Using Seaweed as a Soil Amendment: Effects on Soil Quality and Yield of Sweet Corn (Zea mays L.)

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USING SEAWEED AS A SOIL AMENDMENT:

EFFECTS ON SOIL QUALITY AND YIELD OF

SWEET CORN (ZEA MAYS L.)

BY

ANGELA R. POSSINGER

A THESISSubmitted in partial fulfillment of the
requirements for the degree of

MASTER OF SCIENCE

in

BIOLOGICAL AND ENVIRONMENTAL SCIENCES

UNIVERSITY OF RHODE ISLAND

2011
ABSTRACT

Application of seaweed to manage soil fertility is a traditional practice in many coastal regions, utilizing an inexpensive, abundant, and nutrient-rich resource. As a practice that re-purposes waste materials, diversifies inputs, and relies on coastal resources, seaweed amendment may be an effective and inexpensive means of strengthening and supporting agriculture in coastal agroecosystems. Putatively, seaweed biomass may be a useful amendment for crop production and soil quality improvement due to provision of plant nutrients (e.g. N, P, K, Ca), and promotion of microbial activity, among other benefits. However, limitations of seaweed application include high sulfur (S), salt, and heavy metal content. The objectives of this study were to: (1) evaluate the effects of seaweed biomass application on soil physical, biological, and chemical properties important for agricultural productivity, maintenance of soil quality, and conservation of soil resources; (2) determine the sweet corn \textit{(Zea mays L.)} yield obtained by implementing seaweed amendment as a soil fertility management practice; and (3) assess the economic feasibility of seaweed amendment for sustainable agriculture through synthesis of experimental findings and cost-benefit comparison between seaweed application and pre-formulated fertilizer use.

Low-dose seaweed (LDS), high-dose seaweed (HDS), and pre-formulated 8-1-9 (N-P-K) organic fertilizer (PFF) fertilizer treatments were employed in a sweet corn production field experiment from October 2011 to November 2012. Pre-seeding N application rates were 42, 84, and 45 kg total N/ha for LDS, HDS, and PFF, respectively. All fertilizer treatments received side-dress N at a rate of 68 kg total
N/ha. Seaweed was collected and applied in November 2011 and May 2012, and was analyzed for carbon (C), nitrogen (N), heavy metal, and nutrient (e.g. K) content. To determine seaweed effects on soil quality, soil properties were assessed prior to seaweed application (October 2011), and repeatedly throughout the 2012 sweet corn growing season. Soil properties evaluated were aggregate stability, bulk density, infiltration, available water capacity (AWC), nitrate (NO$_3^-$), phosphate (PO$_4^{3-}$), extractable potassium (K$^+$), extractable calcium (Ca$^{2+}$), heavy metals (Pb, Cd, Cr, Zn, and As), total K, Fe, Mn, and Ca, electrical conductivity (EC), pH, sulfate (SO$_4^{2-}$), soil organic matter (SOM), active C, potentially mineralizable N (PMN), and earthworm abundance. Soil properties were determined using recommended national and regional protocols. To assess the effects of seaweed amendment on crop production, the yield and quality of sweet corn was determined by measurement of yield (hundredweight/ha and bushels/ha), above-ground biomass, average ear weight, and dissolved soluble solids (DSS) content.

Seaweed amendment had no significant effects on soil physical properties. No significant differences in NO$_3^-$ and PO$_4^{2-}$ were observed in response to seaweed addition. Extractable K$^+$ levels were higher, indicating that primary nutrient provision was equivalent or improved with seaweed addition. In contrast, soil pH decreased and EC and SO$_4^{2-}$ increased significantly as a result of seaweed amendment, and these effects varied in persistence. For instance, in May 2012, pH decreased from 6.0 to 5.6, EC increased from 42 to 329 microsiemens (µS)/cm, and SO$_4^{2-}$ increased from 1.4 to 8.7 ppm between the PFF and HDS fertilizer treatments, respectively, but these values returned to PFF levels at the end of the growing season. No effects were observed in
extractable Ca$^{2+}$, total heavy metals, or total K, Fe, Mn, and Ca. Significant increases in active C in both seaweed treatments were observed in the later part of the growing season, with average active C of 608 mg C/kg dry soil in LDS and HDS in August 2012, compared to 492 mg C/kg dry soil in the PFF fertilizer treatment. In contrast, PMN decreased in seaweed treatments compared to PFF in July 2012. Soil organic matter and earthworm abundance did not differ significantly as a result of seaweed amendment.

The average yield (45 hundredweight/ha), above-ground biomass (0.5 kg dry weight/plant), and DSS (15 °Brix) did not differ among fertilizer treatments, but the average weight of fresh corn ears was significantly greater in the LDS fertilizer treatment (0.22 kg) compared to the PFF treatment (0.19 kg). Overall, these results suggest that seaweed amendment as a means of partially replacing total N supply (38% and 55% for LDS and HDS, respectively) may be a viable agricultural practice. However, the implementation of this practice must be viewed in light of financial requirements (e.g. labor and transportation) and potential yield enhancement, as well as persistence and magnitude of soil quality changes. A preliminary analysis showed that the additional costs of labor and transportation may not be offset by increases in yield and decreases in fertilizer cost. For improved financial viability of seaweed amendment, these expenses may be reduced by improved coordination of seaweed collection and application. With improvements in collection efficiency and prediction of nutrient supply, seaweed amendment is recommended, primarily due to improvements in soil biological quality (active C) and sweet corn quality (average ear weight).
ACKNOWLEDGMENTS

I am grateful for the support of my major advisor, José Amador. In particular, I greatly appreciate his initial support when I first presented the idea to evaluate seaweed as an amendment material, and continued guidance in preparation of a graduate student grant to fund the project and development of research design and analysis goals. Throughout the time spent working in the lab, I have greatly appreciated the opportunity to ask questions (maybe too many at times) about the day-to-day research process, as well as discuss many aspects of science and education from a broader perspective. I am indebted to Dr. Amador for facilitating an atmosphere of both dedicated scholarship and enjoyable collegiality, and for continuously keeping track of my progress and overall well-being.

My thesis committee members Dr. Rebecca Brown and Dr. Steven Alm have been instrumental in developing the agronomic aspects of this project, with input towards crop management practices, analysis techniques, and statistical analysis, as well as guidance in preparation of the proposed research. At the Greene H. Gardner Crops Research Center, I would like to especially thank Carl Sawyer and Tim Sherman for help with equipment and crop management, as well as the farm crew. In the Laboratory of Soil Ecology and Microbiology, I would like to thank Janet Atoyan for laboratory skill and instrument training. My undergraduate assistants Andrew Giguere and Nathan Winkler were essential in completing the field and laboratory work necessary for this project. Special thanks to Andrew and Nate for the time spent collecting and spreading seaweed, taking soil respiration readings, counting earthworms, and husking corn – your time, effort, and positive attitude made an
enormous difference. Thank you to the rest of the laboratory and field helpers: Bianca Peixoto, Abra Clawson, Mike Badziemerowski, Ethan Sneesby, and Joshua Sargent. Many thanks to my lab mates, Alissa Becker and Jen Cooper – dinners, Downton Abbey, knitting circles, walks, Thai food, and many entertaining conversations have been an important part of my graduate school experience.

In the Department of Natural Resources Science, I am thankful for the frequent help and lunch conversations with Deb Bourassa. There are many instances of sharing knowledge, tools, and instruments among various laboratories in the department, and I would like to especially thank Dr. Mark Stolt, Andy Paolucci, Brett Still, and Jonathan Bakken in the Laboratory of Pedology and Soil Environmental Science for assistance with many analysis procedures.

Funding for this study was provided by a Sustainable Agriculture Research and Education Graduate Student Grant (GNE-026).
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CHAPTER 1

INTRODUCTION

Soil amendment with organic materials is a common component of soil fertility management for crop production, with the aim of providing essential plant nutrients and improving overall soil physical, chemical, and biological quality (Diacano and Montemurro, 2010). Marine macroalgae, or seaweed, has been historically used as a soil amendment material, and may have application for modern agriculture as a low-cost source of nutrient-rich biomass (Angus and Dargie, 2002; Cuomo et al., 1995). While seaweed compost and extract products have been widely evaluated for agricultural applications (Woznitza and Barrantes, 2005; Khan et al., 2010), evaluation of unprocessed seaweed biomass as an amendment material is limited, particularly with regard to soil quality. Application of seaweed material may uniquely affect soil quality parameters as a result of its chemical characteristics, including carbon (C) and nitrogen (N) composition, and salt, sulfur (S), heavy metal, and trace element content. In this study, the putative benefits of seaweed amendment for crop growth and production were assessed in a sweet corn (Zea mays L.) field experiment, including analysis of soil physical, biological, and chemical properties.

Historical use of seaweed in agriculture

In coastal regions, collection and application of seaweed is a traditional soil fertility management strategy, especially where agriculture relies on use of local resources (Cuomo et al., 1995). As a readily-available, low-cost material to supplement soil fertility, application of seaweed biomass is often an integral
component of traditional, small-scale, diversified agriculture (Angus and Dargie, 2002). For instance, agriculture in the Machair region of the Scottish Outer Hebrides Islands involves a rotation-intensive system that integrates the application of locally-available seaweed biomass (Angus and Dargie, 2002; Kent et al. 2003). Traditional agriculture of the Machair, practiced for at least 1,000 years before present (YBP), relies on a “crofting” system that generally includes an intensive rotation of livestock grazing, field crop cultivation, and two years of fallow, with hypothesized effects on soil biodiversity (Angus and Dargie, 2002; Vink et al., 2009). Soil fertility is still largely maintained by the traditional practice of application of manure and seaweed, primarily the brown alga *Laminaria digitata* (Angus and Dargie, 2002), which is collected and piled onshore for 1-2 weeks prior to application. Promotion of seaweed application as a part of sustaining small-scale, diversified agriculture is supported by Scottish Natural Heritage, a governmental conservation organization, as well as local conservation group efforts (Angus and Dargie, 2002).

In addition to the Machair region, historical accounts of seaweed use in agriculture range from the British Isles, to coastal mainland Europe, to the northeastern region of the United States, including New York, Maine, and Rhode Island (Fussel, 1973; Smith et al., 1989; Cuomo et al., 1995). For example, prior to the adoption of synthetic fertilizer, potato production in Rhode Island incorporated seaweed collection as a means of maintaining soil fertility, including for agricultural research at the University of Rhode Island Agricultural Experiment Station (R. Casagrande, personal communication).
Seaweed in the modern agricultural context

In organic or reduced-input cropping systems, both in the U.S. and worldwide, seaweed-based agricultural products (e.g. extracts for foliar application and composts) are commonly employed (Khan et al., 2009). However, application of unprocessed biomass is less prevalent. To reduce dependence on application of inorganic fertilizers, make use of an abundant (sometimes over-abundant) resource, and improve soil quality, the traditional practice of seaweed application may have modern application in coastal regions. Because adding seaweed to soil can increase plant macro- and micronutrients, and may improve soil biological, chemical and physical properties (Khan et al., 2009), the practice may be an additional strategy to manage soil fertility and quality that addresses the dual problems of reliance on inorganic chemical fertilization and wasting of valuable, nutrient-rich biomass.

Inorganic fertilizer inputs account for a large fraction of conventional farm expenses, energy consumption, and carbon emissions (Lal, 2004). Application of inorganic fertilizers without addition of organic amendments, cover crop use, or use of alternative tillage practices can result in depletion of soil organic matter (SOM), with concomitant negative effects on many soil properties important for crop productivity (e.g. nutrient retention, moisture-holding capacity, aggregate formation, and microbial activity) (Brock et al., 2012; Franzluebbers, 2012). Furthermore, levels of nutrient elements other than N, P, and K (e.g. Ca, Mg, Mo, B, and S) are generally low in inorganic fertilizers, and are of increasing concern for crop quality and nutritional value (Welch and Graham, 2012). Consequently, reliance on inorganic fertilizer as a sole source of fertility is often questioned as a sustainable management strategy, and
diversification of inputs is encouraged, particularly inputs that provide not only primary nutrients (i.e. N, P and K), but also organic matter and trace elements (Lal, 2004). Organic amendments used to improve soil fertility include traditional (e.g. animal manure) and non-traditional (e.g. industrial by-products) materials (Power et al., 2000). Seaweed, which contains primary nutrients, organic C, and other nutrient elements, is thus a good candidate organic amendment material as part of a diversified soil fertility management strategy.

In addition to the potential crop nutrition benefits of seaweed amendment, the prevalence of seaweed biomass in coastal areas as a result of both natural phenomena and anthropogenic impacts may allow for use of seaweed with minimal cost. Nutrient (N and P) enrichment of coastal waters – sometimes attributed to fertilizer runoff from agriculture and home use – can cause excessive seaweed growth (Morand and Merceron, 2005). In addition to detrimental ecological impacts (e.g. oxygen depletion), the accumulation of seaweed biomass on beaches can have negative economic consequences (RI DEM, 2010). For instance, in the summer of 2012, accumulation of the red seaweed *Polysiphonia* sp. on Massachusetts beaches required mechanical removal and disposal in order to maintain beaches for public use, costing money for equipment use and labor, as well as preventing beach use. Beach-cast biomass is often removed and disposed of in landfills. Although the species composition and properties of beach-cast seaweed varies based on location and environment (e.g. estuarine vs. marine), the coordination of accumulated seaweed biomass removal with agricultural application may provide a low-cost, locally-available resource for soil fertility management. To initiate this arrangement for
coastal regions, characterization of seaweed biomass in terms of location and abundance, species composition, and chemical characteristics relevant to soil quality and plant nutrition is required. Additionally, quantification of seaweed biomass effects on soil quality and crop production is required to validate putative benefits or negative effects of seaweed amendment practices.

**Integrated soil quality**

To account for potential impacts of seaweed amendment on factors beyond the standard soil fertility measures (e.g. primary nutrients, physical properties), integrated soil quality should be assessed. An integrated approach to soil quality includes physical, chemical, and biological soil characteristics, including both common soil test parameters (e.g. pH and primary nutrient levels) and less-common parameters developed as indicators of overall soil health and potential for crop production (Gugino *et al.*, 2009). In many cases, a change in one soil property (e.g. increased stability of soil aggregates) affects other soil quality parameters (e.g. infiltration and bulk density). In particular, soil quality indicators are most useful when they are supported by evidence of correlation with increased yield, and reflect both rapid and long-term changes in soil quality as a result of management practice (Weil *et al.*, 2003; Gugino *et al.*, 2009). In addition to common soil test parameters, such as pH and primary nutrients, levels of active C and potentially mineralizable N (PMN) are considered effective indicators of soil biological quality, with evidence supporting correlation with improved crop productivity (Weil *et al.*, 2003; Gugino *et al.*, 2009).
Sweet corn production

In addition to assessment of effects on overall soil quality, effective evaluation of agricultural management practices is improved by determining effects on measurable factors relevant to farm economic viability – namely changes in crop yield – with direct effect on income. For comparatively small-scale vegetable production – the scale at which seaweed amendment is likely most applicable – direct market crops such as sweet corn are common. In Rhode Island, approximately 445 ha (1100 acres) were planted to sweet corn in 2007, with an economic value of about $2,000,000 USD (USDA NASS, 2013). In contrast, only approximately 200 ha (500 acres) were planted to potatoes, another historically important Rhode Island vegetable crop (USDA NASS, 2013). Sweet corn has relatively high nutrient requirements, and typically receives both broadcast and side-dress fertilization (UMass Cooperative Extension, 2013).

