The Effects of a Novel, Slow-Release Application Method for an *Ascophyllum nodosum* Seaweed Extract Biofertilizer on Maize (*Zea mays* L.) Growth and Nutrient Accumulation

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Abstract

Applications of *Ascophyllum nodosum* seaweed extracts (ANE) in a liquid formulation improves yield in some crops by increasing nutrient uptake. However, the repeated applications of liquid formulations needed to induce these effects can be expensive and time-consuming. A one-time application of a slow-release ANE formulation may be a more efficient application method. In the current study, slow-release ANE formulations were developed, and it was hypothesized that applications of these formulations would result in greater growth in maize (*Zea mays* L.) than the liquid formulation. The twelve slow-release formulations were created by combining ANE and two organic compounds in differing ratios. These formulations were molded into small spheres, with one to three of these "capsules" positioned above the maize kernels at planting. After 10 weeks within a greenhouse (October-December 2018), shoot fresh/dry weight and root dry weight of all plants, as well as nutrient concentrations of shoots/roots from selected treatments were collected. Plant height was also collected halfway through and at the end of this 10-week period. Data from slow-release treatments was compared to controls of liquid ANE, no additives, and a 50/50 composite of the two organic compounds without ANE. Analysis of variance and mean separation tests revealed that certain formulations (i.e. formulation C) and lower application rates of the slow-release biofertilizer resulted in increases in shoot dry weight of up to 47% compared to the liquid seaweed extract control. The data also indicated that higher application rates and higher concentrations of the ANE resulted in decreases in shoot dry weight of up to 33% compared to the liquid seaweed extract control. Maize with the greatest dry weights had significantly lower nutrient concentrations when compared to maize with the lowest dry weights, suggesting that increasing physiological nitrogen use efficiency could have been the potential mode of action for enhancing growth.

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LIST OF ABBREVIATIONS

ANE: *Ascophyllum nodosum* extract

ANOVA: Analysis of variance

DW: Dry weight

FW: Fresh weight

PGPR: Plant growth-promoting rhizobacteria

PNUE: Physiological nitrogen use efficiency

TK: Tukey-Kramer

1. INTRODUCTION

1.1 Fertilizers

Fertilizers are products applied to soils to restore nutrients removed by crops. One of the most prominent fertilizers used in agriculture today are nitrogen fertilizers, due to the role of nitrogen in many critical plant processes, including photosynthesis (Bassi et al. 2018). With an exponential increase in crop output needed to fulfill growing food demands, the use of nitrogen fertilizers worldwide is expected to triple over the next three decades (Tilman et al. 2002). While nitrogen fertilizers are effective, they have the potential to negatively impact the environment (Vitousek et al. 2009; Drinkwater and Snapp 2007), through air pollution, greenhouse gas emissions, soil acidification, and ground/surface-water contamination (reviewed by Chen et al. 2018). Presently, half of all fertilizing nitrogen is not absorbed by crops, leaking into the surrounding environment as a pollutant (Ladha et al. 2000; Galloway et al. 2008; Lassaletta et al. 2014). Additionally, crop soils experience decreased microbial diversity after excessive inorganic fertilizer treatments, as some symbiotic microorganism populations diminish (Mäder et al. 2002). A greater dependency on inorganic fertilizers may also develop through repeated usage due to disturbances in nutrient cycling (Singhalage et al. 2019).

1.2 Biofertilizers

Biofertilizers are an increasingly popular technology that could help minimize agricultural runoff by increasing nutrient use efficiency (Kloepper and Schroth 1978; Suslow et al. 1979; reviewed by Vessey 2003). Biofertilizers as defined by Vessey (2003) encompass any additive with a living component which increases nutrient uptake by the plant and subsequently improves growth. The term “biofertilizer” can be misleading as not

all provide primary nutrients to the plant like a traditional fertilizer (reviewed by Vessey 2003). For the purposes of this thesis, biofertilizers will be defined as any living or non-living organic substance that can enhance plant growth through biological activity. This includes biostimulants, which are described as "any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content" by du Jardin (2015).

One of the most prevalent biofertilizer forms are plant growth-promoting rhizobacteria (PGPR), as they can benefit plants in a variety of ways (reviewed by Bhattacharyya and Jha 2011). Rhizobacteria include the genera *Azospirillum*, *Azotobacter*, *Bacillus*, *Pseudomonas*, and *Serratia*, and occupy the rhizosphere or interior of the plant (Vessey 2003; Bashan et al. 2013). The rhizosphere is the soil surrounding the plant roots – it is chemically and biologically influenced by the roots (Philippot et al. 2013) and contains life which influences the physiology and development of the host plant (Igiehon and Babalola 2017). The mechanisms underlying the benefits of PGPR include the conversion of atmospheric nitrogen into usable forms, the promotion of longer and thinner roots for more efficient nutrient uptake, the introduction of new symbioses between the plant and microorganisms, and improving the accessibility of existing nutrients via solubility changes (reviewed by Bhattacharyya and Jha 2011). Biofertilizers are not solely derived from bacteria, as seaweed extracts can also contribute to enhanced growth through a variety of physiological mechanisms.

1.3 Seaweed extract biofertilizers

The use of seaweeds as a crop enhancer has been practiced throughout history. Archaic agricultural methods involved burying seaweed as a raw material or as a composite

with an organic medium (reviewed by Craigie 2010), resulting in better soil nutrition and crop yield (Thivy 1964). Seaweed fertilizers experienced widespread adoption due to their favourable effects (Chapman 1980; Nelson and Van Staden 1984), and the use of seaweed as a modern crop resource was solidified by European agronomists in the 1950s to improve soil quality (Rao 1992). Seaweed biofertilizers could play an important role in sustainable agriculture as the abundance of seaweeds may help fulfill the need for organic fertilizers worldwide (Jeswani 1999).

Seaweeds encompasses multiple aquatic multicellular eukaryotes including red, green, and brown macro-algae (Chapman 1980), all of which can be used to create biofertilizers. The growth benefits provided by seaweed extract biofertilizers include changes in root morphology to increase nutrient uptake (longer, thinner roots), germination promotion, and increased resistance to environmental stressors (reviewed by Nabti et al. 2016). These advantages are the result of an abundance of nutrients, hormones, and organic compounds present inside these seaweeds (Zodape et al. 2010). For example, seaweeds are rich in many polysaccharides that are contained within their cell walls and organelles (Murata and Nakazoe, 2001; Mwalugha et al. 2015). Polysaccharide composition varies between algae groups (Chojnacka et al. 2012) and these molecules have been shown to prevent infection by Sultana et al. (2005) whereby a spray comprised of different algae was applied to okra. At a more fundamental level, seaweeds are a plentiful source of essential soil nutrients (i.e. N, P, and K) (Imbamba 1972; Tay et al. 1987; Sethi 2012; Mirparsa et al. 2016) and contain more minerals on a dry weight basis than terrestrial plants (Manivannan et al. 2008; Kumar et al. 2009).

Research into the interactions between seaweed extracts and soil microorganisms has been underway since 1917, especially in the field of infection suppression (Oppermann 1953). Several seaweed extracts can minimize the effects of pathogens (Featonby-Smith and Staden 1983) and the presence of pathogens within soil (Al-Haj et al. 2009), subsequently improving crop growth. For example, *Ascophyllum nodosum* extracts have been shown to severely reduce the reproductive capacity of nematodes (Whapham et al. 1994).

One appealing aspect of using seaweeds in agriculture are the variety of phytohormones they provide, including cytokinin (Kingman and Senn 1977) and gibberellins (Brink and Cooper 1947). Research has established that cytokinin from seaweed extracts induce root growth that increases crop yield by enhancing absorption of water and nutrients (Russo and Berlyn 1991). As well, the gibberellic acid of red algae promotes germination by stimulating the release of growth-promoting sugars within the endosperm (Sun and Gubler 2004).

Seaweed phytohormones can also help plants recover from drought damage by promoting plant development and physiological processes that counteract oxidative damage (Kasim et al. 2015). Additionally, seaweed fertilizers are beneficial in saline conditions as they can minimize osmotic stress via osmoregulatory compounds, including sorbitol, proline, polyamines, and betaines (reviewed by Nabti et al. 2016). This supports both the plant and its symbiotic microorganisms in the rhizosphere (reviewed by Nabti et al. 2016). In short, seaweed extracts are productive fertilizers that can provide various benefits for plant welfare (Mathur et al. 2015) and add considerable nutrients to the soil (Ramarajan et al. 2012).

1.4 *Ascophyllum nodosum* extract

Brown algae extracts are a prevalent seaweed product used in modern agriculture. Of these algae, *Ascophyllum nodosum* is the most well-researched species (reviewed by Craigie 2010). *Ascophyllum nodosum* seaweeds propagate near North Atlantic shores (Keser et al. 2005) including the Nova Scotian coastline, where there are over 70 tonnes of seaweed per hectare of seafloor (Ugarte et al. 2010). Additionally, *A. nodosum* fronds possess exceptional regenerative capabilities which strengthens its potential as an industrial resource (Ugarte et al. 2010).

Ascophyllum nodosum extracts are referred to as biostimulants in most scientific literature as they are typically used to stimulate plant growth (reviewed by Craigie 2010). Even at extremely dilute (micromolar) concentrations, *A. nodosum* enhances plant growth (Crouch and van Staden, 1993; Khan et al. 2009), which may be attributed to the variety of plant hormones within this alga (Zodape et al. 2010). Two of the most prominent hormones within *A. nodosum* are cytokinins and abscisic acid (Wally et al. 2012). Cytokinins are thought to improve nutrient uptake efficiency (N, P, K, etc.) by beneficially changing roots to be longer and thinner (Russo and Berlyn 1991) and can stimulate chlorophyll production (Savasangari et al. 2011). Abscisic acid has novel effects on plants when applied as a biostimulant (Verslues et al. 2006) and can reduce the risk of desiccation by closing leaf stomata (reviewed by Craigie 2010). However, its functional effects in *A. nodosum* are unclear (Rensing et al. 2008).

Hormones are not the only beneficial substance in *A. nodosum* extracts. Other growth stimulators from this seaweed, such as various betaines, have been shown to combat common plant infections like bean rust (Tyihák 2006) on top of improving the amount of

stored chlorophyll (Blunden et al. 1996). Additionally, a large portion of *A. nodosum* is comprised of phlorotannin polymers (Ragan and Glombitza 1986) which neutralized harmful yeast fungi in an experiment using phlorotannins from other brown algae (Lopes et al. 2013).

1.5 Fertilizer formulations

Regardless of whether a biofertilizer contains living or non-living active ingredients, its performance, efficiency, and perceived marketability is ultimately determined by its formulation (reviewed by Vessey, 2003). Biofertilizer formulations can vary in terms of carrier type (e.g. liquid or granular), stabilizer (e.g. buffers), and coatings or sticking agents (reviewed by John et al. 2010). PGPR biofertilizers are formulated by incorporating a microorganism into a carrier and pairing it with a protective stabilizer before application (Xavier et al. 2004). To create these fertilizers, PGPR are proliferated in a specially-designed liquid environment then added to a medium such as soil or mineral grains (Herrmann and Lesueur 2013).

No biofertilizer composition is optimal in all environments and for all crops (Herrmann and Lesueur 2013), so the type of microorganism and the formulation used in a PGPR biofertilizer is important for maximizing the growth of different crops. For example, the ability of the introduced microorganism to compete with other microorganisms could be poor, leading to extirpation of the introduced species, or too great, leading to extirpation of native species (Herrmann and Lesueur 2013). To ensure maximum crop growth, an ideal composition must facilitate the optimal conditions for microorganism function upon application (McQuilken et al. 1998).

The types of formulations most commonly used for seaweed biofertilizers include liquids and powders (Alam et al. 2013), which are applied by coating the seed surface (Ben Rebah et al. 2007). Powders such as peat are usually sterilized before the inoculant is added to ensure compatibility, though Hassan et al. (2018) found that sterilizing halophyte root powder lowered its ability to decrease soil salinity. Liquids are relatively popular due to their ease of manufacture and use (Albareda et al. 2008). One shortcoming of this formulation is an increased vulnerability of the inoculant to outside forces (Singleton et al, 2002; Tittabutr et al. 2007; Albareda et al. 2008).

1.6 Slow-release formulations

Slow-release formulations are a promising application technique for fertilizers and have advantages over fast-release methods (i.e. liquids and powders). Pollution from fast-release fertilizer runoff can be attributed to the timing of application – much of the fertilizer is not absorbed by the crop during the early stages of growth due to their low nutrient requirements (reviewed by Azeem et al. 2014). Slow-release formulations are designed to retard the supply of active ingredients so that the needs of the crop are fulfilled by one application (reviewed by Timilsena et al. 2014). This ensures that the active ingredients are accessible when crop requirements are greatest, improving nutrient use efficiency and harvest returns (Shaviv 2001). These formulations are also more economic as they require fewer applications at lower amounts when compared to fast-release formulations (reviewed by Azeem et al. 2014). The benefits of using a slow-release formulation are numerous, from enhancing substrate quality and germination (reviewed by Azeem et al. 2014) to minimizing browning and osmotic stress (Shaviv 2001; Trenkel 2010).

Organic or mineral coatings can be applied to the exterior of slow-release fertilizer particles, with uncoated formulations having a greater presence in modern agriculture (Fan and Li 2010). The diffusion of fertilizers from uncoated formulations can be mediated by chemical or biological processes such as decomposition and the release of fertilizers from coated formulations are highly influenced by coating composition (reviewed by Timilsena et al. 2014). Formulation, coating characteristics, and the environment itself all influence fertilizer release (reviewed by Azeem et al. 2014).

"Eco-friendliness" is a priority when designing slow-release formulations (Blouin and Rindt 1967) due in part to the importance of plant symbionts (Celsia and Mala 2014). In fact, microbe activity can drive the release of active ingredients from many different slow-release formulations. For instance, the amount of PGPR in the soil can directly influence the emission rate of inorganic fertilizers by increasing solubility (Celsia and Mala 2014). Additionally, slow-release fertilizers have applications in the field of bioremediation. The precision of slow-release formulations means that soil microorganisms can be supplied with the exact amount of nutrients required to sustain restoration (Reis et al. 2013).

1.7 Objectives

Conventional liquid formulations of *A. nodosum* biofertilizers improve plant growth in a variety of crops (Battacharyya et al. 2015) but require frequent applications (e.g. monthly applications during the growing season), presenting a significant expense for some farmers (Timilsena et al. 2014). Alternatively, slow-release formulations enhance the effects of inorganic fertilizers by increasing the availability of active ingredients and require fewer applications (reviewed by Azeem et al. 2014; Timilsena et al. 2014). The

overall goal of this research is to develop a formulation that will make ANE more effective as a biostimulant by increasing its exposure time to roots. To this author's knowledge, slow-release formulations represent an unexplored approach to agriculture as an equivalent seaweed extract release method has not existed heretofore.

