SOIL AND PLANT RESPONSES TO LIPID-EXTRACTED ALGAE

A Dissertation

by

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ABSTRACT

Although algae is much more productive per area of cultivation compared to first-generation biofuel feedstocks, algae production may not be economically sustainable without high value coproducts. One of many possible coproducts may be algae residue following lipid extraction that might be used as a soil amendment for agricultural production.

The overall objective of this series of experiments was to determine the feasibility and management strategies required to best utilize lipid-extracted algae (LEA) as an organic fertilizer and soil conditioner. Effects of LEA on nutrient availability, soil C storage, aggregate stability, soil acidity and salinity, greenhouse gas (GHG) loss, changes in soil microbial activity and community composition, and forage growth were assessed.

Soil organic C (SOC) measured 392-d after amending soil with 1.5 and 3.0% LEA for a microcosm incubation was increased by approximately 0.2 and 0.3% OC, respectively, compared to the control. Approximately 10% of added LEA-C was mineralized and lost as CO_2 compared with 15% of added wheat straw-C. Lipid-extracted algae enhanced aggregate formation and soil SOC storage in microaggregates at 0-15 cm depth over a12-month field incubation with greater mean weight diameter by 12 months and approximately 42 and 66% of total SOC from 1.5 and 3.0% LEA treatments, respectively.

With glass house and field studies, nutrient availability was enhanced with LEA amendments; however, LEA applied at a 3.0% rate decreased seedling emergence of foxtail millet (*Setaria italica*) and salt-tolerant ryegrass (*Lolium multiflorum*), and thus, herbage mass (HM) and nutrient uptake were also decreased. Soil amended with 1.5% LEA, however, increased HM of pearl millet (*Pennisetum glaucum*), salt-tolerant ryegrass, and a sorghum-sudangrass hybrid [(*Sorghum bicolor* (L.) Moench× *Sorghum sudanese* (P.)].

Soil LEA-application should be a significant source of organic nutrients for microbial transformation and usage and plant uptake, and thus, may reduce inputs of inorganic fertilizer. The potential for LEA amendments enhancing aggregate formation, and consequently soil C storage, was indicated by mean weight diameter and SOC within macro- and microaggregates increasing over time. Lipid-extracted algae application may be a means of mitigating SOC losses due to agricultural production, and also, maintaining or improving soil structure and quality. However, problems with excess soil salinity, sodicity, and nutrients may occur at high LEA addition rates.

DEDICATION

My husband is my love and my best friend and I dedicate this to him. He has not only been my support but also my inspiration. He was so often the encouragement and logic I needed to stay focused and determined. I am so thankful our paths crossed.

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NOMENCLATURE

DAP	Days after planting
DM	Dry matter
EC	Electrical conductivity
GHG	Greenhouse gas
HM	Herbage mass
LEA	Lipid extracted algae
MWD	Mean weight diameter
OM	Organic matter
SMBC	Soil microbial biomass carbon
SOC	Soil organic carbon
SOM	Soil organic matter
WS	Wheat straw

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CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

A project to evaluate the feasibility of applying lipid-extracted algae (LEA) to soils was conducted at the Texas A&M AgriLife Research Station in Beeville, TX, and the Texas A&M University Department of Soil and Crop Sciences. Compared to traditional biodiesel feedstocks, algae offers much greater productivity per area of cultivation. As much as 100 times greater biodiesel can be produced with algae than from soybean [*Glycine max* (L.) Merr.]. Additionally, algae can be produced utilizing brackish water for cultivation. However, algae production currently is not economical without high-value coproducts. If algae is cultivated and harvested in sufficient quantity to provide significant biodiesel for the U.S., then large quantities of LEA will be available to use for animal feeds and soil amendments. Estimates are that 3.6 to 4.5 million metric tons of LEA will result from approximately 4 billion liters of algal biofuels produced.

Increased oil production through algal selection or genetic manipulation and extraction will potentially affect the chemical composition of LEA, causing it to be lower in protein and, therefore, less suitable or profitable as animal feed. However, the C and N, in addition to other macronutrients (P and K) and secondary and micronutrients (Ca, S, Zn, and Fe) in LEA might be economical and suitable for land application. Not only would LEA amendments potentially enhance soil physical and chemical properties, but would also possibly increase soil organic carbon (SOC) accumulation and sequestration.

Nitrogen from LEA will likely be more valuable for animal feed, but there are properties of LEA that could limit its use as an animal feed product making it more practical to use as a soil amendment. Limitations to the use of LEA as animal feed include chemicals or polymers involved with harvesting of algae and extraction of oil as well as the overall chemical composition of LEA. Land application of agricultural coproducts, such as manure, wood chips, compost, poultry litter, and municipal biosolids, are common management practices, but along with LEA, may alter soil quality, microbial community function, and crop production; therefore research on these effects is necessary.

The overall objective of this series of experiments was to determine the feasibility and management strategies required to best utilize LEA as an organic fertilizer and soil amendment. Effects of LEA on nutrient availability, soil C storage, aggregate stability, soil acidity and salinity, GHG fluxes, changes in soil microbial activity and community composition, and growth of forages were assessed.

LITERATURE REVIEW

Next Generation Algae Feedstocks

With continued dependence on foreign oil, increasing global demand, climbing petroleum costs and increasing environmental concerns, the United States has focused attention on biomass-derived fuels. The Energy Independence and Security Act (EISA) of 2007 established a mandatory Renewable Fuel Standard (RFS) requiring transportation fuel sold in the U.S. to contain a minimum of 136 billion liters of renewable fuels by 2022 (U.S. DOE, 2010). The EISA established new categories of renewable fuel, including cellulosic biofuel, biomass-based diesel, advanced biofuel, and total renewable fuel, which must all achieve certain minimum thresholds of lifecycle GHG emission reductions. For example, canola oil produced biodiesel currently meets the reduction threshold of 50%.

In order to meet the EISA standard, cellulosic ethanol is expected to supply the greatest portion of fuel (~57 billion liters) (Perlack et al., 2005), with next generation biofuels demonstrating significant promise. A final ruling released 15 August 2013 by the EPA set the cellulosic-ethanol standards for 2013 production in the U.S. at ~23 million ethanol-equivalent liters, which constitutes a percentage standard of 0.004% (EPA, 2013). The percentage standards represent the ratio of renewable fuel volume to non-renewable gasoline and diesel volume. Advanced biofuel and total renewable fuel production standards were set at 10.4 and 62.6 billion ethanol-equivalent liters, respectively. The production standard for biomass-based diesel was set at 6.4 billion liters (actual). The estimated percentage standards for biomass-based diesel, advanced

biofuel, and renewable fuel in 2013 were estimated at 1.13, 1.62, and 9.74%, respectively. Algae, considered by the EPA as an advanced biofuel feedstock, has the potential to assure that the U.S. complies with the RFS, while at the same time shifting the nation towards energy independence, creating economic opportunities, and providing environmental benefits, such as reduced net C emissions.

Humans have utilized algae, both macro- and microalgae, for centuries as food, feed, and medicines. Algae produced in controlled cultivation processes (open ponds or bioreactors) or harvested from natural environments are utilized as multiple commercial products for human and animal health and nutrition, cosmetic, and industrial applications (U.S. DOE, 2010). As an energy source, microalgae present multiple possibilities for fuel products, such as biodiesel, ethanol, methane, jet fuel, and biocrude.

Compared with traditional feedstocks, algae offers unique advantages, such as utilizing brackish water sources (Christi, 2007), recycling carbon dioxide (CO_2) emissions from flue gas, reducing competition for arable land with food crops (food vs. fuel) (Sheehan et al., 1998) in part because of high productivity per area of cultivation (Table 1.1), and integrated production of fuels and coproducts within biorefineries (U.S. DOE, 2010). Under the biorefinery concept, the production of industrial, high volume and high value chemicals from glycerol, amino acids, and N-containing components of algae biomass, which will be generated as waste or end products from microalgae lipid conversion processes, becomes feasible (Mooibroek et al., 2007) and must be considered in determining the economics of the process.

Table 1.1. Comparison of oil yields from biomass feedstocks [†].

CROP	Oil Yield ($L\ ha^{-1}\ yr^{-1}$)
Soybean	449
Camelina	580
Sunflower	954
Jatropha	1890
Oil palm	5940
Algae	9354-60,800[‡]

[†] Adapted from Christi (2007)

[‡] Estimated yields (DOE, 2010)

Although algae feedstocks have qualities favorable for sustainable biofuel production, several research and development challenges have not yet been met. The oil crisis in the 1970s prompted the U.S. Department of Energy's Office of Fuels Development to fund research for developing algal biodiesel (Sheehan et al., 1998). The program made many advances, such as identifying promising lipid production strains, open production systems (raceway ponds), and principles for photobioreactor design, all of which laid a foundation for present day research, but did not prove to be economically feasible on a large scale (Wijffels and Barbosa, 2010). Current research is focused on minimizing energy requirements and production costs, while maximizing lipid productivity and increasing the value of biomass by making use of individual biomass components, specifically after oil has been extracted.

The conversion of algal oil to biodiesel includes steps that require a multidisciplinary approach given the various technological and system options and their interdependency. Process steps include algal biology and cultivation, harvesting and

dewatering, extraction and fractionation, fuel conversion and coproduct or end product development. Extraction and separation techniques, if too harsh, will break down or contaminate important cell components, which are essential for high value end products; therefore, the chosen lipid extraction methods are a major concern to end product applications. Economical and sustainable production of algae-derived biofuel will likely only be achieved if accompanied with multiple, high revenue yielding by-products, one of which may be use as organic fertilizers and soil conditioners in agricultural production systems.

For the past 50 years, plant and animal oils have been the most common feedstock for biodiesel production, but current sources are not capable of meeting U.S. transportation fuel demands. As estimated by Christi (2007), the amount of existing U.S. cropland required to meet 50% of all U.S. transport fuel needs will be 130 times less using algae feedstock compared to soybean. Photosynthetic components of microalgae cells convert CO_2 to potential biofuels, foods, and high value end products while using sunlight as their sole energy source (U.S. DOE, 2010). Depending on the species and cultivation factors, microalgae produce varying forms and quantities of oil. Algae species producing high quantities of biomass and oil with low growth requirements and high environmental stress tolerance make them a highly desirable biodiesel feedstock.

Photoautotrophs, like algae, require water, light, CO_2, and nutrients for growth. While all components are vital for physiological growth and production, water is an essential but also controversial parameter of algae cultivation. For photosynthesis alone approximately 750 ml water is required per kilogram of biomass produced (Kliphuis et

al., 2010). Additionally, production systems require large volumes of water in order to compensate for evaporation losses in open ponds and to cool closed systems. However, a report by Wijffels and Barbosa (2010) indicated that in order to produce one liter of algae biofuel, 1.5 liters of water is needed compared to 10,000 liters required for traditional bioenergy feedstock production. Algae, unlike most other bioenergy feedstocks, are also capable of being cultivated in water unsuitable for human and animal consumption or crop production, such as salt aquifers, seawater, or waste/runoff water. While this is most definitely an added benefit for algae cultivation and biodiesel production systems, growing algae in brackish, saline, or waste waters will possibly concentrate salts, toxins or contaminants absorbed within the biomass, thus leading to issues using LEA residue as soil amendments, but more importantly the effects of these constituents on food and feed production and quality (U.S. DOE, 2010). In arid and semi-arid ecosystems, salinization is already a major threat to production in agricultural systems (Sumner, 1995).

A possible scenario for microalgae cultivation involves utilizing wastewater rich in OM and nutrients generated by dairy and feedlot operations as a growth media for algae, which could potentially assimilate the dissolved nutrients down to trace concentrations. Thriving in such an environment, the algae would not only be producing lipids for biodiesel conversion, but would also be treating wastewater. Furthermore, high productivity algae ponds have a total cost that is approximately 70% less than creating activated sludge, the leading water treatment technology used in the U.S. (Downing et al., 2002). After lipid extraction and conversion, the LEA residue could then be fed to

dairy or feedlot cattle and/or applied to soil as an organic fertilizer and conditioner. Algae production facilities located near dairies and animal feedlots would potentially decrease input costs of algae production by recycling nutrients and C in addition to reducing transportation and waste disposal costs.

Organic Wastes to Resources

The primary goal of agricultural production systems is to provide the world's population with food, fiber, and fuel. As the global population grows and the demand for food increases, the global agricultural industry, and more specifically producers, will be challenged to enhance crop yields, while protecting the environment and maintaining soil productivity (Godfray et al., 2010). All of these goals must be accomplished with less reliance on non-renewable resources, as well as in a new economic and social setting of growing competition for arable land resources from urban and industrial users.

A potential solution is the use of organic waste products or residues originating within agriculture, municipalities and industry as resources rather than discarding them as waste products (Misselbrook et al., 2012). The U.S. DOE (2010) presented several different options for recovering economic value from LEA residue including, but not limited to: 1) maximum energy recovery from LEA biomass by anaerobic digestion of this material; 2) recovery of protein from LEA biomass for use in food and feed; 3) recovery and utilization of non-fuel lipids; 4) recovery and utilization of carbohydrates from LEA biomass, and glycerol from the transesterfication of lipids to biodiesel; and 5) recovery/extraction of fuel lipids only, with use of the residual biomass as soil fertilizers

and conditioners. According to the U.S. DOE (2010), option five is believed to be the simplest, and due to it being labeled "organic", may be marketed at a premium.

Waste residues may be used on land as organic fertilizers and soil amendments. Depending on the quality of the organic material, it may be possible to maintain or improve soil physical and chemical properties partly as a result of increased soil OM (SOM) and thus enhanced microbial activity. A vital process in ecosystems is microbial decomposition of OM and the subsequent mineralization and liberation of nutrients. Improved soil physical and chemical properties as a result of OM additions may include increased water holding capacity, and cation exchange capacities, enhanced retention of nutrients in the root zone, greater buffering capacity against pH change, improved ability to chelate and form complex ions, and more stable soil structure as a result of aggregate formation (Degens et al., 2000). All of these attributes will likely reduce soil degradation, erosion and compaction, and increase nutrient availability to plants and microorganisms as well as the capacity for C storage in long-term cropping systems (Karami et al., 2012).

Soil Organic Matter and Carbon Cycling Dynamics

An important component of this research used separation of SOC into different OM pools and ^{13}C mass spectrometry to estimate ultimate effects of algae addition on SOC sequestration. Additionally, determining soil gas fluxes of CO_2, nitrous oxide (N_2O), methane (CH_4), and ammonia (NH_3) generated from algae application may further elucidate effects on soil chemical and biological processes as well as enhance our

understanding of impacts that algae applications may have on the soil environment and GHG budgets.

Soil OM is a combination of living, dying, and decomposing biomass including animals, microorganisms, and plant material, plus more recalcitrant products. Soil OM turnover involves a variety of constituents, with mean residence times (MRTs) ranging from days to years and millennia. Mean residence time refers to: 1) the average time an element resides in the pool at steady state or 2) the average time required to completely renew the content of the pool at steady state (Six and Jastrow, 2002). The rate at which SOC is transformed is highly dependent on the degree of stabilization.

Mechanisms responsible for SOC stabilization include biochemical recalcitrance, chemical stabilization, and physical protection (Christensen, 1996). Soil texture and structure plays a dominant role in OM stabilization and protection because even labile OM can be sorbed to clay surfaces or incorporated into aggregates resulting in long-term storage of SOM (Ladd et al., 1993; Tisdall and Oades, 1982). Overall, SOC storage is the net effect of OM inputs and losses through decomposition and decay.

The largest pool within the terrestrial C cycle is SOC (Stockmann et al., 2013). Its accumulation within agricultural soils is affected by management practices, such as the type of residue returned and the type of cultivation, e.g. no-tillage (NT) or conventional tillage (Campbell et al., 2000). In the surface layers of agricultural soils, OM acts as a binding agent for aggregates (Tisdall and Oades, 1982). Aggregation of soil particles offers protection to SOC, thereby enhancing soil C storage dynamics and potentially mitigating GHG emissions. Wright and Hons (2005a) demonstrated that

management practices that increase soil aggregation tend to enhance C sequestration. A study by Jastrow et al. (1996) showed that newly introduced OM is found mostly in larger soil aggregates, making it more susceptible to decomposition because macroaggregates are more likely to be destroyed by agricultural practices compared to microaggregates (Tisdall and Oades, 1982), but in perennial pasture systems this may not be the case.

Soil aggregation and C sequestration are also affected by the amount and quality of residues added to the soil. An agricultural system involving crop rotations will naturally experience differences in quantity and quality of residue returned. Jastrow et al. (1996) observed an increased quantity of macroaggregates resistant to slaking in perennial grass pastures compared to corn [*Zea mays* (L.)] fields. A monoculture study by Wright and Hons (2005b) reported that aggregation was generally greater for wheat [*Triticum aestivum* (L.)] than the other crop species of sorghum [*Sorghum bicolor* (L.) Moench] and soybean. Crops, such as wheat, having low straw N concentration (high C:N ratio) will generally decompose at much slower rates than residues with greater N (Ghidey and Alberts, 1993; Franzluebbers et al., 1995). As a general rule of thumb, OM with a C to N ratio (C:N) less than 25:1 stimulates C and N mineralization, while those with a C:N ratio greater than 25:1 lead to net negative mineralization, or immobilization (Robertson and Groffman, 2007). Slower decomposition generally results in increased SOM and aggregate formation and stability.