Seaweed effects on soil quality

Previous research has addressed the potential for re-purposing of problematic seaweed biomass, usually through production and evaluation of composted seaweed products (Cuomo et al., 1995). Characteristics of seaweed-derived composts vary greatly depending on other ingredients (e.g. inclusion of high C materials such as wood chips) and the properties of the seaweed material (e.g. C:N ratio). Composting processes may be a means to generate a consistent seaweed-derived product in terms of chemical and biological characteristics. However, the relatively low C:N ratio of seaweed biomass (C:N = 18:1) in comparison to terrestrial plant biomass (C:N = ~20 to 100:1) (Lobban and Harrison, 1997) favors N mineralization, so transformation of fresh seaweed biomass to a compost material may result in loss of N, with
concomitant reduction in crop yield in comparison to non-seaweed comports (Cuomo et al., 1995; Wosnitza and Barrantes, 2005). Seaweed-based extracts have been widely evaluated for various agricultural purposes, including the stimulation of plant growth and defense response, soil nutrient enrichment, and promotion of microbial activity and mycorrhizal fungi (Khan et al., 2009). However, most evaluations have been laboratory-based, and the effects of amendment with unprocessed or composted seaweed on soil quality and crop productivity in the field remain understudied.

The seaweed properties with most potential relevance to crop production include (1) elemental composition (e.g. primary plant nutrients, other nutrient elements, heavy metals, S and salts) and (2) organic compound composition (e.g. energy sources for microbial processes). In comparison to terrestrial plants, seaweed generally has higher concentration of Ca, K, Mg, Na, Cu, Fe, I, and Zn (MacArtain et al., 2007). For instance, the Ca content of seaweed varies from 300 to 5750 mg/kg wet weight across the primary algal taxonomic groups, with Ascophyllum nodosum (a common constituent of beach-cast seaweed) representing the highest value. However, as dynamic accumulators of contaminants in the marine environment, seaweed biomass may also be a source of heavy metals (e.g. Pb, Cd, and Cr) when collected from an environment with high levels of these elements (Woznitza and Barrantes, 2005).

Additionally, seaweed biomass can contain higher levels of arsenosugars than terrestrial plants (Castlehouse et al., 2003). Evaluation of Machair soils traditionally amended with Laminaria digitata and Fucus vesiculosus suggests that As may accumulate over time in the form of arsenosugar decomposition products, including
dimethylarsinic acid, arsenate (As(V)), and arsenite (As(III)) (Castlehouse et al., 2003). Furthermore, high S content in seaweed, in the form of organic S compounds (e.g. fucans and carrageenans, sulfated polysaccharides found in brown and red seaweed, respectively) may result in increased S application (Jaulneau et al., 2010). The anaerobic decomposition of organic S results in the production of sulfides (e.g. H₂S) and elemental S, which are subject to microbial oxidation to SO₄²⁻, with net production of hydrogen ions (H⁺) (Brady and Weil, 2008). This can lower soil solution pH, which controls the availability of nutrients. S is also a plant nutrient, and in soils with limited S supply, its addition in seaweed may be beneficial for crop production (Brady and Weil, 2008).

Seaweed may also have a high salt content, which may increase its plant nutrient content (e.g. K⁺) (Rupérez, 2002), but can also contribute to development of saline soil conditions from long-term application. Consequently, historical application of seaweed biomass generally includes a period of rinsing by rain for the purpose of decreasing salt content (Angus and Dargie, 2002). Inorganic ions present in seaweed include Na⁺ and Cl⁻, the most prevalent ions in seawater, as well K⁺, Ca²⁺, and Mg²⁺ (Rupérez, 2002). Increases in soil salinity could result in negative effects on the soil biotic community and crop production through effects on water balance and toxicity of salt ions (primarily Na⁺ and Cl⁻), particularly for salt-sensitive crops such as legumes, which have a salinity threshold of ~1000 microsiemens (µS)/cm (Maas, 1990).

Many of the organic compounds present in seaweed are different from those of terrestrial plants, and vary across the main marine macroalgal taxonomic groups (Jiménez-Escrig and Sánchez-Muniz, 2000). For instance, polysaccharides specific to
seaweed groups include carrageenans, laminarins, and ulvans, specific to red (Rhodophyta), brown (Phaeophycea), and green (Chlorophyta) algae, respectively (Jiménez-Escrig and Sánchez-Muniz, 2000; Jaulneau et al., 2010). Seaweed can also contain persistent compounds commonly found in terrestrial plants, such as cellulose, hemicellulose, and lignin, but the concentration of compounds resistant to microbial degradation (particularly lignin) is less than that of terrestrial plants (Jiménez-Escrig and Sánchez-Muniz, 2000). Consequently, differences in terms of organic C composition between seaweed and terrestrial biomass sources in agriculture (e.g. mulch straw, crop residue) may be associated with: (1) the diversity of carbohydrate compounds, which may expose the soil microbial community to novel sources of organic carbon, and (2) differences in the proportions of readily-degradable compounds (e.g. simple sugars) and compounds more resistant to biodegradation and mineralization (e.g. lignin) by the soil microbial community.

An additional organic component of brown seaweed with potential impact on soil properties is alginate, a gelling polyurinide, which functions in prevention of seaweed dessication (Jiménez-Escrig and Sánchez-Muniz, 2000). The water-holding function of alginate and other gelling polysaccharides may influence soil water holding capacity.

**Objectives**

In order to evaluate the efficacy of seaweed amendment as a practice with application to modern agriculture in Rhode Island, I conducted a field experiment comparing the effects of amendment with seaweed on the yield of sweet corn and soil
quality in comparison to use of organic pre-formulated fertilizer (N-P-K = 8-1-9). The primary objectives of this study were to:

(1) Evaluate the effect of seaweed amendment relative to pre-formulated fertilizer on the yield and quality of sweet corn, an economically important crop for local agricultural production.

(2) Evaluate seaweed amendment effects on physical, chemical and biological soil quality parameters in comparison to a conventional inorganic fertilization treatment.

(3) Assess the economic feasibility of seaweed amendment for sustainable agriculture in coastal New England through synthesis of experimental findings and cost-benefit comparison between seaweed application and pre-formulated fertilizer use.

**Hypotheses**

Evidence from laboratory evaluation of seaweed compost quality known characteristics of seaweed biomass, and putative qualities of seaweed extract products combine to support hypothesized effects on crop yield and soil quality properties (Table 1).
Table 1. Summary of hypothesized effects of seaweed amendment on soil physical, biological, and chemical quality parameters. Relationships between soil parameters and soil productivity were determined based on information from the Cornell Soil Health Assessment Guide (Gugino et al., 2009) and the USDA ARS Soil Quality Assessment Manual (1999).

| Soil Quality Parameter | Hypothesized effect of seaweed amendment | Relationship to soil productivity |
|------------------------|-----------------------------------------|----------------------------------|
| PHYSICAL               |                                         |                                  |
| Aggregate stability    | Increased stability as a result of higher organic matter inputs and fungal biomass | High aggregate stability improves water infiltration and air exchange by decreasing the formation of surface crusts |
| AWC                    | Increased AWC by addition of seaweed moisture-retaining compounds and increased soil organic matter (SOM) | Represents the capacity of soil to store water between rainfall events, especially important during drought periods |
| Infiltration           | Greater infiltration from increased aggregate formation and stability | Limited infiltration can cause long periods of surface saturation, resulting in limited plant nutrient availability and oxygen availability to roots, and increased susceptibility to erosion |
| Bulk density           | Lower bulk density resulting from higher SOM, aggregate formation and stability | High bulk density can limit root growth, and associated plant nutrient and water uptake |
| BIOLOGICAL             |                                         |                                  |
| SOM                    | Increased SOM due to addition of seaweed biomass | Provides nutrients and energy to plants and soil microbial communities |
| Active C               | Increased active carbon due to promotion of microbial activity | Indicates readily available carbon and energy source for the soil microbial community |
| Soil respiration       | Increased respiration due to increased microbial activity | Soil microorganisms decompose organic materials, regulate nutrient cycling, and influence other soil properties such as aeration and composition of soil atmosphere |
| PMN                    | Increased N mineralization due to promotion of microbial activity | Indicates capacity of soil microbial community to transform organic nitrogen into plant-available forms |
| Earthworm abundance    | Decreased earthworm abundance due to toxic effects | Through their feeding, burrowing and casting |
of seaweed (e.g. high levels of osmolytes, sulfur) activities, earthworms improve aggregation, soil drainage, aeration, and soil nutrient availability

| CHEMICAL             | Description                                                                 | Effects                                                                 |
|----------------------|-----------------------------------------------------------------------------|------------------------------------------------------------------------|
| Sulfur/sulfate       | Increased sulfur/sulfate due to high levels in seaweed                      | S oxidation may reduce pH, but S can also be a plant nutrient          |
| pH                   | Reduction in pH as a combination of S oxidation, nitrification, and C mineralization processes | Soil solution pH affects availability of plant nutrients               |
| Primary nutrients (N, P, K) | Increased primary and secondary nutrients (released during decomposition from nutrient-rich seaweed biomass) | Levels and timing of plant nutrients are essential for plant growth and grain production processes |
| Other nutrient elements (Ca, Fe, Mo, Al, Mn, Zn, Cu) | Increased nutrient elements (released during decomposition from nutrient-rich seaweed biomass) | For trace elements small quantities are required, and excessive amounts may have toxic effects. |
| Heavy metals (Pb, Hg, Ni, Cd, Cr, As) | Increased heavy metals in soil, due to possible high levels in seaweed material | High levels of heavy metals may accumulate in soil, which can increase levels in crops and be toxic to soil microflora and fauna |
| Salinity (electrical conductivity) | Increased salinity due to residual salt content | High levels of salts (esp. sodium) in soil can limit plant growth |

**Soil quality.** Hypothesized positive effects of seaweed amendment (i.e. improved soil quality parameters) include increased aggregate stability, infiltration, available water capacity (AWC), SOM, active C, PMN, primary and trace elements, and decreased bulk density (Table 1). Potential negative effects include increased heavy metal content, electrical conductivity (EC), sulfur/sulfate concentration, and decreased pH and earthworm abundance (Table 1).

**Crop yield.** In seaweed-amended plots, sweet corn yield and quality is hypothesized to be at least equal to plots fertilized with pre-formulated organic 8-1-9...
(N-P-K) fertilizer due to the provision of plant nutrients and improvement of diverse soil quality parameters.

**Experiment overview**

I conducted a field experiment was conducted over one growing season to evaluate the effect of seaweed addition on soil quality properties and yield of sweet corn. Soil properties evaluated were: (1) physical (aggregate stability, bulk density, infiltration, and AWC); (2) biological (active C, SOM, soil respiration, PMN, and earthworm abundance); and (3) chemical (sulfur/sulfate, heavy metals, primary and other plant nutrients, pH, and EC). Seaweed material of mixed composition (red, green, and brown species) was collected from Rhode Island beaches in fall 2011 and spring 2012, and applied at two levels: low-dose seaweed (LDS) (42 kg total N/ha) and high-dose seaweed (HDS) (84 kg total N/ha), to replace broadcast fertilization. The seaweed fertilizer treatments were compared to an organic pre-formulated fertilizer (PFF) treatment (45 kg total N/ha). Sweet corn was seeded in May 2012, and side-dress PFF (68 kg total N/ha) was applied to all treatments. At the end of the growing season, corn was harvested and yield determined. Sweet corn quality was assessed by determination of dissolved soluble solids. Soil quality parameters were analyzed for time, overall fertilizer treatments, and interaction effects using Repeated Measures ANOVA, followed by Univariate ANOVA to determine differences among fertilizer treatments at each sampling date. Sweet corn parameters at harvest were analyzed statistically using Univariate ANOVA.
CHAPTER 2

METHODOLOGY

Site description

Twelve field treatment plots (Figure 1) were established in October 2011 at the University of Rhode Island’s Greene H. Gardner Crops Research Center in Kingston, RI. The field was previously planted with butternut winter squash (Cucurbita moschata) in 2011 and disc harrowed prior to initial soil sampling. The soil at the site is in the Enfield series (coarse-silty over sandy or sandy-skeletal, mixed, active, mesic Typic Dystrudepts) (Soil Survey Staff, 2003). Mean annual temperature is 7 to 11°C and mean annual precipitation is 102 to 127 cm (Soil Survey Staff, 2003). Across the site, average (n=3) soil particle size distribution was 40% sand, 49% silt, and 11% clay-sized particles.

Treatments

In order to evaluate the effect of pre-seeding seaweed biomass application in comparison to pre-formulated fertilizer application on the yield of sweet corn (Zea mays L. var. rugosa) and soil quality parameters, 3 fertilizer treatments were employed:

A) Low-dose seaweed (~13,840 kg wet wt/ha) (LDS)

B) High-dose seaweed (~27,680 kg wet wt/ha) (HDS)

C) Organic pre-formulated fertilizer (8-1-9 N-P-K) (PFF)
Fertilizer treatments differed in the form of nutrient addition prior to crop production (broadcast fertilization). For the PFF fertilizer treatment, prior to corn seeding, granulated organic fertilizer (Nature’s Turf 8-1-9, North Country Organics, Bradford, VT) was applied at a rate of 45 kg total N/ha. Of the total N in the PFF treatment, readily-available NO$_3^-$-N composes 27%, with the remaining 73% N in organic forms (e.g. peanut meal, pasteurized poultry litter, and feather meal). Likewise, seaweed biomass was applied at a rate of 42 and 84 kg total N/ha for the
LDS and HDS treatments, respectively, but with the majority of the N in organic forms (0.1% and 0.06% of total N as NO$_3^-$ and NH$_4^+$, respectively). Consequently, the amount of N available is dependent on N mineralization rate. For the PFF treatment, 27% of total N is readily available; of the remaining organic N, ~60% N mineralization is expected within the growing season (Hartz and Johnstone, 2006). Available N from broadcast PFF application is thus estimated as 32 kg N/ha. While no field mineralization values are available for seaweed biomass, N availability for subsequent crops over the growing season for materials with similar N content and low lignin content (e.g. common vetch, *Vicia sativa* L.) are near 50% (Sattell *et al*., 1998), corresponding with a broadcast N application rate of 21 and 52 kg N/ha for LDS and HDS seaweed treatments, respectively.

For each treatment, 4 replicates were employed and arranged in a randomized block design (Figure 1). All crop production and soil quality sampling was conducted within the inner 4.6 x 4.6 m of each plot, excluding border rows (Figure 1).