The objective of this study is to test if slow-release formulations of *A. nodosum* extract will improve the growth of maize compared to a standard ANE liquid application. To achieve this, differing formulations and application rates of an organic treatment were applied to maize (*Zea mays*) alongside controls in a greenhouse. To determine the effect of this slow-release formulation on plant growth and nutrient accumulation, the height, shoot/root weight, and concentration of nutrients in maize were measured after 10 weeks of growth and contrasted against a liquid seaweed extract control.

2. MATERIALS AND METHODS

2.1 Preparation

This experiment tested the effects of four concentrations of *Ascophyllum nodosum* (L.) Le Jolis seaweed extract (ANE; Acadian Seaplants Ltd., Dartmouth, NS) on the growth of maize (*Zea mays* L). These four concentrations were obtained by adding different ratios of the *A. nodosum* seaweed extract to organic compound 1 which created a solid product. Twelve slow-release formulations were then created by combining the ANE and organic compound 1 composite to an organic compound 2 in three different ratios for each ANE concentration (Table 2.1). Note that as these formulations are proprietary information, the concentration of ANE (i.e. SC1, SC2, SC3) used is not provided, and the names of treatment components have been replaced with the generic "compound 1" and "compound 2".

Three control groups were created to compare the effects of our application method on plant growth and nutrient accumulation. Control groups 1 and 2 were treated with no additives or the liquid *A. nodosum* seaweed extract (the latter according to the manufacturer's recommended rates of application; diluted with water to 1 mL/L), respectively, while the control group 3 formulation was comprised of an equal mixture of organic compound 1 and 2 without ANE.

A small metal press was used to mold the biofertilizer formulations into 70 small spheres (hereon referred to as "capsules") to satisfy three application rates. The biofertilizer capsules were tested on maize grown inside a greenhouse at the green roof testing facility of Saint Mary's University (44°37'54"N 63°34'53"W) from October 1st, 2018 until December 10th of the same year. Overhead LR48877 grow lamps (P.L Light Systems,

Beamsville, ON) inside the greenhouse provided supplemental lighting for 16 hours each day (4:00 AM-8:00 PM). Internal temperatures were set to 25°C by supplemental heating and cooling systems in the greenhouse and ranged from 1.60-36.13°C as determined by a HOBO® H08-004-02 hourly temperature logger (Onset Computer Corporation, Bourne, MA) placed in the center of the room.

2.2 Planting

To prevent soil loss through watering, the bottom of all 2.5 L plant pots were covered with a cloth liner. Each pot was then filled with 2 L of Premier® Triple Mix soil (Premier Tech Ltd, Rivière-du-Loup, QC) with space left for water. All pots were organized into two 9 by 23 arrays on benches within the greenhouse and were marked by a unique label which signified treatment. Two-inch holes were dug into the soil of each pot using a test tube, with two maize kernels placed inside each hole to increase the odds of successful emergence. Biofertilizer capsules were put on top of these kernels at three different application rates (one to three capsules). Control groups 1 and 2 were prepared without capsules as they were treated with no additives or liquid seaweed extract, respectively. After the holes were filled with soil, the pots were watered using a hose until water exuded from the bottom.

Pots were originally placed in order of treatment for ease of planting. Seven days after planting the maize had newly emerged and each pot was assigned a number sequentially from 1 to 410. The order of these numbers was then arranged into a completely randomized experimental design using an online list randomizer. Each pot was moved to their newly designated position individually. This was done to eliminate the confounding

variable of greenhouse location (potential fluctuations in temperature/light) on final growth.

2.3 Crop Maintenance

Regular crop maintenance began immediately after planting. Lab ladles were used to apply 100 mL of water to each 2.5 L pot daily in the evening between 4:00 PM-9:00 PM. Eight days after planting, the pots from control 2 were treated with 100 mL of liquid seaweed extract (diluted with water to 1 mL/L), with subsequent applications at the same rate once every 3 weeks thereafter. By this point most seeds had germinated with emerged seedlings. To ensure there was only one test plant per pot, the entire sheath of the smaller plant was removed by cutting just beneath the soil surface. The following week, 100 mL of half-strength Hoagland nutrient solution (Hoagland and Arnon 1950) was added to all 2.5 L pots and was applied twice a week (Monday and Thursday) thereafter. From November 28th onwards, the water and Hoagland solution application rates were increased to 200 mL in response to the advanced growth stage of the maize. After growing for 1 month the pots were removed from the benches and placed on the greenhouse floor to enable more headspace for plant growth (i.e. the top of the shoots were coming too close to the grow lamps and were at risk of heat stress).

2.4 Data Collection

Maize height was determined 1 month after planting the kernels using a tape measure by measuring the distance from the base of the plant to the tip of the longest leaf blade, held erect (Rood 1985). The developmental stage of each plant (Pioneer Hybrid International n.d.) was also identified by counting both the number of leaf collars (indicated

by a sheath notch) and the younger leaves which had not fully emerged (leaf collar not visible).

Plant height and growth stage were recorded again prior to harvest on December 10th and 11th. Harvesting involved the removal of maize shoots from the pots by cutting just above the highest roots with clippers. The shoots were then divided into sections to fit on a Taylor® glass kitchen scale (Taylor Holdco, Oak Brook, IL) to determine individual shoot fresh weight, with shoot sections placed into individual paper bags. Three days after the shoots were removed from the pots, the roots of each plant were taken from the soil, cleaned with water, and placed into individual paper bags. The paper bags containing roots or shoots were dried in an oven for 1 week at 70°C. Final dry weights (DW) of the roots and shoots were calculated by subtracting the weight of a dry paper bag from that of each dried paper bag with its plant contents (roots or shoots). Dry weights were measured using a Denver Instrument PK-352 laboratory scale (Denver Instrument, Bohemia, NY). Adding root and shoot dry weight together produced total dry weight. Using the shoot fresh and dry weight data, the shoot moisture content was calculated with the formula ((shoot FW – shoot DW) / shoot FW) * 100).

Laboratory analyses of shoot, root and soil macro/micronutrient concentration (e.g. N, P, K, Fe, Zn) was carried out by the Nova Scotia Department of Agriculture's Analytical Lab (Dalhousie University, Bible Hill, NS). However, due to cost only a subset of treatments were analysed. The three treatments with the highest dry weights and the three treatments with the lowest dry weights were selected, along with the controls. For shoots, three replicates were created for each treatment by combining the dried shoot sections of 3-4 respective plants. For roots, one replicate was created for each treatment by combining

the dried roots of all 10 respective plants. Three random 100 g soil samples were collected from pots before planting and air dried (Table 2.2).

The amount of nutrients accumulated in the maize shoots (nutrient content) of the three highest dry weight and three lowest dry weight treatments as well as the liquid seaweed extract control were calculated with the formula ((shoot DW * nutrient concentration (%)) / 100) for macronutrients (in g/shoot) and (shoot DW * nutrient concentration (ppm)) for micronutrients (in µg/shoot). Physiological nitrogen use efficiency (PNUE) is a commonly used measure of a plant's ability to produce dry weight relative to the nitrogen content of the plant (Vijayalakshmi et al. 2013). Nitrogen use efficiency can be assessed in various ways (Weih et al. 2010). Here, PNUE was calculated by dividing the average shoot dry weight by the nitrogen content (g shoot DW/g N in shoot) for the three highest/lowest dry weight treatments and the liquid seaweed extract control.

2.5 Statistical Analysis

Analysis of variance with Tukey-Kramer mean separation tests (type II sum of squares; significance level of 0.05) from CoStat (CoHort software) assessed treatment effects on growth and nutrient accumulation.

Table 2.1. Biofertilizer capsule application experimental design. The four seaweed extract concentrations of *A. nodosum* (SC 1, SC 2, SC 3, SC 4) were obtained by combining differing ratios of the seaweed extract and an organic compound 1. Formulations represent differing ratios of the seaweed extract by organic compound 1 composite and an organic compound 2. All slow-release formulations (including control 3) were provided to maize at three application rates (AR).

Treatment		**Number of replicates**			**Total**
Control 1	No additives	10			10
Control 2	Liquid ANE	10			10
		AR 1	AR 2	AR 3	
Control 3	Formulation minus ANE	10	10	10	30
SC 1	Formulation A	10	10	10	30
	Formulation B	10	10	10	30
	Formulation C	10	10	10	30
SC 2	Formulation A	10	10	10	30
	Formulation B	10	10	10	30
	Formulation C	10	10	10	30
SC 3	Formulation A	10	10	10	30
	Formulation B	10	10	10	30
	Formulation C	10	10	10	30
SC 4	Formulation A	10	10	10	30
	Formulation B	10	10	10	30
	Formulation C	10	10	10	30
Total		150	130	130	410

Table 2.2. Average nutrient concentration of Premier® Triple Mix soil collected prior to planting.

Nutrient	**Average Concentration ± SE**
Nitrogen (%) ± SE	2.18 ± 0.04
P_2O_5 (kg/ha) ± SE	90 ± 7.22
K_2O (kg/ha) ± SE	140 ± 10.37
Calcium (kg/ha) ± SE	2363 ± 99.88
Magnesium (kg/ha) ± SE	285 ± 13.30
Sodium (kg/ha) ± SE	54 ± 3.93
Sulfur (kg/ha) ± SE	42 ± 6.24
Aluminum (ppm) ± SE	30.67 ± 1.86
Copper (ppm) ± SE	0.20 ± 0.01
Iron (ppm) ± SE	66.67 ± 3.53
Manganese (ppm) ± SE	3.33 ± 0.33
Zinc (ppm) ± SE	0.66 ± 0.05

3. RESULTS

To explore the effects of slow-release formulations of an *Ascophyllum nodosum* extract (ANE) biofertilizer on maize (*Zea mays* L.) growth and nutrient accumulation, three formulations composed of different ratios of two organic compounds, at four levels of seaweed extract concentration, and at three application rates (1, 2, or 3 capsules) were applied to maize grown for 10 weeks in a greenhouse. Growth parameters measured included shoot height, shoot fresh weight, shoot and root dry weight, and tissue nutrient concentrations.

3.1 Control Analysis

The experimental design tested three unique controls (no additives, liquid seaweed extract, and the slow-release formulation minus *A. nodosum)*. However, an analysis of variance (ANOVA) and Tukey-Kramer (TK) mean separation test among these controls for shoot dry weight (results not shown) found no significant differences, with p-values being less than α (0.05). In terms of biological and agronomic significance, the liquid seaweed extract control is the most relevant control because it is the standard formulation used on commercial crops. Given this, the liquid seaweed extract was the only control used in subsequent statistical analyses.

3.2 Maize Height

The maximum height of each maize plant was determined both midway through and at the end of the growing period, the results of which are shown below (Table 3.1).

Table 3.1. Mean height in centimeters of maize treated with 12 unique formulations of a slow-release *A. nodosum* extract (ANE) biofertilizer over three application rates, as well as three controls. Data was collected halfway through and at the end of the growing period. Treatments consisted of: no additives control (C-NA); liquid ANE control (C-LSE); slow-release formulation minus ANE control (C-FA); four concentrations of ANE (SC1, SC2, SC3, SC4); three formulations (FA, FB, FC); and three application rates (AR1, AR2, AR3). Asterisks are a scale of significance for the analysis of variance, with one asterisk meaning the effect what somewhat significant and three asterisks meaning the effect was highly significant. Additionally, "ns" means that the effect was not significant.

Treatment (control/ANE concentration – formulation – application rate)	Midway mean height (cm) ± SE	Final mean height (cm) ± SE
Control-NA	93.28 ± 5.71	161.70 ± 6.44
Control-LSE	94.41 ± 4.97	158.15 ± 4.99
Control-FA-AR1	100.71 ± 3.33	164.45 ± 4.02
Control-FA-AR2	102.49 ± 5.00	163.90 ± 5.85
Control-FA-AR3	101.54 ± 2.27	166.85 ± 5.64
SC1-FA-AR1	102.87 ± 3.79	164.10 ± 6.92
SC1-FA-AR2	87.31 ± 6.52	156.05 ± 6.90
SC1-FA-AR3	76.26 ± 6.57	147.70 ± 9.07
SC1-FB-AR1	94.93 ± 4.65	166.62 ± 4.51
SC1-FB-AR2	95.72 ± 5.21	169.05 ± 6.14
SC1-FB-AR3	97.30 ± 4.39	166.22 ± 6.54
SC1-FC-AR1	109.35 ± 2.26	175.90 ± 4.93
SC1-FC-AR2	110.17 ± 2.81	182.30 ± 3.60
SC1-FC-AR3	105.27 ± 2.68	164.50 ± 4.23
SC2-FA-AR1	102.93 ± 3.40	172.45 ± 5.00
SC2-FA-AR2	94.11 ± 4.27	167.39 ± 5.14
SC2-FA-AR3	93.19 ± 6.11	171.06 ± 3.83
SC2-FB-AR1	103.51 ± 3.97	173.90 ± 8.79
SC2-FB-AR2	95.25 ± 4.13	164.82 ± 5.32
SC2-FB-AR3	89.281 ± 4.72	163.95 ± 5.59
SC2-FC-AR1	101.85 ± 3.10	166.15 ± 5.08
SC2-FC-AR2	90.23 ± 4.89	164.05 ± 4.94
SC2-FC-AR3	82.23 ± 6.34	152.35 ± 7.59

SC3-FA-AR1	99.31 ± 4.50	166.10 ± 4.78
SC3-FA-AR2	89.28 ± 5.55	165.81 ± 6.60
SC3-FA-AR3	92.70 ± 6.77	171.25 ± 6.67
SC3-FB-AR1	102.81 ± 3.13	169.60 ± 8.11
SC3-FB-AR2	96.90 ± 3.31	166.65 ± 3.84
SC3-FB-AR3	91.23 ± 6.69	166.28 ± 5.78
SC3-FC-AR1	102.81 ± 4.33	168.00 ± 5.61
SC3-FC-AR2	107.12 ± 2.80	179.60 ± 3.77
SC3-FC-AR3	103.32 ± 2.89	170.45 ± 5.13
SC4-FA-AR1	95.56 ± 5.50	161.50 ± 5.48
SC4-FA-AR2	99.76 ± 5.50	165.40 ± 4.42
SC4-FA-AR3	89.22 ± 4.88	172.85 ± 7.36
SC4-FB-AR1	104.20 ± 2.42	162.45 ± 5.19
SC4-FB-AR2	90.93 ± 8.77	153.25 ± 12.03
SC4-FB-AR3	92.71 ± 5.17	158.75 ± 6.35
SC4-FC-AR1	91.82 ± 5.61	161.86 ± 4.86
SC4-FC-AR2	88.46 ± 5.11	158.75 ± 6.05
SC4-FC-AR3	88.46 ± 9.13	155.40 ± 11.59
3-way ANOVA		
Main effects	**p (α = 0.05)**	**p (α = 0.05)**
ANE concentration	0.1353, ns	0.1543, ns
Formulation	0.0176, *	0.6917, ns
Application rate	0.0000, ***	0.4960, ns
Interaction		
ANE concentration * Formulation	0.0000, ***	0.0034, **
ANE concentration * application rate	0.5016, ns	0.3247, ns
Formulation * application rate	0.6461, ns	0.2770, ns
ANE concentration * Formulation * application rate	0.1206, ns	0.8493, ns

The average height of the maize was 96.6 centimeters halfway through the growing period and 166.3 centimeters at the end of the growing period. The maximum mean height measurement halfway through the growing period was for C formulation at the lowest concentration of *A. nodosum* with the median application rate (SC1-FC-AR2) at 110.17 centimeters. The minimum height of 76.26 centimeters occurred for formulation A at the

same ANE concentration and the highest application rate (SC1-FA-AR3). Mean height collected at the end of the growing period ranged from 182.3 centimeters (SC1-FC-AR2; the same as midway) to 147.7 centimeters (SC1-FA-AR3). This represents a 1.7- and 1.9-fold difference in maximum and minimum mean height, respectively, between time periods.

