals used to disinfest the soil in strawberry production are Chloropicrin, 1- 3 Dichloropropene, Di-Methyl-Di-Sulfide, and Methyl Isothiocyanate (Metam-Sodium, Metam-Potassium) (Melanson, 2021). Depending on the chemical used, farmers must allow for two to three weeks between fumigation and planting (Poling et al., 2005).

Strawberry transplants are planted into the raised beds after plastic mulch and drip tape are laid down and enough time has passed between fumigation and planting date Strawberry plug plants and bare-root plants are both common transplants in NC (Poling et al., 2005). Planting date is dependent on the region of NC. In the mountains growers aim to plant in mid to late September, in the Piedmont growers aim to plant in late September or early October, and

growers in the coastal plains aim to plant in mid-October, however all planting dates are dependent on sourcing transplants in a timely manner (McWhirt et al., 2020). After planting, strawberry establishment encompasses the development of root system, canopy, and crown (Poling et al., 2005). Depending on the region and the winter temperatures, strawberry plants usually go dormant between November and February.

With the formation of the first flowers in late February into April, spring frost can cause injuries to blossoms, and has the potential to decrease yields. Growers use floating row covers, overhead irrigation or a combination of both to protect strawberry blossoms from frost and freeze damage in spring (Poling, 2016). In the majority of NC strawberry growing regions, growers will start to apply the liquid fertilizer calcium nitrate in early March, through all of harvest, often into the first week of June (Hicks et al., 2015). Spring fertigation is often applied weekly and based on plant nutrient content, assessed through tissue sample analysis (Hicks et al., 2015). In the piedmont and coastal plains of NC, harvest generally starts in mid-April and can go through the first week of June (Poling, 2016).

Nutrient Needs and Fertilizer Practices in NC Strawberry Production

N must be applied at a proper rate and supplied to the plant as needed throughout the different growth stages of the crop. Many variables can affect N balance, including soil conditions, age of the crop, irrigation system, and water quality (Cameira and Mota, 2017). The current N recommendations for pre-plant strawberries is 67 kg/ha while the current recommendation for spring fertigation is to apply 5.88 kg/ha of N each week and to adjust based on NO_3 concentration in the petiole (Hicks et al., 2015). However, a study conducted at the Central Crops Research Station in Clayton, NC found that the optimal pre-plant N rate to be 34 kg/ha (Miner et al., 1997). In the same study the optimal rate for spring fertigation was 0.56

kg/ha per day of N; from Miner et al. calculations spring N fertilization was over 2 times more effective than N applied in the fall (Miner et al., 1997).

Mid-summer soil samples should be sent to agencies, such as the North Carolina Department of Agriculture and Crop Science (NCDA&CS), which will analyze the nutrient content and send specific reports to the grower recommending the application rates (Hicks et al., 2015). Previously, NC strawberry growers were recommended to apply 67 kg/ha of N, 67 kg/ha of phosphorus, and 134.5 kg/ha of potassium (Poling et al., 2005). However, pre-plant N is currently recommended to be applied at a rate of 67 kg/ha, while pre-plant potassium and phosphorous application rates should be dependent on the soil reports and recommendations from the mid-summer soil samples collected (Hicks et al., 2015). Commonly used sources of phosphorous and potassium include diammonium phosphate (DAP), triple superphosphate, potassium chloride, and potassium nitrate. Insufficient potassium supplied to the plant has been linked to low levels of soluble solids and acidity in the fruit. Meanwhile, the oversupply of potassium has been found to cause decreased fruit firmness (Miner et al., 1997).

As of 2015, strawberry growers are recommended to apply 16.8 kg/ha of sulfur as pre-plant. Fertilizers such as potassium sulfate, potash magnesium sulfate, and gypsum are sources of both potassium and sulfur for pre-plant fertilizer application of strawberries (Hicks et al., 2015). Mid-summer soil analysis also provides lime recommendations to amend the soil during tillage (Hicks et al., 2015). Lime most often used is dolomitic lime ($CaMg(CO_3)_2$), or calcitic lime ($CaCO_3$) if soil reports indicate sufficient magnesium concentrations (Hicks et al., 2015). In March, plant tissue sampling is required to plan for fertilization during the spring.

Organic production systems are growing throughout United States agriculture as demand for organic produce from consumers increases (Ogles et. al., 2015). However, organic growers

are more limited by what fertilizers they can use on their farms compared to conventional farmers. Due to the prohibited use of inorganic fertilizers, organic growers largely depend on the use of natural sources such as manure, legume cover crops, animal byproducts, and naturally formed minerals for soil fertility and plant nutrition (Ogles et. al., 2015). Polysulphate has the potential to be another fertilizer source for organic growers and for conventional growers trying to incorporate organic growing methods.

Polysulphate is the product name of the mineral polyhalite, the only known source of polyhalite is the Boulby mine near the North Sea in Cleveland, England, UK. Polysulphate is composed of 48% sulfur trioxide (SO_3) 14% potassium oxide (K_2O), 6% magnesium oxide (MgO), and 17% calcium oxide (K_2O). The product Polysulphate is not a mixture of several salts but a single crystal mineral (Yermiyahu et al., 2017). The product Polysulphate is certified organic in the United States and several other countries (ICL Fertilizers, Cleveland, UK). The use of Polysulphate has great potential as a supplemental fertilizer for the nutrients Ca, Mg, K, and S in soil (Yermiyahu et al., 2017). As an organically certified product, NC strawberry growers who look for organic methods of production, even if they are not a certified organic farm, may be interested in using Polysulphate as a fertilizer for their pre-plant fertilizer.

Impact of Nutrients on Plant Physiology and Growth

Strawberry plants grow from a compressed stem called a crown. Inflorescences, stolons (runners) and leaves grow directly from meristem in the crown (Galletta and Bringhurst, 1990). Stolons produce daughter plants, which are used by the strawberry nursery industry to produce transplant material, which then is sold for fruit production to strawberry growers. Strawberry inflorescences grow from the crown and grow in a branching structure. June-bearing (short-day) cultivars, such as ‘Camarosa’ and ‘Chandler’, are a type of strawberry that produce the majority

of their crop in April and May when grown in NC. June-bearing varieties' growth and flowering are dependent on daylight period (photoperiod) and temperature (Galletta and Bringhurst, 1990). Everbearing (day-neutral) cultivars are able to flower at any daylight period, flower buds can also develop in a wide temperature range (Sønsteby and Heide, 2007). It is common for everbearing cultivars to begin fruiting in the early spring and produce into early summer in NC production (Galletta and Bringhurst, 1990).

Phosphorus and potassium are primary essential macronutrients, meaning that they are both necessary for plant growth in large volume, compared to other essential nutrients. Phosphorus is needed for plants to store energy and for the internal structure of plant cells. Symptoms of phosphorous deficiency can appear as the stunting of new plant growth; the appearance of these symptoms in new growth is due to phosphorus being an immobile nutrient meaning it cannot move throughout the plant. Other symptoms of phosphorus deficiency are dark green coloration of leaves and necrotic spots on the leaves (Taiz and Zeiger, 2006). Potassium is essential for enzymes that are required in respiration and photosynthesis. Potassium deficiencies most often appear as marginal chlorosis (the yellowing of leaves) and necrosis at leaf tips. As a mobile nutrient, deficiencies are most likely to appear in mature leaves first (Taiz and Zeiger, 2006).

Sulfur, calcium, and magnesium are secondary essential macronutrients. Sulfur is part of several amino acids and is necessary for plant metabolism. Sulfur deficiency is similar to N deficiency because both are constituents of protein, however chlorosis due to sulfur deficiency will be present in young and mature leaves, as opposed to just mature leaves for N deficiency (Taiz and Zeiger, 2006). The N to sulfur ratio during spring fertigation is important to account for, if too high (> 18:1) plants may have poor assimilation of both nutrient and cause yellowing

of the leaves (Hicks et al., 2015). Calcium is critical to regulate many processes on the cellular level, including the synthesis of new cell walls, transcription, and cell division. Symptoms of calcium deficiency appears as necrosis of new growth in the leaves and roots (Taiz and Zeiger, 2006). Magnesium helps to activate enzymes that are involved in respiration, photosynthesis, and the synthesis of DNA and RNA. Symptoms of magnesium deficiency include chlorosis between leaf veins which often first occurs in older leaves and pre-mature leaf abscission (Taiz and Zeiger, 2006).

N is essential for plant development and vegetative growth (Taiz and Zeiger, 2006; Poling, 2016). N is a mobile element and can move within the vascular system of the plant from older plant parts to new growth when there is a N deficiency. A common symptom of N deficiency in strawberry is chlorosis, which most commonly occurs in the older leaves of the plant (Taiz and Zeiger, 2006). Thus, younger leaves may not show symptoms of chlorosis or may be a lighter green (Taiz and Zeiger, 2006). N deficiencies can inhibit plant growth causing stunted plant growth and decreased marketable yield (Li et. al., 2013). This negatively affects growers' profitability; however, it is currently unclear what the optimal rate for strawberry pre-plant fertilizer application of N is.

The over-application of N with fertilizers may cause softer fruit and larger plants (Hicks et. al., 2015). Thus, over-application of N fertilizers may negatively affect growers financially in two ways. Firstly, the application of excess fertilizer means that growers are paying for fertilizer that is either lost or not contributing to yield and quality of the crop. Secondly, the quality of the strawberry fruit decreases which can reduce the quantity of produce taken to the market.

Nitrogen Pollution in Agriculture

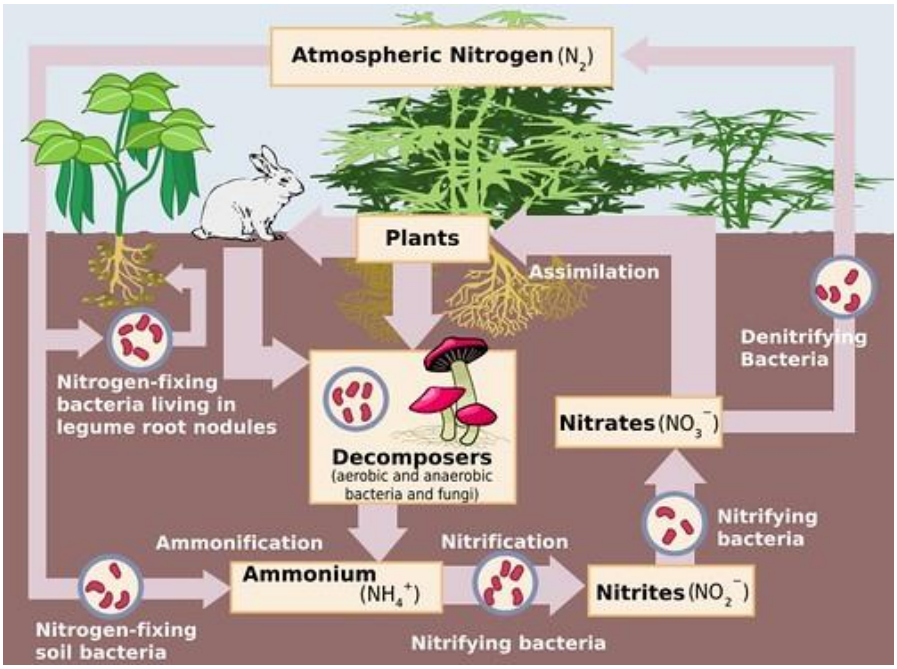

Figure 1.1. The N cycle. "Nitrogen cycle rules!" by sfbaywalk is licensed with Creative Commons BY 2.0.

The earth's atmosphere consists of 78 percent atmospheric nitrogen (N_2), which is converted to ammonia (NH_3) by nitrogen fixing bacteria such as *Azotobacter* and *Rhizobium*. *Azotobacter* is a type of free-living bacteria, meaning it does not form symbiotic relationships, that fixes N_2 into NH_3. Ammonia will often rapidly convert to ammonium (NH_4^+); if ammonia does not convert to ammonium, ammonia can escape the soil system via volatilization. Ammonium is a plant-available form of N and is taken up by plant roots. If ammonium is not taken up by the plant or immobilized, then ammonium will go through the process of nitrification where ammonium transforms into nitrite (NO_2^-) and then nitrate (NO_3^-). Nitrification occurs when specialized bacteria and archaea are in the presence of oxygen. The conversion of ammonium to nitrite is primarily carried out through *Nitrosomonas* and *Nitrosococcus* bacteria

and the conversion of nitrite to nitrate is carried out by *Nitrobacter* and *Nitrococcus* (Ricklefs and Relyea, 2014).

Nitrate is also a plant-available form of N. An oversupply of nitrate often leads to leaching of nitrate into the ground water. Nitrate, together with other fertilizer residues such as phosphates, often finds their way into larger water bodies, leading to over-fertilization of lakes and even seashores (El Mountassir et al., 2021). Nitrate may go through denitrification and enters the atmosphere as nitrous oxide (N_2O) to eventually convert back to atmospheric N (Ricklefs and Relyea, 2014).

The use of irrigation in agricultural systems is critical for plant growth, yet there are several ways that ineffective management of agricultural irrigation can cause negative impacts on the N cycle and environmental pollution. Irrigation supports plant growth by replacing the water lost by the plant through evapotranspiration throughout the day (Poh et al., 2011). Excessive irrigation, fertigation, or heavy rain events can cause an increase in nutrient leaching and runoff (Carson et. al., 2014), thus irrigation methods can directly impact N fertilizer efficiency (De Pascale et. al., 2018). Leaching occurs when nutrients, such as N, are washed down the soil profile, leaving the root zone and entering the ground water (Poh et al., 2011). Runoff occurs on the soil surface when water washes nutrients out of the field and into nearby natural systems that eventually make their way to water bodies.

Excess N in natural water systems can change the growth patterns of aquatic flora. This excess N can cause eutrophication, when algal blooms grow in excess and take up larger than usual amounts of dissolved oxygen in the water, thus killing other aquatic flora and fauna (Cameira and Mota, 2017). Eutrophication is considered a problem worldwide and numerous management programs have been implemented to correct the problem (Christian and Thomas,

2003). Nutrients, such as N, are more likely to escape the soil system when the cation exchange capacity (CEC) of soil is low or when the supply of N, generally through fertilizer, is greater than the CEC can hold even in soils with high CEC. Other variables that can influence nutrient escape include microbial activity, humus content, water content, and which form of N is in the soil, as NO_3^- is more water soluble than NH_4^+ (Ricklefs and Reylea, 2014). Nitrogen severely contributes to eutrophication within American water ways (Christian and Thomas, 2003).

Roughly 60 percent of all coastal rivers and bays in the United States have been at least moderately degraded by N pollution. The Mississippi River Basin has increased in N four-fold due to human activity (Howarth et al., 2002). Specifically in NC, the Neuse Nutrient Strategy has been imposed by state lawmakers since 1997 due to the excessive rates of N and phosphorus in the Neuse River Estuary that have been diminishing water quality since the 1980s. This threatens the ecosystem and population dynamics of the aquatic life in the estuary and downstream due to increased levels of eutrophication (Christian and Thomas, 2003). Because of this, the estuary is listed on the NC impaired waters list (Christian and Thomas, 2003).

Chapter 2: Impact of Different Rates of Fall Applied Polysulphate Combined with Granular Fertilizer on Yield (*F. x ananassa* 'Camarosa') and Nitrogen Distribution in the Soil

Introduction

In NC strawberries are primarily grown in annual hill plasticulture systems. Strawberry fields are usually fertilized before planting (pre-plant) to promote plant establishment, and again in spring to promote flowering and fruiting. As pre-plant fertilizer, often full-spectrum granular fertilizer is broadcasted in the field before fumigation and bed shaping. The actual fertilizer rates depend on soil analysis but are often steered by recommend N broadcast rates of 67 kg/ha (60 lbs/ac) (Hicks et al. 2015). Many small growers apply pre-plant fertilizers as granular full-spectrum fertilizer, such as 6-6-18 or 10-10-10. However, some growers also use custom blends, often consisting of phosphate, potash, and nitrogen. These nutrients are commonly sourced from phosphate-diammonium (18-46-0) or triple superphosphate (0-46-0), potash-potassium nitrate (13-0-44), potassium sulfate (0-0-50), potassium chloride (0-0-60), or ammonium sulfate (21-0-0) (Hicks et al., 2015).

After per-plant fertilizer is applied in fall, beds are shaped and often soil fumigants are applied. The process usually takes place a minimum of 21 days before transplanting, due to fumigation restrictions. Strawberry planting usually takes place either in September or October, however this depends on the region and the availability of plant material (Poling, 2015; McWhirt et al., 2020). Strawberries are planted as transplants (plug plants, bare-roots, cut-offs) into raised beds and irrigated through one or two buried drip lines. Strawberry growers in the mountains and piedmont plant usually mid to late September, while planting dates in Eastern NC range from late September to early October. Plug plants require less overhead watering during plant

establishment compared to other transplants. In general plug plants are faster to establish than other transplant options (Poling, 2015).

After transplanting, plants will develop root mass, establish additional crowns, and establish a larger canopy before entering the dormancy stage (December-February, depending on the temperatures). With rising temperatures, plants will begin to grow again and develop additional branch crowns and canopy. Strawberry plants often begin to flower in mid-March, at the same time liquid fertilizer (fertigation) is injected into the drip irrigation lines to provide nutrients through the harvest stage of the growing season. To assess plant needs for liquid fertilization, petiole samples are collected and analyzed before liquid fertilization is turned on. Liquid fertilizers are applied in the forms of calcium nitrate (15.5-0-0), potassium nitrate (13-0-44), and ammonium sulfate (21-0-0) (Hicks et al., 2015). Depending on N recommendations based on plant tissue analysis reports, between 0-7.85 kg of N/ha/week will be applied. Strawberries are generally harvested in NC between beginning to mid-April to end of May or beginning of June, depending on the weather.

Many growers producing strawberries in plasticulture systems rely on various sources of N to support plant development and growth. Usually, a strategy of fall applied pre-plant fertilizer and drip applied fertigation before and during harvest respectively can provide a production system with reliable yield and high berry quality. Along with the environmental dangers of unbalanced fertilizer regimes (Cameira and Mota, 2017), over-fertilization can cause diminished strawberry yield and fruit quality (Hicks et al., 2015). This decrease in yield is caused by reduced fruit set due to an increase in vegetative growth, which occurs during the uptake of excessive N by the plant (Albornoz, 2016). The overuse of N fertilizer is therefore not cost-effective , due to higher initial costs, but lower fruit quality and yields (Hicks et al., 2015). Pre-plant fertilizer

currently costs roughly $15 per 50 lbs of 6-6-18 fertilizer (Wilson County Farm Supply, Wilson, NC). Fertilizers used for liquid fertilization currently costs around $199 per ton (Wilson County Farm Supply, Wilson, NC). Currently the NCDA&CS recommend the application of 67 kg/ha of pre-plant N for NC strawberry farmers (Hicks et al., 2015).