**Seaweed collection and characterization**

Seaweed biomass for fall application was collected by hand from Watch Hill Beach (WHB), Westerly, RI (41°18′30.27″N, 71°51′48.08″W) in November 2011. Seaweed material was separated by major species groups and identified using a dichotomous key (Villalard-Bohsack, 2003). Additionally, seaweed biomass was collected in late April 2012 from Mackerel Cove, Jamestown, RI, (41°29′18.55″N, 71°23′0.28″W) to supplement fall application. For both applications, the seaweed biomass was piled (~1 m$^3$) near the treatment plots for ~1 week, and received no further processing prior to application. Seaweed biomass was applied by hand.
Randomly selected biomass sub-samples (~1000 g, n=3 for each collection date) were air-dried, ground, and sieved (0.25-mm-mesh) prior to C, N, heavy metal, and trace element analysis. Seaweed C and N content was analyzed using a Carlo Erba EA1108 CHN analyzer (CE Instruments, Inc., Wigan, Ireland). \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) levels were determined in dry, ground seaweed biomass for both collection dates by extraction with 2 M KCl at a 1:5 seaweed-to-extractant ratio. Extracts were shaken for 1 h and gravity filtered with Whatman #42 paper. The filtrate was analyzed for \( \text{NO}_3^- \) and \( \text{NH}_4^+ \) colorimetrically (Doane and Harwath, 2003), with 96-well culture plates and a BioTek PowerWave 340 microplate spectrophotometer (BioTek Instruments, Inc., Winooski, VT). For \( \text{NO}_3^- \) analysis, 100 µL vanadium (III) trichloride solution (saturated vanadium (III) trichloride solution in 1 M HCl, 2% sulfanilamide solution, and 0.2% N-(1-naphthyl)-ethylenediamine dihydrochloride solution) was mixed with a 100 µL aliquot of extract sample and absorption determined at \( \lambda = 540 \) nm after 5 h incubation at room temperature (Doane and Harwatch, 2003). For \( \text{NH}_4^+ \) analysis, 80 µL of a mixed solution of commercial bleach and 6% (w/v) NaOH solution and 80 µL of a sodium salicylate solution were reacted with 40 µL sample (Weatherburn, 1967) and absorption determined at \( \lambda = 650 \) nm after 50 min incubation at room temperature.

**Soil texture**

Soil particle size distribution was determined for samples composited by treatment (n=3) using the hydrometer method (Sheldrick and Wang, 1993).
Soil moisture

Decagon 10-HS sensors (Decagon Devices, Inc., Pullman, WA) were used to measure soil moisture content in all plots in October-November 2011, March-May 2012, and July-September 2012. Sensors (one per plot) were placed within sweet corn rows at a depth of 10 cm and were set to record data on an hourly basis.

Soil physical properties

Soil physical properties were determined in October 2011, May 2012, and September 2012, except for bulk density and infiltration, which were determined in October 2011 and May 2012.

Aggregate stability. Percent wet-aggregate stability (%WAS) was determined for the 1-2 mm aggregate size class using the wet-slaking method (Angers and Mehuys, 1993). For each treatment plot, 3 soil cores (5 cm-dia. x 15 cm length) were collected within the plot sampling area (Figure 1) and mixed with minimal disruption of natural soil aggregates. From each bulk sample, an ~500 g portion of soil was dried at 40°C for 24 h, and sieved to isolate 1-2 mm aggregates, for which water content was determined (W_C) by difference after oven drying (24 h at 105°C). To determine stability after wet slaking, 10 g of 1-2 mm aggregates (W_1) were placed on a #60 sieve (0.25-mm-mesh), saturated by capillary action, and repeatedly submerged in water at a rate of ~30 cycles/min for 10 min. Following slaking, remaining aggregates were dried (24 h at 105°C), weighed (W_2), and dispersed by shaking in 0.5% (w/v) sodium hexametaphosphate solution to determine the weight of primary particles (W_3). Final %WAS was determined using the following equation:

\[
%\text{WAS} = 100 \frac{(W_2 - W_3)}{(W_1/(1+W_C)) - W_3)
\]
**Available water capacity.** AWC represents the difference between water content at field capacity ($\theta_{FC}$) and at the permanent wilting point ($\theta_{PWP}$). AWC was determined by collection of 2 intact soil cores (5 cm-dia. x 15 cm) per treatment plot, with soil held in cores by a cheesecloth cover. In order to approximate saturation (water potential ($\Phi$) $\sim$ 0 kPa), cores were soaked for 24 hr within a container filled to a level 2.5 cm below core top edge. After soaking, cores were removed and allowed to drain for 48-72 hr to approximate $\theta_{FC}$ ($\sim$ 30 kPa). After draining, the $\theta_{FC}$ was determined by measurement of wet and oven-dry (24 h at 105°C) weight of the entire soil core, where:

$$\theta_{FC} = (\text{wet weight (g)} - \text{dry weight (g)}) / \text{(dry weight (g))}$$

Water content at the permanent wilting point ($\theta_{PWP}$) was estimated using a predictive model based on particle size distribution (Saxton *et al.*, 1986). Final AWC ($\text{cm}^3/\text{cm}^3$) was calculated using the equation:

$$\text{AWC} = \theta_{FC} - \theta_{PWP}.$$  

**Bulk density.** Bulk density was determined by collection of 3 separate soil cores (5 cm x 20 cm, volume=271.8 cm$^3$) per plot and measurement of dry weight (24 h at 105°C) of the total soil volume (USDA ARS, 1999).

**Infiltration.** Infiltration rate was determined by measuring the time required for 2.5 cm water to infiltrate at the soil surface (USDA ARS, 1999). A 25-cm-diameter PVC ring (one per plot), with the bottom edge buried ~2.5 cm below the soil surface, was filled to a depth of 2.5 cm with water. The time required for no standing water to remain visible was determined for 2 consecutive additions of water, with the second addition representing the infiltration rate.
Soil chemical properties

Soil chemical analyses were completed on composite bulk soil samples consisting of 5 soil cores (5 cm-dia. x 15 cm) collected from each treatment plot and mixed thoroughly. Properties determined in October 2011, May 2012, and September 2012 include extractable K\(^+\) and Ca\(^{2+}\), total nutrient elements, and heavy metals. Parameters determined monthly (October and November 2011 and April through September 2012) include NO\(_3^−\), PO\(_4^{3−}\), SO\(_4^{2−}\), pH, and EC.

**Extractable potassium and calcium.** K\(^+\) and Ca\(^{2+}\) were extracted from fresh, sieved (2-mm-mesh) soil using Morgan’s solution (0.5 M sodium acetate and 0.5 acetic acid solution adjusted to pH 4.8) at a 1:5 soil-to-extractant ratio. Extracts were shaken for 1 h (low speed), and aliquots of the suspension were transferred to 2 mL plastic microcentrifuge tubes and centrifuged to separate soil particles from the extraction solution (5 min at 13,000 RPM). Following extraction, the supernatant solution was analyzed using inductively-coupled plasma-optical emission spectrometry (ICP-OES) (Optima 8300 Spectrometer, Perkin Elmer, Waltham, MA). Samples were diluted 1:10 in 5% nitric acid for analysis, and analyzed in triplicate (5% nitric acid eluent) with the following wavelengths and view angles: Ca = 317.93 nm, radial view; K = 766.49 nm, radial view.

**Total heavy metal and nutrient element content.** The concentration of total nutrient elements (Ca, Fe, Al, Mn, and Mo) and heavy metals (mercury (Hg), lead (Pb), and cadmium (Cd)) was determined using a Niton XL3t X-Ray Fluorescence Analyzer (ThermoFisher Scientific, Billerica, MA). Prior to analysis, soil was air-dried, ground, and sieved (0.25-mm-mesh).
**Nitrate.** NO$_3^-$ was extracted from fresh sieved soil (2-mm-mesh) 1-2 d after soil bulk sample collection (1-2 d) using a 2 M KCl solution at a 1:5 soil-to-extractant ratio (Gugino et al., 2009). Extraction was completed as described above for extractable K$^+$ and Ca$^{2+}$. The NO$_3^-$ concentration in extracts was determined colorimetrically as described above for seaweed biomass NO$_3^-$ analysis.

**Phosphate.** Extractable soil P was determined for fresh sieved soil (2-mm-mesh) by 0.5 M NaHCO$_3$ extraction (Schoenau and Karamanos, 1993). A 1:10 soil-to-extractant ratio was used, with 30 min shaking and centrifugation as described above for extractable K$^+$ and Ca$^{2+}$. Prior to spectrophotometric PO$_4^{3-}$ analysis, extracts were acidified by addition of 23 µL concentrated sulfuric acid. The PO$_4^{3-}$ concentration in extracts was determined by reaction of 32 µL Murphy-Riley solution with 200 µL sample (Schoenau and Karamanos, 1993). Absorbance was determined at $\lambda=712$ nm after 15 min incubation at room temperature.

**Sulfate.** SO$_4^{2-}$ was extracted from fresh, sieved soil (2-mm-mesh) using a 0.01 M CaCl$_2$ extraction solution at a 1:2 soil-to-extractant ratio (Kowalenko, 1993). After shaking for 30 min at low speed, the extract solution was filtered (#42 Whatman), and ~10 mL filtrate reacted with 0.1 g BaCl$_2$ (LaMotte Company, Chestertown, MD). The concentration of SO$_4^{2-}$ was determined immediately after reaction by measurement of absorbance ($\lambda=420$ nm) using a Shimadzu UV160U UV-Visible Recording Spectrophotometer (Shimadzu Corp., Kyoto, Japan).

**pH.** Soil pH was determined using an UltraBasic pH meter (Denver Instruments, Bohemia, NY) in a 1:1 soil to water soil suspension shaken for 2 min followed by 30 min equilibration time.
**Electrical conductivity.** EC was determined with Traceable® Dual-Display Conductivity Meter (Control Company, Friendswood, TX) in a 1:2 soil to water soil suspension, with automatic instrumental temperature correction.

**Soil biological properties**

Soil biological analyses, with the exception of soil respiration and earthworm abundance, were completed on bulk soil samples composed of 5 soil cores (5 cm x 15 cm) collected from each treatment plot and mixed thoroughly. Analyses were completed monthly for soil samples collected October-November 2011 and April-September 2012.

**Soil organic matter.** SOM was determined by loss-on-ignition at 550°C for 5 hr.

**Active carbon.** Active C represents the fraction of soil C oxidizable by KMnO₄. For each treatment, 2.0 g air-dry, ground soil (0.1-mm-mesh) was reacted with 8 mL 0.2 M KMnO₄, vortexed, and shaken for 2 min at high speed. Aliquots of the suspension were transferred to 2 mL plastic microcentrifuge tubes and centrifuged to separate soil particles from the extraction solution (5 min at 13,000 RPM). The concentration of KMnO₄ in the supernatant remaining after reaction with soil was determined by measurement of absorbance at 550 nm with 1:10 dilution of samples in deionized water (Gugino et al., 2009). The mass of oxidized C (mol) was assumed to be equivalent to mass of KMnO₄ oxidized (mol), calculated using the following equation:

\[
\text{Mol C} = \frac{([\text{average unreacted KMnO}_4] - [\text{sample}])}{0.008 \text{ L}}
\]

The total active C (mg C/kg dry soil) was calculated using the equation:
Active C (mg C/kg dry soil) = (mol C x (12 g/mol C) x 1000 mg/g)/kg dry soil

*Potentially mineralizable nitrogen.* Soil NH$_4^+$ concentration was determined before (Day=0) and after 7 days (Day=7) of anaerobic incubation. For Day 0, NH$_4^+$ was extracted from fresh sieved soil (2-mm-mesh) soon after soil bulk sample collection (1-2 d) using 2 M KCl at a 1:5 soil-to-extractant ratio (Gugino *et al.*, 2009). For Day 7 incubated samples, 8 g fresh, sieved soil (2-mm-mesh) was hand-shaken with 10 mL deionized water, covered, and incubated at 30°C for 7 d. After incubation, NH$_4^+$ was extracted by adding 30 mL 2.67 M KCl, followed by 1 h shaking (low speed) and separation of soil particles by centrifugation as described above. NH$_4^+$ concentration in extracts was determined colorimetrically as described above. The difference in ammonium concentration between Day 0 and Day 7 represents PMN.

*Earthworm abundance.* For each treatment plot, a 30 cm x 30 cm x 30 cm volume of soil was dug and sorted, and earthworms counted and weighed. Additionally, 1 L of mustard solution (1 Tbs. powdered mustard/L) was added to the hole to facilitate the upward movement of deep-burrowing earthworms.

*Soil respiration.* In-field carbon dioxide (CO$_2$) flux measurements were completed using the dynamic closed-chamber method (Richardson, 2006). For each treatment plot, one 25 cm-diameter PVC collar was installed 2.5 cm deep, with an exposed inner-surface collar depth of 9 cm. An air-tight lid was attached, and CO$_2$ concentration within the chamber was measured over a 5 min measurement period using a Li-Cor 6262 infrared gas analyzer (Li-Cor, Lincoln, NE). Measurements of CO$_2$ concentration (µmol CO$_2$/mol air) were recorded at 10 s intervals, and plotted as a function of time. A best-fit linear regression was applied to the data. The mass of
CO₂ within the chamber, n (mol), was determined using the universal gas law,
\[ n = \frac{RT}{PV} \], where \( n = \text{mol CO₂ per mol air} \), \( P = \text{atmospheric pressure (atm)} \), \( V = \text{volume of gas in chamber (L)} \), \( R = \text{universal gas constant (0.0821 L atm/mol K)} \), and \( T = \text{chamber air temperature (K)} \). The rate of CO₂ production per unit area (kg C/ha/day) was determined using the best-fit line slope and calculated volume of air in the chamber and cross-sectional area, following Richardson (2006).

**Sweet corn production**

In May 2012, sweet corn (Zea mays L. cv. “Trinity,” Johnny’s Selected Seeds, Winslow, ME) was seeded by hand at a depth of 2.5 cm and a rate of ~2-4 seeds/20 cm, and later thinned to a final linear plant density of 1 plant/30 cm. Due to uneven germination, corn was re-seeded as necessary through June 5, 2012. Between-plot borders were seeded with perennial ryegrass (Lolium perenne) and mowed weekly. Following standard sweet corn management (UMass Cooperative Extension, 2013), side-dress supplemental N was applied using Nature’s Turf 8-1-9 pre-formulated fertilizer at a plant height of ~30 cm, at a rate of 68 kg total N/ha for all treatments. Throughout the growing season, weeds were removed within the treatment plots by hand cultivation. European corn borer (Ostrinia nubilalis) was controlled by plant and ear-tip application of B. thuringiensis var. kurstaki (Johnny’s Selected Seeds, Winslow, ME).

Corn was harvested by hand at silk dry-down stage in August and September 2012. Immediately after harvest, ears were weighed whole to determine average fresh weight and yield (hundredweight and bushels/ha). Additionally, 20% of the fresh ears were analyzed for dissolved soluble solids (°Brix) using a field refractometer. After
harvest, 33% of the remaining standing stalks (every third stalk) were cut, weighed, and dried (24 h at 60°C) to estimate above-ground biomass.