Animal byproducts, such as dairy manure and poultry litter, are two commonly applied organic residues, but others include oilseed meals and distillers grains, which are

becoming more prevalent due to the increase in biofuel production. Nitrogen is predominately in organic form in organic residues; therefore, OM and organic N generally must be decomposed and mineralized prior to its utilization by plants or microorganisms. It is, therefore, critical to determine the rate of C and N mineralization from OM sources being utilized as soil amendments (Moore et al., 2010).

Dairy manure and oilseed meal (source *Sinapis alba*) with C:N ratios of 10.4 (Moore et al., 2010) and 9.7 (Rothlisberger et al., 2012), respectively, stimulated mineralization, while a material such as corn stover will likely promote immobilization due to its wide C:N ratio of ~60:1 (Robertson and Groffman, 2007). A study by Mills and Alexander (1974) reported C:N ratios for two freshwater, pre-extracted algae cultures of *Ankistrodesmus falcatus* and *Chlamydomonas oblonga* that ranged from 5.4:1 to 7.3:1 and from 5.7:1 to 7.9:1, respectively, depending on the age of the cultures (14 to 28 d). As C:N ratios increased with age, mineralization rates decreased, but even 28-d old algae will likely initiate mineralization because of low C:N ratios. The algae used for biofuel production will likely be cultivated in brackish waters with the oil being extracted prior to soil application, which may cause C and N concentrations to vary slightly from that reported above.

Nutrient Availability

Seaweed and algae have been used for centuries as natural, agricultural fertilizers and soil conditioners (Read, 1849); however, few if any studies have investigated the practicality of using LEA as a soil amendment and nutrient source for agricultural crops and forages. It is unclear whether agricultural crops and forages seeded into LEA-amended soil will benefit from added OM and potentially enhanced nutrient availability or suffer due to the salinity and other characteristics potentially associated with LEA. Dairy manure and poultry litter are raw manure byproducts readily available and commonly applied to agricultural fields and pastures in the U.S as approved organic fertilizers. Approximately 60 – 70% of N in poultry litter is either in the form of ammonia or uric acid, and thus, is readily available or quickly mineralized to an available form of N for plant uptake (Nahm, 2003). A composted material is much slower to mineralize than poultry manure; therefore, compost may provide available N to plants throughout the growing season and potentially even multiple growing seasons.

Moore et al. (2010) estimated the amount of plant-available N (NH_4^+-N + NO_3^--N) after 210 days of incubation to be 61, 56, 44, 29, 2 and -2% of total N in mustard meal, distillers grain, poultry litter, a compost/litter mixture, dairy manure compost, and anaerobically digested fiber, respectively, by using first-order N mineralization equations. Mustard meal and distillers grain were comparable to poultry litter in their total N release but maintained NH_4^+-N concentrations for a longer period after the initial increase. The rate constant of N mineralization (0.03 day $^{-1}$) was lowest in soils amended with mustard meals and distillers grain, indicating a slower release of N, and therefore,

the potential for using biofuel byproducts as slow-release N fertilizers (Moore et al., 2010). A study by Whalen et al. (2001) demonstrated an increased proportion of potentially mineralizable N and P in soil with long-term manure applications.

In order for algae biofuel to be price competitive at the pump while maintaining economic and environmental sustainability, LEA should be used in a way to reduce the overall cost of production. Summarized above, LEA may be useful as a soil amendment; however, additional research is needed to determine the feasibility of and management strategies required to best utilize LEA as an organic fertilizer and soil amendment. Effects of LEA on nutrient availability, soil C storage, aggregate stability, soil acidity and salinity, GHG fluxes, changes in soil microbial activity and community composition, and growth of forages were assessed.

RESEARCH OBJECTIVES

The overall objective of the proposed research was to determine effects of LEA added at various rates on soil properties, soil quality and biochemical reactions as well as plant growth. Specific objectives included:

1) Determination of effects of LEA and wheat straw (WS; *Trifolium* sp.) on soil C and N mineralization as well as changes in soil pH and electrical conductivity (EC) determined *in vivo* over approximately 390 d using laboratory microcosms.
2) Determination of the effect of LEA on: a) warm season forage emergence and seedling survival, b) plant growth (shoot height and weight), c) soil nutrient

availability, d) plant nutrient uptake, and e) soil pH and EC in a glasshouse experiment.

3) Determination of the effect of LEA on: a) cool season salt-tolerant ryegrass (*Lolium multiflorum* Lam.) emergence, b) plant growth (shoot weight), c) soil nutrient availability, d) plant nutrient uptake, and e) soil pH and EC in a glasshouse experiment.
4) Quantification of GHG fluxes from LEA-amended fallow soil in a glasshouse experiment as well as effects on populations of soil bacteria and fungi.
5) Determination of the effects of LEA on soil quality in a field environment by:
    a. isolating and quantifying SOC pools associated with macroaggregates (>250 µm), microaggregates (250-53 µm), and the silt and clay fraction (<53 µm).
    b. determining SOC and total N storage.
    c. investigating the influence of LEA incorporation on aggregate formation.
    d. evaluating the distribution and C sources in aggregate fractions by utilizing natural abundances of the stable isotope $\delta^{13}C$ in soil (C_4) and LEA material (C_3).

CHAPTER II

SOIL CARBON AND NITROGEN DYNAMICS AS AFFECTED BY LIPID-EXTRACTED ALGAE APPLICATION TO SOIL

INTRODUCTION

Organic amendments (e.g. biosolids, manure, and compost) have been suggested as alternative nutrient sources to synthetic inorganic fertilizers, while at the same time possibly increasing C accumulation and storage (Quilty and Cattle, 2011). Organic amendments contain plant nutrients within organic molecular structures, such as proteins and other cellular components, and thus, are not immediately available for plant use. Heterotrophic soil microorganisms begin to degrade macromolecules of recently added organic amendments into their component monomers, and under favorable environmental conditions, these monomers will then be mineralized releasing CO_2 (microbial respiration) and inorganic plant available nutrients, such as N, P, and S. Microbial soil respiration is one of the earliest and most commonly used indexes for assessing microbial activity in soil (Waksman and Starkey, 1924; Franzluebbers et al., 1995). Organic amendments from different sources and with varying chemical compositions may result in different microbial activities (Ng et al., 2014).

The chemical composition of organic amendments, especially C:N ratios and lignin content (or other resistant macromolecules), is important for determining how quickly decomposition proceeds (Vanlauwe et al., 2005). As decomposition of newly added organic amendments progresses and the less stable components are degraded, the

relative proportion of more recalcitrant materials, such as aliphatic macromolecules, increases (Augris et al., 1998; Poirier et al., 2000), which will consequently cause mineralization to decrease. Hydrocarbon molecules of aliphatic nature including algaenans, cutans, and suberans, are insoluble in aqueous media (nonhydrolyzable) and are more resistant to biological and chemical degradation than macromolecular components derived from proteins and polysaccharides (Gelin et al., 1999; Poirier et al., 2000). Cutans and suberans are widely distributed in the plant kingdom, but algaenans are localized in the cell walls of unrelated groups of microalgae. These materials are widely preserved in fossils, and although there is no definite evidence of their contribution to the composition of SOM, it is inevitable that cutans, suberans, and algaenans are present (Gelin et al., 1999), especially if plant residue or LEA is added to soil. By adding a potentially stable C source to soil, such as that from LEA, it may be possible to reduce the net flux of CO_2 to the atmosphere by sequestering C in soil.

Besides the mineralization rate of organic amendments like LEA, timing and rate of application impacts the synchronization of nutrients to plants. It is, therefore, necessary to determine the effect of LEA on microbial activity, mineralization/immobilization and nutrient availability. The primary objective of this laboratory experiment was to determine C and N mineralization due to LEA and WS applications over a 392-d period by measuring microbial respiration (CO_2 evolution) and changes in soil NH_4^+ and NO_3^- in microcosms. Changes in soil pH and electrical conductivity (EC) were also determined. It was hypothesized that LEA would be rapidly

mineralized with a lesser percentage of added LEA-C remaining in soil compared to added wheat straw (WS)-C and greater N availability in LEA-amended soil.

METHODS

Study Area

The soils used for the series of studies described below and in the following chapters were collected from the Texas A&M Agrilife Research Station near Beeville, TX (28º27’30”, 97º 42’21.78”, 75.9 m) and were characterized as either Weesatche or Parrita soil. The average temperature and precipitation for this semi-arid environment was reported to be 21ºC and 81 cm, respectively, by the U.S. Climate Data service. The Weesatche series is described as a sandy clay loam (fine-loamy, mixed, superactive, hyperthermic Typic Argiustolls) with a pH of 6.1 (USDA – NRCS, 2010). This site was previously planted to Kleingrass [*Panicum coloratum* (L.)] and grazed. The Parrita series is as a sandy clay loam with a pH of 6.9 (loamy, mixed, superactive, hyperthermic, shallow Petrocalcic Paleustolls) that consists of shallow, well drained soils that formed in loamy sediments derived from calcareous sandstone of the Goliad Formation of Pliocene age (USDA – NRCS, 2006). The soil collected from both sites was air dried for approximately 28 days, thoroughly mixed and stored until further use. The LEA source for all studies was *Nannochloropsis salina*, a microalgae cultivated in open ponds near Pecos, TX.

Soil and Lipid-Extracted Algae Characterization

Soils, LEA, and WS were analyzed for total organic C and total N by a combustion procedure (Storer, 1984; McGeehan and Naylor, 1988; Schulte and Hopkins, 1996). Soil was analyzed for extractable P, K, Ca, Mg, S, and Na using the Mehlich III procedure (Mehlich, 1978; Mehlich, 1984) with analysis by ICP; micronutrients (Cu, Fe, Mn, and Zn) by extraction with DTPA-TEA, followed by ICP analysis (Lindsay and Norvell, 1978), and extractable NH_4^+-N by the Berthelot reaction involving salicylate and NO_3^--N by cadmium reduction following extraction of both by 1 N KCl using a 1:5 soil to extractant ratio (5 g soil:25 g 1 N KCl), followed by analysis of both using flow injection spectrometry (FIAlab 2600, FIAlab Instruments Inc., Bellevue, WA) (Keeney and Nelson, 1982). Lipid-extracted algae and WS mineral concentrations (B, Ca, Cu, Fe, K, Mg, Mn, Na, P, S, and Zn) were determined by ICP analysis of nitric acid digests (Isaac and Johnson, 1975; Havlin and Soltanpour, 1989). The electrical conductivity of the soil, LEA, and WS were determined in a 1:2 soil or residue to water extract using deionized water with the actual determination made using a conductivity probe (Rhoades, 1982). Soil texture was determined using the hydrometer procedure (Day, 1965).

Lipid Extracted Algae and Wheat Straw Fiber Analysis

Lipid extracted algae and WS samples were weighed and dried at 105°C for 24 h for DM determination. Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially using the method of Van Soest et al.

(1991) and AOAC (1990; method 973.18) in an ANKOM 200 Fiber Analyzer (Ankom Technologies, Macedon, NY). Heat stable α-amylase was used for NDF analysis. Lignin or the more stable C fraction was sequentially measured following ADF using the Ankom (2013) method by incubating the Ankom bags in 72% sulfuric acid in order to solubilize cellulose (Van Soest, 1967). All chemical constituents were reported on a DM basis.

Aerobic Incubation

Microcosms in the laboratory arranged as a RCBD were utilized to measure C respiration and the quantity of N mineralized or immobilized by oxidation of various OM additions to soil. The Weesatche soil was amended with two types of OM, LEA and lignocellulosic WS, and then wetted to 60% water-filled pore space. Lipid-extracted algae was applied at 1.5 and 3.0% on a dry weight basis (g g^{-1}) and WS was applied at 1.5% (g g^{-1}).The control soil was without OM addition. Each treatment was replicated four times totaling 16 microcosms per destructive sample set. The total weight of each dry soil/OM mixture equaled 45 g soil plus the added residue (0.66 and 1.31 g with 1.5 and 3.0% LEA treatments, respectively). The soil water content was maintained throughout the experiment by weighing sample containers and adding deionized (DI) H_2O to a constant weight. Samples were placed in 1liter glass containers along with 10 ml DI H_2O, tightly sealed, and incubated at 30°C in the dark. Aerobic conditions were maintained by venting; microcosm lids were removed for five minutes at least once every seven days. There were five sets of 16 microcosms with one of the sets

destructively sampled at 4, 14, 28, 224 or 392 d following treatment application and wetting.

The fifth set, which was not destructively sampled until the final incubation day (392-d) was used to measure cumulative CO_2 evolution after 1, 4, 7, 14, 28, 56, 112, 168, 224, 280, 336, and 392 d, and therefore, to determine the rate of mineralization as well as the percentage of added LEA-C or WS-C mineralized at each time point. Carbon dioxide was trapped in 1 M KOH and then back titrated with 0.5 M HCl after adding $BaCl_2$ to precipitate the trapped CO_2 as $BaCO_3$. Soil organic C and total N and extractable NH_4^+-N and NO_3^--N soil concentrations of destructive samples were measured throughout the incubation by methods described above.

Soil Microbial Biomass

Chloroform fumigation incubation (CFI) that was used to estimate soil microbial biomass C (SMBC) was determined with some modifications to the original method proposed by Jenkinson and Powlson (1976). The same treatments used for the aerobic incubation detailed above were also used for CFI. Smaller quantities of soil (15 g) were moistened to 50% water-filled pore space, placed into 1 liter glass containers in the presence of 10 ml deionized H_2O, and sealed tightly. Soil was incubated at 30ºC for a period of 14 d in order to establish a steady state of microbial activity prior to fumigation (Franzluebbers et al., 1999). After fumigating samples with ethanol-free chloroform for 24 hr and then removing the fumigant by vacuum, the flush of CO_2-C over a 10-d

incubation period was quantified by titration of the alkali trap with 0.5 M HCl in order to quantify the response of soil microbiota to LEA and WS soil amendments.

The flush of CO_2-C evolved following fumigation was calculated using an efficiency factor of 0.41 and without subtraction of an unfumigated control as suggested by Voroney and Paul (1984), especially in soil which has recently received organic amendments. Franzluebbers et al. (1999) demonstrated much stronger relationships of potential C mineralization and SOC with CFI without subtraction of a control (R^2=0.81 and R^2=0.80, respectively) than with subtraction of a control (R^2=0.30 and R^2=0.38, respectively).

Statistical Analyses

Nonlinear regression was used to depict the relationship between cumulative CO_2-C and time. Lipid-extracted algae- and WS-C mineralized to CO_2-C was calculated as the percent of C added with the amendment. Lipid extracted algae- and WS-N mineralized to inorganic N (N_{inorg}) was also calculated as the percent of added N with the amendment; N_{inorg} is equal to NH_4^+-N plus NO_3^--N.

Statistical analysis was conducted using SAS version 9.3. Effects were analyzed using a linear mixed analysis of variance (ANOVA) procedure at a significance level of $P < 0.05$. Means of significant effects were separated using Fisher's protected LSD. Standard error of the mean was reported for data presented as figures. In SAS, PROC CORR was used to correlate extractable NH_4^+-N and NO_3^--N as a function of soil EC.

RESULTS

Soil and Lipid Extracted Algae Characterization

Soil Characterization

The soil collected from the Texas A&M Agrilife Research Station in Beeville, TX, and used for this incubation experiment is classified as Weesatche sandy clay loam. The Weesatche soil was determined to be 61.5% sand, 28.1% clay, and 10.4% silt, whereas the Parrita soil, also a sandy clay loam was determined to be 60.1% sand, 29.7% clay, and 10.2% silt. The pH of the Weesatche and Parrita soils was 6.1 and 6.9, respectively, while EC values were 0.27 and 0.16 dS m^{-1}, respectively (Table 2).

Soil OC in Weesatche soil (2.5%) was more than 2.5 times that of Parrita soil (0.96%), which is likely the result of different management practices (Table 2). Weesatche soil was under continuous pasture and grazing rotations, whereas the location of Parrita soil sampling was in a conventional cultivation system of annual grasses (species not known). Soil total N of both soils was slightly greater than the average total N in Texas soils (~0.1%).

The Weesatche soil was low in extractable P (13 mg kg^{-1}) and Na (35.6 mg kg^{-1}) but moderate to high in extractable K (358 mg kg^{-1}), Ca (3333 mg kg^{-1}), Mg (237 mg kg^{-1}), S (24 mg kg^{-1}), Fe (20.7 mg kg^{-1}), Zn (3.8 mg kg^{-1}), Cu (3.9 mg kg^{-1}), and Mn (14.9 mg kg^{-1}). Other than high available P (62 mg kg^{-1}), the Parrita soil followed a similar trend to the nutrient availability ratings of Weesatche soil but with lower concentrations of K, Ca, S, Fe, Zn, and Cu. Soil test ratings were based on those of the Soil, Water and

Forage Testing Laboratory of the Texas A&M AgriLife Extension Service, an agency within The Texas A&M University System (College Station, TX).

LEA and WS Characterization

The pH and EC of LEA were determined to be 9.9 and 32.54 dS m^{-1}, respectively and 6.6 and 3.43 dS m^{-1} for WS, respectively. Wheat straw-C (40.4%) was approximately 1.5 times greater than that of LEA (34.3%), but total N was nearly four times greater in LEA (3.2%) than in WS (0.8%) (Table 2.1). The C:N ratio of LEA and WS was 10.8 and 50.5, respectively. Other than K, LEA contained greater mineral concentrations than WS. Both WS and especially LEA should contain sufficient quantities of potentially available nutrients to support most agronomic crops when applied at sufficiently high rates.