Bottoms et al. (2013) and Durner (2020) have investigated N fertilizer rates for strawberries in central California and New Jersey, respectively. Bottoms et al. (2013) found that higher rates of N did not directly lead to high strawberry yields. In 1997, Miner et al. investigated pre-plant N rates in NC through a field study and found that the optimal N rates are closer to 34 kg/ha, nearly half of the current recommended rate. However, current literature on experiments for recommended fertilizer rates for NC strawberry production are lacking (Hicks et al., 2015; Poling, 2015).

Along with lacking recommended rates, further research on alternative sources of fertilizer that could lower N application rates while maintaining rates of other macronutrients are being investigated. Polysulphate may be an alternative source of macronutrients needed in pre-plant fertilization while minimizing growers' application of N in their fields. Polysulphate is a certified organic granular fertilizer, which contains 48 % sulfur, 14 % potassium, 17 % calcium, and 6 % magnesium. These macronutrients are all needed in strawberry production, especially for fruit yield and quality (Hicks et al., 2015). Yermiyahu et al. (2017) claimed that Polysulphate could be one source of fertilizer for horticultural crops, such as strawberries, for growers looking to use organically certified fertilizers. Polysulphate is also an opportunity for strawberry growers who are otherwise limited by the policy regulations, efficiency, or cost of organic fertilizers they can use in their production (Yermiyahu et al., 2017). Polysulphate differs from similar fertilizers because researcher have found that the leaching of calcium, magnesium, potassium, and sulfur

ions in polyhalite appears to be slower compared to the leaching of those ions in other fertilizers (Barbarick, 1991; Vale, 2016). The hypotheses of this study are: (1) combining fall applied pre-plant Polysulphate with lower rates of conventional full-spectrum fertilizer will lead to lower N soil concentrations in raised beds compared to the current conventional pre-plant fertilization practices; (2) fall applied pre-plant Polysulphate in combination with full-spectrum fertilizer will improve strawberry yield and fruit quality in the strawberry cultivar Camarosa, compared to the current standard pre-plant fertilization rate. To investigate these hypotheses, the following objectives of the study were established: (1) evaluate the distribution of N in the raised beds under six different fall applied pre-plant fertilizer rates between transplanting and dormancy; (2) evaluate the impact of six different fall applied pre-plant fertilizer rates co-applied with Polysulphate on the yield and fruit quality of the strawberry cultivar Camarosa.

Materials and Methods

Experimental Design and Field Trials

To investigate the impact of pre-plant fertilizer treatments on the repeated measures of both yield and the concentration of N at different soil depths, two field experiments were conducted over two years (2019-2020; 2020-2021) at two agricultural research stations in NC. The experiments took place at the Piedmont Research Station (PRS) in Salisbury, NC (lat. 35.6967 N long. 80.6227 W, Lloyd clay loam) and the Central Crops Research Station (CCRS) in Clayton, NC (lat. 35.668077 N long. -78.504857 W, Wagram sandy loam).

At both sites in all years the pre-plant treatments applied were Grower Standard (67.25 kg/ha of N applied as 1120.85 kg/ha N-P-K), Low Polysulphate (54.13 kg/ha of N applied as 902.29 kg/ha N-P-K + 28.021 kg/ha Polysulphate), Medium Polysulphate (41.09 kg/ha of N

applied as 684.84 kg/ha N-P-K + 560.43 kg/ha Polysulphate), High Polysulphate (27.98 kg/ha of N applied as 466.27 kg/ha N-P-K + 840.64 kg/ha Polysulphate), and NTC (Non-treated Control). At PRS for both years a sixth pre-plant treatment of Half-Grower Standard (33.63 kg/ha of N applied as 560.43 kg/ha N-P-K) was applied. At CCRS for both years a pre-plant treatment of High N (80.1 kg/ha of N, applied as 1334.93 kg/ha N-P-K) was applied (Table 2.2). Fertilizer was applied before bed shaping (Table 2.1). All experiments were set up as a split plot design, with one plot per treatment and four replicates per plot (Figure 2.1). Each replicate was planted with 20 plug plants (*Fragaria* x *ananassa*, 'Camarosa') (Table 2.1). Potassium rates were similar for the majority of treatments and other nutrients varied as noted in Table 2.3.

Table 2.1. Date of treatment application (application of pre-plant fertilizer), planting of 'Camarosa' plug plants, soil sampling, and plant-tissue sampling at the two field sites, at Piedmont Research Station (PRS) and Central Crops Research Station (CCRS) in the 2019-20 and 2020-21 seasons.

Location & Date	Pre-Plant Application	Planting Date	Soil Sampling Date	Tissue Sampling Date
PRS 2019-2020	6 Sept. 2019	25 Sept. 2019	25 Sept. 2019 15 Nov. 2019 2 Jan. 2020 18 Feb. 2020	15 Nov. 2019 18 Feb. 2020
PRS 2020-2021	16 Sept. 2020	14 Oct. 2020	14 Oct. 2020 10 Nov. 2020 10 Dec. 2020 15 Jan. 2021 24 Feb. 2021	10 Dec.2020 24 Feb. 2021 3 May 2021 25 May 2021
CCRS 2019-2020	12 Sept. 2019	8 Oct. 2019	7 Oct. 2019 26 Nov. 2019 10 Jan. 2020 25 Feb. 2020	26 Nov. 2019 25 Feb. 2020
CCRS 2020-2021	8 Sept. 2020	26 Oct. 2020	26 Oct. 2020 17 Nov. 2020 17 Dec. 2020 22 Jan. 2021 5 March 2021	17 Dec. 2020 5 March 2021 29 April 2021 21 May 2021

Table 2.2. Treatment labels, fertilizer rates, and nitrogen content of the fertilizer rates.

Treatment Name	Fertilizer Rate	Nitrogen Content of Fertilizer Rate
High N	1334.93 kg/ha N-P-K 6-6-18	80.096 kg/ha of N
Grower Standard	1120.85 kg/ha N-P-K 6-6-18	67.251 kg/ha of N
Low Polysulphate	902.29 kg/ha N-P-K 6-6-18 + 280.21 kg/ha Polysulphate	54.137 kg/ha of N
Medium Polysulphate	684.84 kg/ha N-P-K 6-6-18 + 560.43 kg/ha Polysulphate	41.09 kg/ha of N
Half-Grower Standard	560.43 kg/ha N-P-K 6-6-18	33.626 kg/ha of N
High Polysulphate	466.27 kg/ha N-P-K 6-6-18 + 840.64 kg/ha Polysulphate	27.976 kg/ha
NTC	Non-Treated Control	0 kg/ha of N

Table. 2.3. Preplant K, P, Ca, Mg, and S applied through pre-plant fertilizer by treatment. All units below are listed in kg/ha.

Treatment Name	Potassium (K)	Phosphorous (P)	Calcium (Ca)	Magnesium (Mg)	Sulphur (S)
High N	240.288	80.096	80.096	53.397	106.795
Grower Standard	201.753	67.251	67.251	44.834	89.668
Low Polysulphate	201.641	93.367	101.773	52.904	206.685
Medium Polysulphate	201.731	119.55	136.363	61.019	323.791
Half-Grower Standard	100.887	33.626	33.626	22.417	44.834
High Polysulphate	201.619	145.666	170.885	69.089	440.808
NTC	0	0	0	0	0

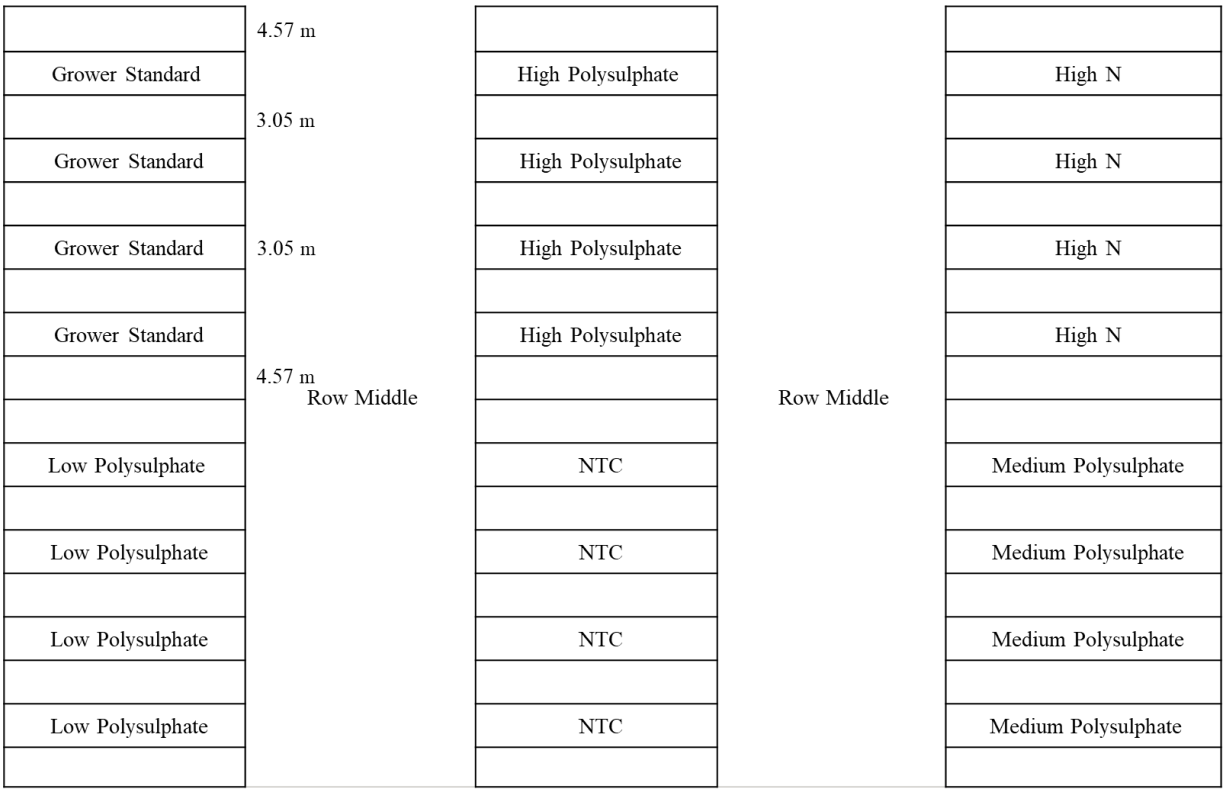

Figure 2.1. Field plot design. The labelled spaces above correlate to the treatments listed above (Table 2.2), each plot had four replicates. Replicates and blank spaces between replicates were 3.05 m, buffer space between treatments and row ends were 4.57 m long. Acronyms of treatments may be referenced in Table 2.2.

Fertilizer Application and Bed Preparation

At PRS on 6 Sept. 2019 and 16 Sept. 2020, six rows were measured at 45.72 m long. At CCRS on 12 Sept. 2019 and 8 Sept. 2020, eight rows were measured at 60.96 m long. While measuring the rows flags were placed at the 22.86 m mark at PRS, and flags were also placed at the 30.48 m mark at CCRS, indicating where one treatment started and the other ended. Then fertilizer was hand broadcasted evenly over the flagged portion of the rows on the same day for both locations and years respectively (Table 2.1).

The fertilizers used at PRS and CCRS was 6-6-18 (Dixie Farm Tested Fertilizers, New Bern, NC), and Polysulphate (ICL Group Ltd., Tel Aviv-Yafo, Israel). Polysulphate is a certified organic fertilizer, mined in the United Kingdom, containing 14% K_2O, 48% SO_3, 17% CaO, and 6% MgO. After fertilizer application, 15 cm high beds were formed at the PRS field site in both years; 20 cm high beds were formed at the CCRS field site both years, at both sites the rows were 1.5 m wide. PRS remained non-fumigated in both years, while CCRS was fumigated with Pic-Clor 60 (shank application, 1,400 L/ha, TriEst, Rocky Mount, NC) on 14 Sept. 2019 and 10 Sept. 2020 respectively. At both locations in both years, one line of drip tape (30 cm perforation, TriEst, Rocky Mount, NC) and plastic mulch (VIF Film, TriEst, Rocky Mount, NC) was laid. Before planting, holes were punched into the plastic and soil using a modified waterwheel at 35 cm distance on the day of planting. *Fragaria x ananassa* ‘Camarosa’ plugs were planted as plug plants in double rows at the dates mentioned above (Table 2.1)

Spring fertilization began on 19 March 2020 and 23 March 2021 at PRS and 30 March 2020 and 22 March 2021 at CCRS. Spring fertilization was applied at a rate of 17 kg/ha/week of 13.6-0-46 N-P-K at PRS and 12.53 kg N/ha/week at CCRS.

Soil Sampling, Processing, and Nitrogen Extraction

To assess the concentrations of soil N in different bed depths, 25.4 cm cores were taken every 4-6 weeks after planting until the first bloom, at both field sites in both years. Three cores per replicate were split in 0-12.7 cm depth and 12.7-25.4 cm depth and combined. Soil samples were stored in plastic containers (Fisher Scientific, Hampton, NH) and kept at -20 ◦C freezer (Frigidaire, Charlotte, NC) to keep N forms stable until samples were able to be processed according to the procedures describe below.

To assess the distribution of N in the soil, frozen soil samples were pushed through a sieve (mesh size of 25 for PRS, 10 for CCRS, Fisher Scientific, Hampton, NH) and weighed to five grams and placed in a sealable container (Fisher, Hampton, NH) with 50 mL of one molarity potassium chloride (1 M KCl) (Sigma Aldrich, St. Louis, MO). Containers of the KCl-soil mixture were placed on a shaker table (New Brunswick Scientific Co., G10 Gyrotory Shaker, Edison, NJ, customized) for one hour. Samples were then removed and filtered using Grade 1 qualitative filter paper (Whatman, Maidstone, UK) through a funnel to fill a specimen jar (Fischer, Hampton, NH). Samples were immediately cooled at +4 ˚C until they were transported to the North Carolina State University (NC State) Environmental and Agricultural Testing Services (EATS). At EATS samples were analyzed for N on the flow injection system Latchat (Hach Co. Quickchem 8500 series 2, Loveland, CO).

Samples were injected into the Latchat QuikChem Flow Injection Analysis system (Lachat Instruments, Milwaukee, Wisconsin) and divided by two modules within the system, to measure NH_4^+ and NO_3^- separately. The $NH4^+$ module mixed injected samples with NaOH/EDTA/Na2HPO4-7H2O and sodium nitroprusside/salicylic acid, a sodium salt color

reagent, the mixture was then heated. The color change of the sample was compared by the Latchat system to standards.

Simultaneously, the $NO3^-$ is analyzed in a second module of the Latchat system which contains a copperized cadmium column that samples flow through as NO_3^- in the sample is reduced to nitrite. The nitrite is then determined by diazotizing with sulfanilamide, then by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water-soluble dye has a magenta color that is read at 520 nm. Both NO_3^- and NH_4^+ levels were assessed in the resulting samples by comparing them to a standard curve and calculated from a regression equation.

Soil wet and dry weights were used to calculate the soil moisture factor for each sample, by dividing the wet weight by the dry weight (Maynard et al., 2006). To assess dry weight, soil samples were placed in a drying oven (Fisher Scientific Isotemp Incubator Model 630D, Hampton, NH) at 40 °C for 24 hours. After 24 hours the samples were taken out of the oven and dry weights were recorded (Mettler-Toledo, PL6001E Classic Light Portable Toploading Balance, Columbus, OH).

Tissue Nutrient Sampling

Strawberry petioles were sampled by collecting the youngest most mature leaf of the plant. Thirty samples were collected as one combined sample among the four replicates of each treatment. Petioles were separated from the leaves and both plant organs were sent to the NCDA&CS for analysis. Plant tissue samples were collected twice at both sites in 2019-2020 and four times in 2020-2021 (Table 2.1).

Strawberry Yield

Marketable and nonmarketable yield were assessed, as well as average fruit weight. Harvest took place at PRS from 17 April 2020 to 22 May 2020 and from 26 April 2021 to 1 June 2021, for 12 and 11 days, respectively. Harvest took place at CCRS from 19 April 2021 to 27 May 2021, for 11 days. Due to university-wide restrictions during the pandemic of COVID-19, in the spring of 2020 strawberry yields could not be assessed at CCRS. Yields were measured by marketable yield and nonmarketable yield (Mettler-Toledo, SB32000 Hi Cap Scale, Columbus, OH). Fruit marketability was judged based on appearance, with imperfections such as heavy deformation, disease symptoms, small size, and water damage all counted as non-marketable.

Average fruit weight was measured by collecting 25 strawberry fruit samples on 3 May 2021, 17 May 2021, and 24 May 2021 at PRS as well as 29 May 2021, 13 May 2021, 20 May 2021, and 24 May 2021 at CCRS. Fruit samples were only collected from the harvest of marketable fruits. Fruit weights were not collected for the 2019-2020 season at either site due to university-wide early pandemic restrictions.

Fruit Chemistry Analysis

Strawberry fruit samples were taken on 3 May 2021, 17 May 2021, and 24 May 2021 at PRS and 29 April 2021, 13 May 2021, and 20 May 2021 at CCRS. Each sample contained 10 strawberry fruit. After sampling, fruit were stored at -20 ◦C freezer (Frigidaire, Charlotte, NC) until chemistry analysis. Fruit chemistry analysis measured the samples' pH, total soluble solids, and acidity.

To perform laboratory analyses, samples were thawed at room temperature. After thawing, samples were then crushed by hand and filtered (Whatman, Maidstone, UK). Filtered juice was tested for pH using a pH/conductivity meter (Apera, PC800, Columbus, OH). Total soluble solids were then tested with a brix-acidity meter (Atago, Pocket Brix-Acidity Meter,

Tokyo, Japan). To assess acidity, samples were diluted at a 1:50 solution (w/w) with deionized water and measured with a brix-acidity meter (Atago, Pocket Brix-Acidity Meter, Tokyo, Japan). The brix-acidity meter was set on standard curve 4 (strawberry).

Statistical Analysis

R studio version 1.4.1103 was utilized to analyze all data. Graphs were made in MS Excel (MS Office, Microsoft, Redmond, WA). Yield, berry weight, and berry chemistry data were analyzed with a two-way ANOVA (alpha = 0.05, SS type I model) by treatment and date. Then, to analyze the ANOVA tests results a Fisher LSD test (alpha = 0.05; agricolae package, de Mendiburu, 2021) was performed. Soil N content (NH_4^+, NO_3^-, and total N) was analyzed by multi-way ANOVA (alpha = 0.05, SS type I model) by date, depth, and treatment. The Grubbs and the Dixon outlier tests were performed on the data for PRS 2020-2021 soil N concentration, 27 samples were identified as outliers. All samples identified as outliers were tested and analyzed again using the methods listed above (section: Soil Sample Processing and Nitrogen Extraction). All samples came back with similar results and were kept in the data set.

Results

Soil Nitrogen Concentration

At PRS in 2019-2020 at the depth of 0-12.7 cm, overall total N was statistically higher in Medium Polysulphate, Grower Standard, and Half-Grower Standard. However, Grower Standard and Half-Grower Standard were statistically similar to Low Polysulphate and High Polysulphate. NTC was the lowest in overall total N but statistically similar to Low Polysulphate and High Polysulphate (Figure 2.2). At PRS in 2019-2020 at the depth of 12.7-25.4 cm there was no significant difference in overall total N between any treatment (Figure 2.2).