**Statistical analyses**

For all soil quality properties except heavy metals and trace elements, data were analyzed for overall effects of sampling month, fertilizer treatment, and interaction effects (sampling month x fertilizer treatment) using Repeated Measures ANOVA ($\alpha=0.05$) in IBM® SPSS® Statistics v. 20 (International Business Machines, Inc., Armonk, NY). When the assumption of sphericity for the time variance-covariance matrix was violated ($p<0.05$), the Greenhouse-Geiser adjusted degrees of freedom (DF) and probability ($p$-value) were used to determine significance (Von Ende, 1993) (**APPENDIX 1**). Univariate ANOVA was used to determine differences among fertilizer treatments at each sampling date. Data violating the assumption of equal variance were transformed logarithmically, and if transformation failed to yield heteroscedasticity, the $p$-value of Levene’s Test for Equal Variance was adopted as the new level of significance, following Underwood (1981) (**APPENDIX 1**). Multiple comparisons for Univariate ANOVA tests were completed using Tukey’s Test ($\alpha=0.05$). Sweet corn yield and quality parameters were analyzed using Univariate ANOVA in SigmaPlot v. 11.0, followed by Tukey’s Multiple Comparison Test ($\alpha=0.05$) (**APPENDIX 2**). For soil levels of total heavy metals and trace elements, Two-Way ANOVA was applied in SigmaPlot v. 11.0 (Systat Software, Inc., Chicago, IL), with sampling month (October 2011 and September 2012) and fertilizer treatment as variables (**APPENDIX 3**).
Economic evaluation

In order to evaluate the economic implications of seaweed application, expenses throughout the sweet corn production process were recorded for both seaweed and pre-formulated fertilizer treatments (e.g. seed, labor, agricultural chemicals, transportation, and fertilizer costs). Additionally, the difference in expected income was assessed by estimation of corn market value based on data from the USDA National Agricultural Statistics Service (NASS).
CHAPTER 3

RESULTS

Seaweed characterization

Species composition. Seaweed biomass collected in fall 2011 from Westerly, RI, was largely composed of brown and red seaweed species, including *Ascophyllum nodosum* (12.5% dry weight (DW), *Laminaria digitata* (2% DW), *Chondrus crispus* (15.2% DW), *Fucus vesiculus* (8.2% DW), assorted filamentous red algae (10.5% DW), and mixed, non-algal plant material (e.g. eelgrass, 51.5% DW). Seaweed collected in spring 2012 from Jamestown, RI included *Saccharina saccharina* (0.5% DW), *A. nodosum* (9.5% DW), *Fucus* sp. (11.6% DW), *Grinellia americana* (2.5% DW), *C. crispus* (3.3% DW), *Ulva* sp. (0.95% DW), assorted filamentous red algae (69.7% DW), and mixed non-algal plant material (1.7% DW).

Carbon and nitrogen content. Seaweed C content and C:N ratio varied between collection dates, with material collected in fall 2011 having a higher C content and C:N ratio than material collected in spring 2012 (Table 2). Additionally, seaweed material had a higher dry matter content at the time of application in fall 2012. KCl-extractable NH$_4^+$ and NO$_3^-$ accounted for 0.06% and 0.1% of the total N in seaweed material, respectively.

Total heavy metal and nutrient element content. Cd, Cr, Hg, and Cu were not present above the instrumental limit of detection (LOD) (Table 3). Pb, As, and Zn were detected at low concentrations in seaweed from both collection sites (Table 3).
Total K, Ca, Fe, Mn, and S were detected in seaweed biomass at relatively high levels in comparison to terrestrial plants (Table 4.)

**Table 2.** Average (a, n=6; b, n=4; c, n=12, and d, n=3) seaweed C, N dry matter content, and C:N ratio (±standard deviation) for Westerly and Jamestown.

| Sampling location | Date    | Dry matter content  | C     | N     | C:N   |
|-------------------|---------|---------------------|-------|-------|-------|
|                   |         | %  | g/kg dry matter | %     | g/kg dry matter | %     | g/kg dry matter | %     | g/kg dry matter | %     | g/kg dry matter |
| Westerly Nov 2011 | 22.3^a  | ±6.9         | 213.7^c | ±10.8 | ±3.9   | ±5.4   |
| Jamestown April 2012 | 16.5^b | ±4.0         | 153.9^d  | ±26.1 | ±2.5   | ±0.29  |

**Table 3.** Average (n=3) seaweed heavy metal content (±standard deviation) for Westerly and Jamestown.

| Sampling location | Date    | Pb     | Cd    | Cr    | Zn     | Hg     | Cu     | As     |
|-------------------|---------|--------|-------|-------|--------|--------|--------|--------|
|                   |         | mg/kg DW |       |       |        |        |        |        |
| Westerly Nov 2011 | 10.3    | <LOD   | <LOD  | 58.7  | <LOD   | <LOD   | 9.6    | ±3.2   |
| Jamestown April 2012 | 14.2 | <LOD   | <LOD  | 68.4  | <LOD   | <LOD   | 9.8    | ±1.8   |

**Table 4.** Average (n=3) seaweed total element content (±standard deviation) for Westerly and Jamestown.

| Sampling location | Date    | K     | Ca    | Fe    | Mn    | S     |
|-------------------|---------|-------|-------|-------|-------|-------|
|                   |         | g/kg DW |       |       |       |       |
| Westerly Nov 2011 | 31.7    | 23.8  | 4.1 ± | 0.14  | 22.4  |
| Jamestown April 2012 | 50.8  | 31.9  | 7.4   | 0.60  | 23.0  |
Soil moisture

For the weeks with continuous volumetric water content measurement (10/20/2011-11/24/2011, 4/4/2012-5/14/2012, and 7/25/2012-9/14/2012), average weekly volumetric water content did not differ significantly among fertilizer treatments (Figure 2, Table 5). Additionally, gravimetric water content at the time of soil nutrient extraction was equivalent among fertilizer treatments (Figure 2, Table 5). Both weekly volumetric and gravimetric water content differed significantly over time, but no significant interaction effects were present.

Figure 2. Average (n=4) weekly volumetric water content for all fertilizer treatments. Error bars represent one standard deviation.
Figure 3. Average (n=4) gravimetric water content at the time of nutrient (e.g. NO$_3^-$ and PO$_4^{3-}$) analysis. Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

Table 5. Summary of soil moisture main effects (time, fertilizer treatment and time X fertilizer treatment) from Repeated Measures ANOVA. Factors with $p$-values below level of significance ($p<0.05$) are indicated with (*).
Soil physical properties

No statistically significant differences in soil physical properties were detected as a result of seaweed treatment at any sampling date (Table 6, Table 7). For aggregate stability and infiltration, time was significant as a main effect, but the same trends were detected for all fertilizer treatments (Table 6, Table 7). Overall aggregate stability for all treatments was greater in September 2012, and infiltration rate was increased in May 2012 compared to October 2011.

Table 6. Average (n=4) values for soil physical properties (±standard deviation). Within columns, values with the same letter are not significantly different.

| Fertilizer treatment | Wet-aggregate stability (| (%) | Bulk density (g/cm³) | Infiltration rate (cm/min) | Available water capacity (g/g) |
|----------------------|-------------------------|----------|----------------------|-----------------------------|-----------------------------|
| LDS                  | Oct 23.6 ±8.1 | May 26.2 ±13.3 | Sep 25.2 ±2.8 | Oct 1.15 ±0.03 | May 1.16 ±0.02 | Sep 0.42 ±0.02 | Oct 0.23 ±0.02 | May 0.23 ±0.02 | Sep 0.21 ±0.03 |
| HDS                  | Oct 31.6 ±3.9 | May 34.4 ±4.6 | Sep 33.4 ±3.1 | Oct 1.15 ±0.07 | May 1.14 ±0.01 | Sep 0.13 ±0.07 | Oct 0.22 ±0.01 | May 0.22 ±0.02 | Sep 0.20 ±0.03 |
| PFF                  | Oct 32.7 ±8.3 | May 46.7 ±10.8 | Sep 43.9 ±7.8 | Oct 1.15 ±0.05 | May 1.17 ±0.02 | Sep 0.23 ±0.02 | Oct 0.23 ±0.37 | May 0.21 ±0.05 | Sep 0.28 ±0.15 |

Table 7. Summary of physical property main effects (time, fertilizer treatment and time X fertilizer treatment) from Repeated Measures ANOVA. Factors with p-values below level of significance (p<0.05) are indicated with (*).

| Parameter          | Time (sampling date) | F statistic | DF | p-value | F statistic | DF | p-value | F statistic | DF | p-value |
|--------------------|----------------------|-------------|----|---------|-------------|----|---------|-------------|----|---------|
| Aggregate stability|                      | 23.62       | 2  | <0.001* | 0.975       | 2  | 0.418   | 0.57        | 4  | 0.688   |
| AWC                |                      | 0.169       | 1  | 0.691   | 0.341       | 2  | 0.72    | 1.394       | 2  | 0.297   |
| Bulk density       |                      | 0.083       | 1  | 0.78    | 0.139       | 1  | 0.872   | 0.152       | 2  | 0.861   |
| Infiltration       |                      | 12.13       | 1  | <0.01*  | 2.74        | 2  | 0.117   | 2.31        | 2  | 0.155   |

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Soil chemical properties

Nitrate. Levels of NO$_3^-$ varied significantly over time, but did not vary overall with fertilizer treatment as a main effect (Figure 3, Table 10). No interaction effects between sampling month and fertilizer treatment were detected. However, in May 2012, NO$_3^-$ levels were significantly higher in the HDS treatment than the LDS or PFF treatments. For all treatments, NO$_3^-$ increased after addition of seaweed and PFF, reaching maximum levels in July 2012.

![Nitrate-N (NO$_3^-$-N) (µg/g dry soil)](image)

**Figure 3.** Mean (n=4) NO$_3^-$-N concentration as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

Phosphate. PO$_4^{3-}$ levels differed significantly over time, and no interaction effects were detected between sampling month and seaweed treatment (Figure 4,
Table 10). Although no overall main effects of seaweed treatment were identified, PO$_4^{3-}$ levels were significantly lower in the HDS treatment in September 2012 relative to PFF and LDS treatments.

![Graph showing PO$_4^{3-}$ levels over time for different treatments](image)

**Figure 4.** Mean (n=4) PO$_4^{3-}$-P levels as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

**Extractable potassium and calcium.** K$^+$ levels did not differ significantly over time, but did differ with seaweed treatment, and significant interaction effects were detected (Figure 5, Table 10). In May 2012, after seaweed application, K$^+$ levels increased with seaweed addition, following the order: HDS>LDS>PFF. Extractable Ca$^{2+}$ levels decreased significantly between October 2011 and May 2012 sampling, but did not differ uniformly as a function of fertilizer treatment (Figure 6, Table 10).
In addition, interaction effects were present between sampling month and seaweed treatment.

**Figure 5.** Mean (n=4) extractable K⁺ concentration as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.
**Figure 6.** Mean (n=4) extractable Ca$^{2+}$ concentration as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

**Total elements.** The heavy metals Cd, Hg, Ni, Co, and Cu were not present in soil above the limit of detection for any treatment. As and Zn were detected, but did not increase between October 2011 and September 2012 (before and after seaweed addition) for any fertilizer treatment (Table 8, Table 11). Cr was also detected, and increased significantly between October 2011 and September 2012, but no significant effect of seaweed addition was detected (Table 8, Table 11). Although Pb was significantly lower in the PFF treatment plots in October 2011, this difference is presumably due to field heterogeneity, and Pb was not significantly different at the
end of the growing season (Table 8, Table 11). The trace nutrient Mo was not present above the instrumental limit of detection. Total K, Ca, Fe, and Mn did not change over time, or as a function of fertilizer treatment, and no interaction effects were detected (Table 9, Table 11).

Table 8. Average (n=4) total heavy metal content of soil (± standard deviation) prior to seaweed addition (October 2011) and at the end of the growing season (September 2012). Within columns, values with the same letter are not significantly different.

| Fertilizer treatment | Pb  | Cr  | Zn  | As  |
|----------------------|-----|-----|-----|-----|
|                      | mg/kg dry soil | Oct | Sep | Oct | Sep | Oct | Sep | Oct | Sep |
| LDS                  |     |     |     |     |     |     |     |     |     |
| 23.1 ± 1.3           | 22.8 ± 13       | 39.8 ± 6.9    | 50.6 ± 7.7    | 26.5 ± 1.8    | 26.1 ± 7.2    | 22.8 ± 6.6    | 22.2 ± 2.4    |
| HDS                  |     |     |     |     |     |     |     |     |     |
| 19.4 ± 1.7           | 18.9 ± 4.5      | 40.7 ± 4.9    | 52.8 ± 8.3    | 24.2 ± 3.8    | 32.3 ± 5.6    | 25.1 ± 1.7    | 22.5 ± 0.62   |
| PFF                  |     |     |     |     |     |     |     |     |     |
| 16.9 ± 5.3           | 19.7 ± 2.2      | 26.5 ± 1.8    | 50.4 ± 14     | 26.4 ± 2.0    | 28.7 ± 2.9    | 21.4 ± 4.3    | 25.1 ± 5.0    |

Table 9. Average (n=4) total nutrient element content of soil (± standard deviation) prior to seaweed addition (October 2011) and at the end of the growing season (September 2012). Within columns, values with the same letter are not significantly different.

| Fertilizer treatment | K    | Ca   | Fe   | Mn   |
|----------------------|------|------|------|------|
|                      | g/kg dry soil | Oct | Sep | Oct | Sep | Oct | Sep | Oct | Sep |
| LDS                  |     |     |     |     |     |     |     |     |     |
| 14.8 ± 0.49          | 15.0 ± 0.23   | 8.08 ± 0.31    | 7.93 ± 0.21    | 19.7 ± 1.3    | 18.6 ± 0.15    | 0.330 ± 0.0025    | 0.351 ± 0.0019   |
| HDS                  |     |     |     |     |     |     |     |     |     |
| 15.0 ± 0.59          | 15.2 ± 0.31   | 8.02 ± 0.20    | 7.96 ± 0.39    | 19.3 ± 0.47    | 18.5 ± 0.23    | 0.314 ± 0.0026    | 0.302 ± 0.0024   |
| PFF                  |     |     |     |     |     |     |     |     |     |
| 15.3 ± 0.15          | 15.0 ± 0.20   | 8.21 ± 0.39    | 7.94 ± 0.007   | 18.8 ± 1.3    | 19.0 ± 0.30    | 0.314 ± 0.0057    | 0.351 ± 0.0044   |

Sulfate. Soil $\text{SO}_4^{2-}$ levels differed significantly over time and as a function of seaweed treatment, and interaction effects were detected (Figure 7, Table 10). $\text{SO}_4^{2-}$
levels in the HDS treatment increased significantly compared to the LDS and PFF treatments in May 2012 (after spring seaweed application), and remained significantly higher in June and July 2012. By the end of the growing season, SO$_4^{2-}$ levels decreased in the HDS and LDS treatments, but were still significantly higher in the HDS fertilizer treatment at the end of the growing season.

**Figure 7.** Mean (n=4) SO$_4^{2-}$-S concentration as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

**pH.** Soil pH varied significantly over time, with significant interactions between sampling month and seaweed treatment (Figure 8, Table 10). Although no overall main effects attributed to seaweed treatment were detected, pH was
significantly lower for both seaweed treatments in November 2011, April 2012, and May 2012, with values following the order: LDS<HDS<PFF. After May 2012, pH values in LDS and HDS treatments increased to those observed for the PFF treatment.

Figure 8. Mean (n=4) pH as a function of fertilizer treatment. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

**Electrical conductivity.** EC varied significantly over time and as a function of seaweed treatment (Figure 9, Table 10). Significant interaction effects between seaweed treatment and sampling date were detected. EC did not differ among fertilizer treatments in October 2011 or at the end of the growing season (September 2012), but was significantly higher in seaweed-amended treatments than the PFF treatment in both November 2011 and April 2012. Across sampling dates with differences in EC,
values for the HDS treatment were consistently higher than for the LDS treatment, which was consistently higher than the PFF treatment (Figure 9).