Fiber analysis demonstrated potential differences in degradability/stability between LEA and WS. Neutral detergent fiber, ADF, and lignin or the most stable C fraction in LEA measured 29.1%, 17.3%, and 13.4% (DM basis), respectively, while these components represented 71.9%, 43.4%, and 3.5% (DM basis) of WS, respectively.

Table 2.1. Soil, LEA, and WS chemical characteristics.

		Electrical	Organic	Total	Soil Extractable Nutrients† & LEA/WS Total Minerals‡									
	pH	Conductivity	C	N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	----	dS m^{-1}	---------%---------		----------mg kg^{-1} ----------									
Soil														
Weesatche	6.1	0.272	2.5	0.18	13	358	3333	237	24	35.6	20.7	3.8	14.9	3.9
Parrita	6.9	0.158	0.96	0.14	62	314	2667	278	12	58.8	6.0	0.5	16.5	0.5
Amendments														
LEA	9.9	32.54	34.3	3.18	4339	6997	62666	7212	9282	52922	3664	28.5	78.5	14.0
WS	6.6	3.43	40.4	0.8	800	14400	3200	800	992	533	39	20	17	5.0

† Soil nutrients (P, K, Ca, Mg, and S) and Na are Mehlich III extractable and DTPA (Fe, Zn, Mn, and Cu) extractable.
‡ Lipid extracted algae (LEA) and wheat straw (WS) were analyzed for total concentrations.

Carbon Dynamics

Soil Organic Carbon

Soil amended with 3.0% LEA demonstrated significantly different C cycling dynamics over the 392-d incubation compared to the control and 1.5% WS treatment. Soil OC measured 4, 14, 28, 224, and 392 d after treatment initiation was significantly greater with the 3.0% LEA treatment than all other treatments (Fig. 2.1). After 392 d of incubation, SOC in soil amended with 1.5% LEA was significantly greater than the control and 1.5% WS treatment, but until this point SOC in 1.5% LEA treated soil was less than or equal to SOC in the 1.5% WS treatment.

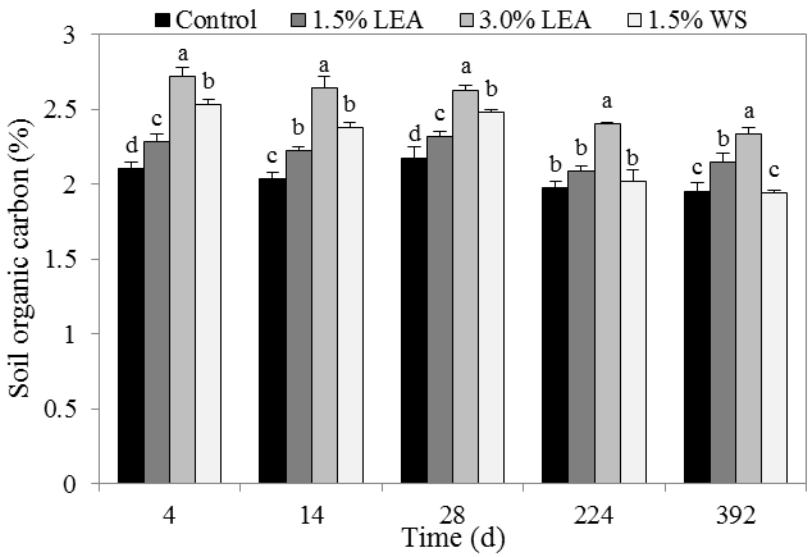

Fig. 2.1. Soil organic carbon over 392 d of incubation as affected by amendments of lipid extracted algae (LEA) and wheat straw (WS) residues. Mean percentages followed by the same letter within incubation date are not different at $P < 0.05$ by Fisher's protected LSD.

Carbon Mineralization

The amount of C mineralized at each sampling event was measured as evolved CO_2-C and cumulatively added in order to determine the total amount of C mineralized. Carbon dioxide-C evolution from amended soils was greater than that from the control throughout the experiment, indicating that regardless of the source, organic amendments enhanced microbial activity (Fig. 2.2). A significantly greater amount of cumulative C was mineralized and lost from soil treated with 3.0% LEA compared to any other treatment and control over the 392-d incubation, except for 1.5% WS at 280 d (Fig. 2.2).

The rate of C mineralization from 1 d to 4 d was greatest for 3.0% LEA (16.5 mg CO_2-C 45 g^{-1} soil d^{-1}) followed by 1.5% LEA (15.1 mg CO_2-C 45 g^{-1} soil d^{-1}), 1.5% WS (9.1 mg CO_2-C 45 g^{-1} dry soil d^{-1}), and then the control (3.7 mg CO_2-C 45 g^{-1} soil d^{-1}). The rate of C mineralization in 1.5% LEA treated soil decreased after 4 d while that in 1.5% WS treated soil increased; therefore, cumulative CO_2-C measured at 28 d was not significantly different between the two treatments. At 56 d of incubation and thereafter, CO_2-C evolution from the 1.5% WS treatment was significantly greater than that of the 1.5% LEA treatment.

Nonlinear regressions were fitted to CO_2-C evolution with time in response to treatment. The r^2 values ranged from 0.9220 to 0.9891 for the control and the 1.5% LEA treatment, respectively. The regression for each treatment demonstrated that a slower and more stable state of respiration had been reached by approximately 56 d.

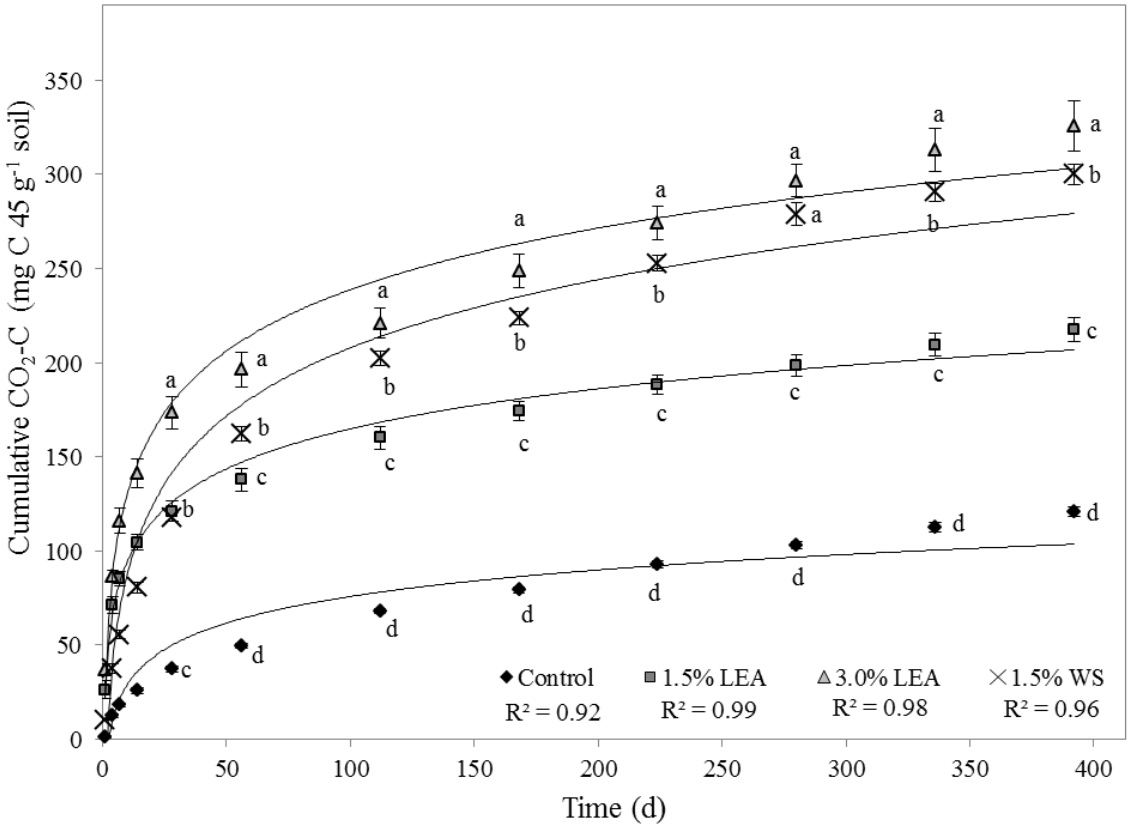

Fig. 2.2. Soil CO_2-C evolution over a 392-d incubation period. Inset shows days 1 - 14, while the main graph shows all data. Mean values followed by the same letter within incubation date are not different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw are denoted as LEA and WS, respectively.

Although more CO_2-C was mineralized and lost as CO_2 from soil amended with 3.0% LEA (Fig. 2.2), a greater percentage of added WS-C was mineralized compared to that with either 1.5 or 3.0% LEA applications (Fig. 2.3). Approximately 1.5 times more C was added with 3.0% LEA (411 mg C 45 g^{-1} soil) compared to the 1.5% WS treatment (269 mg C 45 g^{-1} soil).

The percentage of added C mineralized by day 7 was greatest with the 1.5% LEA (32.0%) treatment followed by 3.0% LEA (23.7%) and 1.5% WS (13.8%), but at 56 d, no difference was observed between 1.5% LEA and 1.5% WS (Fig. 2.3). The percentage of mineralized C with 1.5 and 3.0% LEA treatments (45.7% and 44.1%, respectively) was significantly less than the 1.5% WS treatment (59.6%) by 224 d; moreover, the percent C mineralized with 1.5% WS treated soil remained significantly greater than 1.5 and 3.0% LEA treatments until the end of the 392 d incubation.

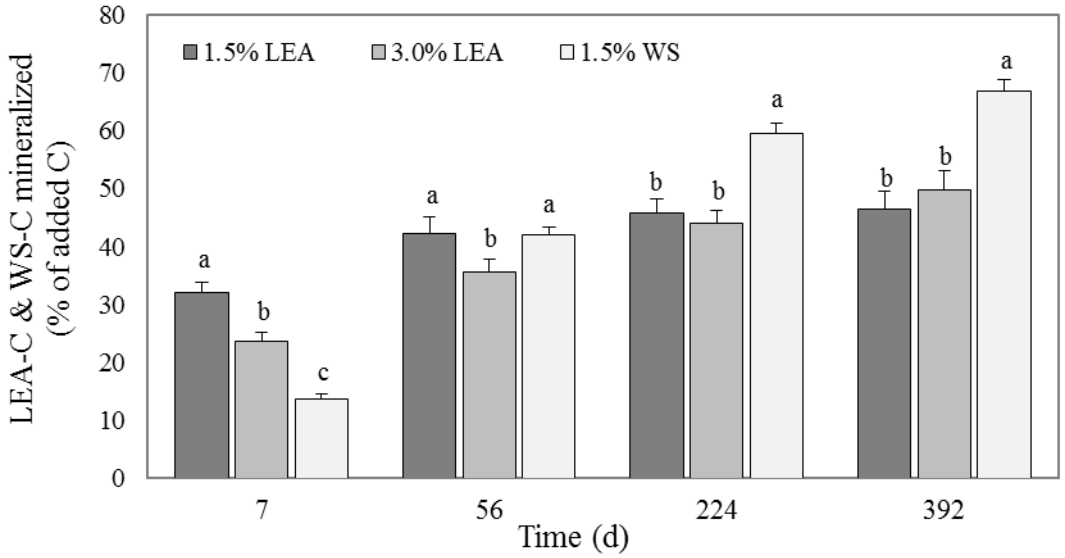

Fig. 2.3. LEA-C and WS-C mineralized after 7, 56, 224, and 392 d of incubation. Mean values represent the percentage of added C from either lipid extracted algae (LEA) or wheat straw (WS) that was mineralized and are not different at $P < 0.05$ by Fisher's protected LSD when followed by the same letter within incubation day.

Soil Microbial Biomass Carbon

Significant differences in SMBC were observed between treatments ($P = 0.01$). Soil microbial biomass C was significantly less for the control compared to either 3.0% LEA or 1.5% WS treatments, but it was not significantly different from the 1.5% LEA treatment (Fig. 2.4). No differences were found between organic amendment treatments; however, numerically the 3.0% LEA treatment resulted in the greatest SMBC (2063 mg C kg^{-1} soil) followed by 1.5% WS (1996 mg C kg^{-1} soil) and 1.5% LEA (1704 mg C kg^{-1} soil).

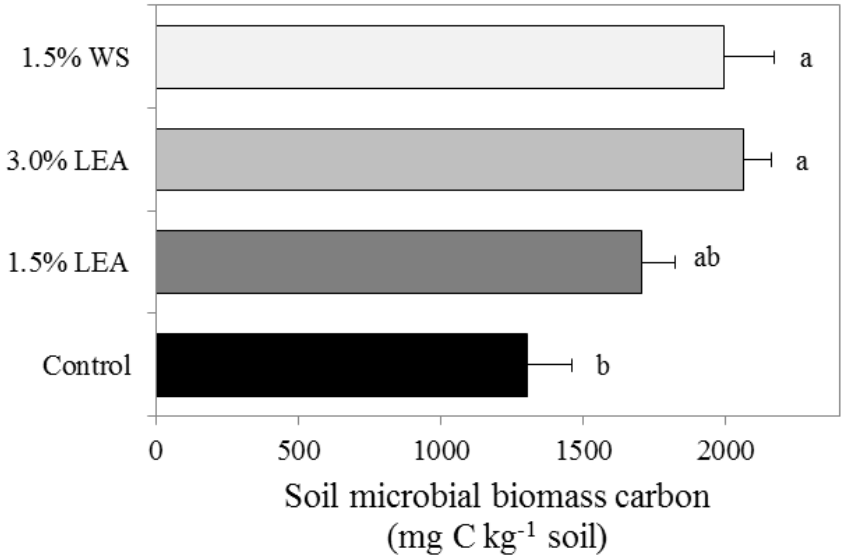

Fig. 2.4. Soil microbial biomass carbon determined using chloroform fumigation incubation. Mean values are not different at $P < 0.05$ by Fisher's protected LSD when followed by the same letter. Bars on columns represent standard error of the mean. Lipid extracted algae and wheat straw are denoted as LEA and WS, respectively.

Nitrogen Concentrations and Dynamics

Total Soil Nitrogen

Significant treatment differences ($P < 0.05$) were observed for soil total N within each of the sampling time points (Fig. 2.5). The 3% LEA treatment contained significantly greater soil total N than any other treatment throughout the 392-d incubation and increased total N by 36.1% compared to the control. Except at 4 d, the 1.5% LEA treatment was also significantly greater than the control and 1.5% WS treatment and by 392 d had 20.6% greater soil total N compared to the control. Compared to the control, the 1.5% WS amendment did not increase soil total N over the 392-d incubation.

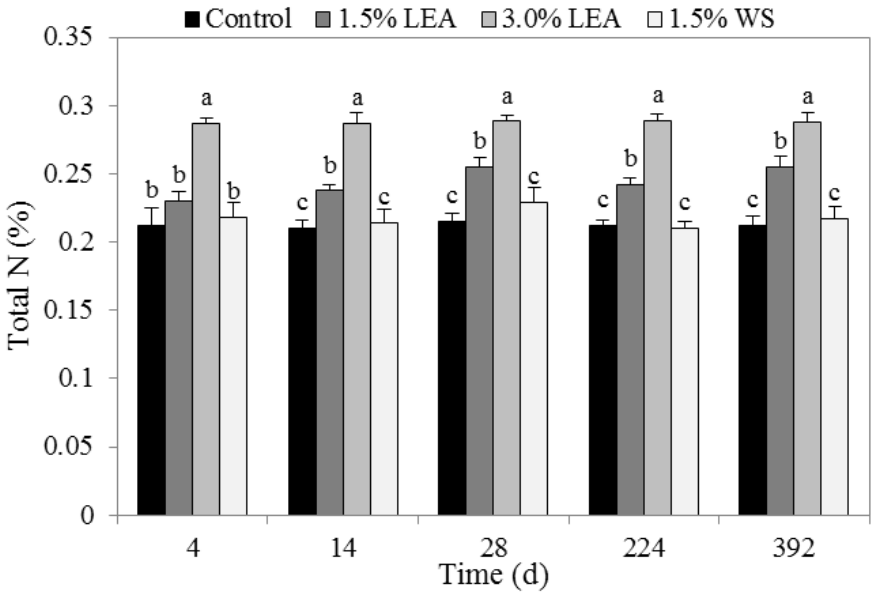

Fig. 2.5. Soil total N measured over the 392-d incubation as affected by amendments of lipid extracted algae (LEA) and wheat straw (WS) residues. Mean percentages followed by the same letter within incubation day are not different at $P < 0.05$ by Fisher's protected LSD.

Nitrogen Transformations

Soil NH_4^+-N extracted at 4, 14, and 392 d of incubation showed significantly different treatment effects (Fig. 2.6a). Four-days after treatment initiation, no difference in NH_4^+-N was observed between LEA treatments and both were significantly greater than the control or 1.5% WS treatments. However, NH_4^+-N measured after 14 d was greatest with the 3.0% LEA treatment and was not different between the control, 1.5% LEA, and 1.5% WS treatments. Approximately 63% of the total extractable inorganic N present in the 3.0% LEA treatment at 14 d was NH_4^+-N rather than NO_3^--N, which was also the predominant form of available N in the 1.5% LEA treatment at this time (Fig. 2.6a,b). Extractable NH_4^+-N decreased for all treatments by 28 d, with no significant differences observed between treatments from then until the end of the incubation. The 1.5% WS treatment was significantly greater in extractable NH_4^+-N than the other treatments by 392 d.