At PRS in 2020-2021 at the depth of 0-12.7 cm, overall total N was statistically higher in Grower Standard and Low Polysulphate. The statistically lowest overall total N was NTC. Medium Polysulphate and High Polysulphate were statistically similar to Half-Grower Standard, which was also statistically similar to Low Polysulphate (Figure 2.3). At PRS in 2020-2021 at the depth of 12.7-25.4 cm, overall total N was statistically higher in Grower Standard, Half-Grower Standard, and Medium Polysulphate. NTC had the lowest overall total N but was statistically similar to Low Polysulphate, High Polysulphate, and Medium Polysulphate (Figure 2.3).

At CCRS in 2019-2020 at the depth 0-12.7 cm overall total N was highest in High N. All other treatments were statistically similar in overall total N (Figure 2.4). At CCRS in 2019-2020 at the depth 12.7-25.4 cm overall total N was highest in High N and Grower Standard. NTC, Low Polysulphate, High Polysulphate, and Medium Polysulphate were significantly lower, however Grower Standard and Medium Polysulphate were statistically similar (Figure 2.4).

At CCRS in 2020-2021 at the depth 0-12.7 cm overall total N highest was in High N, all other treatments were lower and statistically similar (Figure 2.5). The overall total N at CCRS in 2020-2021 at the depth 12.7-25.4 cm was highest in High N and was lowest in NTC, High Polysulphate, Medium Polysulphate, and Low Polysulphate (Figure 2.5). In both years at CCRS N declined steadily and faster than N movement at PRS.

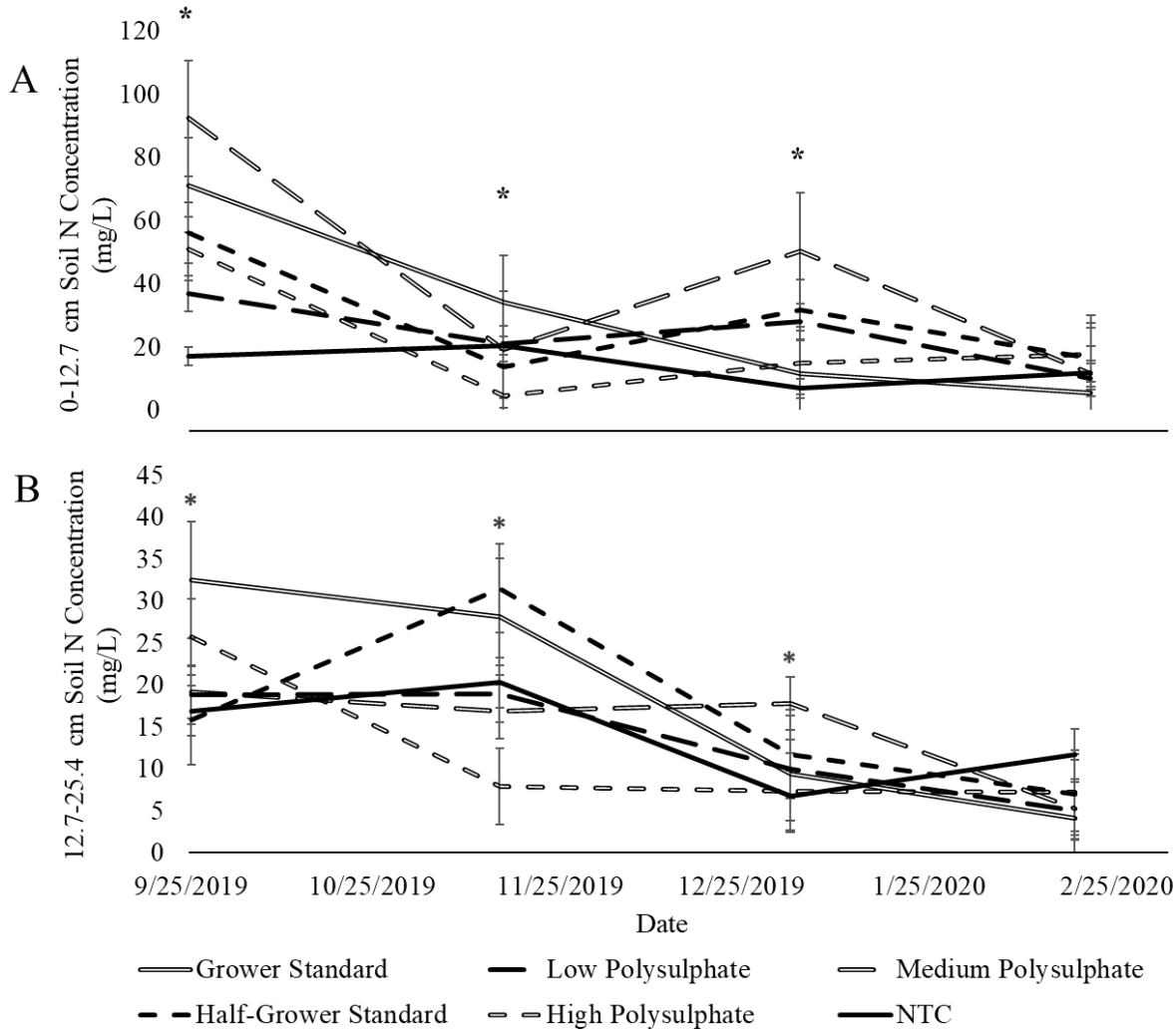

Figure 2.2. PRS total average N in 2019-2020 at A) the depth 0-12.7 cm and B) the depth 12.7-25.4 cm. Error bars represent standard error of the mean. The symbol '*' represents a statistical difference between treatments on that date.

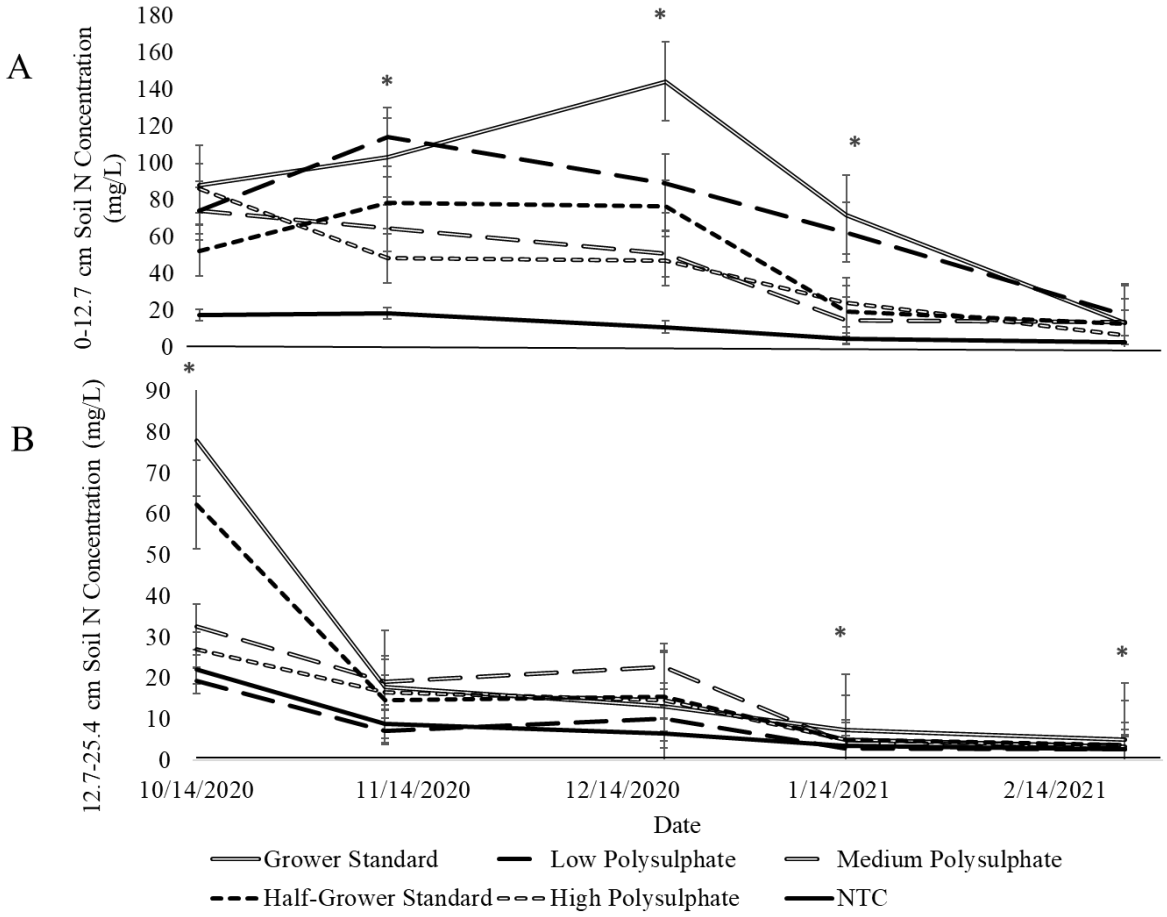

Figure 2.3. PRS total average N in 2020-2021 at A) the depth 0-12.7 cm and B) the depth 12.7-25.4 cm. Error bars represent standard error of the mean. The symbol '*' represents a statistical difference between treatments on that date.

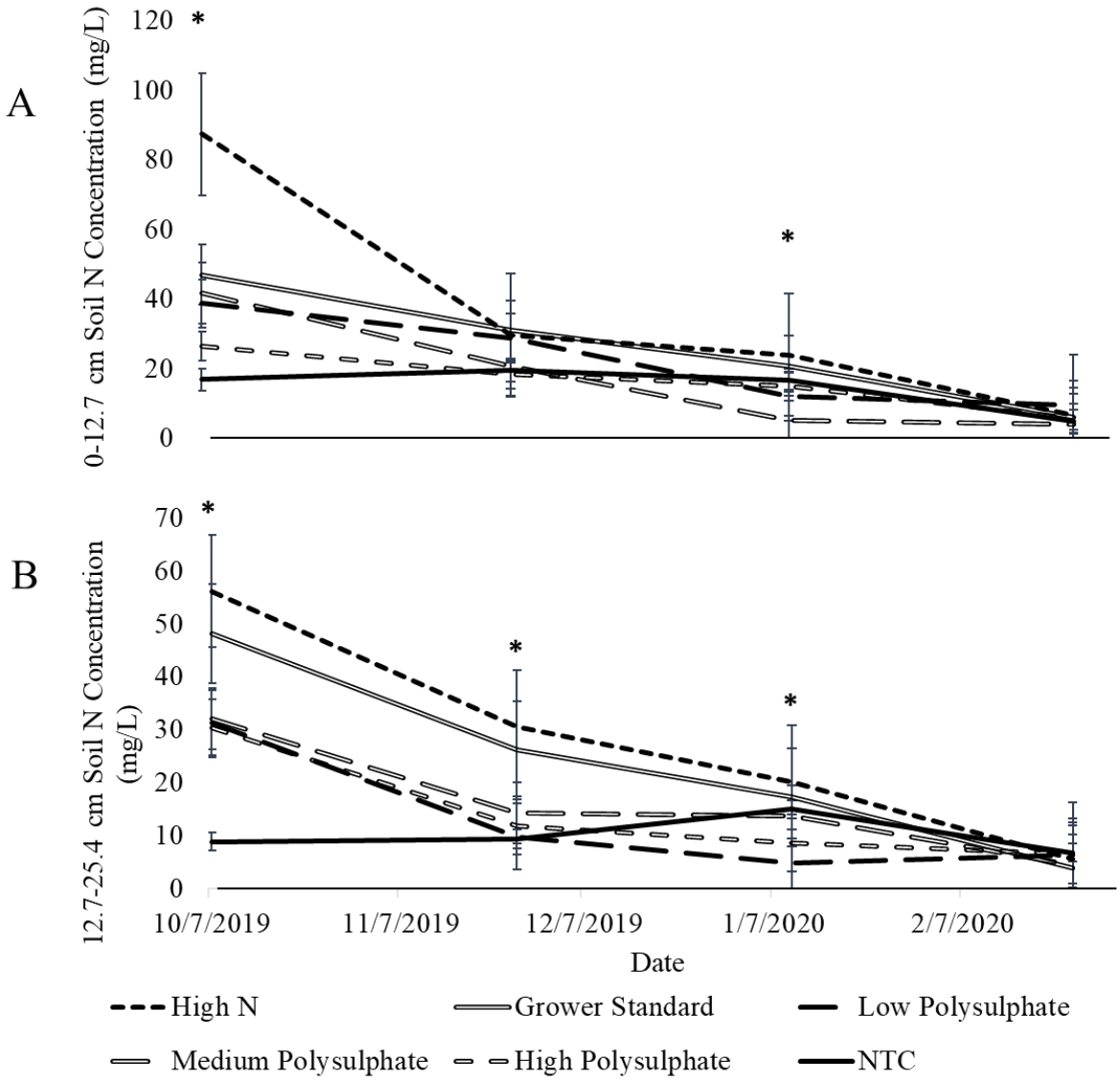

Figure 2.4. CCRS total average N in 2019-2020 at A) the depth 0-12.7 cm and B) the depth 12.7-25.4 cm. Error bars represent standard error of the mean. The symbol '*' represents a statistical difference between treatments on that date.

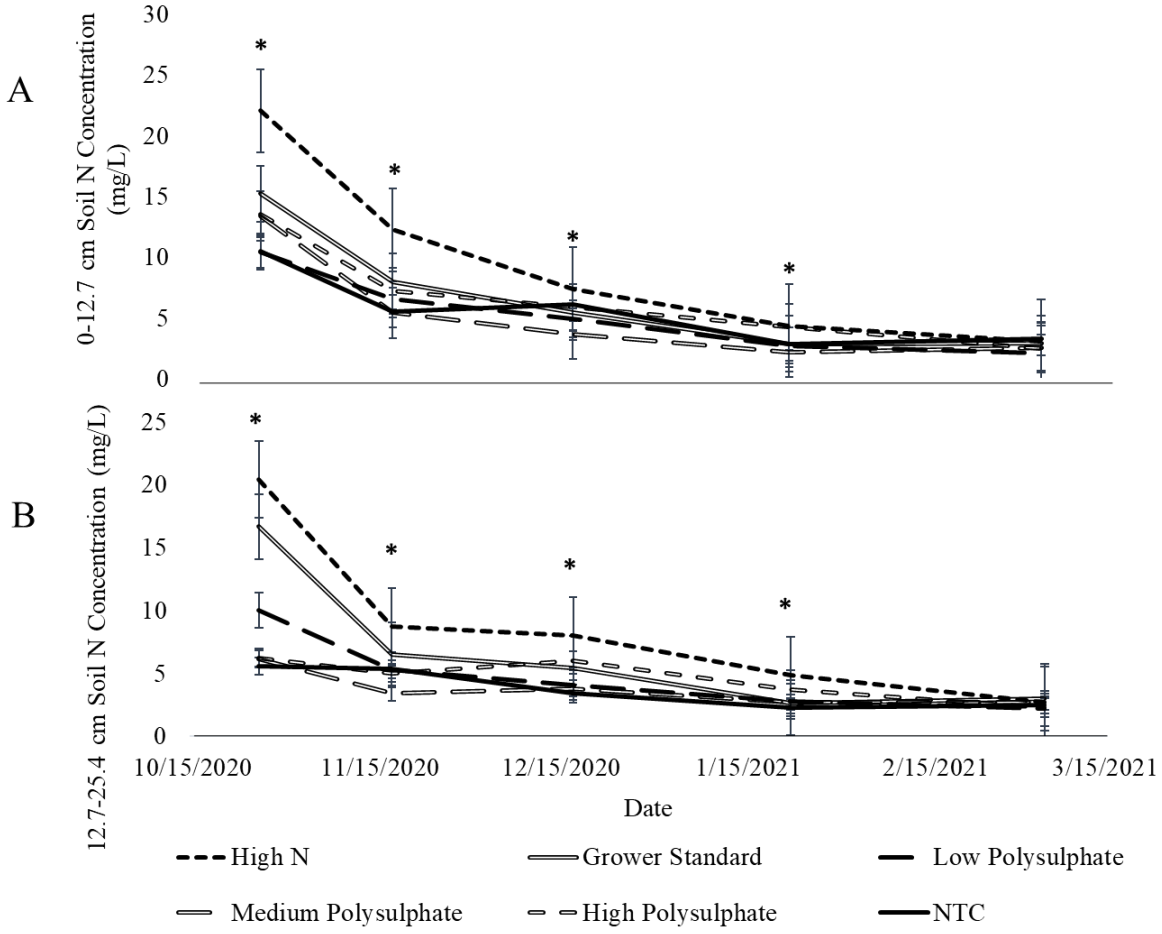

Figure 2.5. CCRS total average N in 2020-2021 at A) the depth 0-12.7 cm and B) the depth 12.7-25.4 cm. Error bars represent standard error of the mean. The symbol '*' represents a statistical difference between treatments on that date.

Petiole Nutrient Concentrations

During the overall samples at PRS 2019-2020 Medium Polysulphate and High Polysulphate had the highest N concentration of the petiole samples however were not significantly different to all other treatments except NTC. At PRS in 2020-2021 for the overall N concentration of samples, there were no statistical differences in N concentration of the petiole samples taken.

At CCRS in 2019-2020 there were no statistically significant differences in overall N concentration between treatments for the field season. At CCRS in 2020-2021 there were no statistically significant differences between any treatment in the overall season. Further data on petiole nutrient concentration (K, P, Ca, Mg, and S) can be found in Appendix N.

Table 2.4. Piedmont Research Station (PRS) 2019-2020 N concentration (ppm) of petiole samples (n=1). No petiole samples were collected during the harvest stage of the season due to university wide pandemic restrictions.

	Petiole N Content (ppm)	
Treatments	**15 November 2019**	**18 February 2020**
Grower Standard	3.05	2.54
Low Polysulphate	3.38	2.57
Medium Polysulphate	3.35	2.81
High Polysulphate	3.51	2.81
Half-Grower Standard	3.10	2.82
NTC	3.07	2.25

Table 2.5. Piedmont Research Station (PRS) 2020-2021 N concentration of petiole samples (ppm) (n=1).

	Petiole N Content (ppm)			
Treatments	**10 December 2020**	**24 February 2021**	**3 May 2021**	**25 May 21**
Grower Standard	3.20	2.54	1.99	1.83
Low Polysulphate	3.39	2.81	2.00	2.16
Medium Polysulphate	3.16	2.57	1.81	1.91
High Polysulphate	3.23	2.81	2.02	2.09
Half-Grower Standard	3.31	2.82	2.07	2.12
NTC	3.40	2.25	2.30	2.25

Table 2.6. Central Crops Research Station (CCRS) 2019-2020 N concentration of petiole samples (ppm) (n=1). NTC sample was not collected on 26 November 2019 due to insufficient plant sizes. No petiole samples were collected during the harvest stage of the season due to university wide pandemic restrictions.