**Figure 9.** Mean (n=4) electrical conductivity as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.
Table 10. Summary of chemical property main effects (time, fertilizer treatment, and time X fertilizer treatment) from Repeated Measures ANOVA. Factors with $p$-values below level of significance ($p<0.05$) are indicated with (*).

| Property | Time (sampling date) | Fertilizer treatment | Interaction effects |
|----------|----------------------|----------------------|---------------------|
|          | $F$ statistic  | DF  | $p$-value | $F$ statistic | DF  | $p$-value | $F$ statistic | DF  | $p$-value |
| NO$_3^-$ | 64.73              | 2.8 | <0.001*  | 0.209        | 2.9 | 0.815     | 2.47          | 5.7, 26 | 0.052     |
| PO$_4^{3-}$ | 46.72          | 1.8 | <0.001*  | 0.18         | 2.9 | 0.835     | 1.48          | 3.7, 17 | 0.253     |
| K$^+$    | 1.55              | 2   | 0.242     | 15.12        | 2.8 | <0.005*   | 3.06          | 4.16, <0.05* |
| Ca$^{2+}$ | 58                | 2   | <0.001*   | 2.772        | 1.6 | 0.147     | 6.29          | 2.12, <0.05* |
| SO$_4^{2-}$ | 7.59            | 1.7 | <0.05*    | 5.93         | 2.6 | <0.05*    | 3.89          | 3.5, 10  | <0.05*    |
| pH       | 89.68             | 1.9 | <0.001*   | 0.557        | 2.9 | 0.592     | 4.42          | 2.9, <0.05* |
| EC       | 25.13             | 1.6 | <0.001*   | 165.8        | 2.9 | <0.001*   | 5.78          | 3.3, <0.05* |
Table 11. Summary of Two-Way ANOVA main effects (time, fertilizer treatment, and time X fertilizer treatment) for heavy metals and nutrient elements. Factors with p-values below level of significance (p<0.05) are indicated with (*).

| Element | Time (sampling date) | Fertilizer treatment | Interaction effects |
|---------|----------------------|----------------------|---------------------|
|         | F statistic | DF | p-value | F statistic | DF | p-value | F statistic | DF | p-value |
| Pb      | 0.268       | 1,18 | 0.611 | 4.775       | 2,18 | <0.05* | 0.619       | 2,18 | 0.549 |
| Zn      | 2.997       | 1,18 | 0.101 | 0.351       | 2,18 | 0.709 | 1.771       | 2,18 | 0.199 |
| As      | 0.013       | 1,18 | 0.909 | 0.215       | 2,18 | 0.809 | 1.317       | 2,18 | 0.293 |
| Cr      | 9.303       | 1,18 | <0.05* | 0.0642      | 2,18 | 0.938 | 0.0784      | 2,18 | 0.925 |
| K       | 0.012       | 1,18 | 0.918 | 0.65        | 2,18 | 0.534 | 0.963       | 2,18 | 0.401 |
| Ca      | 1.897       | 1,18 | 0.185 | 0.205       | 2,18 | 0.817 | 0.296       | 2,18 | 0.747 |
| Fe      | 2.625       | 1,18 | 0.123 | 0.235       | 2,18 | 0.793 | 1.702       | 2,18 | 0.21  |
| Mn      | 1.094       | 1,18 | 0.309 | 1.834       | 2,18 | 0.188 | 0.981       | 2,18 | 0.394 |

Soil biological properties

Soil organic matter. SOM differed significantly over time, with no significant interaction effects detected between sampling month and seaweed treatment (Figure 10, Table 12). The main effect of seaweed treatment showed no significant differences in SOM. In July 2012, both LDS and HDS treatments were higher in SOM, but these differences were not significant at the adopted level of significance (p<0.005, Levene’s p-value for transformed data) (Univariate ANOVA: F<sub>2,9</sub>=9.254, p=0.007) (Figure 10, Appendix 1).
Figure 10. Mean (n=4) soil organic matter as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

Active carbon. Active C varied significantly over time and as a function of seaweed treatment (Figure 11, Table 12). Significant interaction effects were detected between sampling month and seaweed treatment. Significant positive effects of seaweed treatment were found in July, August, and September 2012. In July 2012, the LDS and PFF treatments differed significantly, while in August 2012, both the LDS and HDS treatments were significantly higher than PFF. In September 2012, the LDS treatment was significantly higher than both the HDS and PFF treatments.
Figure 11. Mean (n=4) active carbon as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

**Potentially mineralizable nitrogen.** PMN varied significantly both over time and as a function of seaweed treatment (Figure 12, Table 12). Additionally, significant interaction effects between sampling month and seaweed treatment were detected. In July 2012, PMN for the PFF treatment nearly 10 times greater than for either LDS or HDS treatments (Figure 12, Table 12).
Figure 12. Mean (n=4) potentially mineralizable nitrogen as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

Soil respiration. Soil respiration varied significantly over time, reaching a minimum value in November 2011 (Figure 13, Table 12). For some months, average soil respiration was greater in HDS plots, but these differences were not significant as a main effect. Interaction effects between sampling month and seaweed treatment were not detected.
Figure 13. Mean (n=4) soil respiration as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.

**Earthworm abundance.** Earthworm abundance did not differ significantly over time or as a function of seaweed treatment, and interaction effects were not detected (Figure 14, Table 12). Earthworm abundance was consistently low across the study site, with an average population density of 1.6 ± 3 earthworms/m³ across all fertilizer treatments and sampling dates.
Figure 14. Mean (n=4) earthworm abundance as a function of fertilizer treatment and time. Months with significant differences among fertilizer treatments are indicated with (*). Error bars represent one standard deviation. Solid and dashed arrows represent seaweed and PFF application dates, respectively.
Table 12. Summary of soil biological property main effects (time, fertilizer treatment, and time X fertilizer treatment) from Repeated Measures ANOVA. Factors with p-values below level of significance (p<0.05) are indicated with (*).

| Property          | Time (sampling date) | Fertilizer treatment | Interaction effects |
|-------------------|----------------------|----------------------|---------------------|
|                   | F statistic | DF | p-value | F statistic | DF | p-value | F statistic | DF | p-value |
| SOM               | 9.55       | 1.2, 10.4 | <0.05* | 1.17       | 2.9 | 0.353 | 0.113       | 2.3, 10.4 | 0.917 |
| Active C          | 15.87      | 7, 56   | <0.001* | 6.6        | 2.8 | <0.05* | 3.2         | 14, 56 | <0.005* |
| PMN               | 101.2      | 2.3, 21 | <0.001* | 5.93       | 2.9 | <0.05* | 8.13        | 4.6, 20.8 | <0.001* |
| Soil respiration  | 42.26      | 7, 49   | <0.001* | 2.25       | 2.7 | 0.176 | 0.996       | 14, 49 | 0.472 |
| Earthworm abundance | 1.06       | 6, 56   | <0.05* | 0.067      | 2.9 | 0.936 | 1.37        | 12, 56 | 0.21 |

Sweet corn production

Average sweet corn yield, measured both as hundredweight/ha and bushels/ha, was greater in LDS and HDS fertilizer treatments, but the increase was not statistically significant (Figure 15, Figure 16, Table 13).
Figure 15. Mean (n=4) sweet corn yield (in hundredweight/ha) as a function of fertilizer treatment. Treatments with the same letter were not significantly different. Error bars represent one standard deviation.
Figure 16. Mean (n=4) sweet corn yield (in bushels/ha) as a function of fertilizer treatment. Treatments with the same letter were not significantly different. Error bars represent one standard deviation.
Similarly, above-ground plant biomass was greater in LDS and HDS fertilizer
treatments, but the increase in biomass was not statistically significant (Figure 17,
Table 13).

![Bar graph](image_url)

**Figure 17.** Mean (n=4) above-ground plant biomass as a function of fertilizer
treatment. Treatments with the same letter were not significantly different. Error bars
represent one standard deviation.
Although overall yield measures were not significantly different, average sweet corn ear fresh weight was greater in the LDS fertilizer treatment than either the HDS or PFF treatments (Figure 18, Table 13).

![Bar chart showing average fresh ear weight as a function of fertilizer treatment. Treatments with the same letter were not significantly different. Error bars represent one standard deviation.](image)

Figure 18. Mean (n=4) ear fresh weight as a function of fertilizer treatment. Treatments with the same letter were not significantly different. Error bars represent one standard deviation.

Additionally, dissolved soluble solids, a measure of sweet corn sweetness in °Brix, were not significantly different among fertilizer treatments (Figure 19, Table 13).
**Figure 19.** Mean (n=4) dissolved soluble solids as a function of fertilizer treatment. Treatments with the same letter were not significantly different. Error bars represent one standard deviation.

**Table 13.** Summary of Univariate ANOVA) corn production parameters. Factors with $p$-values below level of significance ($p$<0.05) are indicated with (*).

| Parameter                | F statistic | DF | p-value |
|--------------------------|-------------|----|---------|
| Fresh ear weight         | 6.47        | 2,9| <0.05* |
| Hundredweight/ha         | 2.91        | 2,9| 0.106   |
| Bushels/ha               | 1.82        | 2,9| 0.217   |
| DSS                      | 0.725       | 2,9| 0.511   |
| Above-ground biomass     | 2.87        | 2,9| 0.109   |
Economic analysis

Between the seaweed and PFF fertilizer treatments, quantifiable differences in cost were associated only with seaweed collection (transportation and labor), estimates of potential yield improvement, and additional cost of fertilizer (Table 14). All economic estimations are based on the total fertilizer treatment area for this experiment (0.01 ha). For labor, the Rhode Island minimum wage in 2013 was used ($7.75/hr). Based only on these factors, the expense of seaweed amendment was approximately 3.5 and 0.5 times greater for the HDS and LDS treatments, respectively.

Table 14. Comparison of costs and benefits of seaweed amendment compared to use of only pre-formulated fertilizer.

| Fertilizer treatment | Costs | Benefits |
|----------------------|-------|----------|
|                      | Amount ($) | Amount ($) |
| LDS                  |       |          |
| Round-trip mileage   | 28 mi @ $0.55/mi | 15.40 |
|                      |       | Increased yield |
|                      |       | 0.18 hundred-weight (Cwt) @ $35/Cwt |
|                      |       | 6.30 |
| Collection labor     | 4.5 hrs @ $7.75/hr | 31.00 |
|                      |       | -- |
|                      |       | -- |
| HDS                  |       |          |
| Round-trip mileage   | 28 mi @ $0.55/mi | 15.40 |
|                      |       | Increased yield |
|                      |       | 0.32 Cwt @ $35/Cwt |
|                      |       | 11.55 |
| Collection labor     | 9 hrs @ $7.75/hr | 69.75 |
|                      |       | -- |
|                      |       | -- |
| PFF                  | Addl. fertilizer cost | 38.8 lb Nature's Turf 8-1-9 @ $35/50 lb. | 27.16 |
|                      |       | -- |
|                      |       | -- |
|                      |       | -- |
CHAPTER 4

DISCUSSION

Seaweed collection

In this study, fall and spring seaweed collection was completed at local sites with frequent and reliable accumulation of beach-cast seaweed biomass. Watch Hill Beach and Mackerel Cove are relatively protected inlets, which often supports high beach deposition of seaweed by natural currents and wave action. However, seaweed proliferation as a result of anthropogenic nutrient inputs may be less at these sites than sites located further from the open ocean. At the time of collection for this study, sites with excessive seaweed accumulation presumably due to anthropogenic causes were scarce, due in part to the season during which collection took place, since seaweed biomass usually reaches the highest levels in July and August (Thornber et al., 2008), as well as beach-clearing weather events, such as Hurricane Rita in September 2011. Consequently, the seasonal variation of excess seaweed accumulation may require monitoring and communication among beach managers and farmers in order to make optimal use of this resource. In lieu of using often hard-to-predict “problematic” seaweed biomass, beach sites with high seaweed accumulation due to inherent geographic or environmental conditions may offer a more consistent and reliable source.
Seaweed characteristics

In comparison to terrestrial plant biomass, the seaweed collected for this study can be characterized as a high-moisture material with relatively high N content (15.5 g/kg DW) and low C:N ratio (10:1 to 13:1). For instance, values of N content and C:N ratios of common crop residue materials are in the range of 4-5 g N/kg plant DW and C:N ratio of ~100:1 for stem biomass, and 12-15 g N/kg plant DW and C:N ratio of ~30:1 for leaf biomass, based on values for soybean (*Glycine max*), corn (*Zea mays*), and switchgrass (*Panicum virgatum*) stems and leaves (Johnson *et al.*, 2007). Additionally, the total calcium (Ca) and potassium (K) content of the seaweed material was higher than many terrestrial plants, on the order of 4 times greater Ca content and 2 times greater K content (Tian *et al.*, 1992). For comparison, biomass of the cover crop velvet bean (*Mucuna pruriens*) contains approximately 5-7 g Ca/kg DW and 18 g K/kg DW (Tian *et al.*, 1992), whereas levels in the seaweed biomass in this study were 21-38 g Ca/kg DW and 31-50 g K/kg DW.

Elements of concern (e.g. heavy metals) may also be found at higher levels in seaweed relative to terrestrial plant biomass; however, in the context of field application guidelines, these were not sufficiently high to raise concerns in this study. For example, As was present in seaweed biomass (maximum 9.8 ± 1.8 mg/kg DM), but at much lower levels than those for *F. vesiculosus* and *L. digitata* biomass collected from coastal Scotland (25 ± 7 and 74 ± 2 mg/kg DM, respectively) (Castlehouse *et al.*, 2003). For sewage sludge, an agricultural amendment with particular risk of heavy metal contamination, the US EPA regulatory limit for As for soil application is 75 mg/kg (USDA NRCS, 2000). Although the seaweed biomass

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collected in this study does not pose a concern in terms of exceeding regulatory limits for As application, consideration of As is warranted for seaweed application, particularly when brown algal species such as *F. vesiculosus* and *L. digitata* constitute a higher percentage of the biomass applied. US EPA yearly application limits and long-term maximum cumulative loading also provide guidelines and perspective for potential As application risks. In the present study, the As level in seaweed biomass would result in an annual loading of 0.03 kg/ha/yr for the HDS treatment, well below the EPA regulatory limit of 2 kg/ha/yr. Additionally, the total maximum cumulative loading for As is 41 kg/ha, which would require ~1,300 years of seaweed application at the HDS rate to reach the maximum load, assuming no losses of As from the soil.

Cadmium (Cd), chromium (Cr), mercury (Hg), and copper (Cu) were not present above the limit of detection. Pb and Zn were detected, but at concentrations substantially lower than the US EPA regulations for sewage sludge application. For Pb and Zn, the maximum levels present in seaweed (14.2 ± 2.8 and 68.4 ± 11.0 mg/kg, respectively), are ~30 times lower than the US EPA limits of 420 and 2500 mg/kg for Pb and Zn, respectively, in sewage sludge. Consequently, the heavy metal content of the seaweed used in this study does not pose a concern in terms of long-term accumulation of heavy metals. Since many seaweed species or ecotypes are tolerant of high levels of heavy metals (e.g. Cu in anti-fouling paint) (Reed and Gadd, 1990), amendment with seaweed biomass collected from areas likely to be affected by heavy metal contamination may require pre-application analysis. Seasonal variation in heavy metal levels in seaweed may also be important for timing of collection, since higher concentrations are generally found in winter and early spring, and lower
concentrations in summer and autumn (Caliceti et al., 2002). Additionally, species- or
group-specific variation in heavy metal accumulation suggests selection of a variety of
seaweed taxonomic groups to avoid over-application of heavy metals. For instance,
higher heavy metal concentrations in are generally found in brown seaweed species
(Phaeophyceae) as a result of increased metal sorption capacity in the alginate cell
wall matrix (Figuera et al., 2000).