Extractable soil NO_3^--N was significantly different between treatments within each of the incubation days (Fig. 2.6b). After 4 d of incubation, the control had the greatest concentration of NO_3^--N compared to the organically-amended soils. The 1.5% LEA treatment contained the greatest amount of NO_3^--N (148 mg kg^{-1} dry soil) by 14 d, whereas the NO_3^--N concentration for the 3.0% LEA treatment was not different from the control. The WS treatment at this time was significantly lower in NO_3^--N, indicating possible N immobilization.

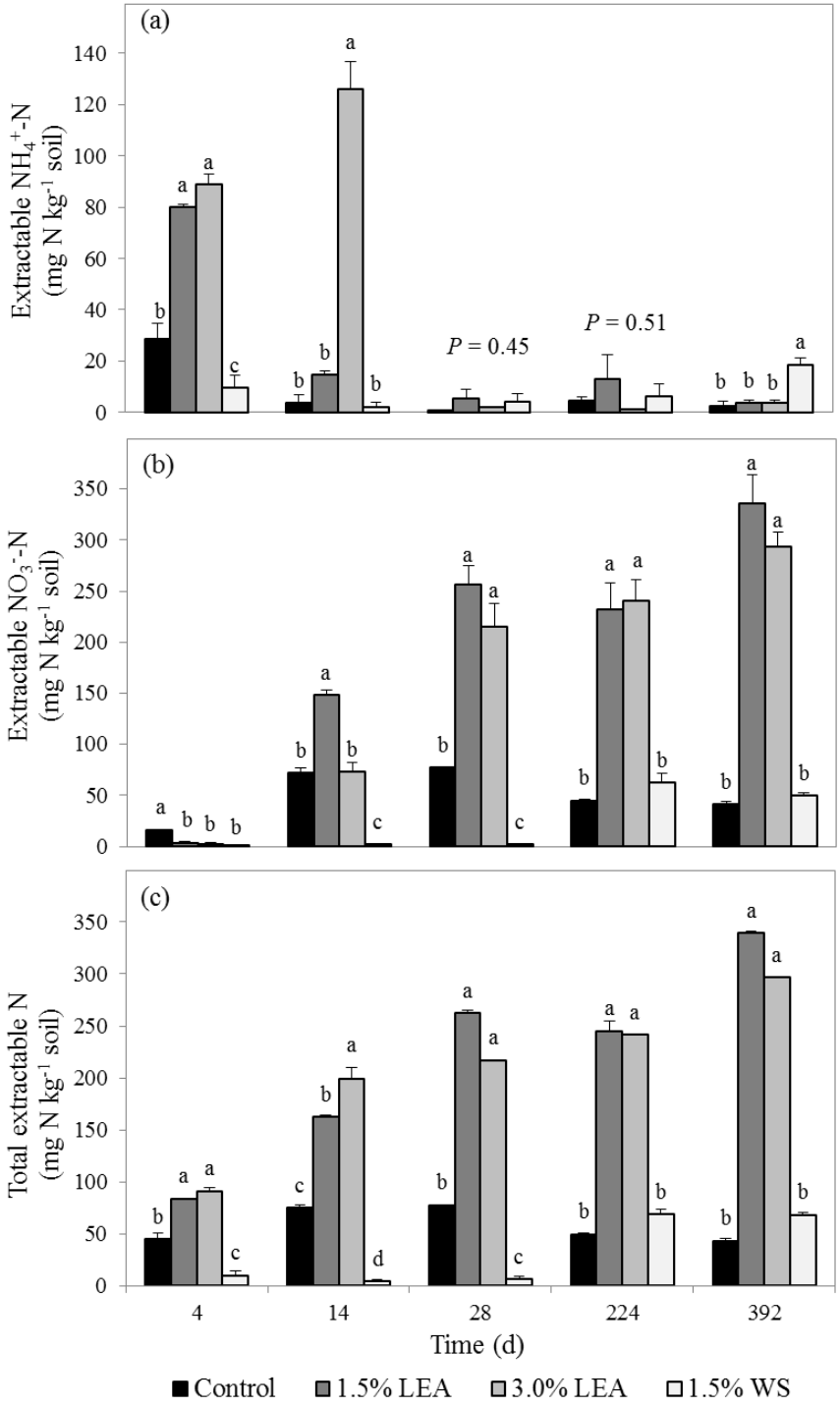

Fig. 2.6. Soil extractable inorganic N determined over the 392-d incubation as (a) extractable NH_4^+-N, (b) extractable NO_3^--N, and (c) total extractable N. Mean values followed by the same letter within N form and incubation day are not different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw are denoted as LEA and WS, respectively.

From 14 to 28 d, the amount of extractable NO_3^--N increased for both LEA treatments, so that by 28 d there was no difference in NO_3^--N between these treatments. This trend remained throughout the remainder of the study with 336 and 293 mg NO_3^--N kg^{-1} soil produced with 1.5% LEA and 3.0% LEA treatments, respectively, by the end of the incubation. Extractable NO_3^--N in the 1.5% WS treatment was significantly less than the control at 4, 14, and 28 d, but no differences between these two treatments were observed at 224 and 392 d of incubation.

Total inorganic N released as NH_4^+-N and NO_3^--N after 14 d of incubation was greatest from the 3.0% LEA treatment, even though this amount was only 13% of the N added with this treatment (Fig. 2.6c). After 392 d of incubation, 1.5% LEA and 3.0% LEA treatments released statistically similar concentrations of inorganic N that were significantly greater than both the control and 1.5% WS treatments. The percentage of added N mineralized and in the form of either NH_4^+- or NO_3^--N by the end of the study was 63%, 27%, and 21% from 1.5% LEA, 3.0% LEA, and 1.5% WS treatments, respectively.

The percentage of added LEA-N mineralized at 4 d with 1.5 and 3.0% LEA was not different, but both were greater than the percentage of WS-N mineralized (Fig. 2.7). Wheat straw amendment resulted in net immobilization at 4, 14, and 28 d after treatment application, demonstrated in the figure (Fig. 2.7) as negative values. By 14 d, a greater percentage of N in 1.5% LEA-amended soil had mineralized compared to the that in 3.0% LEA-treated soil. No difference in the fraction of added N mineralized existed between 1.5% WS and 3.0% LEA at 224 and 392 d, but both treatments were less than

the fraction of N mineralized from the 1.5% LEA treatment. Approximately two times the percentage of added LEA-N was mineralized with 1.5% LEA (63.0%) than 3.0% LEA (27.3%) at 392 d.

Soil pH and Electrical Conductivity Responses to LEA and WS Application

Soil pH was affected differently by treatments within each time point except at 28 d ($P = 0.16$) (Table 2.2). No differences in soil pH between the 1.5 and 3.0% LEA treatments occurred, other than at 14 d. Furthermore, soil pH increased from 5.1 for the control to 6.9 and 7.1 by 392 d in response to 1.5% LEA and 3.0% LEA applications, respectively.

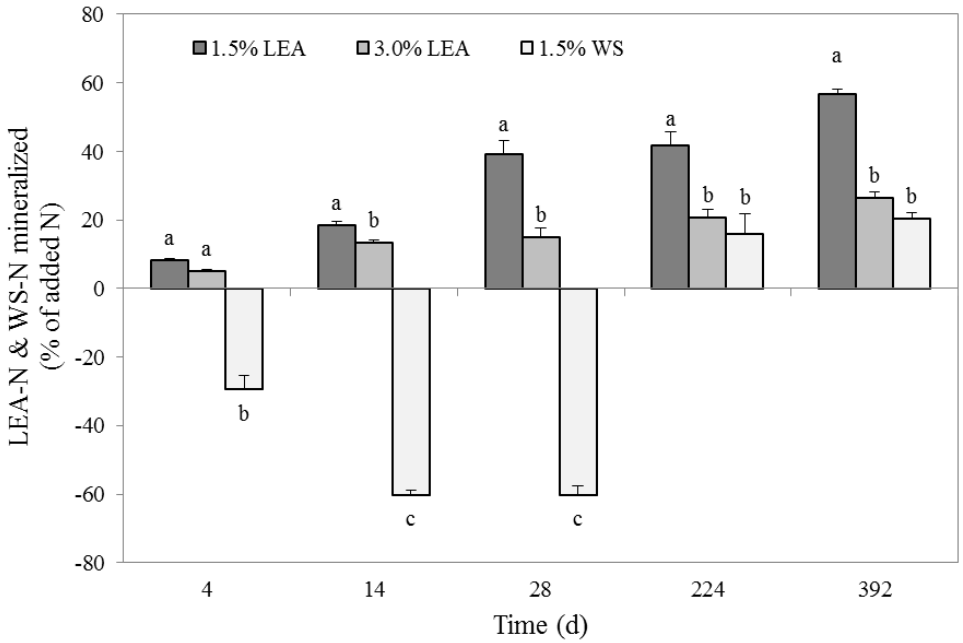

Fig. 2.7. LEA-N and WS-N mineralized after 4, 14, 28, 224, and 392 d of incubation. Mean values represent the percentage of added N from either lipid extracted algae (LEA) or wheat straw (WS) that was mineralized and are not different at $P < 0.05$ by Fisher's protected LSD when followed by the same letter within incubation day.

Table 2.2. Soil pH and EC in response to lipid-extracted algae and wheat straw amendments measured over 392-d incubation.

	Incubation Time (d)				
Treatment	4	14	28	224	392
	Soil pH				
Control	5.1b[†]	5.2c	6.1	4.9c	5.1b
1.5% LEA[‡]	7.7a	7.4b	7.0	7.0a	6.9a
3.0% LEA	8.0a	8.0a	7.1	7.3a	7.1a
1.5% WS[§]	5.7b	5.3c	6.3	5.4b	4.9b
p-value	<0.0001	<0.0001	0.1642	<0.0001	<0.0001
	EC (dS m^{-1})				
Control	0.2c	0.3c	0.4c	0.5c	0.5c
1.5% LEA	0.9b	1.1b	1.2b	1.5b	1.2b
3.0% LEA	1.7a	1.7a	1.9a	1.9a	2.0a
1.5% WS	0.2c	0.2c	0.2d	0.4c	0.5c
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001

[†] Mean pH and EC values followed by the same letter within incubation day are not different at $P < 0.05$ by Fisher's protected LSD.
[‡] LEA denotes lipid-extracted algae.
[§] WS denotes wheat straw.

The EC measured over incubation time demonstrated significantly different responses to treatments. For each time point, EC increased by 3.6 to 9 times for the 3.0% LEA treatment in comparison to the control. The 1.5% LEA treatment resulted in an EC consistently greater than the control but less than the 3.0% LEA treatment. Regressions of increasing soil EC with extractable inorganic N (NH_4^+ and NO_3^-) at 14 d demonstrated different trends; NH_4^+-N increased with increasing EC (R^2=0.83) and NO_3^--N decreased (R^2 = 0.84) (Fig. 2.7). By 28 d, relationships between EC and extractable N were no longer observed (R^2 < 0.2).

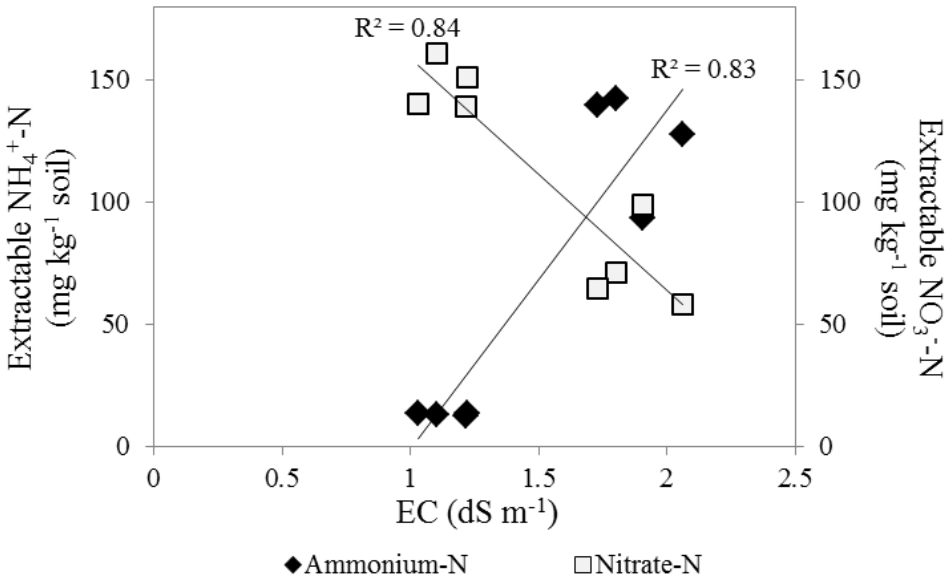

Fig. 2.8. Extractable NH_4^+-N and NO_3^--N 14 d after lipid-extracted algae application (1.5 and 3.0%) as a function of soil electrical conductivity (EC).

DISCUSSION

Based on the C:N ratios of LEA and WS (10.8 and 50.5, respectively), WS was expected to be more stable and store greater amounts of C, while LEA was expected to decompose faster, and therefore, contribute less to SOC. Even though the cumulative C losses were greatest with 3.0% LEA compared to the other treatments (Fig. 2.2), greater percentages of the C added with both this and the 1.5% LEA treatment were stabilized as SOC (Figs. 2.1 and 2.3). One possible explanation for greater SOC stabilization with LEA treatment may be due to LEA's macromolecular composition.

Fiber analysis of LEA and WS determined that LEA was comprised of a larger stable C fraction compared to the lignin in WS, which may explain greater C accumulation and stabilization in LEA-treated soil. *Nannochloropsis salina*, the microalgae used in this experiment, has been reported to contain aliphatic macromolecules known as algaenans, which are more resistant to chemical and biological decay than other macromolecular compounds derived from proteins, polysaccharides, and even lignin (Gelin et al., 1999; Poirier et al., 2000); therefore, it is suggested that algaenans may be responsible for greater SOC stabilization with LEA application to soil.

Unlike C loss, the total inorganic N that was mineralized to NH_4^+-N and then nitrified to NO_3^--N over the 392-d incubation was numerically greater with the 1.5% LEA treatment than the 3.0% application of LEA (Fig. 2.6). Moreover, a greater percentage of added LEA-N was mineralized at 392 d with 1.5% LEA compared to the 3.0% LEA application. LEA, because of its low C:N, ratio was hypothesized to result in

net N mineralization, with the greatest application rate supplying greater quantities of available N. However, the 3.0% LEA treatment demonstrated a short-term inhibition of nitrification which may possibly be attributed to the salinity generated with this treatment. Between 4 and 14 d, NH_4^+-N accumulated in soil treated with 3.0% LEA, while NH_4^+-N decreased and NO_3^--N accumulated in soil treated with 1.5% LEA. It was not until 28 d after treatment initiation that NH_4^+-N decreased and NO_3^--N increased for the 3.0% LEA treatment. A study by Megda et al. (2014) reported a similar nitrification inhibition with increasing rates of NH_4Cl to soil. Soil EC appeared to affect available N (NH_4^+ or NO_3^-) 14 d after LEA (1.5 or 3.0%) was applied, with the trend that as soil EC increased, NH_4^+-N increased ($R^2 = 0.83$) but NO_3^--N decreased ($R^2=0.84$) (Fig. 2.8).

CHAPTER III

SALINITY ASSOCIATED EFFECTS OF LIPID-EXTRACTED ALGAE RESIDUE ON A RANGE OF SALT TOLERANT FORAGES

INTRODUCTION

Soil salinity is one of the major environmental factors and abiotic stresses restricting plant growth and productivity especially in arid and semi-arid areas. It affects approximately 20% of irrigated arable land, and is responsible for damage to plant development, particularly at the seedling stage (Flowers and Yeo, 1995). The deleterious effects of salinity on plant growth may be associated with plant metabolism, nutrient deficiencies, osmotic stress, specific ion toxicities, or the combination of these factors (Ashraf, 1994; Hasegawa et al., 2000). Although LEA exhibits potential use as a source of nutrients, there may be negative effects on plant growth associated with salinity when applied to soil.

Nutrient deficiencies or imbalances in saline soil may be due to the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+}, and NO_3^- (Hu and Schmidhalter, 2005). As a consequence of having to complete with excess Na for entry into plant cells, reduced K uptake is among the major harmful effects caused by salinity (Rubio et al., 1995; Hafsi et al., 2007). Application of nitrogen fertilizer has been reported to mitigate the adverse effects caused by salt stress on a number of crops (Lewis et al., 1989; Leidi et al., 1992). Plants grown in saline media and supplemented with NO_3^- rather than NH_4^+ had greater productivity, even though NH_4^+ had less costly N assimilation (Lewis et al.,

1989). Past research reported wheat and maize (*Zea mays* L.) to be more sensitive to salinity as the ratio of NH_4^+: NO_3^- increased (Leidi et al., 1991; Botella et al., 1997).