Three-Way analysis of variance for seaweed extract concentration, formulation, and application rate

Analysis of variance for midway height with the three experimental factors (ANE concentration, formulation, and application rate; Table 3.1; see Appendix for full ANOVA table) and the liquid ANE control revealed a significant effect for formulation ($p = 0.0176$), application rate ($p < 0.0001$), and the interaction between ANE concentration and formulation ($p < 0.0001$). This interaction was also the only significant effect found within the "end height" ANOVA ($p = 0.0034$; Table 3.1; see Appendix for full ANOVA table). Additionally, the Tukey-Kramer mean separation test (see Appendix for full TK table) found the midway heights of both the lowest application rate (AR1) and formulation C to be significantly higher than that of the highest application rate (AR3) and formulation A, respectively.

3.3 Maize Weight

Shoot fresh weight and all dry weights are given in Table 3.2 below.

Table 3.2. Mean shoot weight (fresh/dry), root dry weight, and total dry weight for maize (*Zea mays*) treated with 12 unique formulations of a slow-release *A. nodosum* extract (ANE) biofertilizer over three application rates as well as three controls. See Table 3.1 for legend.

Treatment (control/ANE concentration – formulation – application rate)	Mean shoot fresh weight (g) ± SE	Mean shoot dry weight (g) ± SE	Mean root dry weight (g) ± SE	Total dry weight (g) ± SE
Control-NA	136.25 ± 16.74	18.23 ± 2.91	1.65 ± 0.23	19.88 ± 2.96
Control-LSE	144.25 ± 12.49	19.92 ± 2.17	1.94 ± 0.20	21.87 ± 2.23
Control-FA-AR1	160.20 ± 9.98	23.20 ± 2.11	2.29 ± 0.19	25.49 ± 2.14
Control-FA-AR2	159.00 ± 11.12	23.91 ± 2.30	2.33 ± 0.20	26.24 ± 2.32
Control-FA-AR3	165.40 ± 6.47	23.31 ± 1.34	2.39 ± 0.18	25.70 ± 1.34
SC1-FA-AR1	166.20 ± 13.25	23.25 ± 2.44	2.43 ± 0.23	25.68 ± 2.52
SC1-FA-AR2	135.40 ± 18.98	17.19 ± 3.05	1.91 ± 0.31	19.10 ± 3.17
SC1-FA-AR3	110.20 ± 17.65	13.29 ± 2.70	1.46 ± 0.28	16.29 ± 2.62
SC1-FB-AR1	148.60 ± 13.78	19.87 ± 2.47	2.14 ± 0.31	22.01 ± 2.59
SC1-FB-AR2	151.00 ± 15.22	21.35 ± 2.88	1.91 ± 0.29	23.26 ± 2.96
SC1-FB-AR3	152.70 ± 22.01	20.38 ± 3.34	2.11 ± 0.34	24.98 ± 2.63
SC1-FC-AR1	177.40 ± 11.32	26.64 ± 1.80	2.75 ± 0.16	29.39 ± 1.84
SC1-FC-AR2	198.80 ± 9.68	29.27 ± 1.99	2.55 ± 0.25	31.82 ± 2.08
SC1-FC-AR3	154.25 ± 19.70	21.67 ± 2.93	2.48 ± 0.35	26.83 ± 1.69
SC2-FA-AR1	173.50 ± 9.05	24.78 ± 1.91	2.48 ± 0.15	27.26 ± 1.95
SC2-FA-AR2	153.50 ± 14.13	20.26 ± 2.38	1.80 ± 0.20	22.07 ± 2.42
SC2-FA-AR3	129.90 ± 25.93	17.10 ± 3.78	1.29 ± 0.29	22.98 ± 2.94
SC2-FB-AR1	179.10 ± 13.25	25.20 ± 2.32	2.49 ± 0.16	27.69 ± 2.31
SC2-FB-AR2	148.75 ± 13.16	19.49 ± 2.45	1.95 ± 0.21	21.44 ± 2.48
SC2-FB-AR3	149.10 ± 17.53	18.89 ± 3.04	1.75 ± 0.26	20.64 ± 3.11
SC2-FC-AR1	160.45 ± 11.60	21.58 ± 2.15	2.18 ± 0.18	23.76 ± 2.16
SC2-FC-AR2	144.15 ± 10.81	18.47 ± 1.99	1.69 ± 0.20	20.16 ± 2.04
SC2-FC-AR3	123.30 ± 17.46	14.86 ± 2.81	1.54 ± 0.25	16.41 ± 2.89
SC3-FA-AR1	172.50 ± 10.04	24.14 ± 2.15	2.12 ± 0.19	26.26 ± 2.19
SC3-FA-AR2	157.75 ± 17.49	20.54 ± 2.78	1.65 ± 0.24	22.18 ± 2.84
SC3-FA-AR3	169.15 ± 14.57	22.77 ± 2.52	2.01 ± 0.16	24.78 ± 2.51
SC3-FB-AR1	179.45 ± 13.07	24.99 ± 2.57	2.37 ± 0.14	27.36 ± 2.51
SC3-FB-AR2	166.40 ± 12.17	22.55 ± 2.26	2.13 ± 0.22	24.68 ± 2.34

SC3-FB-AR3	140.30 ± 21.00	17.69 ± 3.2	1.64 ± 0.28	21.48 ± 2.78
SC3-FC-AR1	168.50 ± 7.93	21.92 ± 1.78	2.10 ± 0.16	24.02 ± 1.81
SC3-FC-AR2	190.30 ± 8.11	27.83 ± 1.42	2.29 ± 0.20	30.12 ± 1.44
SC3-FC-AR3	174.00 ± 9.25	23.74 ± 1.85	2.25 ± 0.17	25.99 ± 1.87
SC4-FA-AR1	162.40 ± 12.35	22.19 ± 2.39	2.18 ± 0.24	24.37 ± 2.45
SC4-FA-AR2	155.50 ± 10.68	20.82 ± 2.11	1.93 ± 0.19	22.75 ± 2.14
SC4-FA-AR3	159.35 ± 14.63	20.61 ± 2.44	1.64 ± 0.16	22.25 ± 2.45
SC4-FB-AR1	160.45 ± 7.24	22.92 ± 1.83	2.36 ± 0.20	25.28 ± 1.85
SC4-FB-AR2	143.30 ± 17.92	18.43 ± 2.54	1.67 ± 0.22	22.29 ± 1.57
SC4-FB-AR3	111.70 ± 23.61	14.38 ± 3.32	1.40 ± 0.30	19.73 ± 2.78
SC4-FC-AR1	141.55 ± 12.80	19.43 ± 2.55	1.88 ± 0.29	21.31 ± 2.64
SC4-FC-AR2	139.00 ± 11.69	18.17 ± 2.35	1.67 ± 0.19	19.84 ± 2.39
SC4-FC-AR3	147.00 ± 20.34	20.00 ± 3.36	1.76 ± 0.27	24.14 ± 2.78
3-way ANOVA				
Main effects	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**
ANE concentration	0.0123, *	0.0293, *	0.0014, **	0.0267, *
Formulation	0.5781, ns	0.3451, ns	0.0923, ns	0.3125, ns
Application rate	0.0830, ns	0.0099, **	0.0000, ***	0.0056, ***
Interaction				
ANE concentration * Formulation	0.0009, ***	0.0004, ***	0.0027, **	0.0003, **
ANE concentration * application rate	0.5639, ns	0.4935, ns	0.2291, ns	0.4855, ns
Formulation * application rate	0.3842, ns	0.2646, ns	0.2218, ns	0.2728, ns
ANE concentration * Formulation * application rate	0.2196, ns	0.2598, ns	0.5132, ns	0.2711, ns

Shoot fresh weight ranged from 198.80 grams (SC1-FC-AR2) to 110.20 grams (SC1-FA-AR3), with an average value of 159.37 grams. Similarly, the maximum value for mean shoot dry weight was 29.27 grams (SC1-FC-AR2) with a minimum value of 13.29 grams (SC1-FA-AR3) and an average of 20.99 grams. The range of mean root dry weight values were from 2.75 grams (SC1-FC-AR1) to 1.40 grams (SC4-FB-AR3), averaging at 2.05 grams. The average total dry weight was 23.65 grams, with a minimum value of 16.29 grams (SC1-FA-AR3) and a maximum value of 31.82 grams (SC1-FC-AR2).

Mean shoot fresh weight was at least five times that of mean shoot dry weight for each treatment. It is interesting to note that the mean shoot dry weight values for the slow-release treatments (21.61 g) was larger than that of the liquid ANE control (19.92 g). ANE concentration, application rate, and the interaction between ANE concentration and formulation had a significant effect in the three-way ANOVA of dry weights.

Three-way analysis of variance for seaweed extract concentration, formulation, and application rate

Shoot Dry Weight

To determine the significance of the three experimental factors (ANE concentration, formulation, and application rate) on shoot dry weight, a three-way ANOVA was carried out (Table 3.2; see Appendix for full ANOVA table) which showed a significant effect for ANE concentration ($p = 0.0293$) and application rate ($p = 0.0099$), but not formulation ($p = 0.3451$). There were no significant interactions besides ANE concentration and formulation ($p = 0.0004$).

Root Dry Weight

For root dry weight the three-way ANOVA (Table 3.2; see Appendix for full ANOVA table) found significant effects for ANE concentration ($p = 0.0014$) and application rate ($p < 0.0001$), as well as for the interaction between ANE concentration and formulation ($p = 0.0027$). In contrast to shoot weight, the Tukey-Kramer mean separation test (see Appendix for full TK table) showed that there was a significant difference between the maximum and minimum values of both ANE concentration (SC1 vs. SC4) and application rate (AR1 vs. AR3).

Total Dry Weight

Total dry weight effects with significance (Table 3.2; see Appendix for full ANOVA table) were ANE concentration ($p = 0.0267$) and application rate ($p = 0.0056$), while formulation was not significant ($p = 0.3125$). Between these factors, only the interaction between ANE concentration and formulation was significant ($p = 0.0003$).

Shoot Fresh Weight

A statistical analysis (Table 3.2; see Appendix for full ANOVA table) testing the effects of experimental factors on maize shoot weight immediately after harvest was also performed, the only significant effects being ANE concentration ($p = 0.0123$) and its interaction with formulation ($p = 0.0009$).

Two-way analysis of variance for application rate and a combination of formulation and seaweed extract concentration

The ANOVA for dry weight showed formulation having a non-significant effect individually but was highly significant when interacting with ANE concentration (Table 3.2). Because of this, it was decided to combine these factors and test them in two-way ANOVA with application rate to see if the mean separation tests produced any significant differences. As stated previously, the only control included in these analyses were the liquid seaweed extract.

Table 3.3. Two-way analyses of variance (seaweed extract concentration by formulation combination and application rate) for the mean shoot, root, and total dry weight for maize (*Zea mays*) treated with slow-release *A. nodosum* extract (ANE) biofertilizers and a liquid seaweed extract control.

2-way ANOVA			
Treatment	**Mean shoot dry weight (g)**	**Mean root dry weight (g)**	**Total dry weight (g)**
Main effects	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**
ANE concentration by formulation	0.0383, *	0.0020, **	0.0352, *
Application rate	0.0126, *	0.0000, ***	0.0074, **
Interaction			
ANE concentration by formulation * Application rate	0.5145, ns	0.2688, ns	0.5082, ns

For all three dry weight measures, the two-way ANOVA showed a significant effect for ANE concentration by formulation and application rate.

Shoot Dry Weight

A significant difference for shoot dry weight was found (Table 3.3; see Appendix for full ANOVA table) among ANE concentration by formulation ($p = 0.0383$) and application rate ($p = 0.0126$), though these factors did not have a significant interaction ($p = 0.5145$).

Root Dry Weight

For root dry weight significant effects were found (Table 3.3; see Appendix for full ANOVA table) for ANE concentration by formulation ($p = 0.0020$), as well as for application rate ($p < 0.0001$). There was no significance between interacting factors ($p = 0.2688$), and the maximum and minimum measures within both factors (ANE concentration 1 versus 4; application rate 1 versus 3) were significantly different as determined by the Tukey-Kramer mean separation test (see Appendix for full TK table).

Total Dry Weight

The total dry weight factors of ANE concentration by formulation ($p = 0.0352$) and application rate ($p = 0.0074$) were designated as significant effects (Table 3.3; see Appendix for full ANOVA table) but were not significant as an interaction ($p = 0.5082$).

Two-way analysis of variance for application rate and seaweed extract concentration within formulation C only

Formulation C produced the highest overall values for shoot, root, and total dry weight (Table 3.2) and was the only formulation with significant effects (results not shown). To more thoroughly examine the effects of ANE concentration and application rate on dry weight within formulation C, two-way ANOVAs were conducted within this single formulation.

Table 3.4. Two-way analysis of variance (seaweed extract concentration and application rate) within formulation C for the mean shoot, root, and total dry weight for maize (*Zea mays*) treated with slow-release *A. nodosum* extract (ANE) biofertilizers and a liquid seaweed extract control.