Soil quality

*Physical properties*. For all physical properties, no statistically significant
differences were detected among the fertilizer treatments. Consequently, the
hypothesized positive effects of seaweed amendment on soil physical quality
(increased aggregate stability, infiltration and available water capacity, and reduced
bulk density) were not supported in this study. Overall, aggregate stability of soil at
the study site is rated as moderate to poor, ranging from ~30-40% (Gugino et al.,
2009), a value that could be expected for an agricultural soil with repeated, frequent
cultivation. Available water capacity, at 0.224 g/g, is rated as medium to good
(Gugino et al., 2009).

Many soil physical properties, including aggregate stability, require several
years after management changes before appreciable improvements are observed (Islam
and Weil, 2000). The duration of this study was likely insufficient for development of
uniform observable, significant improvements in aggregate stability across the study
site. In order to provide a better perspective for adoption of alternative management
strategies, including amendment with seaweed, it is important to recognize that many
changes in soil physical quality may not be observed immediately after implementation of a management change.

In contrast to aggregate stability, AWC, bulk density, and infiltration rate are more ephemeral properties, and may be expected to respond within days of change in management (Islam and Weil, 2000). Rapid changes in these parameters are generally associated with tillage (i.e. mechanical disruption and aeration), although they may also be affected by substantial inputs of organic matter (Gugino et al., 2009). In this study, the amount of seaweed biomass applied resulted in a layer approximately 0.25 – 1 cm thick, a small amount of biomass relative to the volume of soil in the plow layer, which is on the order of 15 cm. Additionally, the treatments were uniformly subject to mechanical tillage following seaweed application, potentially masking any changes in AWC, bulk density or infiltration as a result of organic matter addition. High variability in infiltration rates across the treatment replicates may also have precluded the development or detection of significant differences.

**Chemical properties.** Significant increases in SO$_4^{2-}$, EC, and exchangeable K$^+$ with seaweed addition support hypothesized effects of seaweed amendment. Changes in these properties were transient, with increases observed soon after seaweed addition, returning to PFF fertilizer treatment levels by the end of the growing season. Additionally, a reduction in pH was hypothesized in response to seaweed amendment, and this effect was observed for a short period (~ 1 month) after addition. By contrast, NO$_3^-$ and PO$_4^{3-}$ levels did not increase in seaweed treatments (LDS and HDS) compared to the PFF treatment, supporting the hypothesis of equivalent provision of
primary nutrients. In contrast, hypothesized increases in total trace elements (Ca, Mn, Fe) and heavy metals (Pb, Cd, Cr, Zn, Hg, Cu, As) in soil were not observed.

Equivalent or increased provision of primary nutrients (in this case, NO$_3^-$, PO$_4^{3-}$, and K$^+$) is an essential factor for successful adoption of an alternative fertilizer management practice, because limitation of these essential nutrients generally results in the most recognizable, quantifiable differences in crop growth, yield, and ultimately, economic viability. Prior to seaweed or PFF addition, soil levels of both PO$_4^{3-}$ and extractable K$^+$ were relatively high (~60 and 150 µg/g dry soil for PO$_4^{3-}$ and K$^+$, respectively). Based on the Cornell Soil Health Guide, extractable K$^+$ at the site is rated as very good, exceeding the published rating chart (Gugino et al., 2009). Potassium does not pose a leaching or toxicity risk, and does not contribute to poor soil quality at high concentrations.

In contrast, PO$_4^{3-}$ and NO$_3^-$ can be a concern at excessive levels due to leaching risk, and improvements in soil quality decrease above maximum concentrations, reaching “poor” rating at concentrations above 30 µg/g dry soil for both PO$_4^{3-}$-P and NO$_3^-$-N (Gugino et al., 2009; Marx et al., 1999; Heckman, 2003). For the soils at the study site, high natural abundance of Fe oxides and hydroxides generally allows for substantial retention of phosphate by metal-P complex formation, and the amount of PO$_4^{3-}$ in the soil solution (i.e. water-extractable PO$_4^{3-}$) was negligible (N. Winkler, unpublished data), suggesting that the majority of PO$_4^{3-}$ extracted with NaHCO$_3$ was previously loosely sorbed to Fe and Al oxide surfaces (Schoenau and Karamananos, 1993). In contrast to PO$_4^{3-}$ and K$^+$, NO$_3^-$ was consistently low (close to 0) across fertilizer treatments prior to seaweed application in October 2011, and early in the
growing season (April and May 2012). Presumably, as availability of N from the seaweed biomass and PFF increased, NO$_3^-$-N levels increased to moderate (10-15 µg N/g dry soil), but leaching risk was likely minimal due to rapid crop uptake.

Addition and subsequent decomposition of seaweed biomass high in total S (~23 g/kg DM) resulted in significant differences in SO$_4^{2-}$, but returned to control levels by the end of the growing season. Increases in SO$_4^{2-}$ could have conflicting effects on soil quality and crop production, influencing both pH and plant nutrition. For example, microbial S oxidation to sulfate results in the production of hydrogen ions (H$^+$), reducing soil pH, with the sulfate contributing to the soluble salt content (Janzen, 1993; Germida, 2005). Alternatively, as a component of amino acids (cysteine, cystine, and methionine) and vitamins (e.g. vitamin A), S is also be a plant nutrient, and may have positive effects on crop production.

Reduction in pH was observed in this study soon after addition of seaweed biomass. In addition to S oxidation as a potential influence on pH, other microbial processes contributing to reduced pH as a result of organic matter addition include (1) C mineralization and carbonic acid (H$_2$CO$_3$) production (Simunek and Suarez, 1993), and (2) ammonia oxidation via nitrification (Myrold, 2005). With respect to plant growth, the pH at the site was initially low, rated as “poor”, with an average pH ~5.3 in October 2011, and increased to “moderate” in the spring, with an average pH ~6.0 in April 2012 (Gugino et al., 2009). pH is a critical variable for soil quality, particularly in relation to nutrient availability; consequently, with a low initial pH, acidification as a result of seaweed addition may be of particular concern (Gugino et al., 2009). Distinction between pH reduction specifically related to seaweed addition
(i.e. S oxidation) and acidification as a result of organic matter addition and decomposition is necessary for elucidation of potential pH effects of seaweed amendment.

As hypothesized, EC was significantly higher in seaweed amended plots, with higher values in HDS than in LDS. EC, which represents the ability of the soil solution to conduct an electrical charge, has a well-supported relationship with soil salinity and crop growth (Janzen, 1993). While Na\(^+\) and Cl\(^-\) are the predominant ions accounting for soil salinity, Ca\(^{2+}\), Mg\(^{2+}\), and SO\(_4\)\(^{2-}\) are also important components of total soil salinity (Janzen, 1993), and are considered plant nutrients. However, the negative effects of increased inorganic ions on plant and microorganism physiology are generally of greatest concern, with negative effects observed at levels above 2000 µS/cm (Janzen, 1993). In this study, EC reached a maximum of ~350 µS/cm as a result of seaweed addition, well below the risk for crop damage, even for especially salt-sensitive crops. For instance, beans (*Phaseolus vulgaris*), have an EC threshold of 1000 µS/cm (Maas, 1990), and sweet corn is moderately salt-sensitive, with a threshold of 1700 µS/cm (Maas, 1990). Thus, although significant EC increases were observed with seaweed addition, they did not reach levels known to have a negative effect on crop physiology. In temperate climates, dissolved salts are generally mobile in the soil, as evidenced by EC returning to PFF fertilizer treatment levels at conclusion of the growing season. Consequently, long-term accumulation may not be of concern, but increased EC remains a potential short-term negative effect.

**Biological properties.** Among the biological properties analyzed, only active carbon (C) was affected by seaweed application. Soil respiration, earthworm
abundance and total SOM content varied over the growing season, but the same trends over time were observed for all fertilizer treatments. PMN was consistent among the fertilizer treatments throughout the growing season, with the exception of a significantly greater value in the PFF treatment in July 2012.

Soil respiration, a measure of overall microbial activity, was hypothesized to increase as a result of seaweed addition due to the provision of C substrates for microbial metabolism, some of which may be new to the microbial community and readily-degradable. In some cases (e.g. May, June, and July 2012), average soil respiration was greater in the HDS fertilizer treatment, but these changes were not consistent over time, and high variability within treatment replicates precluded identification of significant differences among fertilizer treatments. Consequently, the hypothesis of increased soil respiration in response to seaweed amendment was not supported. Similarly, earthworm abundance – hypothesized to decrease due to changes in soil EC – was highly variable across treatment replicates, and overall abundance of earthworms was very low across the study area. Although significant differences in earthworm abundance were not identified, some changes in chemical properties (e.g. increased EC, reduced pH, and increased heavy metal content) would be expected to affect earthworm abundance, particularly through effects on osmotic balance. Earthworms, with high surface area exposed to the soil-water environment, are particularly sensitive to changes in soil EC and other chemical properties (Lee, 1985), and their population density and biomass would be a useful continued indicator of the soil environment’s suitability for macrofauna.
Potentially mineralizable N is a measure of the microbial community capacity to mineralize organic N to NH$_4^+$, a plant-available form. This process is dependent on the presence of microorganisms involved in N mineralization and availability of organic N for mineralization (Myrold, 2005). With addition of N-rich organic matter, PMN levels would be expected to increase with seaweed amendment, but hypothesized increases in PMN were not observed. The opposite effect (decreased PMN in comparison to the PFF fertilizer treatment) was observed on one sampling date (July 2012), wherein PMN in both seaweed treatments was significantly reduced. Overall, PMN was very low for all fertilizer treatments (5-10 µg N/g dry soil/week), corresponding to a rating of “poor” to “moderate” (Gugino et al., 2009), except for the sampling date following the addition of side-dress N, with values increasing to “moderate” to “good” in this treatment. In general, PMN may be correlated with factors such as organic matter, active C, and aggregate stability (Gugino et al., 2009); given moderate to low ratings in these properties in this study, low levels of PMN are a reasonable finding. In some cases, negative values of PMN were observed, indicating net immobilization of NH$_4^+$ resulting from an N-limited environment.

The hypothesized increases in active C were observed on sampling dates closer to the end of the growing season. However, while increases in active C were observed with seaweed addition, these did not correspond with seaweed biomass quantity. The LDS fertilizer treatment had either equivalent or greater values than the HDS treatment on the dates when differences between seaweed and non-seaweed treatments were observed. The time required for disintegration processes (e.g. physical reduction of seaweed particle size) may be a factor in the lag in active C changes, with increases
apparent approximately 3 months after seaweed biomass application (July, August, and September 2012). The average active C level at the study site across all fertilizer treatments (~600 mg/kg dry soil) is rated as “moderate” and follows the general trend of other related biological properties (e.g. SOM and PMN) (Gugino et al., 2009).

In comparison to other measures of soil C (e.g. total SOM), active C responds relatively quickly to changes in management (Weil et al., 2003), representing the fraction of soil C oxidizable by dilute KMnO₄. This fraction includes C in both living and dead microbial biomass, as well as C in compounds readily available to the microbial community (e.g. C in functional groups at the edge of complex organic molecules) (Weil et al., 2003). Active C is generally correlated with increased microbial activity, and is well-supported as an indicator variable of potential crop yield improvement (Weil et al., 2003). Consequently, changes in active C may represent a substantial benefit for overall soil quality and potential yield improvement as a result of seaweed amendment.

**Sweet corn production**

As a soil fertilizer management practice, replacing a part of the total N supply with seaweed was equally effective in terms of yield and above-ground biomass production as using only pre-formulated fertilizer. In this study, seaweed biomass was used to replace fertilizer added at the pre-seeding, or broadcast, fertilization step. At this step, PFF fertilizer was applied at a rate of 45 kg N/ha. Due to high N requirements of sweet corn, an additional side-dress N application is usually required based on soil test results (UMass Cooperative Extension, 2013). Since NO₃⁻ and NH₄⁺ levels were uniformly low - indicating N deficiency - prior to the sidedress stage
(plant height ~30 cm), an additional 68 kg N/ha was applied to all fertilizer treatments, according to standard recommended management practices (UMass Cooperative Extension, 2013). Thus, sweet corn and soil quality production results must be viewed with the perspective of seaweed as providing a portion of overall N supply.

Sweet corn yield was either equivalent or significantly greater for seaweed treatments compared to the PFF fertilizer treatment for all yield measures (average ear biomass, hundredweight, bushels/ha, and above-ground biomass). For average ear biomass, the LDS fertilizer treatment was significantly higher than either the HDS or PFF treatments. The remaining yield measures, as well as ear quality (dissolved soluble solids), were statistically equivalent across all fertilizer treatments. While differences in yield were not statistically significant, a trend towards higher yield in seaweed-amended plots was observed for all yield measures, which may have economic relevance. For instance, the average increase in hundredweight on a per-hectare basis is equivalent to an additional $575 in income. Regardless of potential economic impact, the equivalent yield supported by seaweed amendments may be sufficient for consideration of adopting this as an alternative management practice, assuming additional costs are not restrictive.

Yield across all fertilizer treatments in this study was lower than average yield for sweet corn in Rhode Island in 2007 (~45 vs. 60 hundred weight) (USDA NASS, 2013). Consequently, it is likely that supply of nutrients was equally limited for all treatments. Since extractable PO$_4^{3-}$ and K$^+$ levels were generally high across treatments, NO$_3^-$ is more likely the limiting nutrient. K$^+$ was significantly higher in seaweed-amended treatments after spring application, but returned to PFF treatment...
levels at the conclusion of the growing season. At an initial (October 2011) average K level of ~150 µg/g dry soil across all fertilizer treatments, K$^+$ was unlikely to be initially limiting, with an Cornell Soil Health overall soil quality rating of over 100% (Gugino et al., 2009).