	Petiole N Content (ppm)	
Treatments	**26 November 2019**	**25 February 2020**
Grower Standard	3.45	2.90
Low Polysulphate	3.68	2.63
Medium Polysulphate	3.70	2.71
High Polysulphate	3.77	2.95
High N	3.77	2.98
NTC	-	2.73

Table 2.7. Central Crops Research Station (CCRS) 2020-2021 N concentration of petiole samples (ppm) (n=1).

	Petiole N Content (ppm)			
Treatments	**17 December 2020**	**5 March 2021**	**29 April 21**	**21 May 2021**
Grower Standard	2.74	3.39	3.12	2.25
Low Polysulphate	2.90	3.27	3.09	2.15
Medium Polysulphate	3.02	2.84	3.31	2.09
High Polysulphate	3.08	3.79	2.95	2.26
High N	2.96	3.64	3.10	2.23
NTC	3.01	3.49	3.17	2.47

Yield and Berry Quality

In 2019-2020 at PRS, cumulative marketable yield was significantly higher in all treatments, compared to treatment NTC (920.58 ± 1.87 g/plant). At PRS, cumulative total yield was significantly higher in all treatments compared to treatment NTC (1135.55 ± 1.61 g/plant). (Figure 2.6). Due to university-wide pandemic restrictions, there were no data collected for average fruit weight, pH, acidity, and soluble solids during the 2019-2020 season.

The cumulative marketable yield of 2020-2021 at PRS was overall lower than the 2019-2020 PRS cumulative yields. Grower Standard, Medium Polysulphate, and High Polysulphate had significantly less cumulative marketable yield than all other treatments. Half-Grower Standard and Low Polysulphate had the highest cumulative yield in 2020-2021 at PRS (Figure 2.6, Figure 2.7). Average fruit weight at PRS in 2020-2021 was highest in Half-Grower Standard and NTC (Table 2.8). In 2020-2021 at PRS there was no statistical difference in the average acidity or pH of fruits (Table 2.9). The average soluble solids in 2020-2021 at PRS was higher in

all treatments except NTC, however NTC was statistically similar to Grower Standard, Low Polysulphate, and Half-Grower Standard (Table 2.9).

The cumulative marketable yield at CCRS in 2020-2021 was highest in Medium Polysulphate, Low Polysulphate, NTC, and Grower Standard. High N and High Polysulphate had the lowest cumulative marketable yield of all treatments (Figure 2.6, Figure 2.7). In 2020-2021 at CCRS Grower Standard was highest and yet statistically similar to High N, Low Polysulphate, High Polysulphate. Medium Polysulphate was the lowest average fruit weight treatment, but statistically similar to NTC, High N, and Low Polysulphate (Table 2.8). In 2020-2021 at CCRS the average soluble solids of fruits were highest in High N but statistically similar to all treatments except for the NTC. NTC was the lowest in average soluble solids of fruits but statistically similar to all treatments except High N (Table 2.10). The average fruit acidity in 2020-2021 at CCRS was statistically higher in High N, High Polysulphate, and Growers Standard. The average fruit acidity was statistically lower in Low Polysulphate, Medium Polysulphate, and NTC, however Grower Standard was statistically similar to Low Polysulphate and Medium Polysulphate (Table 2.10). In 2020-2021 at CCRS the average pH was statistically higher in High Polysulphate, Grower Standard, and High N. The average pH was statistically lower in Medium Polysulphate, NTC, and Low Polysulphate, however Grower Standard and High N were statistically similar to Low Polysulphate (Table 2.10).

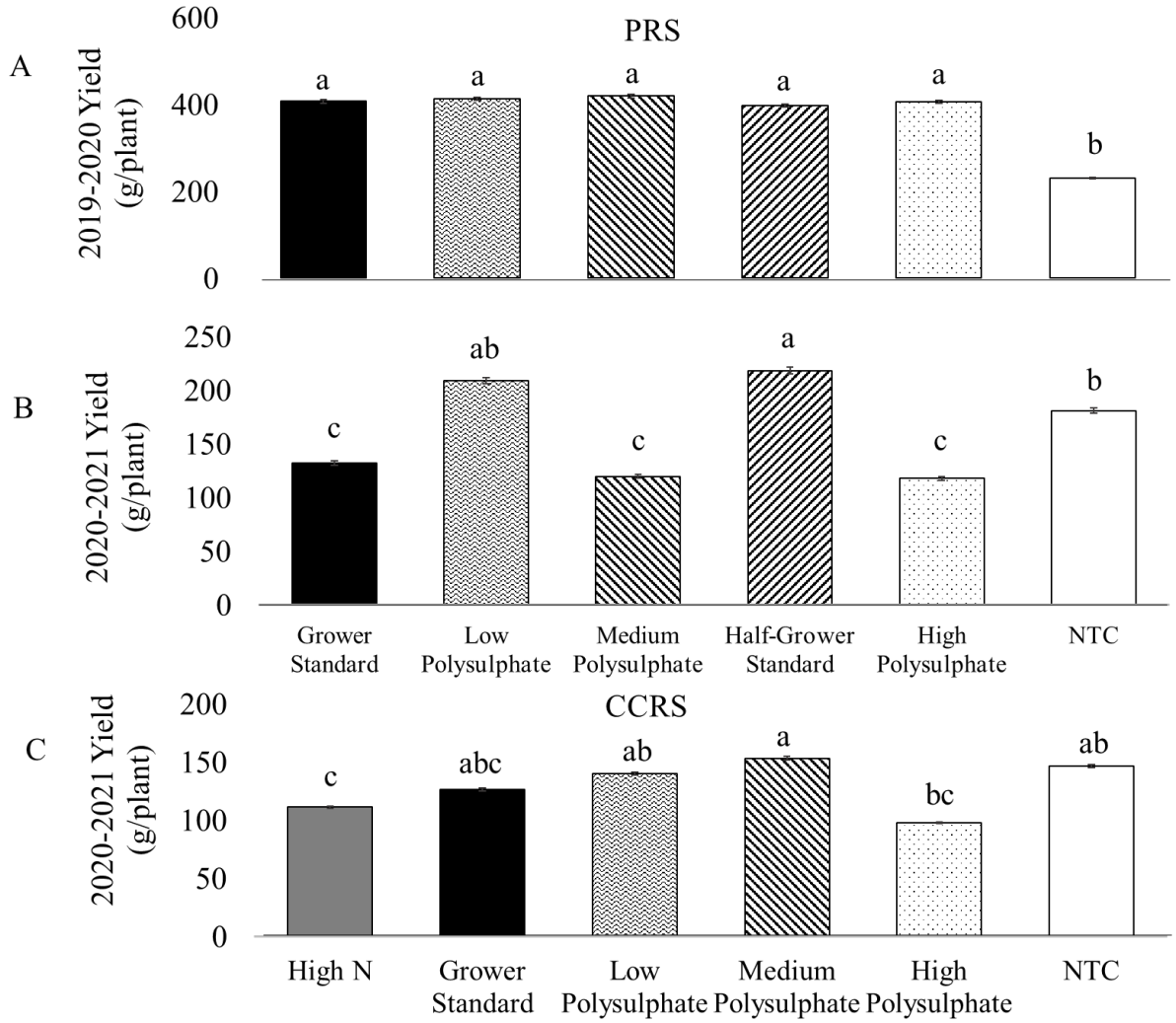

Figure 2.6. Cumulative yield at PRS 2019-2020, PRS 2020-2021, and CCRS 2020-2021. Treatments shown in order of decreasing N concentrations. Standard error of the means shown in lines on the top of each bar. Letters above the bar express statistical difference between treatments.

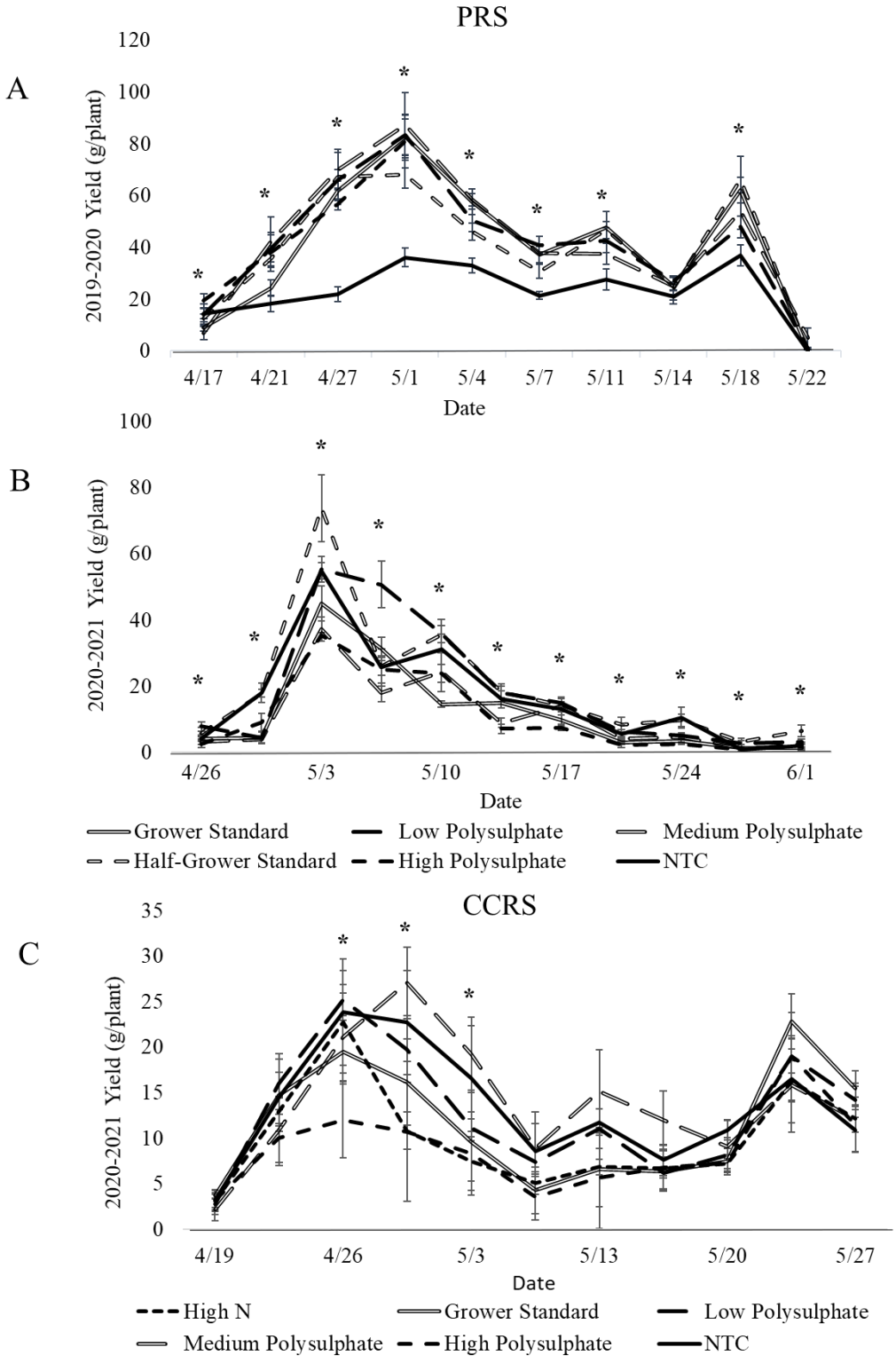

Figure 2.7. Average marketable yield curves for the years harvested at PRS and CCRS. Bars represent the standard error of the mean. The symbols '*' represent a significant difference between treatments on that date.

Table 2.8. Average fruit weight in PRS and CCRS in 2020-2021. Standard error of the means of fruit weight is shown. Blank spaces are due to some treatments not being applied at both research stations.

Treatment	PRS Average Fruit Weight (g)	CCRS Average Fruit Weight (g)
High N	-	20.11 ± 1.67 abc
Grower Standard	13.51 ± 2.15 c	22.05 ± 1.46 a
Low Polysulphate	13.95 ± 2.35bc	20.20 ± 0.61 abc
Medium Polysulphate	13.13 ± 2.52 c	18.78 ± 1.07 c
Half-Grower Standard	15.87 ± 2.61 a	-
High Polysulphate	12.72 ± 1.71 c	21.16 ± 0.33 ab
NTC	15.14 ± 2.41 ab	19.40 ± 1.03 bc

Table 2.9. PRS 2020-2021 strawberry fruit sample chemistry: average total soluble solids, average acidity, and average pH. Standard error of the means for each variable shown.

Treatments	Average Total Soluble Solids	Average Acidity	Average pH
Grower Standard	6.71 ± 0.31 ab	0.33 ± 0.02	3.78 ± 0.03
Low Polysulphate	6.76 ± 0.47 ab	0.33 ± 0.01	3.81 ± 0.03
Medium Polysulphate	6.45 ± 0.43 a	0.33 ± 0.03	3.82 ± 0.02
Half-Grower Standard	6.49 ± 0.41 ab	0.34 ± 0.2	3.79 ± 0.03
High Polysulphate	6.69 ± 0.53 a	0.33 ± 0.03	3.76 ± 0.05
NTC	6.42 ± 0.30 b	0.33 ± 0.02	3.79 ± 0.03

Table 2.10. CCRS 2020-20221 strawberry fruit sample chemistry: total soluble solids, acidity, and pH. Standard error of the means shown.

Treatments	Average Total Soluble Solids	Average Acidity	Average pH
High N	6.93 ± 0.37 a	0.4 ± 0.03 a	3.67 ± 0.03 ab
Grower Standard	6.49 ± 0.4 ab	0.35 ± 0.03 ab	3.67 ± 0.04 ab
Low Polysulphate	6.88 ± 0.48 ab	0.33 ± 0.03 bc	3.62 ± 0.02 bc
Medium Polysulphate	6.88 ± 0.32 ab	0.29 ± 0.02 bc	3.57 ± 0.03 c
High Polysulphate	6.73 ± 0.5 ab	0.4 ± 0.03 a	3.71 ± 0.03 a
NTC	6.26 ± 0.47 b	0.26 ± 0.02 c	3.59 ± 0.02 c

Discussion

Impact of Fall Applied Pre-Plant Polysulphate Co-applied with Different Rates of Granular Fertilizer on Strawberry Yield

The objectives of this study were to assess the N distribution in raised beds under six different fall applied pre-plant fertilizer rates during transplanting and dormancy, and to investigate the impact of six different fall applied pre-plant fertilizer rates which were co-applied with Polysulphate on yield and fruit quality of strawberry. The main finding of this study was that fall applied pre-plant fertilizer co-applied with Polysulphate can lower N concentration and distribution in the soil compared to conventional standards, however fertilizer rates co-applied with Polysulphate did not show a consistent improvement in yield or fruit quality compared to conventional pre-plant fertilizer rates.

Miner et al. (1997) investigated the effects of pre-plant N and spring drip N application rates on strawberry (*Fragaria* x *ananassa* 'Chandler') on yield and quality, specifically to find the optimum rate of N for strawberries grown in NC. Miner et. al. (1997) set up a factorial experimental design in 1992 and 1993, at the CCRS in NC, with varying rates of preplant N (0, 34, and 67 kg/ha) in the form of NH_4NO_3, which were similar to this study's pre-plant N rates NTC, Half-Grower Standard, and Grower Standard treatments. As well as varying rates of N for liquid spring fertilization (1993: 0, 0.19, 0.37, 0.56, and 0.75 kg/ha/d; 1992: 0, 0.37, 0.75, and 1.12 kg/ha/d). Miner et. al (1997) found that the optimal rate of preplant N for NC to optimize yield was 34 kg/ha. This is similar to treatment Half-Grower Standard in this study. In 2019-2020 and 2020-2021 Half-Grower Standard had significantly higher yield than NTC. Results from our study support the conclusion of Miner et. al. (1997) that an optimum N pre-plant fertilizer rate may be closer to 34 kg/ha versus the current grower recommendation of 67 kg/ha of N in pre-plant fertilizer (Hicks et al., 2015).

Miner et al. (1997) also observed that marketable strawberry yield increased with increasing N rates fitting a linear plateau. Non-marketable (cull) yield increased linearly with pre-plant N rate in 1993 but was not affected in 1992. In 1992 average fruit weight was significantly increased by pre-plant N, however in 1993 average fruit weight was not affected by pre-plant N. In our study, treatment Half-Grower Standard at PRS (2020-2021) had the highest average fruit weight among treatments. The results of the 2020-2021 PRS and CCRS support Miner et. al. (1997) data from 1993 as both had an overall fruit size that did not increase with increasing pre-plant N.

Polysulphate contains 48% sulfur (S), 14% potassium (K), 6% magnesium (Mg), and 17% calcium (Ca). Those elements are essential for fruit development and fruit quality in plants and strawberries (Hicks et al., 2015). By combining Polysulphate with different rates of 6-6-18, a commonly used pre-plant fertilizer in NC, we were able to maintain a consistent rate of pre-plant potassium. Potassium is important for the quality of strawberry fruit because insufficient potassium can decrease fruit firmness (Miner et al., 1997).

We observed a significant difference in yield between NTC and all other treatments in 2019-2020 at PRS, but no significant impact of Polysulphate on strawberry yield or fruit quality. In 2020-2021 at both field sites, yield results were not as clear due to overall low yield. The low yields on both sites in 2020-2021 may have been caused by a combination of late planting date, suboptimal weather conditions and extraordinarily poor conditions of the received transplant material. Fall applied Polysulphate in combination with lower rates of conventional granular fertilizer did not lead to increased yields or fruit quality in ‘Camarosa’. The lack of consistent yield increase may be due to the varying rates of other nutrients (calcium, sulfur, magnesium, and phosphorus) applied in the trials (Table 2.3).

In current conventional industry production, it is expected for plants to produce roughly 544 g per plant (Poling, 2015), however the 2020-2021 yields at both locations never surpassed a cumulative yield of 250 g/plant for any treatment. Despite these low yields, the overall trend of our results is similar to the findings of Bottoms et al. (2013), who developed optimum Diagnosis and Recommendation Integrated System (DRIS) leaf blade and petiole nutrient ranges for the California strawberry industry. The DRIS approach is an analysis of the differences in foliar nutrient concentrations and nutrient ratios between high-yielding and low-yielding fields to find the degree in which various nutrients may limit yield, either in excess or deficiency (Bottoms et. al., 2013). Bottoms et. al. (2013) observed that fields ranged from ~2 to 8.1 hectacres (~5 to 20 acres). Total seasonal N fertilizer rates ranged from 132.3-475.2 kg/ha (118-424 lbs/a) with a median N rate of 229.8 kg/ha (205 lbs/a). Bottoms et. al. (2013) collected soil samples, petiole samples, and yield data in the field of 53 commercial strawberry (*Fragaria* x *ananassa*, 'Albion') farms in the coastal valleys of central California in the years 2010 and 2011.