2-way ANOVA			
Treatment	**Mean shoot dry weight (g)**	**Mean root dry weight (g)**	**Total dry weight (g)**
Main effects	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**
ANE concentration	0.0000, ***	0.0000, ***	0.0000, ***
Application rate	0.2910, ns	0.4472, ns	0.3648, ns
Interaction			
ANE concentration * Application rate	0.0544, ns	0.3857, ns	0.0647, ns

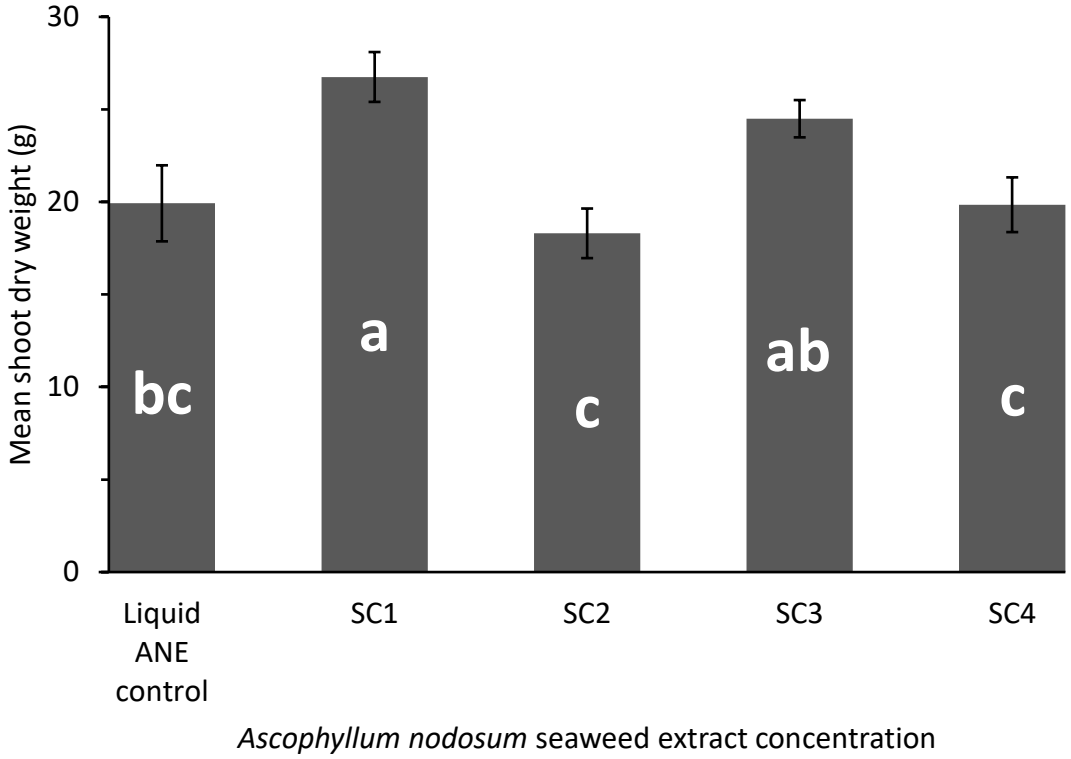
The three-way ANOVA for formulation C (Table 3.4) had a highly significant effect ($p \leq 0.00001$) for ANE concentration in all three dry weight measures.

Tukey-Kramer mean separation tests for seaweed extract concentration

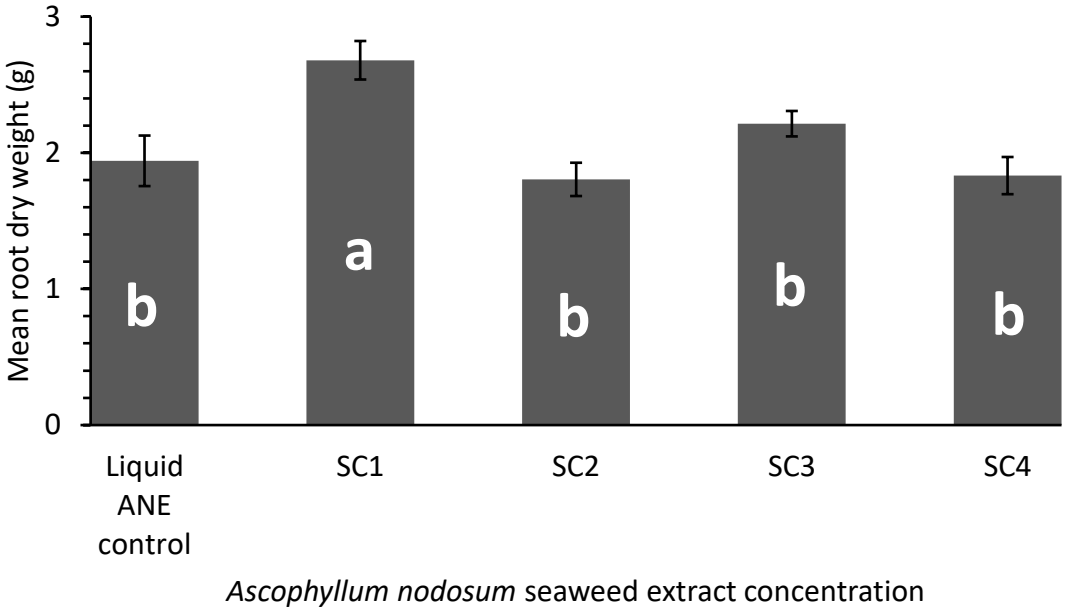
ANE concentration had a large effect on all dry weights ($p \leq 0.05$) in the two-way ANOVA (Table 3.4; see Appendix for full ANOVA tables), though application rate did not ($p \geq 0.05$). Additionally, the interaction between these two factors was not significant

($p \geq 0.05$). The Tukey-Kramer mean separation test also showed significant differences within ANE concentration (Figure 3.1).

A.



B.



C.

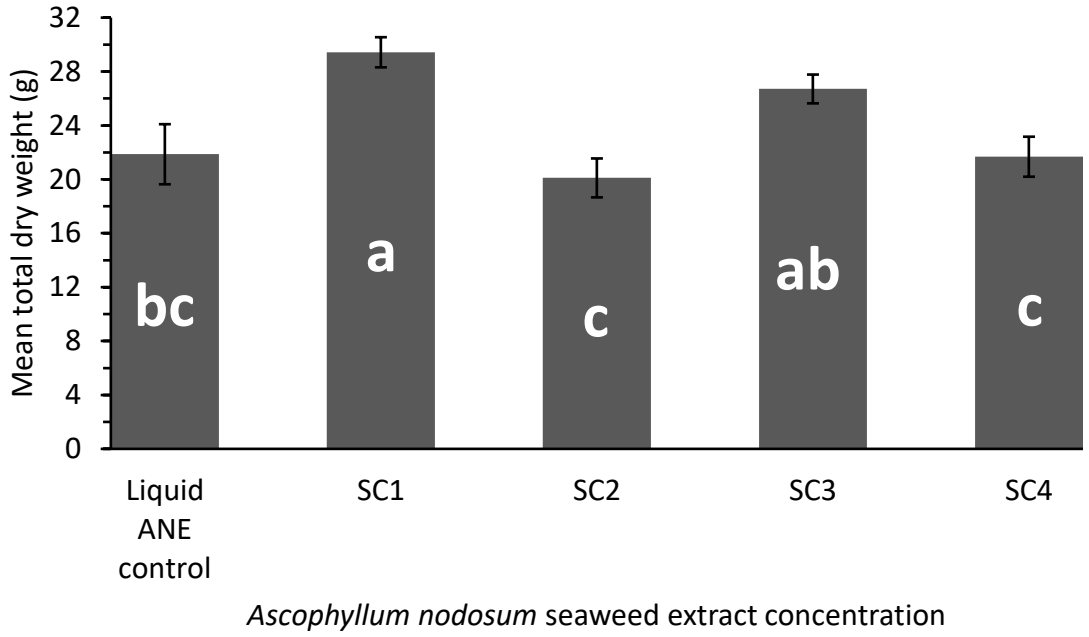


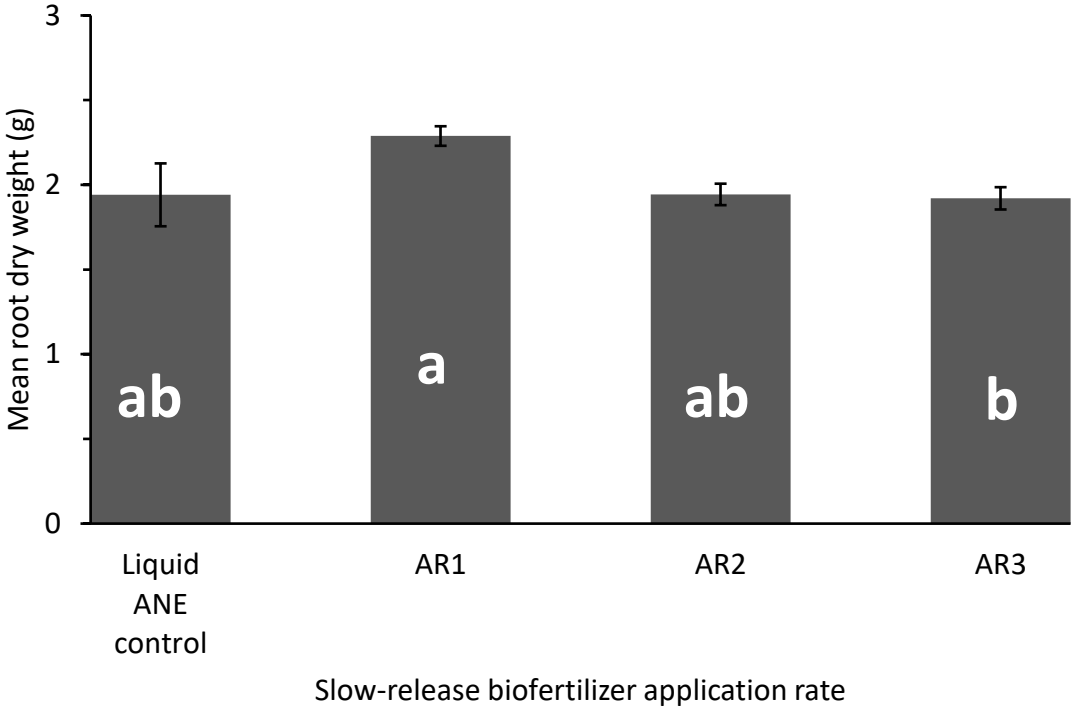
Figure 3.1. Effects of increasing *A. nodosum* seaweed extract concentrations (SC; lowest: SC1 to highest: SC4) from the formulation C slow-release biofertilizer on the mean shoot (A.), root (B.) and total (C.) dry weight of maize (*Zea mays*) compared to the liquid *A. nosodum* extract (ANE) control. Seaweed concentrations labelled with the same letter were not significantly different from each other ($\alpha = 0.05$). Error bars represent standard error.

For plants treated with formulation C, the Tukey-Kramer mean separation test indicated that the mean shoot dry weight for plants treated with the lowest ANE concentration (SC1) were significantly different than that of the liquid ANE control as well as concentrations SC2 and SC4 ($p < 0.0001$).

Tukey-Kramer mean separation tests for application rate

The effect of increasing application rate on the dry weight of the maize shoots and roots were determined by a Tukey-Kramer mean separation test which ran concurrent to the initial three-way analysis (see Appendix for full TK table). The tendency of root dry weight to decrease in response to higher application rates was shown through the significant difference between AR1 and AR3 for root dry weight (A., Figure 3.2) but was absent from the shoot dry weight (B., Figure 3.2).

A.


Mean root dry weight (g)
3
2
1
0
ab
a
ab
b
Liquid ANE control
AR1
AR2
AR3
Slow-release biofertilizer application rate

B.

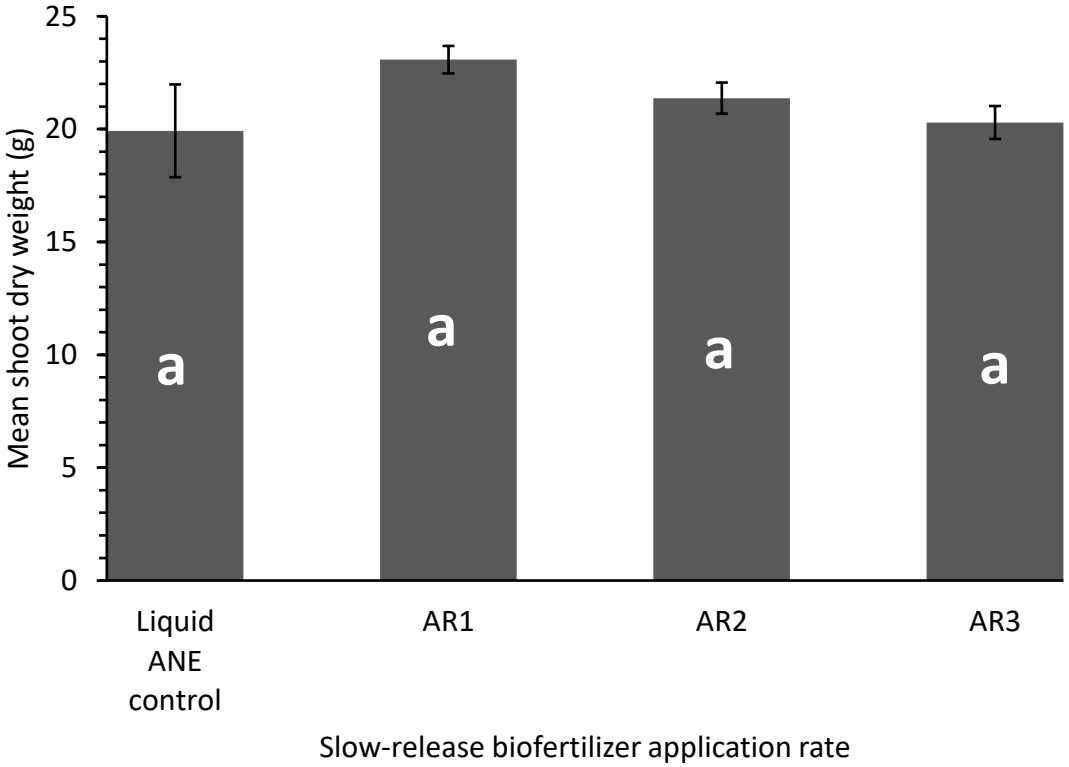

Mean shoot dry weight (g)
25
20
15
10
5
0
a
a
a
a
Liquid ANE control
AR1
AR2
AR3
Slow-release biofertilizer application rate

Figure 3.2. Effects of increasing application rate (AR; lowest: AR1 to highest: AR3) of a slow-release *A. nodosum* extract biofertilizer on the root (A.) and shoot (B.) dry weight of maize (*Zea mays*) compared to the liquid seaweed extract control for all combined formulations and seaweed extract concentrations. Application rates labelled with the same letter were not significantly different from each other ($\alpha = 0.05$). Error bars represent standard error.

The Tukey-Kramer mean separation test indicated that the mean root dry weight for plants treated with the lowest application rates (AR1) of the slow-release biofertilizer was significantly different than that of an application rate of 3 ($p < 0.0001$).

The Tukey-Kramer mean separation test indicated that the mean shoot dry weight was not significantly different between application rates ($p = 0.0099$).

3.4 Maize Shoot Moisture Content

A three-way ANOVA using moisture content values was run in order to determine if the three experimental factors had a significant effect on the water uptake of maize. Through the three-way ANOVA (see Appendix for full ANOVA table) a significant effect was found in application rate both individually ($p = 0.0004$) and when interacting with ANE concentration ($p = 0.0018$). Values of water content relative to the two highest application rates (AR2 and AR3) were larger than the lowest (AR1) as calculated by the Tukey-Kramer mean separation test (see Appendix for full TK table).

3.5 Maize Nutrient Tissue Concentrations

Shoot and root tissue samples of treatment groups with the greatest average dry weight (SC1-FC-AR1, SC1-FC-AR2, and SC3-FC-AR2) and the treatment groups with the lowest average dry weight (SC1-FA-AR3, SC2-FC-AR3, and SC4-FB-AR3) as well as maize treated with the liquid ANE control were analyzed for their concentrations of several macro (N, Ca, K, Mg, P) and micronutrients (Fe, Zn). Root biomass was not substantial

enough to have individual replicates, so all ten replicates from each treatment group were analysed as one sample.