In contrast, the timing of N availability is critical for sweet corn growth, and initial values of NO$_3^-$ were uniformly low across the study site. While no obvious signs of N limitation were observed in the corn crop, addition of seaweed biomass and PFF may have not been sufficient to maximize crop growth. The potential for N limitation underscores the importance of reliable predictions of N availability, based on material decomposition rates, nitrification and denitrification processes, leaching losses, volatilization, and other components of N cycling relevant to plant-available N supply. A large body of research regarding prediction of N supply as a function of material composition and climatic factors has been developed over several decades, including laboratory and field evaluations (De Neve and Hofman, 1996; Trinsoutrot et al., 2000). For seaweed biomass, the prediction of N mineralization in the field is largely unknown. For this study, a general value of mineralized N of 50% of total N was assumed based on mineralization for high-N legumes (Fox et al., 1990; Sattell et al., 1998), but this value can differ greatly depending on climatic variables (e.g. precipitation, temperature) and does not take into account losses due to leaching. As a means of strengthening N availability predictions for seaweed biomass, stable isotope techniques (e.g. enrichment of seaweed biomass with $^{15}$N) could be implemented in the field to trace the fate and mineralization rate of applied organic N.
Economic analysis

Examination of quantifiable differences in costs and benefits between seaweed and non-seaweed treatments resulted in a net expenses 3.5 and 0.5 times greater than PFF treatments for LDS and HDS seaweed treatments, respectively. These estimates take into account both mileage and wear-and-tear for transportation ($0.55/mile), assume Rhode Island minimum wage ($7.75/hr) for collection labor, and are based on the area of each amendment treatment (0.01 ha) employed in this study. Consequently, the transportation and labor involved in collecting seaweed as a fraction of overall nutrient supply on the scale utilized in this study does not support the practice based solely on basic, quantifiable costs. However, several factors should be considered in application of economic findings to seaweed amendment on a larger scale. First, the increase in income associated with increased harvest area may not be proportional to the increase in cost for application on a larger scale, following the principles of "economies of scale." Second, this economic evaluation does not account for externalities associated with either seaweed or PFF application, such as environmental impacts (e.g. energy requirements for fertilizer production). These externalities can be included as monetary factors in life-cycle assessment procedures, a style of evaluation with increasing use in the agricultural sciences (Haas et al., 2000). For a more inclusive estimation of total costs and benefits of seaweed amendment, future studies would benefit from life-cycle assessment or a similar analysis procedure. Finally, the monetary value of increased yield is based on Rhode Island wholesale value for sweet corn on a hundredweight basis, a value that may increase for direct farm sales, especially of organically-certified sweet corn.
CHAPTER 5

CONCLUSIONS

Seaweed biomass collected for this study was composed of a mixture of red, brown, and green algal species, and in comparison to terrestrial plant biomass, was relatively high in elements important for crop growth (e.g. N, K, Ca, and Fe). Primary plant nutrients were either equivalent (N and P) or greater (K) with seaweed amendment, so the potential for realizing target crop nutrition requirements is supported. No effects, either negative or positive, were observed for the soil physical properties evaluated, which may be a function of time required for physical property change to be observed. For chemical properties, hypothesized effects on pH, EC, and SO₄²⁻ were detected, with short-term decreased pH and increased EC and SO₄²⁻ after seaweed addition. Decreased pH, as a critical variable for soil productivity, may be of concern for farm application, but the observed decrease is also associated with decomposition of any organic amendment. Increased EC, which at high levels may negatively affect crop growth, did not reach levels of concern for this season. Finally, SO₄²⁻ production may play a part in decreasing pH, but S is also a plant nutrient, so effects may be contradictory.

No effects on the biological properties of soil respiration, SOM, or earthworm abundance were observed. PMN was significantly higher in the PFF treatment in July 2012, while at the same date seaweed treatments had net N immobilization, so the prediction and consistency of N supply may be a limitation of seaweed application, as
is the case for most organic amendment materials. However, a positive biological quality effect was increased active C, a soil quality indicator with good correlation to plant productivity and crop yield. Overall, effects on soil quality are both negative (e.g. decreased pH and increased EC) and positive (e.g. increased active C), but should be viewed in light of the persistence of effects, as well as the distinction between seaweed-specific and general organic matter addition effects.

When applied as a fraction of overall N crop requirements, no significant differences in above-ground biomass or yield were observed, indicating that equivalent crop productivity could be obtained by implementing seaweed amendment. Additionally, while no differences in dissolved soluble solids were observed, the average weight of corn ears was greater in seaweed-amended treatments, so potential for improved ear quality and marketability may be a positive benefit of seaweed amendment. However, labor requirements and transportation costs may limit the economic viability, especially when balanced with the limited financial benefit of increases in yield. The balance of potential for increased crop success and material costs requires facilitation of improved methods and timing of collection. Additionally, predictability of nutrient supply is a critical issue that requires further evaluation, especially of seaweed mineralization rates in the field. In future studies, evaluation the fate of C and N and rate of cycling may contribute to a better-informed use of this unique biomass source.
Appendix 1. Summary of Repeated Measures ANOVA (RM ANOVA) values for sphericity of overall time and time x fertilizer treatment main effects. If the assumption of sphericity was violated (Mauchley’s Test for Sphericity \( p \)-value < 0.05), the Greenhouse-Geiser (G-G) adjusted probability was used (Von Ende, 1993). Within sampling months, Levene’s Equality of Error Variance test was used to determine homogeneity of variance, and if violated \( (p<0.05) \), data were transformed logarithmically. If transformation failed to produce homogeneity, the \( p \)-value of the Levene Test was adopted as the new level of significance (Underwood, 1981). For comparisons within sampling month (Tukey’s Multiple Comparison Test), rows with the same letter are not significantly different.

| Aggregate stability | Sampling month | Interaction effects |
|---------------------|----------------|---------------------|
|                     | Sphericity     | Original \( p \)-      | G-G adjusted \( p \)- | Original \( p \)- | G-G adjusted \( p \)- |
| p-value             | assumed \( Y/N \) | value \( (Y/N) \)   | value \( (Y/N) \)   | value \( (Y/N) \) | value \( (Y/N) \) |
|---------------------|----------------|------------------------|-----------------------|-------------------|-----------------------|
| 0.944               | Yes            | <0.001                 | N/A                   | 0.688             | N/A                   |

Comparisons within sampling month

| Levene’s Test for Error Variance p-value | Transforme Levene’s p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|-----------------------------------------|-----------------------------|------------------------------|-----|-----|-----|
| Oct-11 \( <0.05 \)                     | 0.05                        | 0.407                        | a   | a   | a   |
| Nov-11 \( / \)                         | /                           | /                            | /   | /   | /   |
| Apr-12 \( / \)                         | /                           | /                            | /   | /   | /   |
| May-12 \( 0.619 \)                     | N/A                         | 0.641                        | a   | a   | a   |
| Jun-12 \( / \)                         | /                           | /                            | /   | /   | /   |
| Jul-12 \( / \)                         | /                           | /                            | /   | /   | /   |
| Aug-12 \( / \)                         | /                           | /                            | /   | /   | /   |
| Sep-12 \( 0.86 \)                      | N/A                         | 0.124                        | a   | a   | a   |
### Available water capacity

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|-------------------|--------------------------|------------------|----------------------|------------------|----------------------|
| <0.05             | No                       | 0.846            | 0.691                | 0.276            | 0.297                |

#### Comparisons within sampling month

|          | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------|------------------------------------------|------------------------------|------------------------------|-----|-----|-----|
| Oct-11   | 0.239                                    | N/A                          | 0.739                        | a   | a   | a   |
| Nov-11   | */                                        | */                           | */                           | /   | /   | /   |
| Apr-12   | */                                        | */                           | */                           | /   | /   | /   |
| May-12   | 0.23                                     | N/A                          | 0.739                        | a   | a   | a   |
| Jun-12   | */                                        | */                           | */                           | /   | /   | /   |
| Jul-12   | */                                        | */                           | */                           | /   | /   | /   |
| Aug-12   | */                                        | */                           | */                           | /   | /   | /   |
| Sep-12   | <0.05                                    | 0.119                        | 0.418                        | a   | a   | a   |

### Bulk density

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|-------------------|--------------------------|------------------|----------------------|------------------|----------------------|
| None              | No                       | 0.78             | 0.78                 | 0.861            | 0.861                |

#### Comparisons within sampling month

|          | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------|------------------------------------------|------------------------------|------------------------------|-----|-----|-----|
| Oct-11   | 0.659                                    | N/A                          | 0.99                         | a   | a   | a   |
| Nov-11   | */                                        | */                           | */                           | /   | /   | /   |
| Apr-12   | */                                        | */                           | */                           | /   | /   | /   |
| May-12   | 0.242                                    | N/A                          | 0.274                        | a   | a   | a   |
| Jun-12   | */                                        | */                           | */                           | /   | /   | /   |
| Jul-12   | */                                        | */                           | */                           | /   | /   | /   |
| Aug-12   | */                                        | */                           | */                           | /   | /   | /   |
| Sep-12   | */                                        | */                           | */                           | /   | /   | /   |
## Infiltration

| Sphericity p-value | Sphericity assumed (Y/N) | Sampling month | Interaction effects |
|--------------------|--------------------------|----------------|---------------------|
| None               | No                       | <0.05          | <0.05               | 0.155 | 0.155 |

### Comparisons within sampling month

|                       | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|-----------------------|------------------------------------------|------------------------------|-----------------------------|-----|-----|-----|
| Oct-11                | <0.05                                    | 0.173                        | 0.681                       | a   | a   | a   |
| Nov-11                | /                                        | /                            | /                           | /   | /   | /   |
| Apr-12                | /                                        | /                            | /                           | /   | /   | /   |
| May-12                | <0.05                                    | 0.245                        | <0.05                       | a   | a   | a   |
| Jun-12                | /                                        | /                            | /                           | /   | /   | /   |
| Jul-12                | /                                        | /                            | /                           | /   | /   | /   |
| Aug-12                | /                                        | /                            | /                           | /   | /   | /   |
| Sep-12                | /                                        | /                            | /                           | /   | /   | /   |

## Nitrate

| Sphericity p-value | Sphericity assumed (Y/N) | Sampling month | Interaction effects |
|--------------------|--------------------------|----------------|---------------------|
| <0.05              | No                       | <0.001         | <0.001              | <0.001 | <0.05 |

### Comparisons within sampling month

|                       | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|-----------------------|------------------------------------------|------------------------------|-----------------------------|-----|-----|-----|
| Oct-11                | 0.065                                    | N/A                          | 0.055                       | a   | a   | a   |
| Nov-11                | 0.328                                    | N/A                          | 0.802                       | a   | a   | a   |
| Apr-12                | 0.005                                    | N/A                          | 0.54                        | a   | a   | a   |
| May-12                | 0.19                                     | N/A                          | <0.005                      | a   | b   | a   |
| Jun-12                | 0.114                                    | N/A                          | 0.081                       | a   | a   | a   |
| Jul-12                | 0.578                                    | N/A                          | 0.129                       | a   | a   | a   |
| Aug-12                | 0.578                                    | N/A                          | 0.719                       | a   | a   | a   |
| Sep-12                | 0.2                                      | N/A                          | 0.267                       | a   | a   | a   |
### Phosphate

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|-------------------|--------------------------|------------------|----------------------|------------------|----------------------|
| <0.05             | No                       | <0.001           | <0.001               | <0.001           | <0.05                |

#### Comparisons within sampling month

| Sampling month | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------------|------------------------------------------|------------------------------|----------------------------|-----|-----|-----|
| Oct-11         | 0.959                                    | N/A                          | 0.811                      | a   | a   | a   |
| Nov-11         | 0.814                                    | N/A                          | 0.932                      | a   | a   | a   |
| Apr-12         | 0.471                                    | N/A                          | 0.909                      | a   | a   | a   |
| May-12         | 0.266                                    | N/A                          | 0.411                      | a   | a   | a   |
| Jun-12         | 0.134                                    | N/A                          | 0.305                      | a   | a   | a   |
| Jul-12         | 0.856                                    | N/A                          | <0.05                      | a   | a   | a   |
| Aug-12         | 0.034                                    | 0.014*                       | 0.277                      | a   | a   | a   |
| Sep-12         | 0.368                                    | N/A                          | <0.05                      | a   | b   | ab  |

*Adopted as new level of significance

### Potassium

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|-------------------|--------------------------|------------------|----------------------|------------------|----------------------|
| 0.668             | Yes                      | 0.242            | N/A                  | 0.047            | N/A                  |

#### Comparisons within sampling month

| Sampling month | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------------|------------------------------------------|------------------------------|----------------------------|-----|-----|-----|
| Oct-11         | 0.248                                    | N/A                          | 0.05                        | a   | a   | a   |
| Nov-11         | /                                        | /                            | /                           | /   | /   | /   |
| Apr-12         | /                                        | /                            | /                           | /   | /   | /   |
| May-12         | 0.567                                    | N/A                          | <0.001                      | a   | b   | c**|
| Jun-12         | /                                        | /                            | /                           | /   | /   | /   |
| Jul-12         | /                                        | /                            | /                           | /   | /   | /   |
| Aug-12         | /                                        | /                            | /                           | /   | /   | /   |
| Sep-12         | <0.001                                   | 0.003*                       | 0.237                       | a   | a   | a   |

*Adopted as new level of significance

**Outlier value excluded from PFF May-12 by the Grubb's Outlier Test at α=0.05
### Extractable calcium

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|--------------------|--------------------------|------------------|----------------------|------------------|----------------------|
| <0.05              | No                       | <0.001           | <0.001               | <0.05            | <0.05               |

**Comparisons within sampling month**

| Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|-----------------------------------------|-----------------------------|-----------------------------|-----|-----|-----|
| Oct-11                                  | 0.401                       | N/A                         | 0.067 | /*  | a    | a    |
| Nov-11                                  | /                           | /                           | /    | /   | /    |
| Apr-12                                  | /                           | /                           | /    | /   | /    |
| May-12                                  | 0.089                       | N/A                         | 0.123 | a   | a    | a    |
| Jun-12                                  | /                           | /                           | /    | /   | /    |
| Jul-12                                  | /                           | /                           | /    | /   | /    |
| Aug-12                                  | /                           | /                           | /    | /   | /    |
| Sep-12                                  | 0.074                       | N/A                         | <0.005 | a  | b    | b    |

*LDS Oct-11 data excluded due to instrument error

### Sulfate

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|--------------------|--------------------------|------------------|----------------------|------------------|----------------------|
| <0.05              | No                       | <0.001           | <0.05                | <0.001           | <0.05               |

**Comparisons within sampling month**

| Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|-----------------------------------------|-----------------------------|-----------------------------|-----|-----|-----|
| Oct-11                                  | <0.001                      | <0.001*                     | 0.163 | a   | a    | a    |
| Nov-11                                  | 0.208                       | N/A                         | 0.174 | a   | a    | a    |
| Apr-12                                  | <0.001                      | <0.001*                     | 0.599 | a   | a    | a    |
| May-12                                  | 0.082                       | N/A                         | <0.05  | a   | b    | ab   |
| Jun-12                                  | 0.074                       | N/A                         | <0.05  | ab  | a    | b    |
| Jul-12                                  | 0.054                       | N/A                         | <0.05  | ab  | a    | b    |
| Aug-12                                  | 0.505                       | N/A                         | 0.165  | a   | a    | a    |
| Sep-12                                  | <0.05                       | 0.959                       | <0.05  | ab  | a    | b    |

*Adopted as new level of significance
| Sphericity | Sphericity | Original p- | G-G | Original p- | G-G |
|-----------|-----------|-------------|-----|-------------|-----|
| p-value   | assumed   | value       | p-value | adjusted p-value | p-value |
| <0.05     | No        | <0.001      | <0.001 | <0.001      | <0.05 |

Comparisons within sampling month

| Sampling month | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------------|------------------------------------------|-----------------------------|-------------------------------|-----|-----|-----|
| Oct-11         | <0.05                                   | 0.053                       | 0.52                          | a   | a   | a   |
| Nov-11         | 0.189                                   | N/A                         | <0.001                        | a   | b   | c   |
| Apr-12         | 0.568                                   | N/A                         | <0.05                         | a   | b   | ab  |
| May-12         | 0.939                                   | N/A                         | <0.005                        | a   | ab  | b   |
| Jun-12         | 0.466                                   | N/A                         | 0.248                         | a   | a   | a   |
| Jul-12         | 0.096                                   | N/A                         | 0.875                         | a   | a   | a   |
| Aug-12         | <0.05                                   | 0.055                       | 0.299                         | a   | a   | a   |
| Sep-12         | 0.456                                   | N/A                         | 0.904                         | a   | a   | a   |

Electrical conductivity

| Sphericity | Sphericity | Original p- | G-G | Original p- | G-G |
|-----------|-----------|-------------|-----|-------------|-----|
| p-value   | assumed   | value       | p-value | adjusted p-value | p-value |
| <0.05     | No        | <0.001      | <0.001 | <0.001      | <0.05 |