Bottoms et al. (2013) found that N fertilizer application rates (pre-plant and seasonal) did not correlate with fruit yield, this is under Bottoms et al (2013) assumption that pre-plant makes up half of all N applied. From this it can be inferred that the impact on yield by rate of N can be limited by other nutrient and environmental needs of strawberry plants. In our fields we have two sites that had lesser quality planting material that may have impacted yield, however further research will be required to investigate the limitations on yield caused by quality of planting material. At the same time, the wide variation of macronutrient rates, especially in secondary macronutrients, in our experiment could have caused deficiencies in some treatments leading to the significant differences in yield and fruit quality. Bottoms et al. (2013) concluded that blade

optimum ranges were lower than previously published for N and zinc needs, while other nutrients needs were similar to previously published sufficiency ranges.

Bottoms et al. (2013) varied in production methodology compared to our study because California, strawberry harvest lasts 7-8 months longer than the 6-8 weeks that are common for NC. Also, the cultivar studied in Bottoms et al., Albion, is an ever-bearing cultivar which requires higher N compared to the June-bearing cultivar, Camarosa, used in our field experiments (Galletta and Bringhurst, 1990). Despite these differences, we can still relate similar findings, such as essential nutrients were within a sufficient range in plant tissue to support plant growth. However, to establish the optimum ranges of nutrients (K, P, Ca, Mg, and S) applied as pre-plant fertilizer in NC will take further research.

Nitrogen Concentrations in Soil and Plant Tissues after Pre-Plant Fertilizer Application

Soil nitrogen levels shortly after pre-plant application were highest in those treatments with the highest rates of 6-6-18 in both locations and in both years. At both field sites in both years, the total N concentration in the soil at 12.7-25.4 cm depth was at least half of, if not equivalent to, the 0-12.7 cm depth at the time of planting for the treatments Growers Standard and High N. At CCRS in both years, total N concentration in the soil declined steadily, and faster than total N concentration at PRS. By January (3 months after planting) in both years, at CCRS we measured half the total N concentration in the soil than was measured at planting at both depths. By January (3 months after planting) in both years, at PRS there was not as consistent a trend in total N concentration in the soil. These variations between sites could partially be explained by CCRS being fumigated at the start of the season while PRS was never fumigated. Conventional fumigation significantly reduces soil microbial populations (McWhirt et al., 2015),

many soil microbes, such as *Azobacter*, impact N and other nutrient concentrations in the soil (Ricklefs and Relyea, 2014).

At CCRS in 2019-2020 and 2020-2021, N decreased rapidly at both depths; N soil concentration declined significantly within 3 months of planting. At PRS in 2019-2020 and 2020-2021 N soil concentration declined gradually compared to CCRS, and even had a slight increase in soil N concentration in the 0-12.7 cm range in mid-December to early January. These differences in N concentration distribution could be due to the different soil types' cation exchange capacity (CEC), because PRS had a higher CEC with clay loam soils, compared to CCRS. CEC is the degree to which soils adsorb and exchange cation, a soil with higher CEC generally has a larger reserve of positively charged mineral nutrients (Taiz and Zieger, 2006). Another possible reason for the difference in N concentration over time at the two sites could be that soils at CCRS were fumigated while soils at PRS were not. This means that PRS had more biological activity during the overall season, while CCRS had to rebuild the population of the soil microorganisms.

At the time of planting, soil N concentration was high at the 0-12.7 cm depth for both years and locations. At CCRS High N and Grower Standard consistently had high concentrations of N at the depth of 12.7-25.4 cm on the day of planting, however roots would not be able to reach N in the 12.7-25.4 cm range at the time of planting. Similarly, at PRS, Grower Standard had high levels of soil N concentration at the depth of 12.7-25.4 cm on the day of planting. All treatments at CCRS and PRS for both years, were statistically similar at both depths by late-February, nearly a month before liquid fertilization began to be applied.

In all field experiments at PRS and CCRS over two years, the petiole N concentration during plant establishment (October – March) did not correlate to soil N concentrations. Bottoms

et.al. (2013) as well as Niskanen and Dris (2002) support those findings. Niskanen and Dris (2002) investigated the nutritional status of strawberry fields and the relationship between soil testing values and leaf nutrient content. Soil and leaf nutrient samples were taken in 338 commercial strawberry fields from 1984-1991. Niskanen and Dris (2002) found that leaf nutrient concentration was not correlated to soil nutrient concentration.

Conclusion

We could not validate our second hypothesis that fall-applied Polysulphate in combination with different rates of granular fertilizer leads to higher yields or fruit quality in 'Camarosa'. However, our results suggest that current recommended pre-plant fertilizer rates are higher than necessary, confirming earlier studies from North Carolina and California.

We could show that fall-applied pre-plant Polysulphate in combination with lower granular fertilizer rates lead to lower N levels in soil, yet to similar yields. N levels decreased rapidly after pre-plant fertilizer application, especially in sandy soil, and are most likely not plant available after a short period of time. We therefore conclude that conventional pre-plant fertilizers can be partially substituted with Polysulphate without loss of yield or fruit quality, but with the benefit of reducing potential environmental N pollution. However, further studies are necessary to evaluate the impact of Polysulphate as source of potassium and sulfur on strawberry plant establishment, yield, and quality.

Chapter 3: Impact of Nitrogen Dose on performance of 'Chandler' Plug Plants in a Soilless Greenhouse System

Introduction

Most plants have developed mechanisms to take up N as NH_4^+ and NO_3^- from the soil, and both N sources are important for plant growth (Cárdenas-Navarro et al., 2006). NO_3^- acts as an osmoticum for plant cells, and when rates of absorption decrease, plant growth can be stunted. When applied together, NH_4^+ is generally taken up by the plant in greater quantities than NO_3^- (Cárdenas-Navarro et al., 2006). Rates of 67 kg/ha (60 lbs/ac) of N are recommended to support strawberry growth during the establishment portion of the season and are applied by growers during the pre-plant phase.

Strawberry plants require N after transplanting to support the plants as they establish in the soil. After planting, strawberry transplants will respond to temperature and photoperiod by first establishing root mass, leaves and crowns. Root mass is established within the first two to three months after planting. The first months of plant establishment after transplanting are important in NC strawberry production because this time allows for strawberry plants to gain vegetative mass. These months are usually October, November, and December. Nitrogen availability can play a major role in achieving a good plant stand at the end of the year (Galletta and Bringhurst, 1990).

Several strawberry cultivars are commonly used in NC strawberry production. 'Ruby June', 'Camarosa', 'Chandler', and 'Sweet Charlie' are the most common cultivars grown in NC. While 'Ruby June' and 'Camarosa' have earlier planting date recommendation, due to their higher requirement of growing degreed days (GDDs) during plant establishment, 'Chandler' and 'Sweet Charlie' are often planted five to seven days later (Poling, 2015). 'Chandler' is more

often grown for pick your own production while ‘Camarosa’ is more commonly grown for pre-picked marketing. Both cultivars ‘Camarosa’ and ‘Chandler’ are short-day strawberries that are recommended for acceptable yield and marketability in the plasticulture system (Poling, 2015).

However, until this study it was unclear what the minimal N needed for strawberry plant establishment was. To investigate this, we developed the hypothesis that strawberry plants do utilize N at an optimal rate during the first weeks of plant establishment. The objective of this study was to conduct a dose response study and evaluate the impact of four rates of pre-applied N fertilizer on strawberry growth in a soilless container system.

Materials and Methods

Experimental Design

To investigate the impact of different rates of nitrogen (N) on strawberry plant growth and physiology, four fertilizer treatments were established in a soil-less container greenhouse experiment. Our study was a dose-response study with a 9.50 mmol/L N as the highest N treatment. High (9.50 mmol/L) was calculated to be similar to the per plant N dose of 67 kg/ha recommended to be applied in the field by converting kg/ha to g/L. Fertilizers used were 6-6-18 N-P-K (Dixie Farm Tested Fertilizers, New Bern, NC) and $NH_4^+NO_3^-$ (Fisher Scientific, Hampton, NH). Treatments, described in Table 3.1, consisted of: (1) 1.19 mmol/L of N, applied as 0.2778 g/L 6-6-18; (2) 2.36 mmol/L of N, applied as 0.2778 g/L 6-6-18 + 0.047 g/L $NH_4^+NO_3^-$; (3) 4.76 mmol/L of N, applied as 0.2778 g/L 6-6-18 + 0.142 g/L $NH_4^+NO_3^-$; and (4) 9.50 mmol/L of N, applied as 0.2778 g/L 6-6-18 + 0.333 g/L $NH_4^+NO_3^-$ (Table 3.1). Initial primary macronutrient concentrations of the substrate, prior to fertilizer application, were 1.23 ppm for inorganic N, 1.06 ppm for NH_4, 0.17 ppm for NO_3, 4.32 ppm for potassium, and 0.41 ppm for phosphorus. Initial secondary macronutrient concentrations of the substrate, prior to

fertilizer application, were 3.20 ppm for calcium, 0.44 ppm for magnesium, and 1.84 ppm for sulfur.

The experiment was a complete randomized design with 14 plants per treatment. Each plant was grown in a 3.6 liter pot (Gro Pro, Burnsville, MN). The experiment took place in a Haygrove Polyethylene plastic greenhouse (Herefordshire, UK) at the NC State Horticulture Field Laboratory (HFL) in Raleigh, NC (lat. 35°47'31.2"N long. 78°41'55.9"W) from 18 Jan. 2021 – 30 April 2021. Greenhouse temperatures during the winter was controlled by a heater (Nortek Global HVAC, O'Fallon, MO) set to 10˚ C. Plants grew under natural light conditions. All plants were irrigated using an automated irrigation system (Rainbird, Azusa, CA) and in 7.62 cm dripper stakes (Netafim, Fresno, CA) per pot. Plants were watered once per day with 0.31 mL of water per pot per day. No drainage was observed during the experiment. No supplemental nutrients were added after planting. Plants received the same concentration of P, K, Ca, Mg, and S (Table 3.2).

Four hand-built gutters were put on two greenhouse benches. Each gutter was 294.4 cm by 20.3 cm wide and contained 14 3.6-liter pots. Substrate was a 50:50 (w/w) coconut coir perlite, created by combining a 70% coconut 30% perlite mix (Mother Earth, Topeka, KS) and 100% perlite (Good Earth, Lancaster, New York). For the mixing process, substrates were weighed using a field scale (Mettler-Toledo, SB32000 Hi Cap Scale, Columbus, OH). Substrate was mixed in a 0.116 cubic meter portable cement mixer (Northern Tool & Equipment Co., Indianapolis, IN) until the media was uniform. Pots were filled to the top with dry substrate and placed on gutters, then watered by hand for 8 days until substrate was saturated. The weight of fertilizer for each treatment was measured (Mettler-Toledo, PL6001E Classic Light Portable Toploading Balance, Columbus, OH) for individual pots. After substrate was saturated, fertilizer

was mixed into individual pots, according to the treatment. Five days after fertilizer was mixed into the pots, strawberry (*Fragaria* x *ananassa*, 'Chandler') plug plants were planted. Plug plants were selected to have two to three leaves, one crown, and no flowers.

Table 3.1. Fertilizer rate, fertilizer source, and N concentration per treatment

Treatment Name	6-6-18 Fertilizer Rate (g/L)	$NH_4^+NO_3^-$ Fertilizer Rate (g/L)	N Content of Total Fertilizer Rate (mmol/L)
Control (1.19 mmol/L)	0.2778	0	1.19
Low (2.36 mmol/L)	0.2778	0.047	2.36
Medium (4.76 mmol/L)	0.2778	0.142	4.76
High (9.50 mmol/L)	0.2778	0.333	9.50

Table 3.2. Total nutrients applied to pots before planting, nutrients were applied one time at the beginning of the experiment. Units are in ppm.

Treatment	Nitrogen (N)	Potassium (K)	Phosphorous (P)	Calcium (Ca)	Magnesium (Mg)	Sulfur (S)
Control (1.19 mmol/L)	16.668	50.004	16.668	16.668	11.112	22.224
Low (2.36 mmol/L)	63.668	50.004	16.668	16.668	11.112	22.224
Medium (4.76 mmol/L)	158.668	50.004	16.668	16.668	11.112	22.224
High (9.50 mmol/)	349.668	50.004	16.668	16.668	11.112	22.224

Table 3.3. – LogPro EN (Hauto, China) data logger collected temperature and relative humidity (RH) over the course of the experiment. From 18 Jan. 2021 – 30 April 2021 LogPro EN collected temperature and RH every 30 minutes.

Month	Average Temperature (◦C)	Average Relative Humidity (%)
January	14.46 ± 0.652	56.64 ± 0.830
February	15.13 ± 0.517	61.33 ± 0.599
March	18.97 ± 0.459	55.89 ± 0.639
April	23.16 ± 0.694	52.55 ± 0.802

Plant Growth and Physiology

The number of leaves per plant were counted once a week. Leaves were only counted if mature and fully unfolded, regardless of size. Crisp leaves were removed and not counted. The number of flowers per plant were counted and then immediately removed from the plant. The number of crowns per plant were counted and recorded on a weekly basis. Crown diameter per plant was measured once a month with a ruler. Chlorophyll measurements were taken with a chlorophyll concentration meter (Apogee Instruments, Logan, UT), and were collected twice from each plant weekly. The chlorophyll readings were taken from the two most recently matured leaves per plant.

At the end of the trial, on day 102 of the experiment (30 April 2021), the remaining plants (12 plants per treatment) were removed from the greenhouse. Six plants per treatment were used to assess fresh and dry weight of above and below ground biomass. Remaining substrate was gently removed from the root system, using water and a fine brush. Above and below ground biomass were placed in the drying oven at 60° C for two weeks. When plant parts were removed from the drying oven, they were weighed (Mettler-Toledo, PL6001E Classic Light Portable Toploading Balance, Columbus, OH) and dry weight was recorded.

Tissue Nutrient Concentrations

To assess nutrient content in substrate and plant tissues, destructive samples were taken on day 50 of the experiment and again at the end of the experiment (after 102 days). On day 50 of the experiment (9 March 2021), two plants per treatment were removed from their pots, and substrate was poured into individually labelled paper bag. Roots were separated from the rest of plant, then cleaned with de-ionized water and set aside to air-dry before being placed in individually labelled paper bags. Plant crowns were separated from the petiole and placed in labelled paper bags, pooled by treatment. All petioles were removed from the plants, petioles were then separated from their leaves and placed in separate bags by treatment. The North Carolina Department of Agriculture and Consumer Service (NCDA&CS) uses an elemental analyzer to measure N in plant tissue via gas chromatography.

At the end of the trial, on day 102 of the experiment (30 April 2021), the remaining plants (12 plants per treatment) were removed from the greenhouse. Six plants per treatment were used to assess nutrient content of plant tissue and substrate, according to the methods describe above. Plants were taken out of their pots, and roots, petioles, and crowns were separated and placed in labelled paper bags. All petioles were harvested from plants then

separated from their leaves and placed in separate bags by treatment. Crowns had to be groups by treatment to have sufficient material to be analyzed. Roots were placed in bags by individual plant. Substrate was gently removed from the root system and placed into labelled paper bags by individual plant.

Substrate Nutrient Concentrations

On day 50 and day 102 of the experiment (9 March 2021 & 30 April 2021) two and six substrate samples, respectively, were collected from the pots and placed in labelled paper bags. Samples were then submitted to the NDCA&CS for nutrient analysis on 17 March 2021 and 5 May 2021. The NCDA&CS extracts N forms from substrate via saturated media extraction then uses a Skalar (spectrophotometric) test to analyze the sample.

Statistical Analysis

Statistical data was analyzed in R studio version 1.4.1103. Graphs were made in MS Excel (MS Office, Microsoft, Redmond, WA). For the parameters of number of leaves, chlorophyll, number of crowns, crown diameters, and N content of plant organs (petioles, crowns, and roots) and substrate were analyzed by a two-way ANOVA (alpha = 0.05; SS type I model), factors were treatment and date. Then to analyze the ANOVA tests results, a Fisher LSD test (alpha = 0.05; agricolae package, Felipe de Mendiburu (2021).) was performed for each of the aforementioned parameters. For the parameters of above and below ground biomass a one-way ANOVA was used for analysis. To find the statistical differences within the ANOVA (alpha = 0.05; SS type I model), factor tested was treatment; to analyze the results a Fisher LSD (alpha = 0.05) test was performed for each parameter. Linear regression models for number of crowns,

number of leaves, and average chlorophyll were created in MS Excel (MS Office, Microsoft, Redmond, WA).

Results

Plant Growth and Physiology

The average number of leaves were significantly different over the entire experiment between all treatments. High (9.50 mmol/L) treatment had significant more leaves at 6.02 ± 0.563 than Low (2.36 mmol/L) at 5.47 ± 0.44 and Medium (4.76 mmol/L) at 5.61 ± 0.49. All treatments had more leaves than Control (1.19 mmol/L) at 4.86 ± 0.36 (Appendix I). At the end of the experiment there was a positive correlation between number of leaves and increased rate of N (R square = 0.892; Figure 3.1).

The overall average number of crowns was statistically different during the experiment. These differences are due to earlier crown development in High (9.50 mmol/L), compared to all other treatments. Low (2.36 mmol/L) was statistically similar to both Medium (4.76 mmol/L) and Control (1.19 mmol/L), however Medium (4.76 mmol/L) were had a higher number of crowns than Control (1.19 mmol/L) (Appendix J). However, the average number of crowns at the end of the experiment was statistically similar in all treatments (R squared = 0.920; Figure3.2).

There was also no significant difference in the average crown diameter between the four treatments at the end of the experiment (R squared = 0.894; Figure3.3, Appendix K).