Foxtail millet [*Setaria italica* (L.)] and pearl millet [*Pennisetum glaucum* (L.)] are particularly important food and fodder grain crops grown in arid and semi-arid regions. Pearl millet and its wild relatives are rated to be fairly tolerant to salinity (Ashraf and McNeilly, 1987); however, pearl millet at germination stage seems to be sensitive at EC of 16 dS m^{-1} and greater (Dua, 1989). As soil salinity increased from 3 to 7 dS m^{-1}, the relative yield of foxtail millet was reported to decrease sharply from approximately 95% to 40% of the control yield; and at 9 dS m^{-1}, yield decreased to 20% of the control (Ravikovitch and Yoles, 1971). Thus, compared to pearl millet, foxtail millet would be considered much more sensitive. Both sorghum [*Sorghum bicolor* (L.) Moench] and sudangrass (*S. sudanese*) are considered moderately salt tolerant with thresholds (i.e. the maximum salinity that does not reduce yield below that obtained under non-saline conditions) of 6.8 and 2.8 dS m^{-1}, respectively (Bower et al., 1970; Francois et al., 1984). Based on peer-reviewed literature, it was hypothesized that pearl millet would have greater salt tolerance, followed by sorghum-sudangrass and foxtail millet; however, tolerance was thought to possibly vary over developmental stages.

The objective of this glasshouse experiment was to determine the effect of LEA on: a) warm-season grass seedling survival of: foxtail millet, pearl millet, and a sorghum-sudangrass hybrid, b) plant growth and health, c) plant nutrient uptake, d) soil nutrient availability, and e) soil pH and EC.

METHODS

The experiment was arranged as a RCBD within forage type. Sample size was 60 growth columns, including: 3 grasses, 4 treatments (and control), and 4 replications. Growth columns (33 cm length) were constructed using PVC pipe with an I.D. of 10 cm. The bottoms of the columns were capped to prevent excessive loss of water and nutrients, but drainage holes were drilled in caps to prevent the soil from becoming anaerobic. Weights of empty columns and soil filled columns were measured and recorded.

Unamended Weesatche soil was added to the bottom half (15 cm) of all columns. The remaining upper half of the column was filled with dry soil amended with one of the following on a dry weight basis: 1) control (N and P fertilizer), 2) 1.5% LEA, 3) 3.0% LEA, 4) 1.5% LEA + 1.5% WS, and 5) 1.5% WS plus N and P fertilizer. Inorganic N (NH_4NO_3) and P [$Ca(H_2PO_4)_2 \cdot H_2O$] (561 kg N ha^{-1} and 112 kg P ha^{-1}), LEA, and WS were added and incorporated by mixing thoroughly in dry soil. Fertilizer was added to the control and 1.5% WS treatment in order to prevent N and P limitations and consequently immobilization. Nitrogen was applied as a split application; 280.5 kg N ha^{-1} was added initially and then the same amount was applied several weeks after the second harvest, which was the first appearance of plant nutrient deficiency symptoms. Columns were filled with amended soil to a bulk density of ~ 0.8 g cm^{-1}. Deionized H_2O was added to each column so that the soil gravimetric water content was approximately 0.28 g g^{-1}; constant weight was maintained over time by water addition.

Soil-filled columns were left to incubate undisturbed, except when water was added, at 32º to 35°C for 14 d, at which time foxtail millet, pearl millet, and sorghum-sudangrass were planted (20, 10, and 10 seed per column, respectively) at 1 cm depth. Emergence was monitored and recorded daily for 14 d, after which time columns were thinned to two plants. At 49 d after planting (DAP), plants were uniformly cut to 7 cm stubble height; sorghum-sudangrass and pearl millet were again cut 89 and 127 DAP. Foxtail millet was replanted immediately after cutting and cut again at 61 DAP.

Plant chlorophyll was measured as a plant health indicator once N deficiency symptoms were visually observed in sorghum-sudangrass and pearl millet, which was after the first harvest or prior to the second application of N was added to the control (+N,+P) and 1.5% WS (+N,+P) treatment. Before the second application of N fertilizer was added to the control (+N,+P) and 1.5% WS (+N,+P) treatment, plant chlorophyll was estimated using a SPAD-502 meter (Minolta Camera Co. Ltd., Japan) for sorghum-sudangrass and pearl millet; foxtail millet leaves did not have a large enough surface area for measuring. Chlorophyll measurements were also taken 10 d post-fertilization and prior to the second and third harvests.

Harvested plant material was weighed prior to and after drying to constant weight at 65º C, ground (0.5-mm) in a cyclone mill (Udy Cyclone Sample Mill 3010-030; Fort Collins, CO, USA), and then analyzed for N, P, K, Ca, Mg, Na, Fe, Zn, Cu, and S by the same methods previously reported. Nutrient uptake for each harvest was calculated by multiplying the mineral concentration of harvested herbage by the amount of HM per pot. Since treatments were only applied to the top 15 cm of soil in columns,

all columns were sampled to a 15 cm depth after final harvest. Samples were then either incubated for SMBC determination or dried for nutrient analysis.

Soil collected for nutrient analysis was dried at 65º C to constant weight. Samples were then ground (< 2 mm) with a flail grinder and analyzed for extractable NH_4^+- and NO_3^--N, and P, K, Ca, Mg, S, and micronutrients by the same methods described previously. Samples for soil total C and N were further ground (< 150 μm) in a ring and puck mill prior to analysis. Soil microbial biomass C and N were determined by chloroform fumigation incubation (Franzluebbers et al., 1999).

Statistical Analyses

Statistical analysis was conducted using SAS version 9.3. Effects were analyzed using a linear mixed analysis of variance (ANOVA) procedure at a significance level of $P < 0.1$ for seedling emergence and $P < 0.05$ for all other analyses. Means of significant effects were separated using Fisher's protected LSD.

RESULTS

Forage Seedling Emergence, Herbage Mass, and Nutrient Uptake

Seedling Emergence

No treatment differences were observed for foxtail millet or sorghum-sudangrass seedling emergence, but pearl millet seedling emergence was affected ($P < 0.1$) by LEA application (Table 3.1). Pearl millet seedling emergence was most reduced in soil treated with 3.0% LEA, while the 1.5% LEA was not different from the control (+N,+P).

Foxtail millet emergence ranged from 27.5% to 43.8% and was much less than sorghum-sudangrass or pearl millet emergence. Also, the coefficient of variation determined for foxtail millet emergence (32.5%) was two times that of the other forages (average 16.2%).

Table 3.1. Foxtail millet, pearl millet, and sorghum-sudangrass seedling emergence 14 d after planting as a percent of the seed planted and the corresponding test statistics within forage type.

	Forage type		
	Foxtail millet	Sorghum-sudangrass	Pearl millet
	Seedling emergence		
	---------% of seed planted---------		
Treatment			
Control (+N,+P)	37.5	90.0	90.0a[†]
1.5% LEA[‡]	36.3	72.5	85.0ab
3.0% LEA	43.8	82.5	65.0b
1.5% LEA + 1.5% WS[§]	27.5	80.0	90.0a
1.5% WS (+N,+P)	30.0	97.5	82.5ab
	ANOVA		
Test statistics			
p-value	0.32	0.15	0.098
CV, %	32.47	16.24	16.19

[†] Means followed by the same letter within column are not different at $P < 0.1$ by Fisher's protected LSD.
[‡] LEA denotes lipid-extracted algae.
[§] WS denotes wheat straw.

Forage Herbage Mass

No treatment differences were detected for yield from either harvest or total HM of foxtail millet (Fig. 3.1a). Pearl millet HM from the first and second harvests was not different among treatments or the control (+N,+P), but was different at the third harvest (Fig. 3.1b). Herbage mass of pearl millet grown in 3.0% LEA-amended soil was greater than that of other treatments or the control (+N,+P).

Treatment differences existed for sorghum-sudangrass HM at the second harvest and for total HM, but not the first or third harvests (Fig. 3.1c). At the second harvest, greater sorghum-sudangrass herbage was collected from 3.0% LEA-amended soil compared to the control (+N,+P) and all other treatments except 1.5% LEA. Total sorghum-sudangrass HM from 1.5 and 3.0% LEA-amended soil (25.8 g and 28.3 g, respectively) was greater than the other amendment treatments, but not the control (+N,+P).

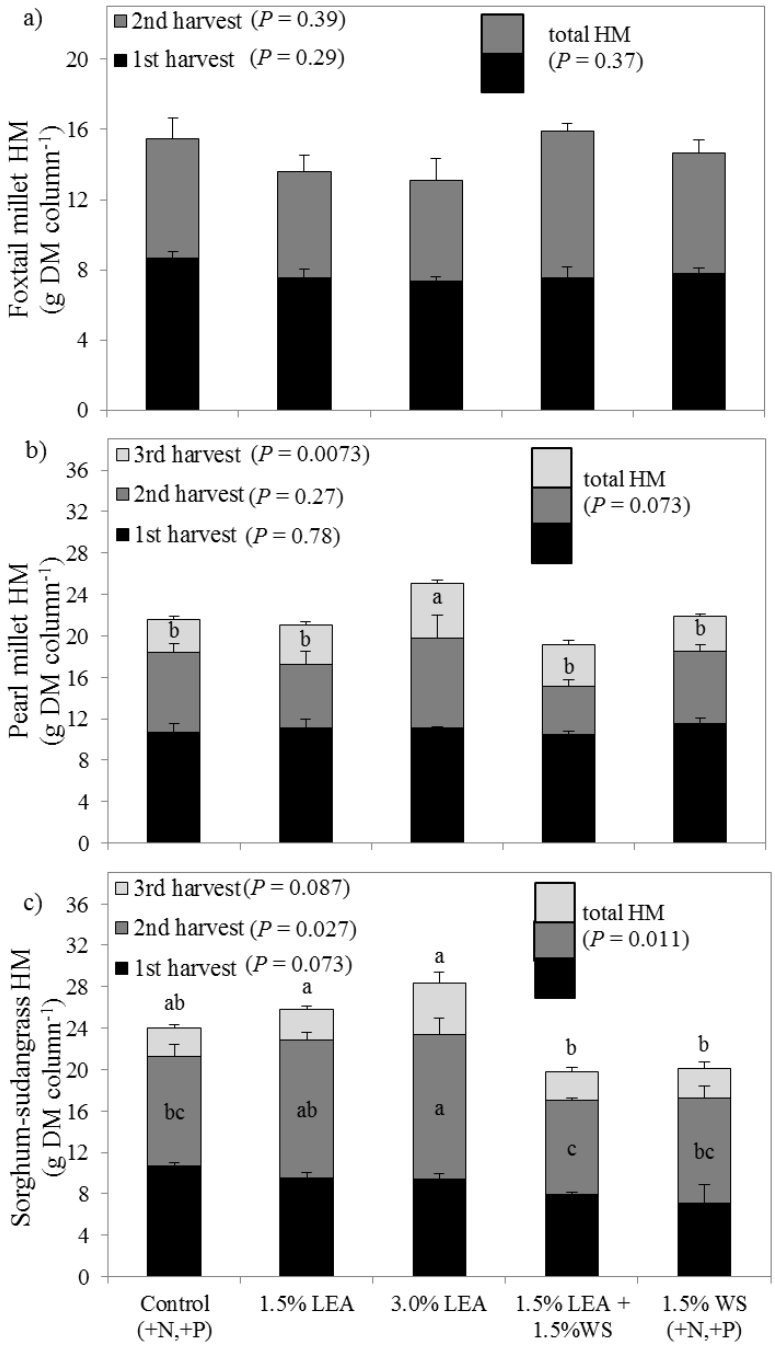

Fig. 3.1. Herbage mass (HM) of a) foxtail millet, b) pearl millet, and c) sorghum-sudangrass collected at harvests. Total HM was calculated as the sum of each harvest and represented by the height of treatment columns. Means followed by the same letter within harvest or total harvest are not significantly different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

Foxtail Millet Nutrient Concentration and Uptake

Foxtail millet nutrient concentrations were significantly different at both harvests, except for Fe and Cu at the first harvest and Fe and Mn at the second harvest (Table 3.2). Nitrogen concentrations were greater from 3.0% LEA-amended soil compared to that from the control (+N,+P) and amendment treatments, except 1.5% LEA at both harvests. This was also the case with P, except that no P difference was detected at the first harvest between the control (+N,+P) and 1.5% and 3.0% LEA treatments.

Potassium was greatest in foxtail millet grown in 1.5% WS (+N,+P) compared to all other treatments or the control (+N,+P) at the second harvest, but at the first harvest was only greater than the control (+N,+P). The 3.0% LEA treatment resulted in the greatest plant Ca concentration for foxtail millet at the second harvest, but only greater than 1.5% WS (+N,+P) at the first harvest. Foxtail millet harvested from 3.0% LEA-amended soil had greater Mg, S and Na concentrations compared to the control (+N,+P) and 1.5% LEA + 1.5% WS and 1.5% WS (+N,+P) treatments at both harvests. Plant Mn from the first harvest was greater for the three LEA treatments compared to the control (+N,+P), while Cu was greater with only the 3.0% LEA treatment compared to the control (+N,+P) at the second harvest. Plant Na concentrations were greatest from 3.0% LEA treatments for both harvests.

Table 3.2. Nutrient concentrations of foxtail millet (dry matter basis) at the first and second harvests.

Treatment	TN	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	%	mg kg^{-1} DM									
	1st harvest										
Control (+N,+P)	2.5 bc[†]	1490 ab	49772 b	3789 ab	3365 c	1711 bc	121 c	55	46 c	58 b	7.2
1.5% LEA[‡]	2.7 ab	1599 ab	57566 a	5069 a	4000 b	1967 ab	2054 b	56	78 a	77 a	8.9
3.0% LEA	3.0 a	1983 a	63161 a	4921 a	4516 a	2043 a	4926 a	53	59 bc	79 a	9.3
1.5% LEA + 1.5% WS[§]	2.4 bc	1173 b	63299 a	3661 ab	3280 c	1682 bc	1407 b	53	66 ab	63 ab	9.2
1.5% WS (+N,+P)	2.2 c	1395 b	60720 a	2901 b	2833 d	1634 c	144 c	48	63 ab	54 b	8.5
p -value	0.0032	0.049	0.0018	0.04	<0.0001	0.033	<0.0001	0.90	0.012	0.039	0.084
	2nd harvest										
Control (+N,+P)	1.5 b	1553 b	36648 b	3996 c	2329 cd	1211 c	156 b	259	33 c	52	6.7 b
1.5% LEA	2.7 a	1843 ab	39707 b	4961 b	3322 b	1659 b	918 b	67	54 a	44	8.6 ab
3.0% LEA	2.8 a	2470 a	38225 b	6634 a	4038 a	2003 a	3380 a	65	52 a	58	10.1 a
1.5% LEA + 1.5% WS	1.9 b	1276 b	40767 b	4277 c	2856 bc	1446 bc	802 b	85	46 ab	44	6.6 b
1.5% WS (+N,+P)	1.6 b	1611 b	46457 a	4241 c	2233 d	1408 bc	189 b	63	43 b	53	6.2 b
p -value	0.0041	0.038	0.0054	<0.0001	<0.0001	0.0014	<0.0001	0.20	0.0008	0.076	0.022

[†] Means followed by the same letter within harvest and column are not significantly different at $P < 0.05$.
[‡] LEA and TN denote lipid-extracted algae and total N, respectively.
[§] WS denotes wheat straw.

Nutrient uptake by foxtail millet was different among treatments for all nutrients except K, Fe, and Cu at the first harvest; whereas, Na uptake was the only element with treatment differences at the second harvest (Table 3.3). Nitrogen, Ca, S, and Mn uptake by foxtail millet was similar for the control (+N,+P) and 1.5% and 3.0% LEA treatments. Phosphorus uptake was greater for 3.0% LEA-amended soil compared to the other treatments, but not the control (+N,+P). Moreover, foxtail millet uptake of Mg and Na was greatest in soil amended with 3.0% LEA. At the first and second harvests, approximately 2.5 and 3.5 times more Na, respectively, were taken up and concentrated in foxtail millet with the 3.0% LEA treatment compared to 1.5% LEA (Table 3.2 and 3.3).

Pearl Millet Nutrient Concentrations and Uptake

Pearl millet N concentration was greater with 3.0% LEA (3.5%) compared to any other treatment or the control (+N,+P) (mean = 1.8%), which was greater than the 1.5% LEA + 1.5% WS and 1.5% WS (+N,+P) treatments (1.4% and 1.1%, respectively) but less than 1.5% LEA (2.3%) (Table 3.4). At the second harvest, N was greater for the control (+N,+P) and 1.5% WS (+N,+P) treatment compared to any LEA treatment, which ranged from 1.0% to 1.2% N. No differences were detected for N between treatments at the third harvest. Phosphorus concentration was less for 1.5% LEA compared to the control (+N,+P) at the first harvest. At the first harvest, pearl millet concentrations of K, Mg, S, and Na were greatest with 3.0% LEA, and Na was also greater at the second and third harvests for this treatment.

Table 3.3. Nutrient uptake by foxtail millet measured at the first and second harvests.