Table 3.5. Average root and shoot nutrient concentration of maize (*Zea mays*) tissues treated with slow-release *A. nodosum* biofertilizers and a liquid seaweed extract control. Each shoot treatment was an average of three replicates, with each replicate being composed of three to four plant shoots. Root dry weight was insufficient for replicates, each treatment composed of 10 individual plant roots. See Table 3.1 for legend.

Treatment	Nitrogen (%) ± SE	Calcium (%) ± SE	Potassium (%) ± SE	Magnesium (%) ± SE	Phosphorus (%) ± SE	Iron (ppm) ± SE	Zinc (ppm) ± SE
Shoots							
Control-LSE	0.907 ± 0.083	0.229 ± 0.006	1.850 ± 0.211	0.203 ± 0.009	0.239 ± 0.011	18.30 ± 0.34	8.98 ± 0.24
SC1-FC-AR1	0.737 ± 0.045	0.224 ± 0.010	1.545 ± 0.048	0.209 ± 0.011	0.217 ± 0.008	21.25 ± 1.65	8.36 ± 0.60
SC1-FC-AR2	0.703 ± 0.058	0.221 ± 0.011	1.577 ± 0.120	0.197 ± 0.007	0.201 ± 0.012	19.25 ± 0.91	7.59 ± 0.65
SC3-FC-AR2	0.707 ± 0.057	0.198 ± 0.006	1.701 ± 0.112	0.177 ± 0.006	0.200 ± 0.009	19.40 ± 0.75	8.01 ± 0.43
SC1-FA-AR3	1.177 ± 0.162	0.256 ± 0.019	2.928 ± 0.325	0.185 ± 0.013	0.302 ± 0.037	25.65 ± 0.30	12.88 ± 0.76
SC2-FC-AR3	1.187 ± 0.048	0.260 ± 0.013	2.822 ± 0.104	0.195 ± 0.014	0.315 ± 0.020	25.41 ± 0.91	12.17 ± 0.57
SC4-FB-AR3	1.060 ± 0.161	0.252 ± 0.023	2.738 ± 0.352	0.188 ± 0.015	0.291 ± 0.037	21.55 ± 3.09	10.75 ± 1.47
1-way ANOVA							
Main effect	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**
Treatment	0.0079, **	0.0630, ns	0.0004, ***	0.4803, ns	0.0076, **	0.0132, *	0.0009, **
Roots							
Control-LSE	1	0.439	1.74	0.189	0.15	295.36	35
SC1-FC-AR1	1	0.56	1.584	0.229	0.171	435.46	36.7
SC1-FC-AR2	0.77	0.396	1.58	0.163	0.125	203.68	39.28
SC3-FC-AR2	0.83	0.389	1.661	0.169	0.162	180.24	38.08
SC1-FA-AR3	1.12	0.382	2.423	0.141	0.192	272.7	30.76
SC2-FC-AR3	1.01	0.377	2.227	0.143	0.212	188.75	33.65
SC4-FB-AR3	0.87	0.314	2.033	0.132	0.181	126.61	27.94

To determine whether there were any significant differences in shoot nutrient concentrations between the three largest and smallest treatment groups, one-way ANOVAs

for the concentrations of each macro and micronutrient were tested. As before, these analyses included only the liquid ANE control.

Overall, treatment effects were significant ($p \leq 0.05$; Table 3.5; see Appendix for full ANOVA tables), with the mean separation test (see Appendix for full TK tables) revealing nutrient concentration values for at least treatment SC1-FA-AR3 (low DW) was generally significantly larger than the liquid ANE control, and typically for treatments SC3-FC-AR2 and SC1-FC-AR2 (high DW). Notable exceptions were for iron, where treatments SC1-FA-AR3 and SC2-FC-AR3 were only greater than the liquid ANE control. The macronutrients calcium and magnesium had no significant effect.

3.6 Maize Shoot Nutrient Accumulation

Measures of shoot nutrient amount and physiological nitrogen use efficiency were calculated as per the formulas in the "Materials and Methods" section, the results of which are shown in the table below.

Table 3.6. Average root and shoot nutrient content of maize (*Zea mays*) shoots treated with slow-release *A. nodosum* biofertilizers and a liquid seaweed extract control. Each shoot treatment was an average of three replicates, with each replicate being composed of three to four plant shoots. Root dry weight was insufficient for replicates, each treatment composed of 10 individual plant roots. See Table 3.1 for legend.

Treatment	N (g) ± SE	Ca (g) ± SE	K (g) ± SE	Mg (g) ± SE	P (g) ± SE	Fe (µg) ± SE	Zn (µg) ± SE	PNUE (g DW/g N) ± SE
Shoots								
C-LSE	0.176 ± 0.017	0.045 ± 0.006	0.358 ± 0.038	0.040 ± 0.005	0.047 ± 0.005	356.76 ± 32.50	176.16 ± 21.53	112.04 ± 9.53
SC1-FC-AR1	0.197 ± 0.019	0.060 ± 0.004	0.413 ± 0.022	0.056 ± 0.004	0.058 ± 0.004	566.90 ± 45.23	224.08 ± 24.01	136.81 ± 8.73
SC1-FC-AR2	0.206 ± 0.010	0.065 ± 0.002	0.461 ± 0.022	0.058 ± 0.003	0.059 ± 0.003	563.92 ± 10.61	221.63 ± 11.16	144.11 ± 11.69
SC3-FC-AR2	0.197 ± 0.006	0.056 ± 0.004	0.475 ± 0.006	0.050 ± 0.005	0.056 ± 0.001	543.92 ± 24.97	224.43 ± 11.95	143.24 ± 10.73
SC1-FA-AR3	0.160 ± 0.031	0.037 ± 0.010	0.405 ± 0.090	0.027 ± 0.007	0.042 ± 0.010	376.26 ± 104.44	183.43 ± 46.15	87.91 ± 10.62
SC2-FC-AR3	0.170 ± 0.029	0.037 ± 0.006	0.401 ± 0.059	0.028 ± 0.004	0.045 ± 0.006	370.44 ± 77.54	173.76 ± 27.16	84.54 ± 3.33
SC4-FB-AR3	0.191 ± 0.034	0.045 ± 0.006	0.494 ± 0.087	0.034 ± 0.004	0.052 ± 0.008	386.72 ± 64.98	192.93 ± 31.49	99.52 ± 17.16
1-way ANOVA								
Main effect	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**	**p (α = 0.05)**
Treatment	0 .7854, ns	0 .0233, *	0.6157, ns	0.0008, ***	0.3036, ns	0.0529, ns	0.6349, ns	0.0037, **

Nutrient Content

One-way ANOVA only showed that calcium and magnesium (Table 3.6; see Appendix for full ANOVA table) content had significant effects (Ca p-value = 0.0233; Mg p-value = 0.0008), with treatments SC1-FC-AR2 (0.0578 g) and SC1-FC-AR1 (0.0560 g) having over twice as much magnesium than treatments SC2-FC-AR3 (0.0277 g) and SC1-

FA-AR3 (0.0265 g) as determined by the mean separation test (see Appendix for full TK table). However, there were no significant differences between these treatments for calcium using the same mean separation test. Additionally, these analyses showed no significant effect for the nutrient content of nitrogen, potassium, phosphorous, iron, and zinc.

Physiological Nitrogen Use Efficiency

The one-way ANOVA (Table 3.6; see Appendix for full ANOVA table) found a significant effect for the treatments, with the highest dry weight treatment groups generally having greater PNUE values than the lowest dry weight treatment groups (Figure 3.3).

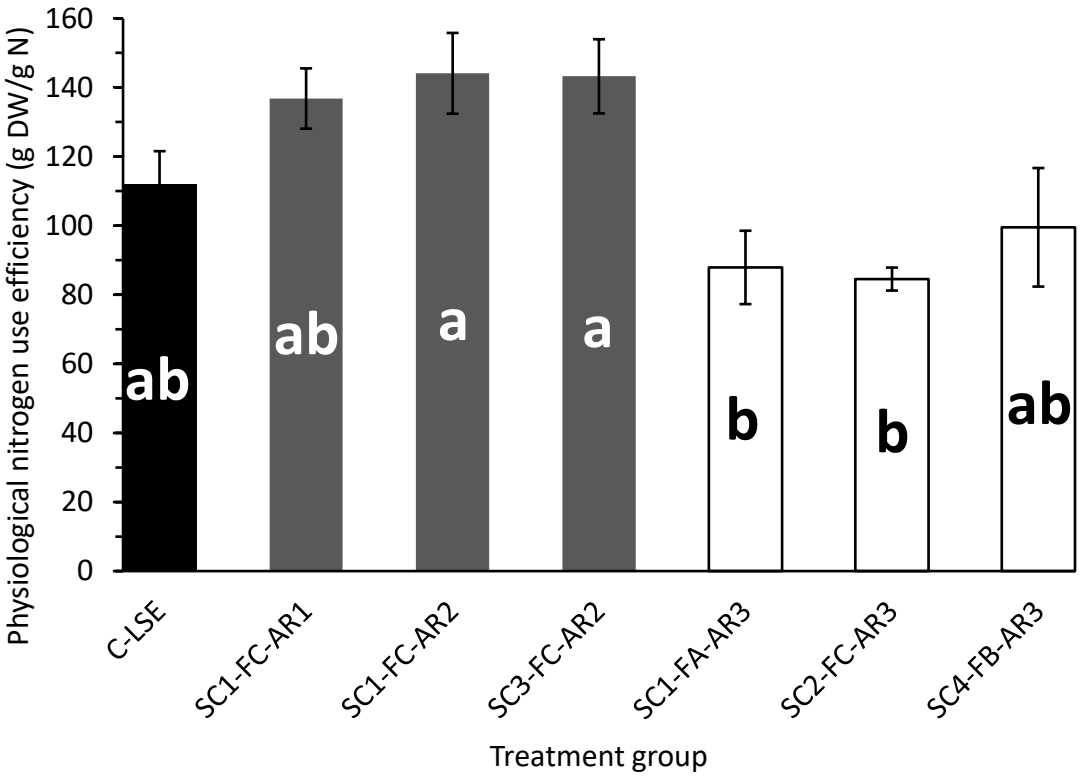


Figure 3.3. Physiological nitrogen use efficiency of the three treatment groups with the highest average dry weight (gray; SC1-FC-AR1, SC1-FC-AR2, and SC3-FC-AR2) and the three treatment groups with the lowest average dry weight (white; SC1-FA-AR3, SC2-FC-AR3, and SC4-FB-AR3) compared to the liquid seaweed extract control (black; C-LSE). Treatment groups with the same letter label show no signifcant difference between each other ($\alpha = 0.05$). Error bars represent the standard error.

According to the Tukey-Kramer mean separation test, the nitrogen use efficiency from the heavier groups SC1-FC-AR2 and SC3-FC-AR2 were significantly different than that of SC1-FA-AR3 and SC2-FC-AR3 from the lighter groups.

4. DISCUSSION AND CONCLUSIONS

4.1 Discussion

The purpose of this study was to test the efficiency of novel slow-release *Ascophyllum nodosum* seaweed extract (ANE) biofertilizer formulations on the growth and nutrient accumulation of maize (*Zea mays*). Over 400 individual maize plants were grown in a greenhouse over a period of 10 weeks. Measures of plant growth and nutrient accumulation were collected throughout the growing period, including plant height, dry weight of roots/shoots, and shoot nitrogen concentration. These data were then evaluated using analysis of variance (ANOVA) and Tukey-Kramer (TK) mean separation tests, especially in relation to the traditional liquid ANE control. Interpretations of these analyses are presented below.

A three-way ANOVA (factors: ANE concentration, application rate, and formulation) on total dry weight indicated significant effects for both ANE concentration and application rate of the slow-release *A. nodosum* biofertilizer (Table 3.2). While formulation did not have a significant effect on plant dry weight, there was a significant interaction between it and ANE concentration (Table 3.2). These results were consistent with the individual measures of both shoot and root dry weight (Table 3.2). One difference of note was that the root dry weight associated with the lowest measures of application rate (AR1) and ANE concentration (SC1) were significantly larger than their maximum respective measures (AR3, SC4) as identified by the post-hoc test (see Appendix for full TK table).

A three-way ANOVA of maize height collected halfway through the growing period generally agreed with dry weight data, such as the highly significant effects of

application rate and the interaction of ANE concentration and formulation (Table 3.1). However, these trends were largely absent from the analysis of final plant height, with the interaction between SC and formulation being the only significant effect (Table 3.1). This was likely due to a height limit imposed by the grow lamps which most maize reached, homogenizing final measurements. Elansary (2017) found herbaceous plant height was significantly enhanced by ANE applications, likely due to metabolic changes from the stimulating organic compounds in ANE. Similarly, beneficial modifications to root morphology by ANE treatments increased tomato plant height by over 35% in an experiment by Ali et al. (2015).

While the three-way ANOVA for dry weight (factors: ANE concentration, application rate, and formulation) demonstrated significant effects for ANE concentration and application rate (Table 3.2), their Tukey-Kramer mean separation tests did not distinguish between the means within these treatment factors, except for the ANE concentration and application rate of root dry weight (see Appendix for full TK table). To refine further analyses it was decided to combine ANE concentration and the non-significant factor of formulation due to their consistently significant interaction. The two-way ANOVA (factors: application rate and ANE concentration by formulation) for all measures of dry weight that followed displayed significant effects for ANE concentration by formulation and again for application rate, with these two factors having a non-significant interaction (Table 3.3). Like before, the Tukey-Kramer mean separation test showed a significant difference between the root dry weight of the upper and lower extents of ANE concentration by formulation and application rate (see Appendix for full TK table).

Because ANE concentration and application rate were factors proven to have a significant effect on dry weight, it was decided that a two-way ANOVA within one formulation (formulation C; factors: application rate and ANE concentration) would be useful to further analyse the influence of these factors on growth effects. Formulation C was selected because it had the greatest effects on dry weight (Table 3.2) and was the only formulation with statistically significant effects. Results showed that the effects of ANE concentration were extremely significant within formulation C, with consistent p-values of less than 0.0001 (Table 3.4). The Tukey-Kramer mean separation test elaborated upon this by showing that, in all dry weight measures, the lowest ANE concentration stimulated growth to an extent greater than that of the liquid ANE control and ANE concentrations 2 and 4 (see Appendix for full TK tables). This increased growth response is consistent with other experiments that tested crop yield under treatments of *A. nodosum*, such as a nearly 10% improvement in strawberry mass due to phytohormone activity seen in an experiment by Mattner et al. (2018). Plant biomass and cob yield of maize was also improved through one late-stage treatment of a *Kappaphycus alvarezii* seaweed extract foliar spray in growth tests by Trivedi et al. (2018). Finally, the physiological benefits of ANE treatments on maize were shown to be highly variable in an experiment by Ertani et al. (2018), with differing effects on root shape and nutrient accumulation from each extract.