Overall average chlorophyll concentration was significantly higher in High (9.50 mmol/L) 19.76 ± 1.79 CCI, compared to Medium (4.76 mmol/L) 17.11 ± 1.92 CCI, Low (2.36 mmol/L) 15.87 ± 1.61 CCI, and Control (1.19 mmol/L) 15.87 ± 1.88 CCI (Appendix L). At the end of the experiment chlorophyll concentration was significantly higher in High (9.50 mmol/L)

11.254 ± 0.351 CCI, compared to Medium (4.76 mmol/L) 9.696 ± 0.334 CCI, Low (2.36 mmol/L) 8.688 ± 0.598 CCI, and Control (1.19 mmol/L) 7.7.38 ± 0.2.55 CCI (R squared = 0.985; Figure3.4)

The average number of flowers during the overall experiment had no statistical difference between treatments (Appendix M). Dry weight of above ground biomass was highest in High (9.50 mmol/L) and Medium (4.76 mmol/L), but Medium (4.76 mmol/L) was statistically similar to Low (2.36 mmol/L) and Control (1.19 mmol/L). Dry weight of below ground biomass was statistically similar between all treatments (Table 3.4)

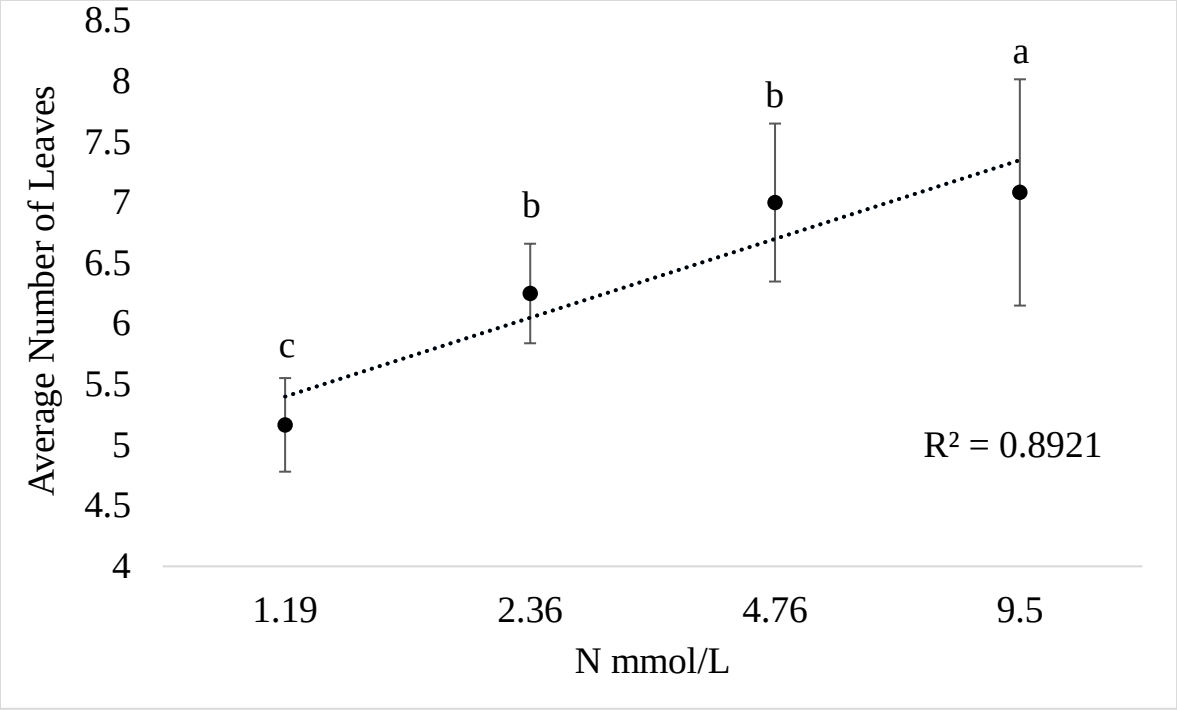

Figure 3.1. Regression curve of the average number of leaves at the end of the experiment, 102 days after planting. The average number of leaves for the experiment was significantly greater in High (9.50 mmol/L) than all other treatments. Error bars show standard error of the means. Letters represent statistical differences between treatments.

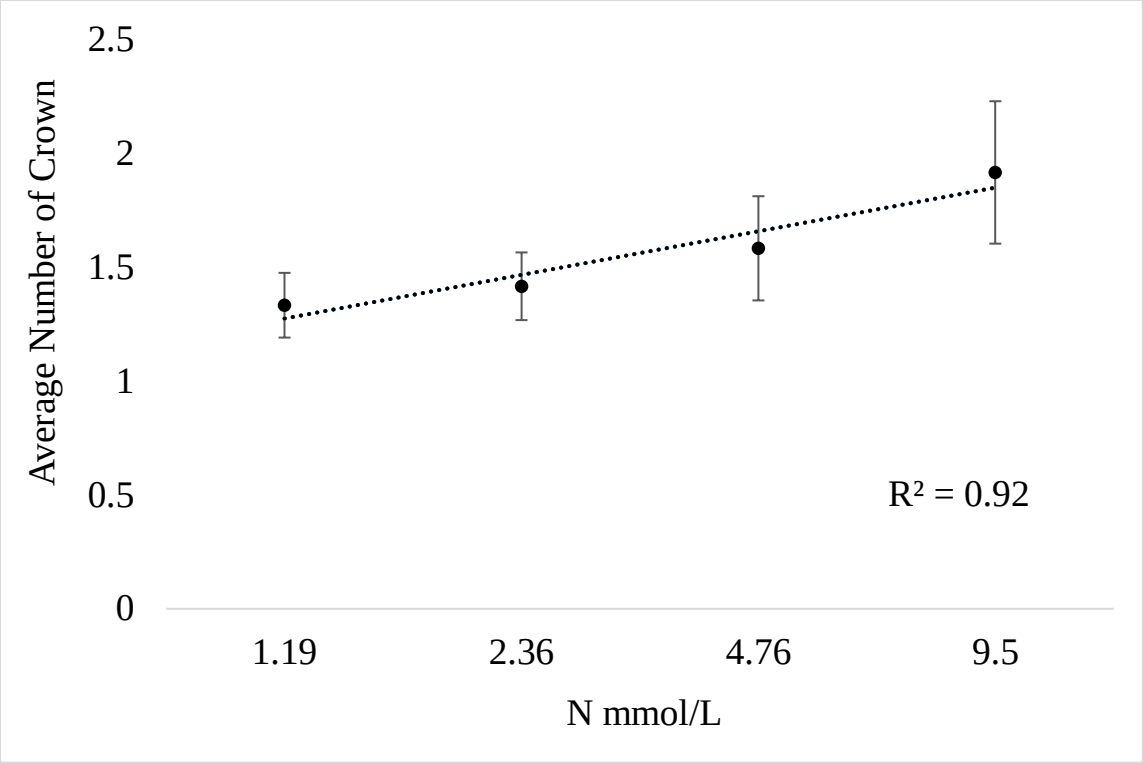

Figure 3.2. Regression curve of average number of crowns at the end of the experiment, 102 days after planting. No significant differences were found. Error bars show standard error of the means.

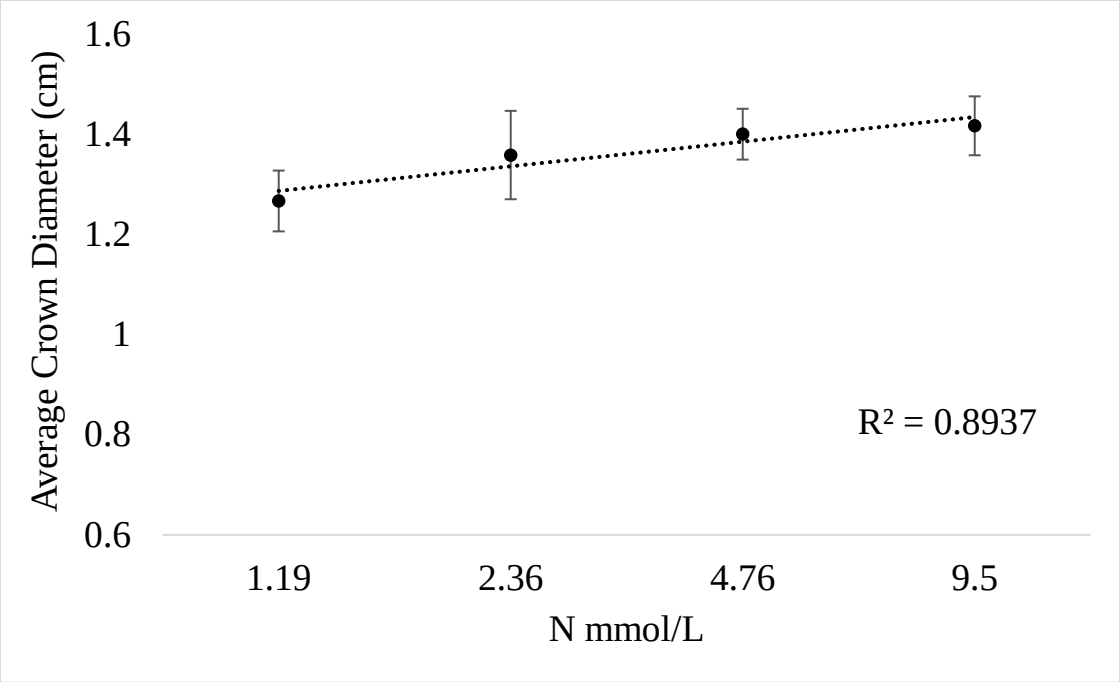

Figure 3.3. Regression curve of average crown diameter at the end of the experiment, 102 days after planting. The average crown diameter was not significantly different for any treatment. Error bars show standard error of the means.

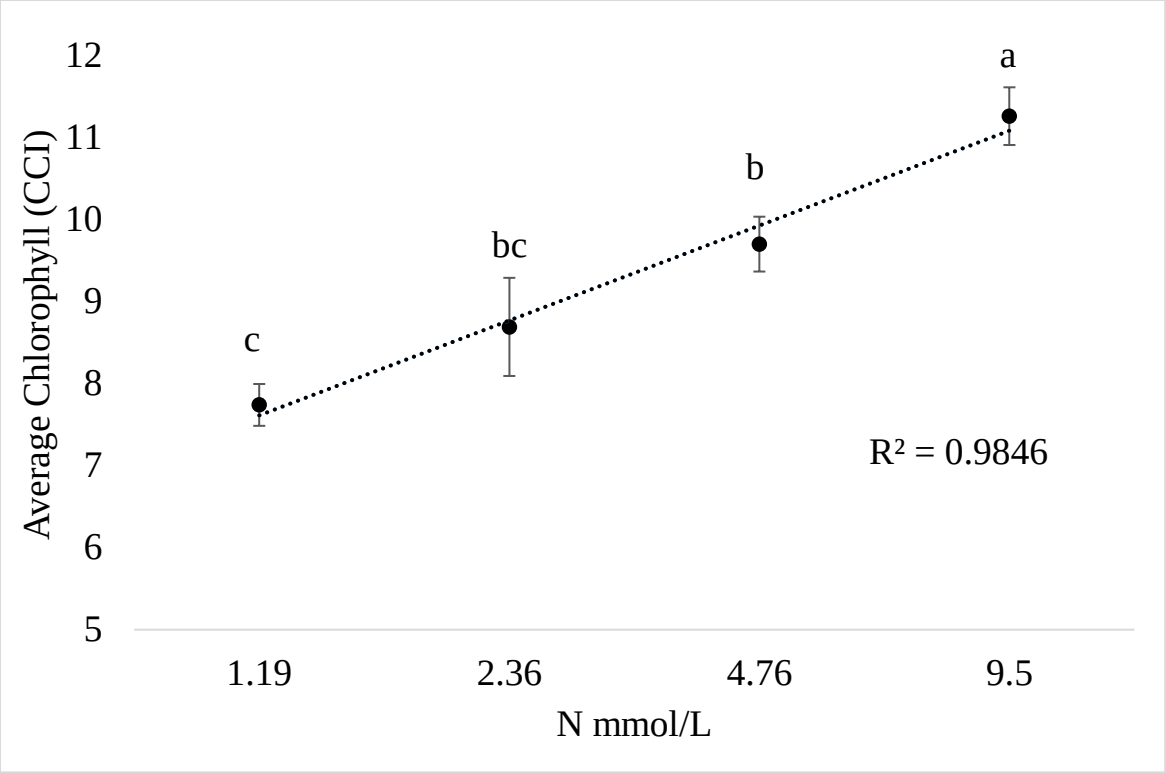

Figure 3.4. Regression curve of the average chlorophyll at the end of the experiment, 102 days after planting. The overall average chlorophyll was significantly more in High (9.50 mmol/L) than all other treatments. Error bars show standard error of the means. Letters represent statistical differences between treatments.

Table 3.4. Average dry weight of the above and below ground biomass and standard error of the mean. Letters represent statistical differences between treatments.

Treatment	Average Above Ground Dry Weight (g)	Average Root Dry Weight (g)
Control (1.19 mmol/L)	2.96 ± 0.168 b	4.14 ± 0.127
Low (2.36 mmol/L)	3.23 ± 0.315 b	4.95 ± 0.309
Medium (4.76 mmol/L)	3.58 ± 0.300 ab	4.35 ± 0.202
High (9.50 mmol/L)	4.26 ± 0.289 a	5.06 ± 0.600

2.3 Nitrogen Content of Plant Tissue

There was no significant difference among any treatments in nitrogen content of the petioles sampled on either 9 March 2021 or 30 April 2021. There was no significant difference among any treatments in nitrogen content of the crowns sampled on either 9 March 2021 or 30

April 2021. At the end of the experiment, 102 days after planting, High (9.50 mmol/L) had statistically greater root N concentrations than any other treatment (Table 2.2.2).

Table 3.5. Plant organs sampled at 50 and 102 days after planting (9 March 2021 and 30 April 2021, respectively) were submitted for N analysis at the NC Department of Agriculture and Consumer Science. Below is the average N concentration in ppm of those sampled plant organs. Standard error not displayed for petiole or crown N content due to samples being grouped by treatment. Letters represent statistical differences between treatments.

Plant Organ	Days after Planting	Control (1.19 mmol/L)	Low (2.36 mmol/L)	Medium (4.76 mmol/L)	High (9.50 mmol/L)
Petiole	50	0.54	0.56	0.71	0.66
	102	0.84	1.09	1.18	1.52
Crown	50	1.03	1.04	1.57	1.57
	102	0.69	0.83	0.76	0.95
Root	50	1.19 ± .345	1.34 ± .350	1.21 ± 0.140	1.34 ± .095
	102	0.67 ± 0.069 b	0.67 ± 0.028 b	0.72 ± 0.073 b	1.05 ± 0.053 a

Table 3.6. Nutrient content of petiole samples, sampled at 50 and 102 days after planting (9 March 2021 and 30 April 2021, respectively) were submitted for analysis at the NC Department of Agriculture and Consumer Science. Below are the average nutrient concentrations in ppm of those sampled plant organs. Standard error not displayed due to samples being grouped by treatment. Units are in ppm.

Treatment	Days after Planting	P	K	Ca	Mg	S
Control (2.36 mmol/L)	50	0.16	2.49	1.04	0.39	0.08
	102	0.23	1.39	1.06	0.26	0.27
Low (2.36 mmol/L)	50	0.13	2.00	1.26	0.49	0.07
	102	0.19	1.19	1.23	0.28	0.28
Medium (4.76 mmol/L)	50	0.21	1.28	1.24	0.57	0.10
	102	0.16	0.99	1.11	0.26	0.28
High (9.50 mmol/L)	50	0.10	2.05	1.36	0.46	0.07
	102	0.15	0.98	1.05	0.27	0.31

2.4 Nutrient Content of Substrate

At 50 days after planting, the High (9.50 mmol/L) treatment had the highest N (432.00 ± 274.00 ppm) remaining in the substrate (Figure 2.2.4). At 102 days after planting High (9.50

mmol/L) treatment had significantly more N (153.91 ± 60.90 ppm) remaining in substrate, compared to Control (1.19 mmol/L) (20.94 ± 7.72 ppm) and Low (2.36 mmol/L) (13.16 ± 5.09 ppm). After 102 days, the N content in Medium (4.76 mmol/L) (94.88 ± 52.92 ppm) was significantly similar to all treatments (Figure 2.2.4).

At the end of the trial there was no statistical differences in the substrate nutrient concentration of potassium, phosphorous, magnesium, or sulfur. High (9.50 mmol/L) was highest in concentration of calcium, however the treatment was significantly similar to Medium (4.76 mmol/L) and Control (1.19 mmol/L). Medium (4.76 mmol/L) and Control (1.19 mmol/L) were also statistically similar to each other and Low (2.36 mmol/L) (Table 3.7).

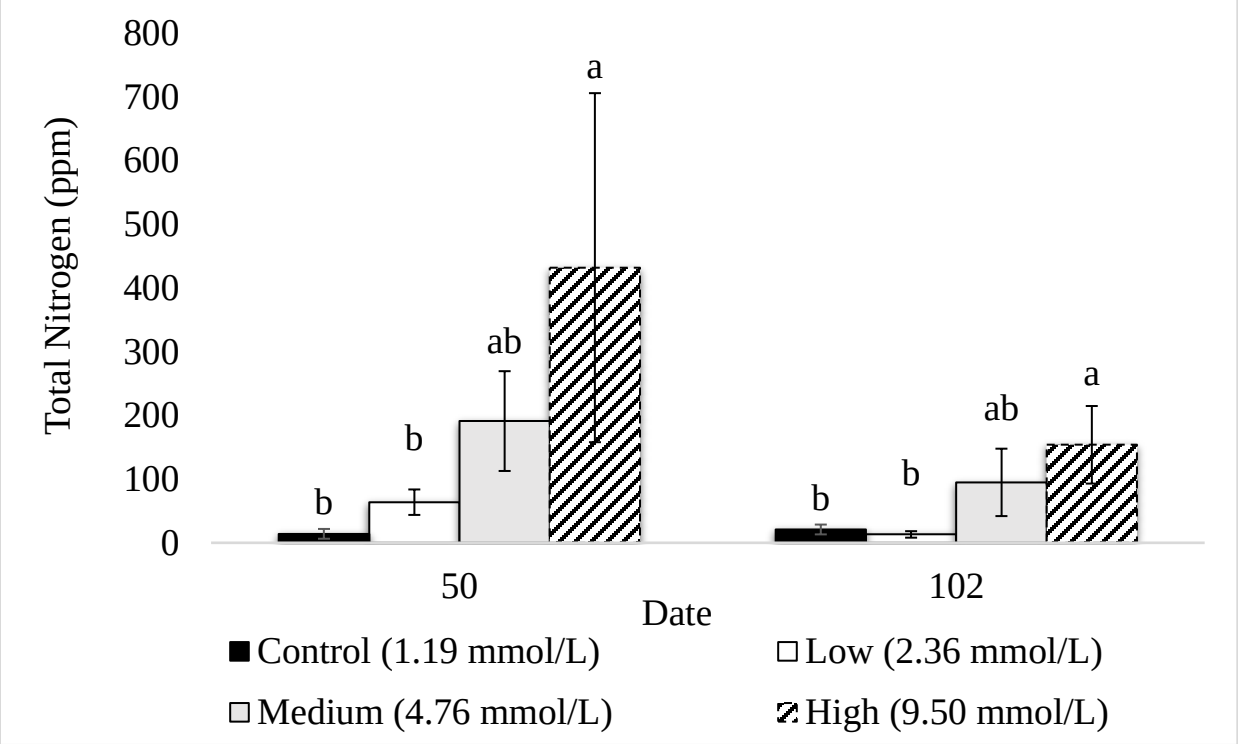

Figure 3.5. Average N content of substrate sampled on day 50 and day 102 of the experiment. Samples were analyzed by the NCDA&CS. Bars represent the standard error of the mean. Letters shown above the bar represent statical differences between treatments.

Table 3.7. Average substrate nutrient concentration from samples collected 102 days after planting. Units are in ppm. Letters shown above the bar represent statical differences between treatments.

Treatments	Potassium (K)	Phosphorous (P)	Calcium (Ca)	Magnesium (Mg)	Sulfur (S)
Control (1.19 mmol/L)	29.1833 ± 4.211	10.132 ± 2.656	40.950 ± 8.250 ab	7.440 ± 1.595	85.750 ± 15.955
Low (2.36 mmol/L)	15.776 ± 7.735	3.754 ± 1.239	12.706 ± 3.793 b	2.306 ± 0.661	40.420 ± 6.947
Medium (4.76 mmol/L)	43.620 ± 22.357	11.356 ± 5.723	37.418 ± 16.475 ab	8.962 ± 4.926	87.300 ± 32.364
High (9.50 mmol/L)	31.575 ± 7.980	8.660 ± 31.575	53.525 ± 19.119 a	9.4775 ± 3.657	74.075 ± 15.514

Discussion

Plant Growth and Physiology

The objective of this study was to evaluate the impact of four rates of pre-applied N fertilizer on strawberry growth in a soilless container system via a dose response study. Our main finding for this study was that strawberry plants will not use all the N given to them at any rate, and especially higher rates such as 4.76 mmol/L of N and 9.50 mmol/L, during a period of plant establishment.