Treatment	TN	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	mg column^{-1}										
	1st harvest										
Control (+N,+P)	211 a[†]	13 ab	430	32 ab	29 b	14.8 a	1 d	0.5	0.40 c	0.5 abc	0.06
1.5% LEA[‡]	199 ab	12 b	433	37 a	30 b	14.6 a	15 b	0.4	0.57 a	0.6 ab	0.07
3.0% LEA	219 a	15 a	465	36 a	33 a	15.0 a	36 a	0.4	0.43 bc	0.6 a	0.07
1.5% LEA + 1.5% WS[§]	177 bc	9 c	473	27 bc	25 c	12.6 b	10 bc	0.4	0.49 b	0.5 bc	0.07
1.5% WS (+N,+P)	171 c	11 bc	473	22 c	22 c	12.7 b	1 cd	0.4	0.49 b	0.4 c	0.07
p-value	0.002	0.0024	0.19	0.0031	<0.0001	0.0024	<0.0001	0.35	0.0008	0.013	0.36
	2nd harvest										
Control (+N,+P)	96	10	244	27	15	8.0	1 d	1.3	0.23	0.3	0.04
1.5% LEA	160	11	236	30	20	9.8	5 bc	0.4	0.31	0.3	0.05
3.0% LEA	150	12	216	37	23	10.9	17 a	0.3	0.28	0.3	0.05
1.5% LEA + 1.5%WS	157	11	340	36	24	12.1	7 b	0.7	0.38	0.4	0.05
1.5% WS (+N,+P)	104	11	316	29	15	9.4	1 cd	0.4	0.29	0.4	0.04
p-value	0.072	0.73	0.078	0.49	0.16	0.27	<0.0001	0.063	0.057	0.77	0.18

[†] Means followed by the same letter within harvest and column are not significantly different at $P < 0.05$.
[‡] LEA and TN denote lipid-extracted algae and total N, respectively.
[§] WS denotes wheat straw.

Table 3.4. Nutrient concentrations of pearl millet (dry matter basis) at the first, second, and third harvests.

Treatment	TN	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	%	mg kg^{-1} DM									
	1st harvest										
Control (+N,+P)	1.8 c[†]	2011 b	46206 bc	5063 a	2600 c	1525 c	121 c	71 abc	92	79 a	7 b
1.5% LEA[‡]	2.2 b	1651 cd	48668 b	4876 ab	3406 b	1718 b	541 b	73 ab	75	60 bc	11 a
3.0% LEA	3.5 a	2305 a	56651 a	5061 a	4375 a	2127 a	1603 a	82 a	75	62 bc	13 a
1.5% LEA + 1.5% WS[§]	1.4 d	1523 d	44938 bc	4094 c	2470 c	1242 d	567 b	55 c	78	56 c	7 b
1.5% WS (+N,+P)	1.1 d	1831 bc	42925 c	4395 bc	1857 d	1048 e	127 c	63 bc	97	70 ab	6 b
p -value	<0.0001	0.0001	0.0010	0.0019	<0.0001	<0.0001	<0.0001	0.031	0.83	0.0055	<0.0001
	2nd harvest										
Control (+N,+P)	1.8 a	5143 a	27325 c	11697 b	3712 bc	1315 c	280 d	125	178	157 ab	7 ab
1.5% LEA	1.0 b	3752 bc	29737 bc	13063 ab	4756 ab	2491 a	1063 c	88	143	153 ab	5 c
3.0% LEA	1.2 b	3167 c	28990 bc	12220 b	5610 a	1796 b	2587 a	60	146	184 a	6 bc
1.5% LEA + 1.5% WS	1.2 b	4024 b	35918 a	16521 a	5327 a	2683 a	1821 b	198	184	128 bc	7 ab
1.5% WS (+N,+P)	1.8 a	5692 a	34493 ab	9265 b	2913 c	1387 c	263 d	111	141	93 c	8 a
p -value	<0.0001	<0.0001	0.023	0.029	0.0004	<0.0001	<0.0001	0.61	0.82	0.016	0.0064
	3rd harvest										
Control (+N,+P)	1.3	5442	30742	11552	3703 b	1971 b	186 b	82	163 ab	84 a	8
1.5% LEA	1.3	4090	28394	8046	3731 b	2326 b	530 b	106	116 b	41 b	8
3.0% LEA	1.4	5243	26324	9032	5925 a	3938 a	8003 a	82	215 a	88 a	10
1.5% LEA + 1.5% WS	1.2	3583	31361	8314	3247 b	2238 b	724 b	61	113 b	37 b	8
1.5% WS (+N,+P)	1.1	4992	33122	11244	3553 b	1726 b	182 b	71	176 ab	74 a	8
p -value	0.7499	0.057	0.12	0.088	<0.0001	0.0034	0.0019	0.22	0.033	0.0005	0.65

[†] Means followed by the same letter within harvest and column are not significantly different at $P < 0.05$.
[‡] LEA and TN denote lipid-extracted algae and total N, respectively.
[§] WS denotes wheat straw.

Soil amended with 1.5% LEA + 1.5% WS resulted in greater K, Ca, Mg, and S concentrations compared to the control (+N,+P) at the second harvest, and except for K this treatment was also greater than 1.5% WS (+N,+P) (Table 3.4). Sodium concentrations at the first, second and third harvests for 3.0% LEA was 9 to 43 times greater than the control (+N,+P), whereas Na was at most 7 times greater for the other LEA treatments compared to the control (+N,+P). Plant Na concentrations also increased with each harvest for the 3.0% LEA treatment.

Nitrogen and P concentrations and uptake followed similar trends at the first and second harvests (Tables 3.4 and 3.5). Potassium, Mg, S, and Na uptake for the 3.0% LEA-treatment was greater than the other amendment treatments or the control (+N,+P). At the third harvest, nutrient uptake was greater for 3.0% LEA or both 1.5% and 3.0% LEA. Sodium uptake by pearl millet receiving 3.0% LEA increased sequentially with harvest.

Table 3.5. Nutrient uptake by pearl millet at the first, second, and third harvests.

Treatment	TN	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	mg column^{-1}										
	1st harvest										
Control (+N,+P)	188 c[†]	21 b	490 b	54	28 c	16 c	1 c	0.8 ab	1.00	0.9	0.08 b
1.5% LEA[‡]	247 b	18 bc	539 b	54	38 b	19 b	6 b	0.8 abc	0.86	0.7	0.12 a
3.0% LEA	383 a	26 a	628 a	56	49 a	24 a	18 a	0.9 a	0.83	0.7	0.15 a
1.5% LEA + 1.5% WS[§]	142 d	16 c	471 b	43	26 c	13 d	6 b	0.6 bc	0.81	0.6	0.07 b
1.5% WS (+N,+P)	130 d	21 b	493 b	51	21 c	12 d	1 c	0.7 c	1.12	0.8	0.07 b
p -value	<0.0001	0.0011	0.0058	0.13	<0.0001	<0.0001	<0.0001	0.061	0.81	0.092	<0.0001
	2nd harvest										
Control (+N,+P)	137 a	39 a	207	86 ab	28 b	10	2 c	0.9	1.29	1.2 b	0.05 a
1.5% LEA	59 b	22 b	176	74 bc	28 b	15	6 bc	0.5	0.86	0.9 bc	0.03 b
3.0% LEA	99 a	27 b	239	96 a	46 a	15	21 a	0.5	1.20	1.5 a	0.05 ab
1.5% LEA + 1.5% WS	52 b	19 b	163	74 bc	24 b	12	9 b	0.8	0.84	0.6 d	0.03 b
1.5% WS (+N,+P)	124 a	40 a	241	64 c	20 b	10	2 c	0.8	0.96	0.6 cd	0.06 a
p -value	0.0008	0.0054	0.19	0.015	0.0044	0.33	<0.0001	0.78	0.49	<0.0001	0.048
	3rd harvest										
Control (+N,+P)	40 b	17 b	96 c	37 b	12 b	6 b	1 b	0.3 b	0.55 b	0.3 b	0.02 b
1.5% LEA	49 b	15 b	106 bc	30 b	14 b	9 b	2 b	0.4 a	0.43 b	0.2 b	0.03 b
3.0% LEA	70 a	27 a	139 a	48 a	31 a	21 a	44 a	0.4 a	1.13 a	0.5 a	0.05 a
1.5% LEA + 1.5% WS	48 b	14 b	123 ab	34 b	13 b	9 b	3 b	0.2 b	0.46 b	0.1 b	0.03 b
1.5% WS (+N,+P)	38 b	17 b	112 bc	38 b	12 b	6 b	1 b	0.2 b	0.60 b	0.3 b	0.03 b
p -value	0.0018	0.0005	0.0071	0.18	<0.0001	0.0010	0.0040	0.012	0.0027	0.0010	0.0003

[†] Means followed by the same letter within harvest and column are not significantly different at $P < 0.05$.
[‡] LEA and TN denote lipid-extracted algae and total N, respectively.
[§] WS denotes wheat straw.

Sorghum-sudangrass Nutrient Concentrations and Uptake

Nutrient concentrations in sorghum-sudangrass were not different among treatments for Ca, Na, or Fe at the first harvest; Ca, Fe, Zn, Mn, or Cu at the second harvest; or N, P, K, Ca, S, Fe, Zn, Mn, or Cu at the third harvest (Table 3.6). Nitrogen, P, Mg, and Mn concentrations of sorghum-sudangrass at the first harvest were greatest in 3.0% LEA amended soil. No differences existed between 1.5% and 3.0% LEA treatments for K and S concentrations at the first harvest, but at the second harvest were greater for 3.0% LEA than 1.5% LEA. The 3.0% LEA treatment resulted in the greatest concentrations of N, Mg, S, and Na at the second harvest; however, plant N for 1.5% LEA was not different from that of the control (+N,+P).

Sorghum-sudangrass P at the second harvest was significantly less for all three LEA treatments than the control (+N,+P) or 1.5% WS (+N,+P) treatment. Magnesium for the 3.0% LEA treatment was greater than the control (+N,+P), 1.5% LEA + 1.5% WS, or 1.5% WS (+N,+P) at all three harvests. At the third harvest plant Na concentration was greatest for the 3.0% LEA treatment, but was not different between the other treatments.

Table 3.6. Nutrient concentrations of sorghum-sudangrass (dry matter basis) at the first, second, and third harvests.

Treatment	TN	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	%	mg kg^{-1} DM									
	1st harvest										
Control (+N,+P)	2.1 c[†]	1630 b	35866 b	4865	1825 cd	1233 b	101	66	38 ab	42 c	7 c
1.5% LEA[‡]	2.5 b	1862 b	39468 a	5140	2562 b	1507 a	101	63	71 b	52 b	9 ab
3.0% LEA	2.9 a	2511 a	40162 a	5671	3027 a	1677 a	269	71	66 a	65 a	10 a
1.5% LEA + 1.5% WS[§]	2.1 c	1791 b	40771 a	4909	2117 c	1319 b	101	59	49 b	52 b	9 b
1.5% WS (+N,+P)	1.7 d	1630 b	37527 ab	4933	1738 d	1173 b	101	60	39 ab	48 bc	7 c
p -value	<0.0001	0.028	0.049	0.16	<0.0001	0.0002	0.23	0.60	0.018	0.0006	<0.0001
	2nd harvest										
Control (+N,+P)	1.3 bc	3789 b	28588 b	9057	2254 c	1186 bc	106 c	95	39	82	7
1.5% LEA	1.2 c	2100 d	24032 c	8235	2689 b	983 d	141 bc	96	58	74	7
3.0% LEA	2.4 a	2604 cd	29505 ab	9013	3563 a	1698 a	209 a	98	73	94	10
1.5% LEA + 1.5% WS	1.3 c	2647 c	26787 bc	7574	2515 bc	998 cd	170 ab	69	55	77	11
1.5% WS (+N,+P)	1.7 b	4433 a	32540 a	8980	2412 bc	1297 b	110 bc	95	55	92	10
p -value	0.0003	<0.0001	0.0016	0.17	<0.0001	<0.0001	0.019	0.92	0.22	0.52	0.20
	3rd harvest										
Control (+N,+P)	1.2	4793	27210	6985	2575 c	1272	103 b	431	29	53	7
1.5% LEA	1.4	4769	26770	7734	3539 ab	1258	103 b	272	34	52	9
3.0% LEA	1.5	3672	28817	8225	3923 a	1173	144 a	114	49	70	8
1.5% LEA + 1.5% WS	1.5	4960	31586	7174	3384 b	1333	103 b	51	33	45	8
1.5% WS (+N,+P)	1.1	4774	29191	6767	2441 c	1101	103 b	173	40	40	7
p -value	0.13	0.13	0.30	0.20	<0.0001	0.39	0.0024	0.65	0.54	0.051	0.73

[†] Means followed by the same letter within harvest and column are not significantly different at $P < 0.05$.
[‡] LEA and TN denote lipid-extracted algae and total N, respectively.
[§] WS denotes wheat straw.

Potassium and Na uptake by sorghum-sudangrass in the first harvest were not affected by treatment, but all other nutrients were (Table 3.7). Moreover, no statistical differences were observed between treatments for K uptake at any harvest. Nitrogen, Mg, and Mn uptake in the first and second harvests were greatest for 3.0% LEA-amended soil, but only Mn uptake was greatest with this treatment at the third harvest. Nitrogen uptake was between 1.2 and 2.2 times greater in 3.0% LEA treated soil compared to the control and other treatments. Phosphorus uptake by sorghum-sudangrass in the first harvest was greatest for 3.0% LEA, followed by 1.5% LEA, but at the second and third harvests, uptake was not different between 3.0% LEA, other treatments or the control (+N,+P).

Calcium uptake in the first harvest was similar between the control (+N,+P) and 1.5 or 3.0% LEA treatments. However, in the second and third harvests Ca uptake was significantly greater for 3.0% LEA, while uptake was not different between the control and 1.5% LEA or 1.5% WS (+N,+P) treatments. At each harvest, the 3.0% LEA treatment resulted in greater Mg uptake, whereas, uptake by 1.5% LEA-treated plants was greater than the control (+N,+P) at only the second harvest.

Table 3.7. Nutrient uptake by sorghum-sudangrass at the first, second, and third harvest.

Treatment	TN	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	mg column^{-1}										
	1st harvest										
Control (+N,+P)	221 b[†]	17 b	383	52 a	19 bc	13 ab	1.1	0.7 a	0.41 bc	0.5 b	0.07 bc
1.5% LEA[‡]	232 ab	18 b	376	49 ab	24 ab	14 a	1.0	0.6 abc	0.66 a	0.5 ab	0.09 ab
3.0% LEA	269 a	23 a	375	52 a	28 a	16 a	2.3	0.7 ab	0.62 ab	0.6 a	0.09 a
1.5% LEA + 1.5% WS[§]	165 c	14 bc	324	39 bc	17 cd	10 bc	0.8	0.5 bc	0.39 c	0.4 b	0.07 bc
1.5% WS (+N,+P)	123 c	12 c	264	34 c	12 d	8 c	0.7	0.4 c	0.28 c	0.3 c	0.05 c
p-value	<0.0001	0.0032	0.092	0.015	<0.0001	0.0004	0.12	0.035	0.0093	0.0056	0.0010
	2nd harvest										
Control (+N,+P)	143 b	40 ab	307	96 bc	24 c	13 b	1.1 c	1.0	0.42	0.9 b	0.07
1.5% LEA	160 b	28 bc	322	110 ab	36 b	13 b	1.9 b	1.2	0.78	1.0 b	0.09
3.0% LEA	332 a	36 abc	415	125 a	49 a	24 a	2.8 a	1.4	1.03	1.3 a	0.14
1.5% LEA + 1.5% WS	115 b	24 c	244	69 c	23 c	9 b	1.5 bc	0.6	0.50	0.7 b	0.10
1.5% WS (+N,+P)	171 b	45 a	331	89 bc	24 c	13 b	1.1 c	0.9	0.54	0.9 b	0.09
p-value	<0.0001	0.019	0.13	0.0058	0.0001	<0.0001	0.0005	0.40	0.098	0.012	0.060
	3rd harvest										
Control (+N,+P)	32	13	75	20 b	7 b	3	0.3 b	1.3	0.08	0.2 b	0.02
1.5% LEA	39	14	78	24 b	10 b	4	0.3 b	0.8	0.09	0.2 b	0.02
3.0% LEA	80	17	143	39 a	20 a	6	0.7 a	0.6	0.22	0.3 a	0.04
1.5% LEA + 1.5% WS	41	13	90	20 b	9 b	4	0.3 b	0.1	0.10	0.1 b	0.02
1.5% WS (+N,+P)	32	14	87	20 b	7 b	3	0.3 b	0.4	0.13	0.1 b	0.02
p-value	0.072	0.51	0.10	0.017	0.0057	0.16	0.0062	0.67	0.055	0.0050	0.071

[†] Means followed by the same letter within harvest and column are not significantly different at $P < 0.05$.
[‡] LEA and TN denote lipid-extracted algae and total N, respectively.
[§] WS denotes wheat straw.

Sulfur, Zn, Mn and Cu uptake were greatest with either the 1.5% or 3.0% LEA treatments at the first harvest, but were not different among treatments by the third harvest for S, Zn, and Cu. Additionally, S uptake was twice as great for 3.0% LEA (24 mg column^{-1}) compared to all other treatments or the control (+N,+P). The three LEA treatments, with Na uptake ranging from 1.8 to 2.5 mg Na column^{-1}, were greater than those for the control (+N,+P) or 1.5% WS (+N,+P) at the second harvest, but no differences existed at the third harvest between the control and 1.5% LEA, 1.5% LEA + 1.5% WS and 1.5% WS (+N,+P) treatments.