Dry weight data presents some interesting trends pertaining to the effects of this slow-release seaweed biofertilizer on the growth of maize. As previously stated, the greater growth effects were stimulated by the lowest level of *A. nodosum* extract (Table 3.2). Root, shoot and total dry weights associated with the highest concentration of *A. nodosum* were consistently among the lowest measures, even when compared to the liquid ANE control

(Figure 3.1). Additionally, the lowest application rate and ANE concentration had significantly higher associated root dry weights compared to the highest application rate and ANE concentration in both the three-way ANOVA and the two-way ANOVA combining ANE concentration and formulation. The roots and shoots of maize treated with an application rate of three were almost always ranked among the lowest for overall weight (Table 3.2), though this difference was not considered significant by most of the Tukey-Kramer mean separation tests, possibly reflecting the highly conservative nature of this test (Saville 2015). These findings suggest that the lowest levels of *A. nodosum* seaweed extract delivered by a slow-release formulation at the lowest application rate stimulated growth better than the traditional liquid *A. nodosum* biostimulant.

Additionally, inhibitory effects on plant growth seem to arise from higher "dosages" of this seaweed extract through increasing its concentration or increasing the application rate of the slow-release formulation biofertilizer. Other experiments on the growth-stimulating properties of *A. nodosum* have found inhibitory effects of the extract at high concentrations. In a study by Alam et al. (2014), concentrations of *A. nodosum* over 10^{-9} M were found to supress development when applied to carrot roots. An explanation given for this inhibition was that of general plant hormone dynamics, wherein the abundant hormones within *A. nodosum* disrupt physiological processes when present in excess (Alam et al. 2014). This phenomenon was studied more thoroughly by Shi et al. (2017) through testing the impacts of *A. nodosum* on different algae species. It was found that potential growth could be reduced by more than 80% when this seaweed extract was applied at concentrations above 1%. The reasoning provided was that the high amounts of phlorotannins within *A. nodosum* completely disables the antioxidant defense system by

deactivating key antioxidative enzymes, leading to hindered growth over time (Shi et al. 2017).

Ascophyllum nodosum has many potential modes of action which could have caused simulating effects on plant growth at lower dosages. Some insight into these mechanisms could be gained through analysing nutrient accumulation (i.e. better growth resulting from higher nutrient content), though it is important to recognize that the following analyses were performed on only six of the 36 treatments, the three with the greatest effects on shoot dry weight and the three with the lowest effects on shoot dry weight.

The non-significant effects obtained by one-way analyses of shoot nutrient content (i.e. the amount of nutrient per plant) revealed that these plants had accumulated similar amounts of nutrients regardless of their size. This suggests that nutrients were not the limiting factor on the growth of maize, and that the mechanism that *A. nodosum* employed to stimulate growth was likely not related to an increase in nutrient acquisition.

Unlike shoot nutrient content, the ANOVA for nutrient concentration (i.e. the amount of nutrient per gram of shoot dry weight) showed a significant inverse effect between nutrient concentration and shoot dry weight. Maize with higher dry weight had lower nutrient concentrations than plants with lower dry weight. This inverse relationship between dry weight and nutrient concentration is known as the dilution effect and was exemplified in a growth experiment performed by Riedell (2010). In this experiment, maize grown in soils with high amounts of nitrogen were larger but had lower phosphorous and potassium concentrations overall when compared to plants grown in low nitrogen soils. While dry weight increased alongside soil nitrogen content, the amount of phosphorous

and potassium macronutrients absorbed by the plants remained comparable regardless of treatment. This occurrence demonstrates that adding dry weight without also enhancing nutrient uptake will result in lower overall plant nutrient concentrations (Riedell 2010). Therefore, *A. nodosum* enhancing growth through improving nutrient uptake is further disproven.

One possible mechanism for the growth benefits of *A. nodosum* in the current experiment could be an increase in physiological nitrogen use efficiency (PNUE), likely by enhancing photosynthesis (Gu et al. 2018). Treatment groups with the highest dry weights had significantly higher nitrogen use efficiency than two of the three groups with the lowest dry weight. What this means is that certain maize grew comparatively larger because the *A. nodosum* extract allowed these plants to create more units of biomass per unit of tissue nitrogen. This line of thinking raises an important question – how could *A. nodosum* improve physiological nitrogen use efficiency?

It is interesting to note that the aforementioned increases in growth relative to nitrogen were also reflected in other nutrients. For this discussion, we will focus on nitrogen use efficiency because it is the most well studied.

An enhancing effect of *A. nodosum* on PNUE was also observed by Trinchera et al. (2014). Through testing liquid applications of this extract on lettuce, it was found that the most diluted extracts produced better nutrient accumulation relative to dry weight and therefore higher yields. Many organic constituents of *A. nodosum* could potentially impact the efficiency of nitrogen use. For example, cytokinins are a substance that influences photosynthesis, allowing this process to persist under high carbon dioxide conditions and influencing chlorophyll concentrations within leaves (Gu et al. 2018). These factors

improve the efficiency of photosynthesis and contribute towards PNUE (Gu et al. 2018), and *A. nodosum* applications are capable of inducing gene expression for the heightened production of cytokinin hormones (Carvalho et al. 2013). Additionally, the nitrogen use efficiency of rice has been improved using liquid sprays of gibberellic acid in an experiment done by Prakash et. al (2015), though its physiological mechanism could not be determined. Gibberellic acids are yet another substance that *A. nodosum* can provide to plants (Sun and Gubler, 2004). Therefore, applications of this seaweed extract could enhance growth through PNUE in the same way as the aforementioned experiment.

4.2 Conclusions

To conclude, this experiment provides evidence that certain combinations of lower ANE concentrations, formulations (i.e. formulation C), and lower application rates of a slow-release *A. nodosum* biofertilizer can enhance growth to a greater extent than the traditional liquid seaweed extract formulation. Because the greatest improvements in growth occurred at the lowest dosages of seaweed extract, it is possible that supra-optimal applications of *A. nodosum* hinders plant growth. While this study was not designed to investigate mode of action, the fact that ANE treatments resulted in an inverse relationship between growth and nutrient concentration in shoot tissue gives insight into how *A. nodosum* enhanced growth in this experiment. One plausible mechanism is a hormone-induced improvement to physiological nitrogen use efficiency, allowing maize to produce more carbon per unit of nitrogen rather than simply increasing the uptake of nutrients. Given the tentative nature of these results, it would be overly speculative to extrapolate the findings of this study on the broader uses of this slow-release formulation on corn field production at this time. Therefore, the findings of this experiment serve as the starting point

for further research into this novel approach to the application of seaweed biostimulants to crop plants.

5. REFERENCES

Alam MZ, Braun G, Norrie J, Hodges DM. *Ascophyllum* extract application can promote plant growth and root yield in carrot associated with increased root-zone soil microbial activity. Canadian Journal of Plant Science. 2014;94(2):337–348. doi:10.4141/cjps2013-135

Alam MZ, Braun G, Norrie J, Hodges DM. Effect of *Ascophyllum* extract application on plant growth, fruit yield and soil microbial communities of strawberry. Canadian Journal of Plant Science. 2013;93(1):23–36. doi:10.4141/cjps2011-260

Albareda M, Rodríguez-Navarro DN, Camacho M, Temprano FJ. Alternatives to peat as a carrier for rhizobia inoculants: Solid and liquid formulations. Soil Biology and Biochemistry. 2008;40(11):2771–2779. doi:10.1016/j.soilbio.2008.07.021

Al-Haj NA, Mashan NI, Shamsudin MN, Mohamad H, Vairappan CS, Sekawi Z. Antibacterial activity in marine algae *Eucheuma denticulatum* against *Staphylococcus aureus* and *Streptococcus pyogenes*. Research Journal of Biological Sciences. 2009;519-524.

Ali N, Farrell A, Ramsubhag A, Jayaraman J. The effect of *Ascophyllum nodosum* extract on the growth, yield and fruit quality of tomato grown under tropical conditions. Journal of Applied Phycology. 2015;28(2):1353–1362. doi:10.1007/s10811-015-0608-3

Azeem B, KuShaari K, Man ZB, Basit A, Thanh TH. Review on materials & methods to produce controlled release coated urea fertilizer. Journal of Controlled Release. 2014;181:11–21. doi:10.1016/j.jconrel.2014.02.020

Bashan Y, Holguin G, De-Bashan LE. *Azospirillum* – plant relationships: physiological, molecular, agricultural, and environmental advances. Canadian Journal of Microbiology. 2013;789-799. doi:10.1139/W04-035

Bassi D, Menossi M, Mattiello L. Nitrogen supply influences photosynthesis establishment along the sugarcane leaf. Scientific Reports. 2018;8(1). doi:10.1038/s41598-018-20653-1

Battacharyya D, Babgohari MZ, Rathor P, Prithiviraj B. Seaweed extracts as biostimulants in horticulture. Scientia Horticulturae. 2015;196:39–48. doi:10.1016/j.scienta.2015.09.012

Ben Rebah F, Prévost D, Yezza A, Tyagi RD. Agro-industrial waste materials and wastewater sludge for rhizobial inoculant production: A review. Bioresource Technology. 2007;98(18):3535–3546. doi:10.1016/j.biortech.2006.11.066

Bhattacharyya PN, Jha DK. Plant growth-promoting rhizobacteria (PGPR): emergence in agriculture. World Journal of Microbiology and Biotechnology. 2011;28(4):1327–1350. doi:10.1007/s11274-011-0979-9

Blouin GM, Rindt DW. Sulfur-coated fertilizer pellet having controlled dissolution rate and inhibited against microbial decomposition. 1967 Sep 19.

Blunden G, Jenkins T, Liu Y-W. Enhanced leaf chlorophyll levels in plants treated with seaweed extract. Journal of Applied Phycology. 1996;8(6):535–543. doi:10.1007/bf02186333

Brink RA, Cooper DC. The endosperm in seed development. The Botanical Review. 1947;13(9):479–541. doi:10.1007/bf02861549

Carvalho MEA, Castro PRD Ce, Gallo LA, Ferraz MVD C. Seaweed extract provides development and production of wheat. Comunicação Científica. 2013;7:166–170.

Celsia A, Mala R. Fabrication of Nano Structured Slow Release Fertilizer System and its Influence on Germination And Biochemical Characteristics of *Vigna Raidata*. International Journal of ChemTech Research. 2014;4497-4503.

Chapman DJ. Seaweeds and their uses. London: Chapman and Hall; 1980.

Chen J, Lü S, Zhang Z, Zhao X, Li X, Ning P, Liu M. Environmentally friendly fertilizers: A review of materials used and their effects on the environment. Science of The Total Environment. 2018;613-614:829–839. doi:10.1016/j.scitotenv.2017.09.186

Chojnacka K. Biologically Active Compounds in Seaweed Extracts - the Prospects for the Application. The Open Conference Proceedings Journal. 2012;3(1):20–28. doi:10.2174/1876326x01203020020

Craigie JS. Seaweed extract stimuli in plant science and agriculture. Journal of Applied Phycology. 2010;23(3):371–393. doi:10.1007/s10811-010-9560-4

Crouch IJ, Staden JV. Evidence for the presence of plant growth regulators in commercial seaweed products. Plant Growth Regulation. 1993;13(1):21–29. doi:10.1007/bf00207588

Drinkwater LE, Snapp SS. Nutrients in Agroecosystems: Rethinking the Management Paradigm. Advances in Agronomy. 2007:163–186. doi:10.1016/s0065-2113(04)92003-2

du Jardin P. Plant biostimulants: Definition, concept, main categories and regulation. Scientia Horticulturae. 2015;196:3–14. doi:10.1016/j.scienta.2015.09.021

Elansary HO. Green roof Petunia, Ageratum, and Mentha responses to water stress, seaweeds, and trinexapac-ethyl treatments. Acta Physiologiae Plantarum. 2017;39(7). doi:10.1007/s11738-017-2444-3

Ertani A, Francioso O, Tinti A, Schiavon M, Pizzeghello D, Nardi S. Evaluation of Seaweed Extracts From Laminaria and *Ascophyllum nodosum* spp. as Biostimulants in *Zea mays* L. Using a Combination of Chemical, Biochemical and Morphological Approaches. Frontiers in Plant Science. 2018;9. doi:10.3389/fpls.2018.00428

Fan XH, Li YC. Nitrogen Release from Slow-Release Fertilizers as Affected by Soil Type and Temperature. Soil Science Society of America Journal. 2010;74(5):1635. doi:10.2136/sssaj2008.0363

Featonby-Smith B, Staden JV. The effect of seaweed concentrate on the growth of tomato plants in nematode-infested soil. Scientia Horticulturae. 1983;20(2):137–146. doi:10.1016/0304-4238(83)90134-6

Galloway JN, Townsend AR, Erisman JW, Bekunda M, Cai Z, Freney JR, Martinelli LA, Seitzinger SP, Sutton MA. Transformation of the Nitrogen Cycle: Recent Trends, Questions, and Potential Solutions. Science. 2008;320(5878):889–892. doi:10.1126/science.1136674

Gu J, Li Z, Mao Y, Struik PC, Zhang H, Liu L, Wang Z, Yang J. Roles of nitrogen and cytokinin signals in root and shoot communications in maximizing of plant productivity and their agronomic applications. Plant Science. 2018;274:320–331. doi:10.1016/j.plantsci.2018.06.010

Hassan TU, Bano A, Naz I. Halophyte root powder: an alternative biofertilizer and carrier for saline land. Soil Science and Plant Nutrition. 2018;64(5):653–661. doi:10.1080/00380768.2018.1509676

Herridge DF. Inoculation Technology For Legumes. Nitrogen-fixing Leguminous Symbioses Nitrogen Fixation: Origins, Applications, and Research Progress.:77–115. doi:10.1007/978-1-4020-3548-7_4

Herrmann L, Lesueur D. Challenges of formulation and quality of biofertilizers for successful inoculation. Applied Microbiology and Biotechnology. 2013;97(20):8859–8873. doi:10.1007/s00253-013-5228-8

Hoagland DR, Arnon DI. The Water-Culture Method for Growing Plans without Soil. University of California Agricultural Experiment Station. 1950;1-39.

Igiehon NO, Babalola OO. Biofertilizers and sustainable agriculture: exploring arbuscular mycorrhizal fungi. Applied Microbiology and Biotechnology. 2017;101(12):4871–4881. doi:10.1007/s00253-017-8344-z

Imbamba SK. Mineral Element Content of Some Benthic Marine Algae of the Kenya Coast. Botanica Marina. 1972;15(2). doi:10.1515/botm.1972.15.2.113

Jeswani KD. We want to provide farmers with updated technology in the field of agriculture. Chemical Engineering World. 1999;23-25.

John RP, Tyagi RD, Brar SK, Surampalli RY, Prévost D. Bio-encapsulation of microbial cells for targeted agricultural delivery. Critical Reviews in Biotechnology. 2010;31(3):211–226. doi:10.3109/07388551.2010.513327

Kasim WA, Hamada E, Shams El-Din N, Eskander SK. Influence of seaweed extracts on the growth, some metabolic activities and yield of wheat grown under drought stress. International Journal of Agronomy and Agricultural Research. 2015:173-189.