The number of crowns in our greenhouse experiment varied by treatment with High (9.50 mmol/L) having significantly more crowns than all other treatments. Durner (2020) investigated the impact of N rate during the conditioning of strawberry plugs (*Fragaria* x *ananasa* ‘Seascape’) on field plant performance. Durner (2020) conditioned plants by providing low rates of N for 1 - 4 weeks then a high rate of N for 1 - 4 weeks to plugs plants in the greenhouse, and then transplanted into the field. Plants receiving any additional N had significantly more new

crowns between July and October compared to plants in the control group (Durner, 2020). This is similar to our finding that increasing N also gradually increases the number of crowns for strawberry plants.

Choi et al. (2010) applied a modified Hogland solution with varying rate of N (0 meq/L, 2.5 meq/L, 5 meq/L, 10 meq/L, and 15 meq/L) for strawberries in a greenhouse setting, while rates for all other nutrients were consistent. Planting material used were plug-grown 'Seolhyang' strawberry seedlings at their three-leaf stage and grown in a perlite substrate, for the first 45 days they were only watered with distilled water. After 45 days plants were watered with the modified Hogland solutions once a week, at all other times they were watered with distilled water. The differences in plant growth and nutrient analysis between treatments were assessed 120 days after planting. Choi et al. (2010) found that the number of leaves was not statistically different between any treatment except for 0 meq/L.

In our greenhouse experiment there was an increase in number of leaves as N increased. This difference in results between Choi et al. (2010) and our greenhouse experiment may stem from the irrigation regime as nutrients were added weekly for strawberries in Choi et al. (2010) meanwhile in our greenhouse experiment fertilizer was applied once at the beginning of the experiment. Another variation in the experiments is the time of sampling; in our experiment on the day of planting fertilizer was already applied in the substrate and number of leaves were assessed for 102 days. Choi et al. (2010) did not fertilizer plugs for the first 45 days and then assessed number of leaves for the next 75 days (for a total of 120 days). Choi et al. (2010) found no statistical difference in crown diameter due to N concentration in the nutrient solution, similar to our findings in the greenhouse project.

Altuntas and Dasgan (2017) aimed to determine the optimum rate of N for greenhouse cultivation of cucumbers in the spring season using organic fertilizer. In a greenhouse using a soil system that had not previously been treated with chemicals (fertilizer or pesticides) the organic fertilizer Pattrone (powder, 10.21% N) was applied through drip as 0 kg/ha, 0.5 kg/ha, 1.5 kg/ha, 2.5 kg/ha, 3.5 kg/ha of pure N. Altuntas and Dasgan (2017) found that the number of leaves for all plants that received an N treatment was higher than the control treatment. The stem diameter of plants in the highest N treatment (3.5 kg/ha) was statistically different than the control treatment. In our greenhouse study there was no non-treated control, this difference along with differing results show that an absolute lack of N can cause deficiency and inhibit growth. However, the addition of N at an undetermined threshold is enough to maintain sufficient growth of a plant.

In our greenhouse there was an increase in the dry weight of above ground biomass as nitrogen increased in the treatments. There was no significant difference in root dry weight across all treatments, root systems were not root bound during the trial. Andriolo et al. (2011) conducted a greenhouse trial in a randomized closed hydroponic system with strawberries (*Fragaria* x *ananassa* 'Arazá') grown in sand. Treatments in the study were 6.5 mmol/L of N, 8.0 mmol/L of N, 9.5 mmol/L of N, 11.0 mmol/L of N, and 12.5 mmol/L of N. There was a linear decrease the dry weight of above ground (shoot) biomass as N increased. Root dry weight also decreased with increased N application. Andriolo et al. (2011) allowed plants to produce fruit and harvested fruit before drying the plant parts. In our study plant parts were dried while the strawberries were flowering but were never able to fruit, this difference in physiological stage may explain some of the difference in dry weight results, however further research will be required.

Choi et al. (2010) collected above ground dry weights. Results showed that the greatest dry weights were in the treatments consisting of the dose 2.5 meq/L, 5 meq/L, and 10 meq/L, 10 meq/L was also statistically similar to the treatment 15 meq/L, all treatments were statistically greater than the treatment 0 meq/L. In our greenhouse experiment we observed that dry weight increased as amount of N applied also increased. These differences in results may be due to the fertilizer regime, implying that consistent low doses of N are enough to sustain similar growth to high doses. However, when fertilizer is applied once as a low dose of N, the N will not remain available either due to being washed out, volatilizing, or moving out of the root zone within the pot, compared to the high doses of N applied once that leave ample amounts in the soil that plant roots can reach.

Nutrient Concentration

In our greenhouse trial, we found no difference in the N concentration of petioles or crowns between any treatment. Cárdenas-Navarro et. al. (2004) attempted to determine the critical nitrogen and nitrogen dilution curve of strawberries (*Fragaria* x *ananassa* 'Duch'). Treatments applied through irrigation were 0.0 mM, 0.01 mM, 0.1 mM, 0.3 mM, 3.0 mM, 9.0 mM, and 30 mM of NO_3^-. During the experiment the N accumulation of plant dry matter was analyzed. Cárdenas-Navarro et. al. (2004) found no statistical difference in petiole N concentration, similar to our own study results. This suggests that applications of higher rates of N to strawberries do not lead to increased utilization of N in vegetative plant organs.

Petioles in Choi et al. (2010) were analyzed for nutrient content 120 days after planting. The petiole nutrient concentrations for total N, potassium, phosphorus, calcium, and magnesium all had statistical differences. This varies from our greenhouse experiment, however in the Choi et al. (2010) experiment, plants were irrigated with distilled water and fertigated once a week,

while our plants were provided a one-time granular application of fertilizer at the beginning of the experiment, thus implying that not only dose but regime can have an impact on petiole nutrient concentration.

Conclusion

In this study, we could show that strawberry plants will not utilize all N supplied at a high dose in the first 102 days after planting. Plants that had higher rates of N applied at the beginning of the study showed more vegetative growth. However, N content in petioles and crowns had no statistical difference, yet there was more than twice the amount of N still left in the substrate of High (95.0 mmol/) than any other treatments. Further research is needed to assess why this occurred and how other essential macro-nutrients impact N utilization during strawberry plant establishment in a soilless containerized system.

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APPENDICES

Appendix A: Total soil nitrogen at PRS 2019-2020, all depths

Appendix B: Total soil nitrogen at PRS 2020-2021, all depths

Appendix C: Total soil nitrogen at CCRS 2019-2020, all depths

Appendix D: Total soil nitrogen at CCRS 2019-2020, all depths

Appendix E: Photos of soil sampling and processing

Appendix F: Photos of greenhouse plants 102 days after planting

Appendix G: n of Parameters for field study

Appendix H: n of Parameters for greenhouse study

Appendix I: Average number of leaves per treatment by date

Appendix J**:** Average number of crowns per treatment by date of data collection.

Appendix K: Average crown diameter (cm) per treatment by date

Appendix L: Average chlorophyll (CCI) per treatment by date

Appendix M: Cumulative average number of flowers per treatment by date

Appendix N: Petiole nutrient (K, P, Ca, Mg, and S) content, both years, both sites

Appendix A

Table A.1. The total soil nitrogen concentration (mg/L) at PRS in 2019-2020 at the depths 0-12.7 cm.

Treatment	25 September 2019	15 November 2019	2 January 2020	18 February 2020
1 (67.25 kg/ha N)	71.16 ± 8.96 ab	33.98 ± 12.75 a	11.36 ± 3.38 b	5.37 ± 1.6 a
2 (54.14 kg/ha N)	36.7 ± 14.29 bc	20.83 ± 9.5 ab	27.89 ± 3.23 ab	9.6 ± 4.34 a
3 (41.09 kg/ha N)	92.33 ± 21.83 a	19.08 ± 8.81 ab	50.31 ± 10.0 a	11.41 ± 3.23 a
4 (27.98 kg/ha N)	50.9 ± 12.28 abc	4.34 ± 1.08 b	14.93 ± 4.6 b	17.53 ± 1.55 a
5A (33.63 kg/ha N)	56.05 ± 16.76 abc	13.55 ± 5.83 ab	31.66 ± 16.89 ab	16.28 ± 13.33 a
6 (NTC)	16.92 ± 3.67 c	20.29 ± 3.88 ab	6.8 ± 0.52 b	11.76 ± 7.76 a

Table A.2. The total soil nitrogen concentration (mg/L) at PRS in 2019-2020 at the depths 12.7-25.4 cm.

Treatment	25 September 2019	15 November 2019	2 January 2020	18 February 2020
1 (67.25 kg/ha N)	32.46 ± 8.18 a	28.18 ± 17.57 a	9.39 ± 5.45 b	4.07 ± 0.99 a
2 (54.14 kg/ha N)	18.80 ± 2.81 bc	18.95 ± 2.41 ab	10.03 ± 0.84 ab	5.01 ± 0.93 a
3 (41.09 kg/ha N)	19.16 ± 4.34 bc	16.82 ± 6.74 ab	17.80 ± 9.32 a	5.29 ± 1.08 a
4 (27.98 kg/ha N)	25.73 ± 3.96 ab	7.89 ± 1.90 b	7.31 ± 2.20 b	7.2 ± 3.74 a
5A (33.63 kg/ha N)	15.87 ± 1.55 bc	15.87 ± 2.05 ab	31.52 ± 7.83 ab	6.96 ±2.33 a
6 (NTC)	8.07 ± 1.19 c	14.67 ± 1.64 ab	6.66 ± 1.4 b	13.2 ± 7.29 a

Appendix B

Table B.1. The total soil nitrogen concentration (mg/L) at PRS in 2020-2021 at the depths 0-12.7 cm.

Treatment	14 October 2020	10 November 2020	10 December 2020	15 January 2021	24 February 2021
1 (67.25 kg/ha N)	88.19 ± 49.0 a	103.06 ± 24.52 a	144.22 ± 21.1 a	71.90 ± 19.17 a	13.15 ± 4.18 a
2 (54.14 kg/ha N)	74.02 ± 13.31 a	114.13 ± 18.73 a	89.01 ± 15.25 b	62.43 ± 15.89 a	13.82 ± 10.34 a
3 (41.09 kg/ha N)	74.04 ± 18.93 a	64.73 ± 20.28 abc	50.74 ± 17.36 bc	14.61 ± 0.55 b	13.95 ± 4.52 a
4 (27.98 kg/ha N)	86.15 ± 17.95 a	48.23 ± 10.62 bc	46.85 ± 12.05 bc	24.31 ± 11.1 b	6.60 ± 0.78 a
5A (33.63 kg/ha N)	52.35 ± 24.32 a	78.42 ± 17.78 ab	76.68 ± 11.45 b	19.66 ± 7.0 b	12.53 ± 8.43 a
6 (NTC)	17.43 ± 1.12 a	18.51 ± 3.24 c	11.06 ± 1.52 c	4.57 ± 0.49 b	2.96 ± 0.19 a

Table B.2. The total soil nitrogen concentration (mg/L) at PRS in 2020-2021 at the depths 12.7-25.4 cm.

Treatment	14 October 2020	10 November 2020	10 December 2020	15 January 2021	24 February 2021
1 (67.25 kg/ha N)	77.91 ± 25.22 a	17.93 ± 9.65 a	13.16 ± 2.07 a	7.30 ± 1.11 a	5.10 ± 1.13 a
2 (54.14 kg/ha N)	23.37 ± 7.48 b	8.33 ± 0.95 a	11.95 ± 3.15 a	4.82 ± 0.51 a	4.20 ± 0.94 ab
3 (41.09 kg/ha N)	32.53 ± 7.88 b	19.08 ± 10.41 a	22.80 ± 13.69 a	4.21 ± 0.28 b	3.68 ± 0.43 ab
4 (27.98 kg/ha N)	26.91 ± 4.63 b	16.50 ± 2.38 a	14.55 ± 6.47 a	4.91 ± 0.79 b	3.26 ± 0.23 ab
5A (33.63 kg/ha N)	62.26 ± 19.93 ab	14.67 ± 2.80 a	15.44 ± 4.69 a	4.95 ± 1.02 b	3.78 ± 0.29 ab
6 (NTC)	22.16 ± 11.16 b	18.51 ± 0.92 a	6.59 ± 1.47 a	3.52 ± 0.14 b	2.71 ± 0.16 b

Appendix C

Table C.1. The total soil nitrogen concentration (mg/L) at CCRS in 2019-2020 at the depths 0-12.7 cm.

Treatment	7 October 2019	26 November 2019	10 January 2020	25 February 2020
1 (67.25 kg/ha N)	46.88 ± 11.17 b	30.90 ± 3.37 a	20.76 ± 3.97 a	5.85 ± 2.47 ab
2 (54.14 kg/ha N)	38.67 ± 12.59 bcd	28.64 ± 14.71 a	11.25 ± 4.79 ab	9.46 ± 3.39 a
3 (41.09 kg/ha N)	41.73 ± 6.63 bc	20.70 ± 4.22 a	5.16 ± 2.86 b	3.90 ± 1.19 b
4 (27.98 kg/ha N)	26.40 ± 2.8 bcd	18.39 ± 4.18 a	14.87 ± 5.32 ab	5.65 ± 0.81 ab
5B (80.1 kg/ha N)	87.35 ± 14.1 a	29.57 ±6.28 a	23.90 ± 7.65 a	6.45 ± 0.96 ab
6 (NTC)	15.39 ± 1.61 d	19.38 ± 2.16 a	16.67 ± 1.56 ab	4.69 ± 0.73 ab

Table C.2. The total soil nitrogen concentration (mg/L) at CCRS in 2019-2020 at the depths 12.7-25.4 cm.

Treatment	7 October 2019	26 November 2019	10 January 2020	25 February 2020
1 (67.25 kg/ha N)	48.13 ± 7.29 ab	26.64 ± 0.61 a	17.25 ± 3.92 ab	3.95 ± 0.73 a
2 (54.14 kg/ha N)	31.36 ± 5.26 bc	9.85 ± 1.84 b	4.97 ± 2.14 c	6.34 ± 1.32 a
3 (41.09 kg/ha N)	32.08 ± 2.34 bc	14.27 ± 4.09 b	13.79 ± 6.79 abc	4.47 ± 1.61 a
4 (27.98 kg/ha N)	30.31 ± 3.70 bc	11.90 ± 1.79 b	8.65 ± 3.02 bc	6.52 ± 1.75 a
5B (80.1 kg/ha N)	56.09 ± 20.10 a	30.63 ± 3.64 a	20.11 ± 2.60 a	5.69 ± 1.15 a
6 (NTC)	9.73 ± 1.04 c	9.43 ± 3.17 b	15.0 ± 1.96 abc	6.82 ± 1.48 a

Appendix D

Table D.1. The total soil nitrogen concentration (mg/L) at CCRS in 2020-2021 at the depths 0-12.7 cm.

Treatment	26 October 2020	17 November 2020	17 December 2020	22 January 2021	5 March 2021
1 (67.25 kg/ha N)	15.24 ± 2.44 b	8.00 ± 1.19 b	5.48 ± 0.23 abc	2.87 ± 0.13 bc	2.90 ± 0.26 a
2 (54.14 kg/ha N)	10.45 ± 1.18 bcd	6.54 ± 1.03 b	4.92 ± 1.12 bc	2.74 ± 0.04 bc	2.13 ± 0.01 a
3 (41.09 kg/ha N)	13.41 ± 4.07 bc	5.44 ± 0.80 bc	3.62 ± 0.81cd	2.18 ± 0.04 c	2.56 ± 0.27 a
4 (27.98 kg/ha N)	13.56 ± 1.89 bc	7.21 ± 0.56 b	5.92 ± 0.43 ab	4.29 ± 0.46 a	2.50 ± 0.40 a
5B (80.1 kg/ha N)	22.05 ± 1.84 a	12.26 ± 1.32 a	7.38 ± 0.8 a	4.37 ± 0.6 a	3.09 ± 0.89 a
6 (NTC)	10.47 ± 1.26 bcd	7.31 ± 1.19 b	6.10 ± 1.02 ab	2.84 ± 0.64 bc	3.31 ± 0.80 a

Table D.2. The total soil nitrogen concentration (mg/L) at CCRS in 2020-2021 at the depths 12.7-25.4 cm.

Treatment	26 October 2020	17 November 2020	17 December 2020	22 January 2021	5 March 2021
1 (67.25 kg/ha N)	16.66 ± 1.24 a	6.48 ± 1.03 ab	5.41 ± 0.74 bc	2.66 ± 0.24 bc	2.98 ± 0.29 a
2 (54.14 kg/ha N)	10.0 ± 1.77 b	5.27 ± 0.95 bc	4.05 ± 0.55 bcd	2.74 ± 0.08 bc	2.20 ± 0.02 a
3 (41.09 kg/ha N)	6.17 ± 2.18 bc	3.42 ± 1.07 cd	3.69 ± 1.05 cd	2.70 ± 0.53 bc	2.72 ± 0.34 a
4 (27.98 kg/ha N)	6.24 ± 0.91 bc	5.03 ± 0.81 bc	6.00 ± 1.06 ab	3.74 ± 0.74 ab	2.27 ± 0.13 a
5B (80.1 kg/ha N)	20.41 ± 3.82 a	8.70 ± 0.76 a	8.01 ± 0.75 a	4.87 ± 0.75 a	2.71 ± 0.41 a
6 (NTC)	5.57 ± 0.54 bc	5.32 ± 0.58 bc	3.39 ± 0.53 cd	2.26 ± 0.07 c	2.51 ± 0.27 a

Appendix E

Figure E.1 Soil probe (left) used at both PRS and CCRS. Soil cores were split in half (0-12.7 cm and 12.7-25.4 cm) and placed in their respective buckets. 3 samples per depth were pooled and mixed, then put in their corresponding sample containers. Photo taken on 26 Oct. 2020.

Figure E.2 PRS samples filtering after being removed from the shaker table. Photo taken on 3 June 2020.