Chlorophyll Measurements

Treatment differences in SPAD values existed for both pearl millet and sorghum-sudangrass prior to the second N fertilizer application, which was approximately 10 d after the first harvest (Fig. 3.2). At this time, SPAD values of both forages were greater for the 3.0% LEA amendment compared to the control (+N,+P), 1.5% LEA + 1.5% WS, or 1.5% WS treatments for sorghum-sudangrass (Fig. 3.2b); whereas, pearl millet 3.0% LEA values were greater than that measured for 1.5% LEA, but not the control (+N,+P) (Fig. 3.2a).

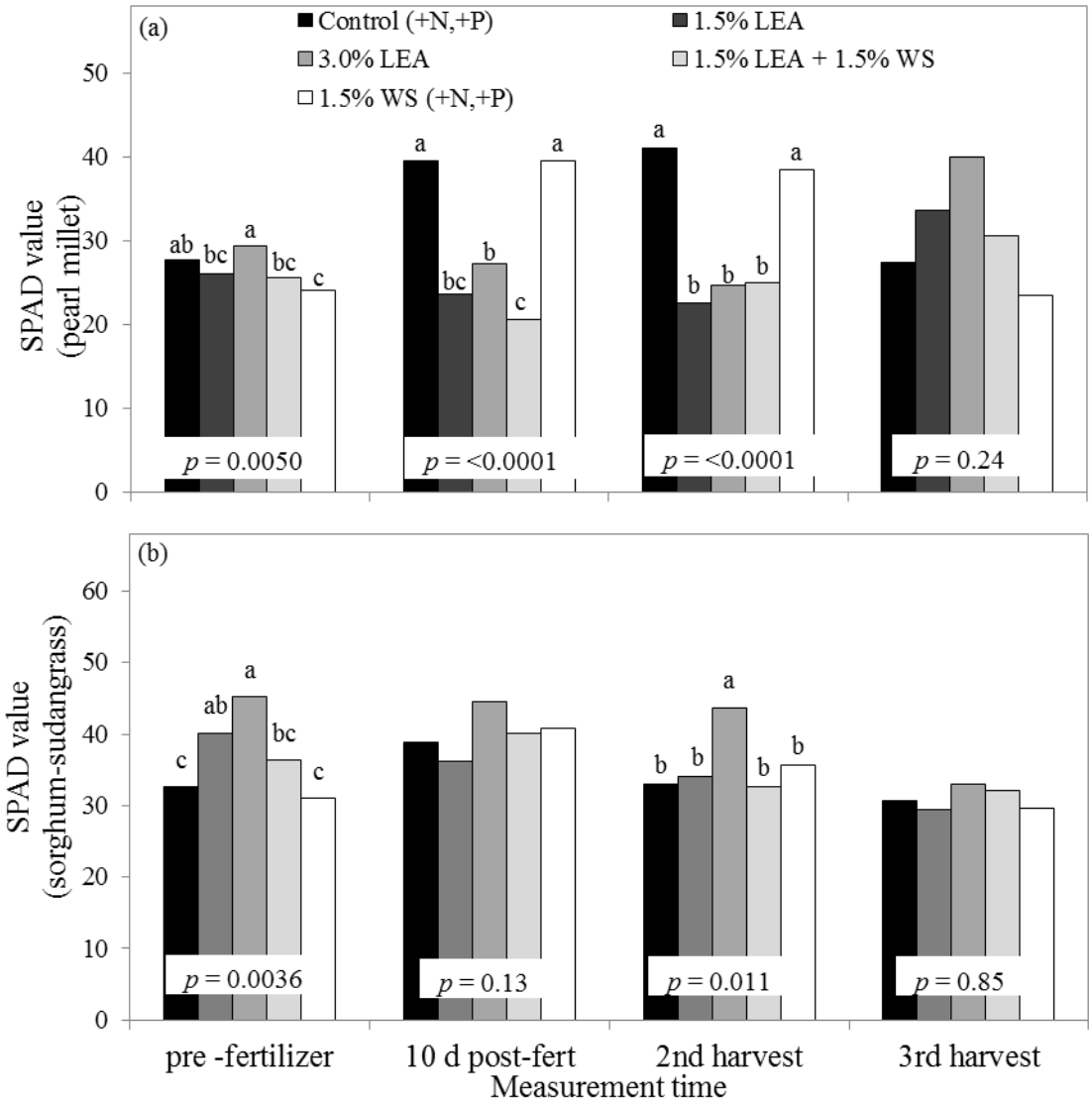

Fig. 3.2. SPAD values of a) pearl millet and b)sorghum-sudangrass measured 10 d after the first harvest and prior to the second N fertilizer application (pre-fertilizer), 10 d after fertilizer application, and prior to the second and third harvests. Mean values followed by the same letter within forage and measurement time are not significantly different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

No differences in SPAD values of sorghum-sudangrass were detected between treatments 10 d after fertilizer application, whereas, pearl millet values were different at this measurement point. The control (+N,+P) and 1.5% WS (+N,+P), both received additional N and resulted in significantly greater SPAD values for pearl millet 10 d after fertilizer application as well as before the second harvest. However, sorghum-sudangrass SPAD values prior to the second harvest were significantly greater for the 3.0% LEA treatment compared to the control and other treatments; the 1.5% LEA value was not different from the control (+N,+P), 1.5% LEA + 1.5% WS, or 1.5% WS (+N,+P) treatments. Chlorophyll measurements taken prior to the third harvest were not different among treatments for either forage.

The relationship between SPAD values and plant N concentrations was positive for both the second and third harvest of pearl millet and sorghum-sudangrass, yet correlation coefficients varied not only between forages but also harvests (Fig. 3.3). The third harvest of pearl millet ($R^2 = 0.66$) had a stronger relationship to the second harvest ($R^2 = 0.76$) (Fig. 3.3a), while a stronger relationship existed for the second sorghum-sudangrass harvest ($R^2 = 0.53$) compared to the third harvest ($R^2 = 0.31$) (Fig. 3.3b). SPAD values and plant N concentrations of pearl millet demonstrated stronger relationships at both harvests in comparison to the relationships of sorghum-sudangrass at each harvest.

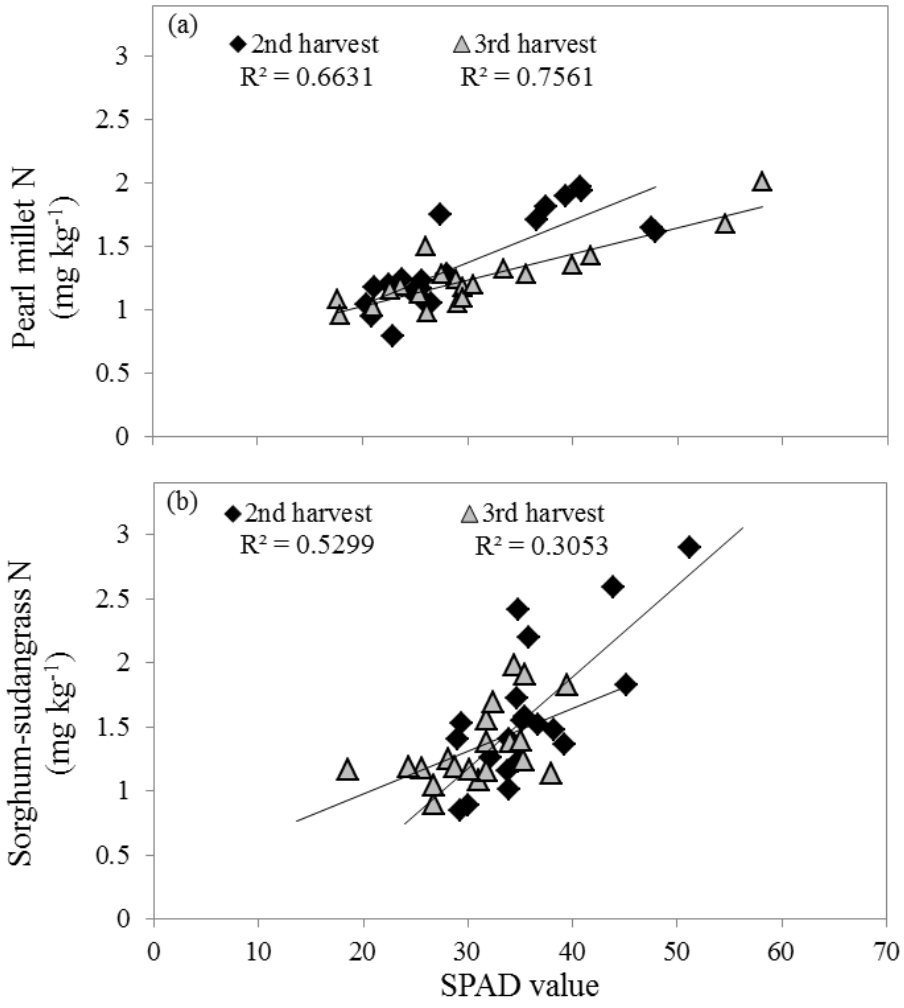

Fig. 3.3. Regression relationship between forages N concentration and SPAD values for the second and third harvests of a) pearl millet and b) sorghum-sudangrass.

Total Nitrogen, Soil Organic Carbon, and Soil Microbial Biomass Carbon

Soil total N of samples taken after the final sorghum-sudangrass and pearl millet harvests exhibited treatment differences, but not those taken from foxtail millet (Table 3.8). Soil amended with 3.0% LEA and 1.5% LEA + 1.5% WS and planted with sorghum-sudangrass contained significantly greater total N compared to the other treatments or control (+N,+P). For soil planted with pearl millet, the control (+N,+P) resulted in significantly less total N (0.23% N) compared to any of the amendment treatments, which were all similar (mean = 0.25% N).

Organic C in soil planted to foxtail millet, sorghum-sudangrass, or pearl millet was different among treatments (Table 3.8). Regardless of which forage had been planted, SOC was significantly greater for the 1.5% LEA + 1.5% WS amendment compared to the control (+N,+P). However, in soil planted to foxtail millet, SOC for 1.5% LEA + 1.5% WS (2.4%) was not only greater than the control (+N,+P) but also any other treatments. The 3.0% LEA and 1.5% LEA + 1.5% WS treatments resulted in greater SOC compared to other treatments for soil planted to sorghum-sudangrass. No treatment differences existed for SOC between organic amended treatments with pearl millet; however, the 3.0% LEA and 1.5% LEA + 1.5% WS amendments were the only treatments which were greater than the control. No treatment differences were observed for SMBC, regardless of forage; nonetheless, soil planted to pearl millet and sorghum-sudangrass had a tendency of greater SMBC for the 3.0% LEA treatment.

Table 3.8. Total N, soil organic carbon (SOC), and soil microbial biomass carbon (SMBC) after the final harvest of foxtail millet, sorghum-sudangrass, and pearl millet.

Treatment	Foxtail millet	Sorghum-sudangrass	Pearl millet
	total N (%)		
Control (+N,+P)	0.22	0.22 c[†]	0.23 b
1.5% LEA[‡]	0.23	0.23 bc	0.25 a
3.0% LEA	0.24	0.27 a	0.25 a
1.5% LEA + 1.5% WS[§]	0.25	0.27 a	0.26 a
1.5% WS (+N,+P)	0.23	0.24 b	0.25 a
p -value	0.067	<0.0001	0.017
	SOC (%)		
Control (+N,+P)	2.06 b	2.01 b	2.13 b
1.5% LEA	2.08 b	2.19 b	2.35 ab
3.0% LEA	2.18 b	2.62 a	2.37 a
1.5% LEA + 1.5% WS	2.40 a	2.61 a	2.48 a
1.5% WS (+N,+P)	2.05 b	2.26 b	2.35 ab
p -value	0.02	0.0006	0.079
	SMBC (mg C g^{-1} soil)		
Control (+N,+P)	2.06	1.58	2.12
1.5% LEA	1.77	2.15	2.24
3.0% LEA	1.92	2.53	3.51
1.5% LEA + 1.5% WS	1.88	2.32	2.59
1.5% WS (+N,+P)	1.29	2.52	3.21
p -value	0.46	0.28	0.12

[†] Means followed by the same letter within analysis and forage type are not significantly different at $P < 0.05$ by Fisher's protected LSD.
[‡] LEA denotes lipid-extracted algae.
[§] WS denotes wheat straw.

Residual Soil Nutrients

Treatment differences existed for extractable NO_3^--N and total inorganic N (N_{inorg} = NH_4^+-N + NO_3^--N) remaining in soil after two harvests of foxtail millet but not for NH_4^+-N (Fig. 3.4a). Nitrate-N was greatest in soil amended with 3.0% LEA (53.1 mg N kg^{-1} soil); however, N_{inorg} was similar between the 1.5% and 3.0% LEA amendments, which were both greater than the control (+N,+P). No differences for extractable NO_3^--N were determined between the control (+N,+P) and 1.5% LEA, 1.5% LEA + 1.5% WS, or 1.5% WS (+N,+P) treatments.

Soil extractable NH_4^+-N, NO_3^--N, and N_{inorg} were different among treatments after the final harvest of pearl millet (Fig. 3.4b). Residual NH_4^+-N was greatest in soil amended with 1.5% LEA + 1.5% WS and 1.5% WS (+N,+P) compared to the control (+N,+P) and 1.5% or 3.0% LEA. Nitrate-N was greatest for 3.0% LEA, and yet N_{inorg} was numerically greater with 1.5% LEA + 1.5% WS and 1.5% WS (+N,+P) treatments. The latter two treatments resulted in greater residual N_{inorg} compared to the control (+N,+P) or 1.5% LEA after three harvests of pearl millet.

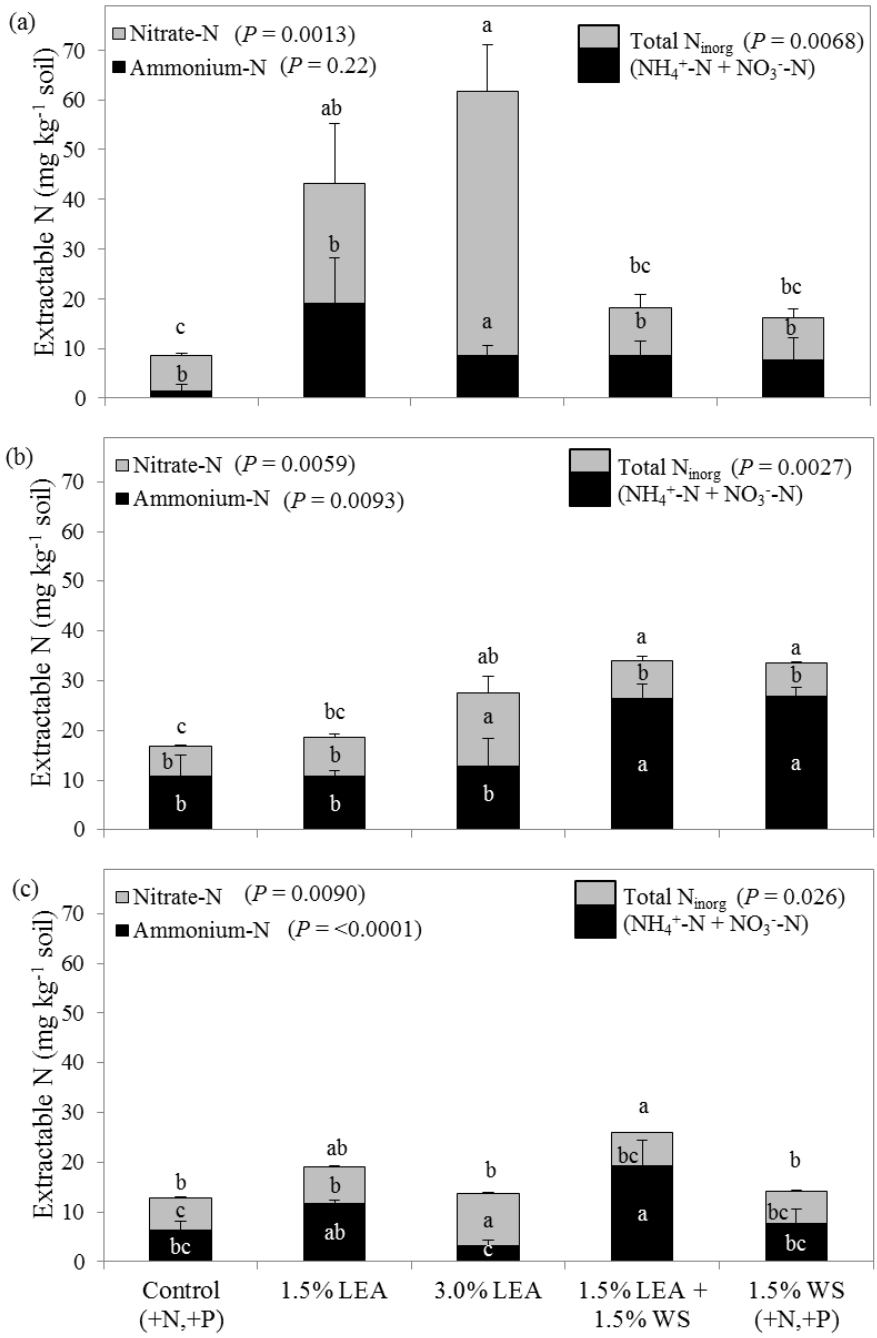

Fig. 3.4. Ammonium-N, NO_3^--N, and total N_{inorg} (NH_4^+-N + NO_3^--N) remaining in soil after the final harvests of a) foxtail millet, b) pearl millet, and c) sorghum-sudangrass. Total N_{inorg} represented by the summed height of treatment columns. Means followed by the same letter within forage-soil and N form are not significantly different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

Treatment differences were determined for extractable NH_4^+-N, NO_3^--N, and N_{inorg} remaining in soil after the third harvest of sorghum-sudangrass (Fig. 3.4c). Both the 1.5% LEA and 1.5% LEA + 1.5% WS amendments resulted in greater NH_4^+-N compared to 3.0% LEA-amended soil after the third harvest of sorghum-sudangrass; however, NO_3^--N was greatest for 3.0% LEA. Additionally, 1.5% LEA resulted in greater NO_3^--N compared to the control (+N,+P). Total N_{inorg} remaining after the third harvest of sorghum-sudangrass was greater in soil amended with 1.5% LEA + 1.5% WS compared to the and 3.0% LEA or 1.5% WS (+N,+P) treatments, but not 1.5% LEA.