Keser M, Swenarton JT, Foertch JF. Effects of thermal input and climate change on growth of *Ascophyllum nodosum* (Fucales, Phaeophyceae) in eastern Long Island Sound (USA). Journal of Sea Research. 2005;54(3):211–220. doi:10.1016/j.seares.2005.05.001

Khan W, Rayirath UP, Subramanian S, Jithesh MN, Rayorath P, Hodges DM, Critchley AT, Craigie JS, Norrie J, Prithiviraj B. Seaweed Extracts as Biostimulants of Plant Growth and Development. Journal of Plant Growth Regulation. 2009;28(4):386–399. doi:10.1007/s00344-009-9103-x

Kingman AR, Senn TL. Bioassay systems to test for plant growth hormones in extracts of *Ascophyllum nodosum*. Suppl Abst. 1977;16.

Kloepper JW, Schroth MN. Plant growth-promoting rhizobacteria on radishes. Proceedings of the 4th International Conference on Plant Pathogenic Bacteria. 1978;879-882.

Kumar RN, Kumar R, Patel K, Viyol S, Bhoi R. Nutrient Composition and Calorific Value of Some Seaweeds from Bet Dwarka, West Coast of Gujarat, India. Our Nature. 2009;7(1):18–25. doi:10.3126/on.v7i1.2565

Ladha JK, Dawe D, Ventura TS, Singh U, Ventura W, Watanabe I. Long-Term Effects of Urea and Green Manure on Rice Yields and Nitrogen Balance. Soil Science Society of America Journal. 2000;64(6):1993. doi:10.2136/sssaj2000.6461993x

Lassaletta L, Billen G, Grizzetti B, Anglade J, Garnier J. 50 year trends in nitrogen use efficiency of world cropping systems: the relationship between yield and nitrogen input to cropland. Environmental Research Letters. 2014;9(10):105011. doi:10.1088/1748-9326/9/10/105011

Liu X, Vitousek P, Chang Y, Zhang W, Matson P, Zhang F. Evidence for a Historic Change Occurring in China. Environmental Science & Technology. 2015;50(2):505–506. doi:10.1021/acs.est.5b05972

Lopes G, Pinto E, Andrade PB, Valentão P. Antifungal Activity of Phlorotannins against Dermatophytes and Yeasts: Approaches to the Mechanism of Action and Influence on Candida albicans Virulence Factor. PLoS ONE. 2013;8(8). doi:10.1371/journal.pone.0072203

Mäder P, Fließbach A, Dubois D, Gunst L, Fried P, Niggli U. Soil Fertility and Biodiversity in Organic Farming. Science. 2002;296(5573):1694–1697. doi:10.1126/science.1071148

Manivannan K, Karthikai Devi G, Thirumaran G, Anantharaman P. Mineral composition of macroalge from Mandapam coastal region, southeast coast of India. American-Eurasian Journal of Botany. 2008;58-67.

Mathur C, Rai S, Sase N, Krish S, Jayasri MA. Enteromorpha intestinalis Derived Seaweed Liquid Fertilizers as Prospective Biostimulant for Glycine max. Brazilian Archives of Biology and Technology. 2015;58(6):813–820. doi:10.1590/s1516-89132015060304

Mattner SW, Milinkovic M, Arioli T. Increased growth response of strawberry roots to a commercial extract from Durvillaea potatorum and *Ascophyllum nodosum*. Journal of Applied Phycology. 2018;30(5):2943–2951. doi:10.1007/s10811-017-1387-9

McQuilken MP, Halmer P, Rhodes DJ. Application of Microorganisms to Seeds. Formulation of Microbial Biopesticides. 1998:255–285. doi:10.1007/978-94-011-4926-6_8

Mirparsa T, Ganjali HR, Dahmardeh M. The effect of biofertilizers on yield and yield components of sunflower oil seed and nut. International Journal of Agriculture and Biosciences. 2016;46-49.

Murata M., Nakazoe J. Production and use of marine algae in Japan. Japan Agricultural Research Quarterly. 2001;281-290.

Mwalugha HM, Wakibia JG, Kenji GM, Mwasaru MA. Chemical Composition of Common Seaweeds from the Kenya Coast. Journal of Food Research. 2015;4(6):28. doi:10.5539/jfr.v4n6p28

Nabti E, Jha B, Hartmann A. Impact of seaweeds on agricultural crop production as biofertilizer. International Journal of Environmental Science and Technology. 2016;14(5):1119–1134. doi:10.1007/s13762-016-1202-1

Nelson WR, Van Staden J. The Effect of Seaweed Concentrate on Wheat culms. Journal of Plant Physiology. 1984;115(5):433–437. doi:10.1016/s0176-1617(84)80042-5

Okon Y, Baker R. Microbial Inoculants as Crop-Yield Enhancers. Critical Reviews in Biotechnology. 1987;6(1):61–85. doi:10.3109/07388558709086985

Oppermann HR. Uber antibiotische Stoffe bei den Grunalgen Stichoccus bacillaris und Protosiphon bomyoides. Archives of Microbiology. 1953;19(4):398–401. doi:10.1007/bf00412158

Philippot L, Raaijmakers JM, Lemanceau P, Van Der Putten WH. Going back to the roots: the microbial ecology of the rhizosphere. Nature Reviews Microbiology. 2013;789–799.

Prakash S, Mahajan G, Sharma N, Sardana V. Enhancing Grain Yield and Nitrogen-Use Efficiency in Rice through Foliarly Applied Gibberellic Acid in Dry-Direct-Seeded Rice. Journal of Crop Improvement. 2015;29(1):65–81. doi:10.1080/15427528.2014.976693

Ragan, MA, Glombitza KW. Phlorotannins, brown algal polyphenols. Progress in Phycological Research.1986;129-241.

Ramarajan S, Henry J, Saravana GA. Effect of seaweed liquid fertilizer on the germination and pigment concentration of soybean. Journal of Crop Science and Biotechnology. 2012;1-5.

Rao R. Seaweeds as biofertilizers in Indian horticulture. Seaweed Research and Utilization. 1992;89-101.

Reis EA, Rocha-Leão MHM, Leite SGF. Slow-Release Nutrient Capsules for Microorganism Stimulation in Oil Remediation. Applied Biochemistry and Biotechnology. 2013;169(4):1241–1249. doi:10.1007/s12010-012-0022-0

Rensing SA, and 69 others. The *Physcomitrella* genome reveals evolutionary insight into the conquest of land by plants. Science. 2008;64-69.

Riedell WE. Mineral-nutrient synergism and dilution responses to nitrogen fertilizer in field-grown maize. Journal of Plant Nutrition and Soil Science. 2010;173(6):869–874. doi:10.1002/jpln.200900218

Rood SB. Application Of Gibberellic Acid To Control Tillering In Early-Maturing Maize. Canadian Journal of Plant Science. 1985;65(4):901–911. doi:10.4141/cjps85-116

Russo RO, Berlyn GP. The Use of Organic Biostimulants to Help Low Input Sustainable Agriculture. Journal of Sustainable Agriculture. 1991;1(2):19–42. doi:10.1300/j064v01n02_04

Savasangari SR, Nagaraj S, Vijayanand N. Influence of seaweed liquid extracts on growth, biochemical and yield characteristics of *Cyamopsis tetragonolaba*. Journal of Phytological Research. 2011;37-41.

Saville DJ. Multiple Comparison Procedures—Cutting the Gordian Knot. Agronomy Journal. 2015;107(2):730. doi:10.2134/agronj2012.0394

Sethi P. Biochemical composition of the marine brown algae *Pedina terastromatica* Hauck. International Journal of Current Pharmaceutical Research. 2012;117-118.

Shaviv A. Advances in controlled-release fertilizers. Advances in Agronomy. 2001:1–49. doi:10.1016/s0065-2113(01)71011-5

Shi P, Geng S, Feng T, Wu H. Effects of *Ascophyllum nodosum* extract on growth and antioxidant defense systems of two freshwater microalgae. Journal of Applied Phycology. 2017;30(2):851–859. doi:10.1007/s10811-017-1287-z

Singhalage ID, Seneviratne G, Madawala H, Wijepala PC. Profitability of strawberry (*Fragaria ananassa*) production with biofilmed biofertilizer application. Scientia Horticulturae. 2019;243:411–413. doi:10.1016/j.scienta.2018.08.033

Singleton P, Keyser H, Sande E. Development and evaluation of liquid inoculants. In DH Herridge, Inoculants and nitrogen fixation of legumes in Vietnam. 2002;52-66. Hanoi: Australian Centre for International Agricultural Research.

Staging Corn Growth [Internet]. DuPont Pioneer website. Available from: https://www.pioneer.com/home/site/us/agronomy/library/staging-corn-growth

Sultana V, Ehteshamul-Haque S, Ara J, Athar M. Comparative efficacy of brown, green and red seaweeds in the control of root infecting fungi and okra. International Journal of Environmental Science & Technology. 2005;2(2):129–132. doi:10.1007/bf03325866

Sun T-P, Gubler F. Molecular Mechanism Of Gibberellin Signaling In Plants. Annual Review of Plant Biology. 2004;55(1):197–223. doi:10.1146/annurev.arplant.55.031903.141753

Suslow, T. V., Kloepper, J. W., Schroth, M. N., & Burr, T. (1979). Beneficial bacteria enhance plant growth rhizobacteria. California Agriculture. 15-17.

Tay SAB, Palni LMS, Macleod JK. Identification of cytokinin glucosides in a seaweed extract. Journal of Plant Growth Regulation. 1987;5(3):133–138. doi:10.1007/bf02087181

Thivy F. Seaweed manure for perfect soil and smiling field. Salt Res Ind. 1964;1-4.

Tilman D, Cassman KG, Matson PA, Naylor R, Polasky S. Agricultural sustainability and intensive production practices. Nature. 2002;418(6898):671–677. doi:10.1038/nature01014

Timilsena YP, Adhikari R, Casey P, Muster T, Gill H, Adhikari B. Enhanced efficiency fertilisers: a review of formulation and nutrient release patterns. Journal of the Science of Food and Agriculture. 2014;95(6):1131–1142. doi:10.1002/jsfa.6812

Tittabutr P, Payakapong W, Teaungroong N, Singleton PW, Boonkerd N. Growth survival and field performance of bradyrhizobial liquid inoculant formulations with polymeric additives. Science Asia. 2007;69-77.

Trinchera A, Marcucci A, Renzaglia M, Rea E. 4th ISOFAR Scientific Conference. Filtrate seaweed extract as biostimulant in nursery organic horticulture. 2014;13-15. Istanbul.

Trivedi K, Anand KV, Vaghela P, Ghosh A. Differential growth, yield and biochemical responses of maize to the exogenous application of *Kappaphycus alvarezii* seaweed extract, at grain-filling stage under normal and drought conditions. Algal Research. 2018;35:236–244. doi:10.1016/j.algal.2018.08.027

Trenkel ME. Slow- and controlled-release and stabilized fertilizers: an option for enhancing nutrient use efficiency in agriculture. Paris: IFA, International fertilizer industry Association; 2010.

Tyihák E. Double immune response of plants to pathogens and its biochemical basis. In JA DaSilva, Floriculture, ornamental and plant biotechnology. 2006;380–387. United Kingdom: Global Science Books.

Ugarte RA, Craigie JS, Critchley AT. Fucoid Flora of the Rocky Intertidal of the Canadian Maritimes: Implications for the Future with Rapid Climate Change. Cellular Origin, Life in Extreme Habitats and Astrobiology Seaweeds and their Role in Globally Changing Environments. 2010:69–90. doi:10.1007/978-90-481-8569-6_5

Vassilev N, Vassileva M, Nikolaeva I. Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Applied Microbiology and Biotechnology. 2006;71(2):137–144. doi:10.1007/s00253-006-0380-z

Verslues PE, Agarwal M, Katiyar-Agarwal S, Zhu J, Zhu J-K. Methods and concepts in quantifying resistance to drought, salt and freezing, abiotic stresses that affect plant water status. The Plant Journal. 2006;45(4):523–539. doi:10.1111/j.1365-313x.2005.02593.x

Vessey JK. Plant growth promoting rhizobacteria as biofertilizers. *Plant and Soil*. 2003;571-586.

Vijayalakshmi P, Kiran TV, Rao YV, Srikanth B, Rao IS, Sailaja B, Surekha K, Rao PR, Subrahmanyam D, Neeraja CN, et al. Physiological approaches for increasing nitrogen use efficiency in rice. Indian Journal of Plant Physiology. 2013;18(3):208–222. doi:10.1007/s40502-013-0042-y

Vitousek PM, Naylor R, Crews T, David MB, Drinkwater LE, Holland E, Johnes PJ, Katzenberger J, Martinelli LA, Matson PA, et al. Nutrient Imbalances in Agricultural Development. Science. 2009;324(5934):1519–1520. doi:10.1126/science.1170261

Wally OSD, Critchley AT, Hiltz D, Craigie JS, Han X, Zaharia LI, Abrams SR, Prithiviraj B. Regulation of Phytohormone Biosynthesis and Accumulation in *Arabidopsis* Following Treatment with Commercial Extract from the Marine Macroalga *Ascophyllum nodosum*. Journal of Plant Growth Regulation. 2012;32(2):324–339. doi:10.1007/s00344-012-9301-9

Weih M, Asplund L, Bergkvist G. Assessment of nutrient use in annual and perennial crops: A functional concept for analyzing nitrogen use efficiency. Plant and Soil. 2010;339(1-2):513–520. doi:10.1007/s11104-010-0599-4

Whapham CA, Jenkins T, Blunden G, Hankins SD. The role of seaweed extract, *Ascophyllum nodosum* in the reduction of fecundity of *Meloidogyne javanica*. Fundamental & Applied Nematology. 1994;181-183.

Xavier IJ, Holloway G, Leggett M. Development of Rhizobial Inoculant Formulations. Crop Manag. 2004;3(1). doi:10.1094/cm-2004-0301-06-rv

Zodape ST, Soumit M, Eswaran K, Reddy MP, Chikara J. Enhanced yield and nutritional quality in green gram (*Phaseolus radiate* L) treated with seaweed (*Kappaphycus alvarezii*) extract. Journal of Scientific & Industrial Research. 2010;468–471.

6. APPENDIX

6.1 Maize Height

Maize expressed little variation in growth stages (data not shown), with most maize at stage V5 development (five visible leaf collars) halfway through the growing period and stage V11 development (11 visible leaf collars) by the end of the growing period.

Three-Way analysis of variance for seaweed extract concentration, formulation, and application rate

Table 6.1. Three-way analysis of variance for midway plant height.