Comparisons within sampling month

| Sampling month | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------------|------------------------------------------|-----------------------------|-------------------------------|-----|-----|-----|
| Oct-11         | 0.083                                   | N/A                         | 0.604                         | a   | a   | a   |
| Nov-11         | 0.17                                    | N/A                         | <0.001                        | a   | a   | b   |
| Apr-12         | 0.385                                   | N/A                         | <0.05                         | ab  | a   | b   |
| May-12         | 0.025                                   | N/A                         | <0.001                        | a   | b   | c   |
| Jun-12         | <0.05                                   | 0.551                       | <0.001                        | a   | a   | b   |
| Jul-12         | 0.074                                   | N/A                         | <0.005                        | a   | b   | a   |
| Aug-12         | 0.065                                   | N/A                         | <0.05                         | a   | b   | a   |
| Sep-12         | 0.282                                   | N/A                         | 0.069                         | a   | a   | a   |
### Soil organic matter

| Sphericity p-value | Sphericity assumed (Y/N) | Sampling month | Interaction effects |
|--------------------|--------------------------|----------------|---------------------|
|                    |                          |                | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
| <0.05              | No                       | <0.001         | <0.05               | 1.00             | 0.917               |

**Comparisons within sampling month**

|          | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------|------------------------------------------|-------------------------------|-----------------------------|-----|-----|-----|
| Oct-11   | 0.18                                     | N/A                           | 0.509                       | a   | a   | a   |
| Nov-11   | /                                        | /                             | /                           | /   | /   | /   |
| Apr-12   | 0.966                                    | N/A                           | 0.669                       | a   | a   | a   |
| May-12   | <0.05                                    | 0.998                         | 0.374                       | a   | a   | a   |
| Jun-12   | <0.05                                    | 0.04*                         | 0.787                       | a   | a   | a   |
| Jul-12   | <0.05                                    | 0.005*                        | 0.007                       | a   | a   | a   |
| Aug-12   | 0.204                                    | N/A                           | 0.647                       | a   | a   | a   |
| Sep-12   | 0.123                                    | N/A                           | 0.915                       | a   | a   | a   |

*Adopted as new level of significance

### Active carbon

| Sphericity p-value | Sphericity assumed (Y/N) | Sampling month | Interaction effects |
|--------------------|--------------------------|----------------|---------------------|
|                    |                          |                | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
| 0.988              | Yes                       | <0.001         | N/A                 | <0.001           | N/A                 |

**Comparisons within sampling month**

|          | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|----------|------------------------------------------|-------------------------------|-----------------------------|-----|-----|-----|
| Oct-11   | 0.402                                    | N/A                           | 0.393                       | a   | a   | a   |
| Nov-11   | 0.997                                    | N/A                           | 0.516                       | a   | a   | a   |
| Apr-12   | 0.453                                    | N/A                           | 0.088                       | a   | a   | a   |
| May-12   | 0.869                                    | N/A                           | 0.457                       | a   | a   | a   |
| Jun-12   | 0.321                                    | N/A                           | 0.08                        | a   | a   | a   |
| Jul-12   | 0.99                                     | N/A                           | <0.05                       | a   | ab  | b   |
| Aug-12   | 0.843                                    | N/A                           | <0.005                      | a   | a   | b   |
| Sep-12   | 0.75                                     | N/A                           | <0.005                      | a   | b   | b   |
### Potentially mineralizable nitrogen

| Sampling month | Interaction effects |
|----------------|---------------------|
|                | Sphericity p-value  | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|                | <0.05               | No                       | <0.001          | <0.001               | <0.001          | <0.001               |

**Comparisons within sampling month**

|                  | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|------------------|------------------------------------------|------------------------------|------------------------------|-----|-----|-----|
| Oct-11           | 0.183                                    | N/A                          | 0.079                        | a   | a   | a   |
| Nov-11           | 0.708                                    | N/A                          | 0.470                        | a   | a   | a   |
| Apr-12           | 0.228                                    | N/A                          | 0.881                        | a   | a   | a   |
| May-12           | 0.063                                    | N/A                          | 0.149                        | a   | a   | a   |
| Jun-12           | 0.216                                    | N/A                          | 0.145                        | a   | a   | a   |
| Jul-12           | 0.7                                      | N/A                          | <0.001                       | a   | a   | b   |
| Aug-12           | 0.063                                    | N/A                          | 0.192                        | a   | a   | a   |
| Sep-12           | 0.096                                    | N/A                          | 0.931                        | a   | a   | a   |

### Soil respiration

|                       | Sampling month | Interaction effects |
|-----------------------|----------------|---------------------|
|                       | Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|                       | 0.114           | Yes                  | <0.001          | N/A               | 0.472          | N/A               |

**Comparisons within sampling month**

|                  | Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|------------------|------------------------------------------|------------------------------|------------------------------|-----|-----|-----|
| Oct-11           | <0.05                                    | 0.08                         | 0.932                        | a   | a   | a   |
| Nov-11           | 0.46                                     | N/A                          | 0.641                        | a   | a   | a   |
| Apr-12           | <0.05                                    | 0.002*                       | 0.068                        | a   | a   | a   |
| May-12           | <0.05                                    | 0.054                        | 0.231                        | a   | a   | a   |
| Jun-12           | 0.216                                    | N/A                          | 0.145                        | a   | a   | a   |
| Jul-12           | 0.185                                    | N/A                          | 0.895                        | a   | a   | a   |
| Aug-12           | 0.376                                    | N/A                          | 0.875                        | a   | a   | a   |
| Sep-12           | 0.058                                    | N/A                          | 0.59                         | a   | a   | a   |

*Adopted as new level of significance
## Earthworm abundance

| Sphericity p-value | Sphericity assumed (Y/N) | Original p-value | G-G adjusted p-value | Original p-value | G-G adjusted p-value |
|-------------------|--------------------------|-----------------|----------------------|-----------------|----------------------|
| 0.055             | Yes                      | 0.4             | N/A                  | 0.21            | N/A                  |

### Comparisons within sampling month

| Levene's Test for Error Variance p-value | Transformed Levene's p-value | Fertilizer treatment p-value | LDS | HDS | PFF |
|------------------------------------------|------------------------------|------------------------------|-----|-----|-----|
| Oct-11                                   | 0.226                        | N/A                          | 0.084 | a   | a   | a   |
| Nov-11                                   | /                            | /                            | /    | /   | /   |
| Apr-12                                   | 0.249                        | N/A                          | 0.932 | a   | a   | a   |
| May-12                                   | <0.05                        | 0.002*                       | 0.323 | a   | a   | a   |
| Jun-12                                   | 0.051                        | N/A                          | 0.537 | a   | a   | a   |
| Jul-12                                   | <0.05                        | 0.007*                       | 0.405 | a   | a   | a   |
| Aug-12                                   | <0.05                        | 0.000*                       | 0.192 | a   | a   | a   |
| Sep-12                                   | 0.06                         | N/A                          | 0.37  | a   | a   | a   |

*Adopted as new level of significance
**APPENDIX 2**

**Appendix 2.** Summary of Univariate ANOVA values for sweet corn production parameters, representing combined harvest data at the end of the growing season. Post-hoc comparisons represent significance of Tukey’s Multiple Comparison Test ($\alpha=0.05$) for comparisons between fertilizer treatments (LDS=low-dose seaweed, HDS=high-dose seaweed, and PFF=pre-formulated fertilizer). Treatments with the same letter within rows are not significantly different.

| Dissolved soluble solids | Test for normality | Test for equal variance | F statistic | DF | p-value |
|--------------------------|--------------------|-------------------------|-------------|----|---------|
|                          | 0.617              | 0.823                   | 3.051       | 2.9| 0.097   |
| Multiple comparisons     | LDS                | HDS                     | PFF         |
|                         | a                  | a                       | a           |

| Above-ground biomass     | Test for normality | Test for equal variance | F statistic | DF | p-value |
|--------------------------|--------------------|-------------------------|-------------|----|---------|
|                          | 0.255              | 0.924                   | 2.869       | 2.9| 0.109   |
| Multiple comparisons     | LDS                | HDS                     | PFF         |
|                         | a                  | a                       | a           |

| Fresh ear weight         | Test for normality | Test for equal variance | F statistic | DF | p-value |
|--------------------------|--------------------|-------------------------|-------------|----|---------|
|                          | 0.98               | 0.709                   | 6.474       | 2.9| <0.05   |
| Multiple comparisons     | LDS                | HDS                     | PFF         |
|                         | a                  | ab                      | b           |

| Yield (hundred weight/ha)| Test for normality | Test for equal variance | F statistic | DF | p-value |
|--------------------------|--------------------|-------------------------|-------------|----|---------|
|                          | 0.174              | 0.4                     | 2.913       | 2.9| 0.106   |
| Multiple comparisons     | LDS                | HDS                     | PFF         |
|                         | a                  | a                       | a           |
| Yield (bushels/ha) | Test for normality | Test for equal variance | F statistic | DF | p-value |
|-------------------|-------------------|-------------------------|-------------|----|---------|
|                   | 0.386             | 0.731                   | 1.819       | 2.9| 0.217   |
| Multiple comparisons | LDS               | HDS                     | PFF         |    |         |
|                   | a                 | a                       | a           |    |         |
Appendix 3. Summary of Two-Way ANOVA results for comparison of total elements (heavy metals and plant nutrients) before and after seaweed addition (October 2011 and September 2012) among fertilizer treatments (LDS=low-dose seaweed, HDS=high-dose seaweed, and PFF=pre-formulated fertilizer). For multiple comparisons, fertilizer treatments with the same letter within rows are not significantly different (α=0.05).

|                  | Test for normality | Test for equal variance |
|------------------|--------------------|-------------------------|
|                  | 0.173              | 0.481                   |

|                  | Sampling month | Fertilizer treatment | Sampling month x fertilizer treatment |
|------------------|----------------|----------------------|---------------------------------------|
| F statistic      | 0.268          | 4.775                | 0.619                                 |
| DF               | 1.18           | 2.18                 | 2.18                                  |
| p-value          | 0.611          | <0.05                | 0.549                                 |

Multiple comparisons

|                | LDS  | HDS  | PFF  |
|----------------|------|------|------|
| Oct-11         | a    | ab   | b    |
| Sep-12         | a    | a    | a    |

Arsenic (log transformed)

|                  | Test for normality | Test for equal variance |
|------------------|--------------------|-------------------------|
|                  | 0.84               | <0.05                   |

|                  | Sampling month | Fertilizer treatment | Sampling month x Fertilizer treatment |
|------------------|----------------|----------------------|---------------------------------------|
| F statistic      | 0.07           | 0.345                | 1.234                                 |
| DF               | 1.18           | 2.18                 | 2.18                                  |
| p-value          | 0.794          | 0.713                | 0.315                                 |

Multiple comparisons

|                | LDS  | HDS  | PFF  |
|----------------|------|------|------|
| Oct-11         | a    | a    | a    |
| Sep-12         | a    | a    | a    |
| Zinc          | Test for normality | Test for equal variance |
|--------------|--------------------|-------------------------|
|              | 0.864              | 0.104                   |
|              | Sampling month     | Fertilizer treatment    | Sampling month x Fertilizer treatment |
| F statistic  | 2.997              | 0.351                   | 1.771                                  |
| DF           | 1.18               | 2.18                    | 2.18                                   |
| p-value      | 0.101              | 0.709                   | 0.199                                  |

Multiple comparisons

|          | LDS | HDS | PFF |
|----------|-----|-----|-----|
| Oct-11   | a   | a   | a   |
| Sep-12   | a   | a   | a   |

| Copper     | Test for normality | Test for equal variance |
|------------|--------------------|-------------------------|
|            | 0.5                | 0.847                   |
|            | Sampling month     | Fertilizer treatment    | Sampling month x Fertilizer treatment |
| F statistic| 1.212              | 0.652                   | 1.068                                  |
| DF         | 1.18               | 2.18                    | 2.18                                   |
| p-value    | 0.289              | 0.536                   | 0.37                                   |

Multiple comparisons

|          | LDS | HDS | PFF |
|----------|-----|-----|-----|
| Oct-11   | a   | a   | a   |
| Sep-12   | a   | a   | a   |

| Iron (log transformed) | Test for normality | Test for equal variance |
|-----------------------|--------------------|-------------------------|
|                       | <0.05              | 0.19                    |
|                       | Sampling month     | Fertilizer treatment    | Sampling month x Fertilizer treatment |
| F statistic           | 2.405              | 0.213                   | 1.734                                  |
| DF                    | 1.18               | 2.18                    | 2.18                                   |
| p-value               | 0.138              | 0.81                    | 0.205                                  |

Multiple comparisons

|          | LDS | HDS | PFF |
|----------|-----|-----|-----|
| Oct-11   | a   | a   | a   |
| Sep-12   | a   | a   | a   |
|                | Test for normality | Test for equal variance |
|----------------|--------------------|-------------------------|
| Manganese      | 0.784              | 0.342                   |
|                | Sampling month     | Fertilizer treatment    | Sampling month x Fertilizer treatment |
| F statistic    | 1.094              | 1.834                   | 0.981                              |
| DF             | 1.18               | 2.18                    | 2.18                               |
| p-value        | 0.309              | 0.188                   | 0.394                              |
| Multiple comparisons | LDS | HDS | PFF |
| Oct-11         | a                  | a                       | a                                  |
| Sep-12         | a                  | a                       | a                                  |

|                | Test for normality | Test for equal variance |
|----------------|--------------------|-------------------------|
| Chromium       | 0.539              | 0.095                   |
|                | Sampling month     | Fertilizer treatment    | Sampling month x Fertilizer treatment |
| F statistic    | 9.303              | 0.0642                  | 0.0784                              |
| DF             | 1.18               | 2.18                    | 2.18                               |
| p-value        | <0.05              | 0.938                   | 0.925                              |
| Multiple comparisons | LDS | HDS | PFF |
| Oct-11         | a                  | a                       | a                                  |
| Sep-12         | a                  | a                       | a                                  |

|                | Test for normality | Test for equal variance |
|----------------|--------------------|-------------------------|
| Calcium        | 0.425              | 0.648                   |
|                | Sampling month     | Fertilizer treatment    | Sampling month x Fertilizer treatment |
| F statistic    | 1.897              | 0.205                   | 0.296                              |
| DF             | 1.18               | 2.18                    | 2.18                               |
| p-value        | 0.185              | 0.817                   | 0.747                              |
| Multiple comparisons | LDS | HDS | PFF |
| Oct-11         | a                  | a                       | a                                  |
| Sep-12         | a                  | a                       | a                                  |
| Potassium | Test for normality | Test for equal variance |
|-----------|--------------------|-------------------------|
|           | 0.236              | 0.575                   |

|                | Sampling month | Fertilizer treatment | Sampling month x Fertilizer treatment |
|----------------|----------------|----------------------|---------------------------------------|
| F statistic    | 0.0108         | 0.65                 | 0.963                                 |
| DF             | 1.18           | 2.18                 | 2.18                                  |
| p-value        | 0.918          | 0.534                | 0.401                                 |

Multiple comparisons

|                | LDS | HDS | PFF |
|----------------|-----|-----|-----|
| Oct-11         | a   | a   | a   |
| Sep-12         | a   | a   | a   |
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