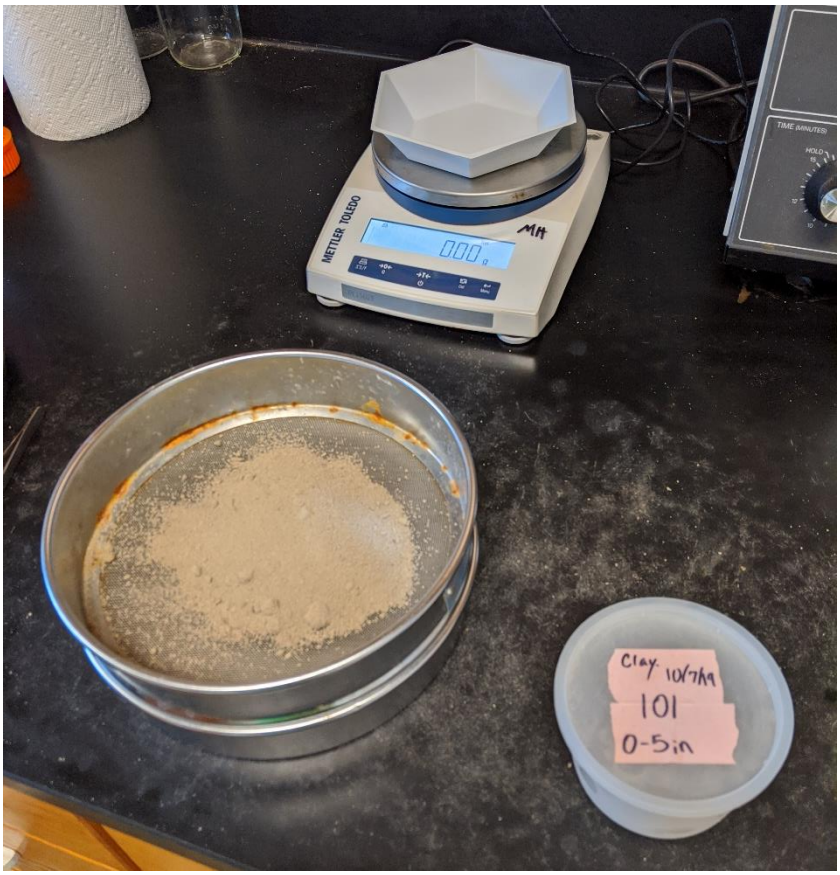

Figure E.3. CCRS soil sample being sieved through 10 mesh sieve before N extraction. Photo taken on 17 Aug. 2020.

Appendix F

Figure F.1. Control (1.19 mmol/L) being prepared for the drying oven. Above and below ground biomass separated, pen for scale. Roots had not been washed before this photo. Photo taken on 1 May 2021.

Figure F.2. Low (2.36 mmol/L) being prepared for the drying oven. Above and below ground biomass separated, pen for scale. Roots had not been washed before this photo. Photo taken on 1 May 2021.

Figure F.3. Medium (4.76 mmol/L) being prepared for the drying oven. Above and below ground biomass separated, pen for scale. Roots had not been washed before this photo. Photo taken on 1 May 2021.

Figure F.4. High (9.50 mmol/L) being prepared for the drying oven. Above and below ground biomass separated, pen for scale. Roots had not been washed before this photo. Photo taken on 1 May 2021.

Appendix G

Table G.1. The n of each parameter in field experiments during 2019-2020 and 2020-2021.

Parameter	Location	Year	n of Parameter
Soil Samples	PRS	2019-2020	16
Petiole Samples	PRS	2019-2020	2
Yield	PRS	2019-2020	48
Soil Samples	CCRS	2019-2020	16
Petiole Samples	CCRS	2019-2020	2
Soil Samples	PRS	2020-2021	20
Petiole Samples	PRS	2020-2021	4
Yield	PRS	2020-2021	44
Average Fruit Weight	PRS	2020-2021	12
Fruit Chemistry	PRS	2020-2021	16
Soil Samples	CCRS	2020-2021	20
Petiole Samples	CCRS	2020-2021	4
Yield	CCRS	2020-2021	44
Average Fruit Weight	CCRS	2020-2021	12
Fruit Chemistry	CCRS	2020-2021	16

Appendix H

Table H.1. The n of each parameter for the 2021 greenhouse experiment.

Parameter	n of Parameter
Number of Leaves	196
Number of Crowns	196
Chlorophyll Concentration	392
Crown Diameter	56
Nutrient Concentration of Petiole (9 March)	1
Nutrient Concentration of Crown (9 March)	1
Nutrient Concentration of Roots (9 March)	2
Nutrient Concentration of Substrate (9 March)	2
Above Ground Biomass	6
Below Ground Biomass	6
Nutrient Concentration of Petiole (30 April)	1
Nutrient Concentration of Crown (30 April)	1
Nutrient Concentration of Roots (30 April)	6
Nutrient Concentration of Substrate (30 April)	6

Appendix I

Table I.1. Average number of leaves per treatment by date of data collection.

Date	Control (1.19 mmol/L)	Low (2.36 mmol/L)	Medium (4.76 mmol/L)	High (9.50 mmol/L)
18 Jan. 2021	2.500 ± 0.151	2.750 ± 0.131	2.750 ± 0.131	2.750 ± 0.131
27 Jan. 2021	3.000 ± 0.123	3.000 ± 0.174	3.000 ± 0.123	2.917 ± 0.149
4 Feb. 2021	3.333 ± 0.188	3.500 ± 0.151	3.333 ± 0.142	3.583 ± 0.149
10 Feb. 2021	3.667 ± 0.142 b	4.167 ± 0.167 a	4.000 ± 0.174 ab	4.167 ± 0.207 a
17 Feb. 2021	4.333 ± 0.142	4.500 ± 0.151	4.667 ± 0.188	4.750 ± 0.179
24 Feb. 2021	4.583 ± 0.149 b	5.167 ± 0.112 ab	5.167 ± 0.207 ab	5.750 ± 0.329 a
3 March 2021	5.250 ± 0.218 b	5.750 ± 0.279 ab	5.750 ± 0.279 ab	6.583 ± 0.468 a
10 March 2021	5.417 ± 0.229 b	6.083 ± 0.336 ab	6.000 ± 0.302 ab	6.750 ± 0.429 a
17 March 2021	5.417 ± 0.193 c	6.333 ± 0.284 bc	6.500 ± 0.314 ab	7.333 ± 0.497 a
26 March 2021	6.417 ± 0.193 b	6.917 ± 0.434 b	7.500 ± 0.500 ab	8.583 ± 0.866 a
31 March 2021	6.417 ± 0.313 b	7.167 ± 0.520 b	7.750 ± 0.552 ab	9.083 ± 0.783 a
9 April 2021	6.667 ± 0.333	7.583 ± 0.358	7.833 ± 0.474	7.667 ± 0.644
16 April 2021	5.917 ± 0.434	7.417 ± 0.417	7.333 ± 0.607	7.500 ± 0.802
23 April 2021	5.167 ± 0.386 b	6.250 ± 0.411 ab	7.000 ± 0.651 a	7.0833 ± 0.933 a

Appendix J

Table J.1. Average number of crowns per treatment by date of data collection.

Date	Control (1.19 mmol/L)	Low (2.36 mmol/L)	Medium (4.76 mmol/L)	High (9.50 mmol/L)
18 Jan. 2021	1 ± 0	1 ± 0	1 ± 0	1 ± 0
27 Jan. 2021	1 ± 0	1 ± 0	1 ± 0	1 ± 0
4 Feb. 2021	1 ± 0	1 ± 0	1 ± 0	1 ± 0
10 Feb. 2021	1 ± 0	1 ± 0	1 ± 0	1 ± 0
17 Feb. 2021	1 ± 0	1 ± 0	1 ± 0	1 ± 0
24 Feb. 2021	1 ± 0 b	1 ± 0 b	1 ± 0 b	1.333 ± 0.188 a
3 March 2021	1.083 ± 0.083 b	1 ± 0 b	1.083 ± 0.083 b	1.500 ± 0.195 a
10 March 2021	1.083 ± 0.083 b	1.250 ± 0.131 ab	1.417 ± 0.193 ab	1.667 ± 0.225 a
17 March 2021	1.083 ± 0.083 b	1.333 ± 0.142 ab	1.417 ± 0.193 ab	1.667 ± 0.225 a
26 March 2021	1.250 ± 0.131	1.333 ± 0.142	1.417 ± 0.193	1.667 ± 0.225
31 March 2021	1.250 ± 0.131	1.417 ± 0.149	1.417 ± 0.193	1.667 ± 0.225
9 April 2021	1.250 ± 0.131	1.417 ± 0.149	1.417 ± 0.193	1.667 ± 0.225
16 April 2021	1.250 ± 0.131	1.417 ± 0.149	1.583 ± 0.228	1.833 ± 0.322
23 April 2021	1.333 ± 0.142	1.417 ± 0.149	1.583 ± 0.228	1.917 ± 0.313

Appendix K

Table K.1. Average crown diameter (cm) per treatment by date of data collection. There was no statistical difference in crown diameter on any day.

Date	Control (1.19 mmol/L)	Low (2.36 mmol/L)	Medium (4.76 mmol/L)	High (9.50 mmol/L)
18 Jan. 2021	0.692 ± 0.061	0.769 ± 0.047	0.767 ± 0.088	0.658 ± 0.029
17 Feb. 2021	1.508 ± 0.061	1.475 ± 0.073	1.375 ± 0.059	1.558 ± 0.071
26 March 2021	1.542 ± 0.038	1.500 ± 0.059	1.492 ± 0.053	1.450 ± 0.058
23 April 2021	1.267 ± 0.061	1.358 ± 0.088	1.400 ± 0.051	1.417 ±0.059

Appendix L

Table L.1. Average chlorophyll (CCI) per treatment by date of data collection.

Date	Control (1.19 mmol/L)	Low (2.36 mmol/L)	Medium (4.76 mmol/L)	High (9.50 mmol/L)
27 Jan. 2021	23.013 ± 0.780	22.817 ± 0.622	24.767 ± 0.966	24.508 ± 0.852
4 Feb. 2021	25.433 ± 1.049 b	25.554 ± 1.036 ab	29.592 ± 1.456 ab	29.829 ± 1.509 a
10 Feb. 2021	24.338 ± 1.042 ab	20.875 ± 1.240 b	25.592 ± 1.535 ab	26.571 ± 1.969 a
17 Feb. 2021	20.304 ± 0.977 b	19.875 ± 0.909 b	20.483 ± 0.994 b	24.888 ± 1.226 a
24 Feb. 2021	20.783 ± 1.525	18.458 ± 1.892	19.925 ± 1.320	21.621± 1.498
3 March 2021	15.492 ± 1.443 b	15.900 ± 1.525 b	15.808 ± 0.720 b	21.858 ± 0.826 a
10 March 2021	14.142 ± 1.258 b	14.329 ± 1.271 b	15.133 ± 0.544 b	20.550 ± 0.628 a
17 March 2021	12.354 ± 0.987 b	13.313 ± 0.506 b	12.958 ± 0.630 b	16.800 ± 0.618 a
26 March 2021	9.929 ± 0.786 c	11.808 ± 0.326 b	11.850 ± 0.374 b	15.167 ± 0.575 a
31 March 2021	9.763 ± 0.784 b	11.525 0.409 b	11.317 ± 0.511 b	14.083 ± 0.421 a
9 April 2021	7.779 ± 0.492 c	7.896 ± 0.552 c	8.958 ± 0.280 b	10.950 ± 0.195 a
16 April 2021	7.113 ± 0.488 c	8.125 ± 0.377 b	8.846 ± 0.348 b	10.313 ± 0.420 a
23 April 2021	7.738 ± 0.255 c	8.688 ± 0.598 bc	9.696 ± 0.334 b	11.254 ± 0.351 a

Appendix M

Table M.1. Cumulative average number of flowers per treatment by date of data collection. There was no statistical difference on any date for cumulative number of flowers.

Date	Control (1.19 mmol/L)	Low (2.36 mmol/L)	Medium (4.76 mmol/L)	High (9.50 mmol/L)
18 Jan. 2021	0 ± 0	0 ± 0	0 ± 0	0 ± 0
27 Jan. 2021	0 ± 0	0 ± 0	0 ± 0	0 ± 0
4 Feb. 2021	0 ± 0	0 ± 0	0 ± 0	0.083 ± 0.083
10 Feb. 2021	1.417 ± 0.452	1.167 ± 0.297	1.750 ± 0.372	1.750 ± 0.351
17 Feb. 2021	3.250 ± 0.750	3.667 ± 0.396	3.583 ± 0.313	3.833 ± 0.441
24 Feb. 2021	6.000 ± 0.801	5.750 ± 0392	6.333 ± 0.396	5.750 ± 0.552
3 March 2021	8.333 ± 0.865	7.917 ± 0.596	8.750 ± 0.629	8.167 ± 0.684
10 March 2021	9.75 ± 0.922	9.364 ± 0.704	10.363 ± 1.146	8.750 ± 0.698
17 March 2021	11.333 ± 0.922	10.846 ± 0.553	11.692 ± 0.835	10.750 ± 0.641
26 March 2021	12.833 ± 0.869	11.333 ± 0.548	13.500 ± 1.019	11.667 ± 0.873
31 March 2021	13.083 ± 0.839	12.167 ± 0.548	13.822 ± 1.224	12.083 ± 0.933
9 April 2021	13.500 ± 0.839	12.833 ± 0.716	14.417 ± 1.240	12.833 ± 0.960
16 April 2021	13.500 ± 0.839	13.250 ± 0.629	14.167 ± 1.308	12.833 ± 0.960
23 April 2021	14.167 ± 0.928	13.750 ± 0.552	15.083 ± 1.240	12.917 ± 0.957

Appendix N

Table N.1 PRS petiole nutrient content 2019-2020.

Treatment	Date	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Grower Standard	15 Nov. 2019	0.38	1.74	0.52	0.35	0.19
	18 Feb. 2020	0.33	1.28	0.50	0.28	0.16
Low Polysulphate	15 Nov. 2019	0.43	1.93	0.47	0.42	0.21
	18 Feb. 2020	0.34	1.41	0.45	0.29	0.16
Medium Polysulphate	15 Nov. 2019	0.44	1.89	0.44	0.39	0.20
	18 Feb. 2020	0.32	1.32	0.42	0.29	0.16
Half-Grower Standard	15 Nov. 2019	0.37	1.80	0.45	0.36	0.20
	18 Feb. 2020	0.34	1.44	0.39	0.30	0.17
High Polysulphate	15 Nov. 2019	0.39	1.83	0.55	0.43	0.21
	18 Feb. 2020	0.33	1.39	0.42	0.31	0.17s
NTC	15 Nov. 2019	0.36	1.59	0.65	0.35	0.18
	18 Feb. 2020	0.30	1.28	0.55	0.28	0.15

Table N.2 PRS petiole nutrient content 2020-2021.

Treatment	Date	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
Grower Standard	10 Dec.2020	0.32	1.49	0.43	0.28	0.15
	24 Feb. 2021	0.31	1.37	0.41	0.29	0.15
	3 May 2021	0.24	1.52	0.81	0.28	0.12
	25 May 2021	0.27	1.39	0.78	0.22	0.09
Low Polysulphate	10 Dec.2020	0.33	1.50	0.41	0.30	0.15
	24 Feb. 2021	0.32	1.47	0.35	0.27	0.14
	3 May 2021	0.27	1.65	0.84	0.29	0.13
	25 May 2021	0.29	1.47	0.64	0.21	0.1
Medium Polysulphate	10 Dec.2020	0.31	1.43	0.39	0.25	0.13
	24 Feb. 2021	0.31	1.44	0.40	0.24	0.14
	3 May 2021	0.26	1.60	0.87	0.29	0.12
	25 May 2021	0.3	1.54	0.57	0.2	0.09
Half-Grower Standard	10 Dec.2020	0.34	1.53	0.41	0.28	0.15
	24 Feb. 2021	0.32	1.39	0.36	0.27	0.15
	3 May 2021	0.30	1.74	0.80	0.29	0.13
	25 May 2021	0.3	1.52	0.53	0.18	0.09
High Polysulphate	10 Dec.2020	0.32	1.48	0.46	0.30	0.16
	24 Feb. 2021	0.29	1.38	0.40	0.25	0.13
	3 May 2021	0.28	1.50	0.82	0.29	0.13
	25 May 2021	0.29	1.5	0.55	0.19	0.1
NTC	10 Dec. 2020	0.33	1.57	0.44	0.28	0.16
	24 Feb. 2021	0.34	1.55	0.39	0.25	0.14
	3 May 2021	0.34	1.65	0.86	0.030	0.14
	25 May 2021	0.3	1.54	0.63	0.2	0.1

Table N.3 CCRS petiole nutrient content 2019-2020.

Treatment	Date	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
High N	26 Nov. 2019	0.58	1.80	0.42	0.52	0.29
	25 Feb. 2020	0.43	1.48	0.51	0.41	0.18
Grower Standard	26 Nov. 2019	0.52	1.62	0.37	0.43	0.23
	25 Feb. 2020	0.42	1.52	0.56	0.47	0.19
Low Polysulphate	26 Nov. 2019	0.56	1.81	0.41	0.49	0.29
	25 Feb. 2020	0.29	1.51	0.64	0.42	0.18
Medium Polysulphate	26 Nov. 2019	0.53	1.87	0.44	0.47	0.36
	25 Feb. 2020	0.38	1.55	0.61	0.41	0.18
High Polysulphate	26 Nov. 2019	0.55	1.82	0.40	0.48	0.29
	25 Feb. 2020	0.42	1.57	0.57	0.39	0.18
NTC	26 Nov. 2019	-	-	-	-	-
	25 Feb. 2020	0.43	1.44	0.60	0.50	0.16

Table N.4 CCRS petiole nutrient content 2020-2021.

Treatment	Date	P (%)	K (%)	Ca (%)	Mg (%)	S (%)
High N	17 Dec 2020	0.41	1.40	0.82	0.47	0.25
	5 March 2021	0.44	1.65	0.38	0.38	0.29
	29 April 2021	0.30	1.74	0.80	0.29	0.13
	20 May 2021	0.29	1.32	0.38	0.27	0.11
Grower Standard	17 Dec 2020	0.39	1.31	0.85	0.45	0.19
	5 March 2021	0.44	1.71	0.37	0.36	0.27
	29 April 2021	0.24	1.52	0.81	0.28	0.12
	20 May 2021	032	1.37	0.35	0.24	0.12
Low Polysulphate	17 Dec 2020	0.38	1.45	0.70	0.40	0.27
	5 March 2021	0.43	1.73	0.44	0.37	0.32
	29 April 2021	0.27	1.65	0.84	0.29	0.13
	20 May 2021	0.28	1.20	0.38	0.23	0.11
Medium Polysulphate	17 Dec 2020	0.39	1.58	0.74	0.40	0.27
	5 March 2021	0.37	1.62	0.49	0.35	0.32
	29 April 2021	0.26	1.60	0.87	0.29	0.12
	20 May 2021	0.25	1.02	0.45	0.24	0.11
High Polysulphate	17 Dec 2020	0.38	1.57	0.78	0.45	0.39
	5 March 2021	0.47	1.97	0.37	0.4	0.48
	29 April 2021	0.28	1.50	0.82	0.29	0.13
	20 May 2021	0.26	1.41	0.39	0.25	0.11
NTC	17 Dec 2020	0.39	1.27	0.77	0.37	0.18
	5 March 2021	0.45	1.66	0.48	0.36	0.24
	29 April 2021	0.34	1.65	0.86	0.30	0.14
	20 May 2021	0.34	1.38	0.36	0.20	0.12