Source	df	Type II sum of squares	Mean square	F-value	P-value	
Main effects						
Seaweed extract concentration	3	1066.37	355.46	1.8656	0.1353	ns
Formulation	2	1559.80	779.90	4.0932	0.0176	*
Application rate	2	3947.58	1973.79	10.3591	0.0000	***
Interaction						
Seaweed extract concentration * formulation	6	6071.34	1011.89	5.3107	0.0000	***
Seaweed extract concentration * application rate	6	1018.62	169.77	0.8910	0.5016	ns
Formulation * application rate	4	475.20	118.80	0.6235	0.6461	ns
Seaweed extract concentration * formulation * application rate	12	3440.48	286.71	1.5047	0.1206	ns
Error	323	61543.48	190.54			
Total	359	79197.49				

Table 6.2. Tukey-Kramer mean separation test for midway plant height at three application rates (AR1, AR2, and AR3) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	AR1	100.9981	120	a
2	AR2	96.0382	119	ab
3	C-LSE	94.4150	10	ab
4	AR3	92.7622	111	b

Table 6.3. Tukey-Kramer mean separation test for midway plant height at three formulations (FA, FB, FC) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	FC	99.0719	118	a
2	FB	96.9546	115	ab
3	C-LSE	94.4150	10	ab
4	FA	94.0568	117	b

Table 6.4. Three-way analysis of variance for final plant height.

Source	df	Type II sum of squares	Mean square	F-value	P-value	
Main effects						
Seaweed extract concentration	3	1504.43	501.48	1.7622	0.1543	ns
Formulation	2	210.00	105.00	0.3690	0.6917	ns
Application rate	2	399.92	199.96	0.7027	0.4960	ns
Interaction						
Seaweed extract concentration * formulation	6	5693.74	948.96	3.3346	0.0034	**
Seaweed extract concentration * application rate	6	1989.24	331.54	1.1650	0.3247	ns
Formulation * application rate	4	1458.95	364.74	1.2817	0.2770	ns
Seaweed extract concentration * formulation * application rate	12	2018.62	168.22	0.5911	0.8493	ns
Error	322	91633.46	284.58			
Total	358	105734.96				

6.2 Maize Weight

Three-way analysis of variance for seaweed extract concentration, formulation, and application rate

Table 6.5. Three-way analysis of variance for shoot dry weight.

Source	df	Type II sum of squares	Mean square	F-value	P-value	
Main effects						
Seaweed extract concentration	3	445.56	148.52	3.0364	0.0293	*
Formulation	2	104.41	52.21	1.0673	0.3451	ns
Application rate	2	458.14	229.07	4.6832	0.0099	**
Interaction						
Seaweed extract concentration * Formulation	6	1255.03	209.17	4.2763	0.0004	***
Seaweed extract concentration * Application rate	6	264.76	44.13	0.9021	0.4935	ns
Formulation * Application rate	4	257.07	64.27	1.3139	0.2646	ns
Seaweed extract concentration * Formulation * Application rate	12	722.86	60.24	1.2315	0.2598	ns
Error	323	15799.11	48.91			
Total	359	19340.75				

Table 6.6. Three-way analysis of variance for root dry weight.

Source	df	Type II sum of squares	Mean square	F-value	P-value	
Main effects						
Seaweed extract concentration	3	6.57	2.19	5.3265	0.0014	**
Formulation	2	1.97	0.99	2.4009	0.0923	ns
Application rate	2	9.97	4.98	12.1331	0.0000	***
Interaction						
Seaweed extract concentration * formulation	6	8.43	1.41	3.4218	0.0027	**
Seaweed extract concentration * application rate	6	3.36	0.56	1.3627	0.2291	ns
Formulation * application rate	4	2.36	0.59	1.4359	0.2218	ns
Seaweed extract concentration * formulation * application rate	12	4.60	0.38	0.9337	0.5132	ns
Error	323	132.70	0.41			
Total	359	170.03				

Table 6.7. Tukey-Kramer mean separation test for root dry weight at four seaweed extract concentrations (SC1, SC2, SC3, and SC4) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SC1	2.2647	87	a
2	SC3	2.0848	89	ab
3	SC2	1.9497	88	ab
4	C-LSE	1.9410	10	ab
5	SC4	1.9170	86	b

Table 6.8. Tukey-Kramer mean separation test for root dry weight at three application rates (AR1, AR2, and AR3) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	AR1	2.2882	120	a
2	AR2	1.9434	119	ab
3	C-LSE	1.9410	10	ab
4	AR3	1.9203	111	b

Table 6.9. Three-way analysis of variance for total dry weight.

Source	df	Type II sum of squares	Mean square	F-value	P-value	
Main effects						
Seaweed extract concentration	3	526.84	175.61	3.1061	0.0267	*
Formulation	2	132.00	66.00	1.1673	0.3125	ns
Application rate	2	595.54	297.77	5.2668	0.0056	**
Interaction						
Seaweed extract concentration * formulation	6	1458.22	243.04	4.2987	0.0003	***
Seaweed extract concentration * application rate	6	309.75	51.63	0.9131	0.4855	ns
Formulation * application rate	4	292.28	73.07	1.2924	0.2728	ns
Seaweed extract concentration * formulation * application rate	12	824.33	68.69	1.2150	0.2711	ns
Error	323	18261.63	56.54			
Total	359					

Table 6.10. Three-way analysis of variance for shoot fresh weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration	3	16356.3	5452.1	3.6868	0.0123	*
Formulation	2	1623.7	811.9	0.5490	0.5781	ns
Application rate	2	7419.1	3709.5	2.5084	0.083	ns
Interaction						
Seaweed extract concentration * Formulation	6	34717.5	5786.2	3.9127	0.0009	***
Seaweed extract concentration * Application rate	6	7173.5	1195.6	0.8085	0.5639	ns
Formulation * Application rate	4	6179.6	1544.9	1.0447	0.3842	ns
Seaweed extract concentration * Formulation * Application rate	12	22983.7	1915.3	1.2952	0.2196	ns
Error	323	477660.1	1478.8			
Total	359	576609.7				

Two-way analysis of variance for application rate and a combination of formulation and seaweed extract concentration

Table 6.11. Two-way analysis of variance (seaweed extract concentration by formulation and application rate) for shoot dry weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration by formulation	3	444.30	148.10	2.8334	0.0383	*
Application rate	2	463.16	231.58	4.4305	0.0126	*
Interaction						
Seaweed extract concentration by formulation * Application rate	6	273.94	45.66	0.8735	0.5145	ns
Error	347	18137.54	52.27			
Total	359	19340.75				

Table 6.12. Two-way analysis of variance (seaweed extract concentration by formulation and application rate) for root dry weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration by formulation	3	6.54	2.18	5.0416	0.0020	**
Application rate	2	10.04	5.02	11.6058	0.0000	***
Interaction						
Seaweed extract concentration by formulation * Application rate	6	3.30	0.55	1.2734	0.2688	ns
Error	347	150.05	0.43			
Total	359	170.03				

Table 6.13. Tukey-Kramer mean separation test for root dry weight at four seaweed extract concentrations (SC1, SC2, SC3, and SC4) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SC1	2.2647	87	a
2	SC3	2.0848	89	ab
3	SC2	1.9497	88	ab
4	C-LSE	1.9410	10	ab
5	SC4	1.9170	86	b

Table 6.14. Tukey-Kramer mean separation test for root dry weight at three application rates (AR1, AR2, and AR3) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	AR1	2.2882	120	a
2	AR2	1.9434	119	ab
3	C-LSE	1.9410	10	ab
4	AR3	1.9203	111	b

Table 6.15. Two-way analysis of variance (seaweed extract concentration by formulation and application rate) for total dry weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration + formulation	3	524.89	174.96	2.8956	0.0352	*
Application rate	2	601.66	300.83	4.9787	0.0074	**
Interaction						
Seaweed extract concentration + formulation * application rate	6	319.72	53.29	0.8819	0.5082	ns
Error	347	20967.10	60.42			
Total	359	22438.28				

Two-way analysis of variance for application rate and seaweed extract concentration within formulation C only

Table 6.16. Two-way analysis of variance (seaweed extract concentration and application rate) within formulation C (FC) for shoot dry weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration	3	1368.21	456.07	11.3329	0.0000	***
Application rate	2	100.44	50.22	1.2479	0.2910	ns
Interaction						
Seaweed extract concentration * Application rate	6	515.80	85.97	2.1362	0.0544	ns
Error	115	4627.96	40.24			
Total	127	6670.46				

Table 6.17. Two-way analysis of variance (seaweed extract concentration and application rate) within formulation C (FC) for root dry weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration	3	14.71	4.90	12.7625	0.0000	***
Application rate	2	0.62	0.31	0.8103	0.4472	ns
Interaction						
Seaweed extract concentration * Application rate	6	2.46	0.41	1.0683	0.3857	ns
Error	115	44.18	0.38			
Total	127	62.31				

Table 6.18. Two-way analysis of variance (seaweed extract concentration and application rate) within formulation C (FC) for total dry weight.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration	3	1655.54	551.85	11.7725	0.0000	***
Application rate	2	95.38	47.69	1.0173	0.3648	ns
Interaction						
Seaweed extract concentration * Application rate	6	576.20	96.03	2.0487	0.0647	ns
Error	115	5390.73	46.88			
Total	127	7785.30				

6.3 Maize Shoot Moisture Content

Table 6.19. Three-way analysis of variance for shoot moisture content.

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Seaweed extract concentration	3	18.17	6.06	1.4862	0.2182	ns
Formulation	2	17.00	8.50	2.0859	0.1259	ns
Application rate	2	65.76	32.88	8.0669	0.0004	***
Interaction						
Seaweed extract concentration * formulation	6	88.35	14.73	3.6130	0.0018	**
Seaweed extract concentration * application rate	6	16.64	2.77	0.6804	0.6656	ns
Formulation * application rate	4	9.49	2.37	0.5823	0.6757	ns
Seaweed extract concentration * formulation * application rate	12	49.72	4.14	1.0166	0.4330	ns
Error	323	1316.44	4.08			
Total	359	1584.17				

Table 6.20. Tukey-Kramer mean separation test for shoot moisture content at three application rates (AR1, AR2, and AR3) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	AR3	87.3468	111	a
2	AR2	86.8983	119	ab
3	C-LSE	86.493	10	ab
4	AR1	86.2778	120	b

6.4 Maize Nutrient Tissue Concentrations

Table 6.21. One-way analysis of variance for shoot Nitrogen concentration (%).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	0.848	0.141	4.7197	0.0079	**
Error	14	0.419	0.030			
Total	20	1.267				

Table 6.22. Tukey-Kramer mean separation test for shoot Nitrogen concentration (%) at four levels of seaweed extract concentration (SE), four slow-release fertilizer formulations (F), three application rates (AR) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SE2-FC-AR3	1.1867	3	a
2	SE1-FA-AR3	1.1767	3	ab
3	SE4-FB-AR3	1.0600	3	ab
4	C-LSE	0.9067	3	ab
5	SE1-FC-AR1	0.7367	3	ab
6	SE3-FC-AR2	0.7067	3	ab
7	SE1-FC-AR2	0.7033	3	b

Table 6.23. One-way analysis of variance for shoot Potassium concentration (%).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	7.161	1.194	8.8687	0.0004	***
Error	14	1.884	0.135			
Total	20	9.045				

Table 6.24. Tukey-Kramer mean separation test for shoot Potassium concentration (%) at four levels of seaweed extract concentration (SE), four slow-release fertilizer formulations (F), three application rates (AR) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SE1-FA-AR3	2.9280	3	a
2	SE2-FC-AR3	2.8217	3	ab
3	SE4-FB-AR3	2.7383	3	ab
4	C-LSE	1.8500	3	bc
5	SE3-FC-AR2	1.7001	3	c
6	SE1-FC-AR2	1.5770	3	c
7	SE1-FC-AR1	1..5450	3	c

Table 6.25. One-way analysis of variance for shoot Phosphorous concentration (%).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	0.044	0.007	4.7714	0.0076	**
Error	14	0.022	0.002			
Total	20	0.066				

Table 6.26. Tukey-Kramer mean separation test for shoot Phosphorous concentration (%) at four levels of seaweed extract concentration (SE), four slow-release fertilizer formulations (F), three application rates (AR) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SE2-FC-AR3	0.3153	3	a
2	SE1-FA-AR3	0.3923	3	ab
3	SE4-FB-AR3	0.2906	3	ab
4	C-LSE	0.2390	3	ab
5	SE1-FC-AR1	0.2173	3	ab
6	SE1-FC-AR2	0.2010	3	b
7	SE3-FC-AR2	0.2003	3	b

Table 6.27. One-way analysis of variance for shoot Iron concentration (ppm).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	156.92	26.15	4.1537	0.0132	*
Error	14	88.15	6.30			
Total	20	245.07				

Table 6.28. Tukey-Kramer mean separation test for shoot Iron concentration (ppm) at four levels of seaweed extract concentration (SE), four slow-release fertilizer formulations (F), three application rates (AR) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SE1-FA-AR3	25.6533	3	a
2	SE2-FC-AR3	25.4100	3	a
3	SE4-FB-AR3	21.5533	3	ab
4	SE1-FC-AR1	21.2533	3	ab
5	SE3-FC-AR2	19.4000	3	ab
6	SE1-FC-AR2	19.2533	3	ab
7	C-LSE	18.2967	3	b

Table 6.29. One-way analysis of variance for shoot Zinc concentration (ppm).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	80.54	13.42	7.6565	0.0009	***
Error	14	24.55	1.75			
Total	20	105.09				

Table 6.30. Tukey-Kramer mean separation test for shoot Zinc concentration (ppm) at four levels of seaweed extract concentration (SE), four slow-release fertilizer formulations (F), three application rates (AR) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SE1-FA-AR3	12.8800	3	a
2	SE2-FC-AR3	12.1700	3	ab
3	SE4-FB-AR3	10.7533	3	abc
4	C-LSE	8.9833	3	bc
5	SE1-FC-AR1	8.3567	3	c
6	SE3-FC-AR2	8.0100	3	c
7	SE1-FC-AR2	7.5900	3	c

6.5 Maize Shoot Nutrient Accumulation

One-Way Analyses of Variance

Table 6.31. One-way analysis of variance for shoot Calcium content (g).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	0.0022	0.0004	3.5696	0.0233	*
Error	14	0.0014	0.0001			
Total	20	0.0036				

Table 6.32. One-way analysis of variance for shoot Magnesium content (g).

Source Main effects	df	Type II sum of squares	Mean square	F-value	P-value	
Treatment	6	0.0031	0.0005	7.6947	0.0008	***
Error	14	0.0009	0.0001			
Total	20	0.0040				

Table 6.33. Tukey-Kramer mean separation test for shoot Magnesium content (g) at four levels of seaweed extract concentration (SE), four slow-release fertilizer formulations (F), three application rates (AR) and the liquid seaweed extract control (C-LSE).

Rank	Mean name	Mean	n	Non-significant ranges
1	SE1-FC-AR2	0.0578	3	a
2	SE1-FC-AR1	0.0560	3	ab
3	SE3-FC-AR2	0.0499	3	abc
4	C-LSE	0.0397	3	abcd
5	SE4-FB-AR3	0.0336	3	bcd
6	SE2-FC-AR3	0.0277	3	cd
7	SE1-FA-AR3	0.0265	3	d

Table 6.34. One-way analysis of variance for physiological nitrogen use efficiency.

Source	df	Type II sum of squares	Mean square	F-value	P-value	
Main effects						
Treatment	6	12087.2009	2014.5335	5.6147	.0037	**
Error	14	5023.1115	358.7937			
Total	20	17110.3124				