Extractable P remaining in soil planted to foxtail millet, sorghum-sudangrass, and pearl millet was greatest with 3.0% LEA (Table 3.9). No differences in extractable K existed between treatments in soil planted to foxtail millet, but differences were detected in soil previously planted to sorghum-sudangrass and pearl millet. Greater residual K was the result of WS amendments as either 1.5% LEA + 1.5% WS or 1.5% WS (+N,+P) treatment, possibly because of the much greater K concentration of WS compared to LEA. Residual extractable soil Ca and Mg was greatest with 3.0% LEA compared to control and all other treatments, except for Ca with 1.5% LEA-amended soil planted to pearl millet. Sulfur was also greatest for 3.0% LEA in soil panted to foxtail millet or sorghum-sudangrass, but in soil planted to pearl millet this treatment had a similar affect as 1.5% LEA + 1.5% WS.

Table 3.9. Extractable nutrients in soil after the final harvest of foxtail millet, sorghum-sudangrass, and pearl millet.

Treatment	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	----------mg kg^{-1} soil ----------									
	Foxtail millet soil									
Control (+N,+P)	29 b[†]	174	3279 b	224 cd	12 bc	67 c	11.8 a	2.1 b	24.6 b	1.2 c
1.5% LEA[‡]	34 b	166	3414 b	240 b	19 b	380 b	7.3 b	2.9 a	15.7 c	1.8 a
3.0% LEA	53 a	182	3808 a	274 a	30 a	690 a	6.3 b	2.1 b	13.2 c	1.1 c
1.5% LEA + 1.5% WS[§]	34 b	201	3406 b	236 bc	17 b	404 b	5.6 b	3.2 a	14.7 c	1.9 a
1.5% WS (+N,+P)	25 b	230	3104 c	223 d	8 c	59 c	10.8 a	2.0 b	30.1 a	1.5 b
p -value	0.0001	0.1800	<0.0001	<0.0001	0.0001	<0.0001	<0.0001	0.023	<0.0001	<0.0001
	Sorghum-sudangrass soil									
Control (+N,+P)	23 b	174 bc	3208 c	218 c	9 b	88 c	14.8 a	1.6 c	28.6 a	1.3
1.5% LEA	14 b	156 c	3462 b	235 b	11 b	390 b	10.9 b	3.1 a	17.3 b	2.1
3.0% LEA	36 a	155 c	3809 a	264 a	17 a	669 a	6.3 c	2.4 abc	15.2 b	1.3
1.5% LEA + 1.5% WS	15 b	202 a	3544 b	238 b	11 b	354 b	5.4 c	2.8 ab	18.2 b	1.7
1.5% WS (+N,+P)	21 b	196 ab	3267 c	225 c	9 b	86 c	13.3 a	2.2 bc	29.7 a	1.5
p -value	0.0018	0.0010	<0.0001	<0.0001	0.0005	<0.0001	<0.0001	0.016	<0.0001	0.10
	Pearl millet soil									
Control (+N,+P)	12 b	138 bc	3351 bc	216 b	6 b	68 c	13.4 a	3.1 ab	33.2 a	1.1 b
1.5% LEA	12 b	136 c	3747 a	225 b	7 b	298 b	8.3 bc	3.3 ab	16.5 b	2.1 a
3.0% LEA	23 a	139 c	3923 a	249 a	13 a	615 a	7.4 c	1.9 b	14.2 b	1.2 b
1.5% LEA + 1.5% WS	13 b	193 a	3491 b	226 b	9 ab	221 b	10.1 abc	4.5 a	21.0 b	2.2 a
1.5% WS (+N,+P)	16 b	165 ab	3215 c	221 b	5 b	71 c	11.2 ab	2.2 ab	34.4 a	1.4 b
p -value	0.0021	0.0003	<0.0001	0.0004	0.011	<0.0001	0.031	0.28	0.0001	<0.0001

[†] Means followed by the same letter within analysis and forage are not significantly different at $P < 0.05$.
[‡] LEA denotes lipid-extracted algae.
[§] WS denotes wheat straw.

Residual extractable soil Na was approximately eight to ten times greater in soil amended with 3.0% LEA compared to the control (+N,+P) or 1.5% WS (+N,+P) treatment regardless of which forage was grown in the soil (Table 3.9). No differences in extractable Na existed in soil amended with 1.5% LEA and 1.5% LEA + 1.5% WS, but both were significantly less than that of 3.0% LEA.

Available Fe was less in soil amended with 1.5% or 3.0% LEA compared to the control (+N,+P), possibly because of increased pH following LEA application. Extractable Zn in soil planted to pearl millet and treated with 1.5% LEA + 1.5% WS was greater than 3.0% LEA, which was similar to all other treatments. However, in soil planted to sorghum-sudangrass, no differences were detected between LEA treatments; nonetheless, 1.5% LEA was the only LEA treatment with extractable Zn greater than both the control (+N,+P) and 1.5% WS (+N,+P) treatment. Conversely, residual extractable Mn after the final harvest of each forage was significantly less with all three LEA treatments compared to the control (+N,+P) or 1.5% WS (+N,+P). Copper availability was greater in 1.5% and 3.0% LEA-amended soil planted to foxtail millet or pearl millet; however, no differences existed for extractable Cu after the final harvest of sorghum-sudangrass.

Soil pH and EC as Affected by Lipid-extracted Algae and Wheat Straw

Soil pH determined after the final harvest of forages was affected by treatment (Fig. 3.5). Soil amended with 3.0% LEA or 1.5% LEA + 1.5% WS and planted to any of these forages resulted in a more alkaline soil pH compared to the control (+N,+P) or other treatments. Compared to the control (+N,+P), the 1.5% LEA treatment resulted in a greater pH in soil planted to pearl millet and sorghum-sudangrass, but not foxtail millet.

Forage effects on soil pH were significant within all treatments except 3.0% LEA (Fig. 3.5). Soil pH for the control (+N,+P) was most alkaline for pearl millet, followed by sorghum-sudangrass, and lowest for foxtail millet. Forage effects within both 1.5% and 3.0% LEA treatments were not different between sorghum-sudangrass and pearl millet. For the 1.5% LEA + 1.5% WS treatment, sorghum-sudangrass resulted in the most alkaline soil pH; whereas, for 1.5% WS (+N,+P), pH in soil planted to sorghum-sudangrass was greater than that for soil from pearl millet, but not foxtail millet.

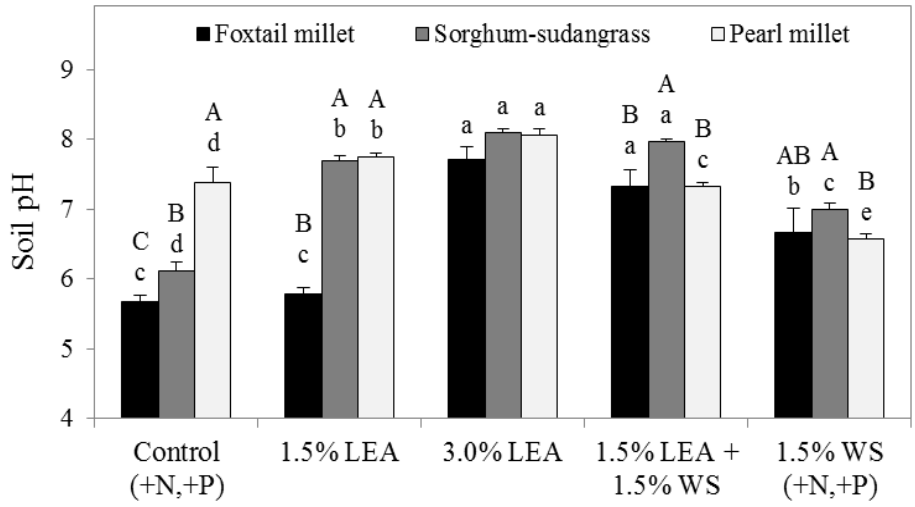

Fig. 3.5. Soil pH measured after the final harvest of foxtail millet, sorghum-sudangrass, and pearl millet. Means followed by the same letter within forage (lowercase letters; forage effect) or treatment (uppercase letters; forage effect) are not significantly different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

Electrical conductivity within forage species was significantly affected by soil amendment; yet, forage only affected EC within 1.5 and 3.0% LEA treatments (Fig. 3.6). Electrical conductivity measured in soil following the second foxtail millet harvest was greatest for the 3.0% LEA amendment (1.8 dS m^{-1}) followed by 1.5% LEA (1.1 dS m^{-1}). Similar to the treatment effects on pH, EC values of soil planted to sorghum-sudangrass were similar between 3.0% LEA and 1.5% LEA + 1.5% WS, and both were greater than the EC for 1.5% LEA. Within 1.5% and 3.0% LEA treatments, EC was greatest in soil previously planted to foxtail millet; however, there was no difference between pearl millet and sorghum-sudangrass.

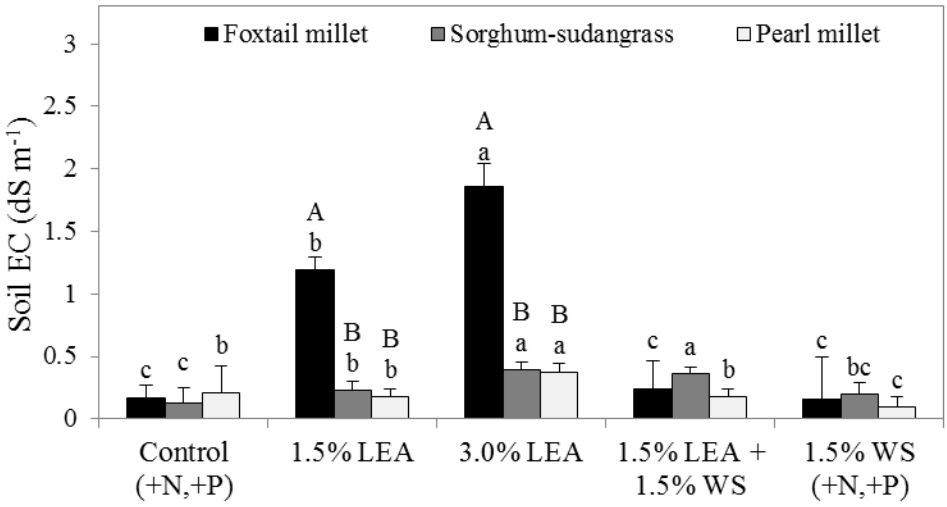

Fig. 3.6. Electrical conductivity (EC) measured following the final harvest of foxtail millet, sorghum-sudangrass, and pearl millet. Means followed by the same letter within forage (lowercase letters; treatment effect) or treatment (uppercase letters; forage effect) are not significantly different at $P < 0.05$ by Fisher's protected LSD. Lipid-extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

DISCUSSION

Forage Seedling Emergence, Herbage Mass, and Nutrient Uptake

Poor seedling emergence of foxtail millet regardless of soil treatment, along with the rather high coefficient of variation may possibly indicate poor seed quality and thus, germination rate was less for this species compared to pearl millet and sorghum-sudangrass. Sorghum-sudangrass demonstrated a tendency to be more tolerant to LEA application than foxtail and pearl millet as demonstrated by greater seedling emergence with sorghum-sudangrass in soil treated with 3.0% LEA. Greater tolerance was expected compared to foxtail millet but not necessarily pearl millet. Nonetheless, these findings agree with studies by Francois et al. (1984) and Dua (1989), who reported grain sorghum

to be significantly more salt tolerant at germination than at later stages of growth, and pearl millet to be more salt sensitive at germination rather than at later stages of growth.

Pearl millet HM was not affected by any treatment compared to the control (+N,+P) at the first and second harvests, but at the third harvest, 3.0% LEA enhanced HM compared to the control (+N,+P) or other treatments (Fig. 3.1b). Pearl millet has been reported to be moderately salt tolerant (Ashraf and McNeilly, 1987); thus, with greater nutrient availability in 3.0% LEA-amended soil, greater HM was produced for this treatment at the third harvest. Sorghum-sudangrass produced less HM when planted in soil treated with 1.5% LEA + 1.5% WS compared to the 1.5% and 3.0% LEA treatments; whereas, HM from 1.5% and 3.0% LEA treatments was not reduced compared to the control (+N,+P) (Fig. 3.1c). Therefore, the reduced HM from soil amended with 1.5% LEA + 1.5% WS was likely the result of net nutrient immobilization, specifically nutrient immobilization due to WS amendment rather than characteristics of LEA.

Plant N concentrations of foxtail millet, pearl millet, and sorghum-sudangrass were consistently greater for the first and second harvests for forages grown in 3.0% LEA-amended soil compared to the control (+N,+P) or 1.5% WS (+N,+P) (Tables 3.2, 3.4, and 3.6) ; therefore, mineralized LEA-N was assimilated by plants more so than added inorganic N. Additionally, SPAD values measured before harvesting sorghum-sudangrass and pearl millet had a strong positive relationship with plant N ($R^2 = 0.53$ and $R^2 = 0.66$, respectively), and thus may be a good measure of N-related plant health. A study by Chapman and Barreto (1997) demonstrated a strong relationship ($r^2 = 0.81$)

between SPAD meter readings and leaf N concentrations of maize (*Zea mays* L.). Plant leaf tissue enzymes are associated with chlorophyll and contain much of the leaf-N; thus, chlorophyll meters can provide a means for estimating leaf N (Chapman and Barreto, 1997).

Plant K concentrations of foxtail millet and pearl millet at the final harvest had a tendency to be less when grown in soil amended with 3.0% LEA compared to 1.5% LEA, 1.5% LEA + 1.5% WS, and 1.5% WS (+N,+P) and even the control (+N,+P) for pearl millet (Tables 3.2 and 3.4). However, sorghum-sudangrass K had a tendency to be less for 3.0% LEA compared to 1.5% LEA + 1.5% WS and 1.5% WS (+N,+P) but greater than 1.5% LEA (Table 3.6). Sodium concentration in any forage was greatest when grown in soil treated with 3.0% LEA (Tables 3.2, 3.4, and 3.6). Less K and greater Na concentrations of plant tissue may be the result of antagonism between Na^+ and K^+ at uptake sites in roots (Rubio et al., 1995; Hafsi et al., 2007).

Soil Dynamics

Soil in which either sorghum-sudangrass or pearl millet was grown had less available N_{inorg} remaining after the final harvest for 1.5% and 3.0% LEA-amended soil than soil in which foxtail millet was grown (Fig. 3.4). This was likely the result of lower foxtail millet HM with these treatments and consequently less N removed from the soil and incorporated into plant tissue compared to sorghum-sudangrass or pearl millet. However, available N_{inorg} after the final harvest of pearl millet was greatest in soil amended with 3.0% LEA and 1.5% LEA + 1.5% WS treatments compared to the control

(+N,+P); whereas, after the final harvest of sorghum-sudangrass, only the 1.5% LEA + 1.5% WS treatment was greater than the control (+N,+P).

Calcium and Na availability following the final forage harvest was significantly greater for the 3.0% LEA amendment (Table 3.9). Excessive Ca and Na may have contributed to increased soil salinity and alkalinity associated with LEA treatments. Both Fe and Mn availability were less with any of the three LEA treatments, which was likely due to increased soil pH with LEA addition. Iron and Mn availability in soil has been documented to be inversely related to changes of soil pH (e.g. as pH increases, Fe and Mn availability decreases) (Soliman et al., 1992).

The 1.5% LEA + 1.5% WS treatment was used in this experiment to compare to the other LEA treatments with the hypothesis that WS may reduce some of the negative aspects associated with LEA, specifically salinity. However, similar EC values with this treatment and 3.0% LEA as well as similar Na availability compared to 1.5% LEA indicated that WS did not aid in mitigating salinity generated with LEA amendment. Compared to soil in which foxtail millet was planted, EC values were significantly less for soil in which pearl millet and sorghum-sudangrass were planted; therefore, it is reasonable to assume pearl millet and sorghum-sudangrass were more effective at remediating LEA-associated salinity.

CHAPTER IV

NUTRIENT AVAILABILITY, GREENHOUSE GAS FLUXES, AND SALT TOLERANT RYEGRASS PRODUCTIVITY AS AFFECTED BY LIPID-EXTRACTED ALGAE SOIL APPLICATION

INTRODUCTION

Organic amendments commonly used in agriculture, such as animal manures, biosolids, municipal solid wastes, yard waste composts, crop residues, seaweeds, blood and bone meal, and humic substances (Thangarajan et al., 2013), have many advantages including improving soil quality and fertility, enhancing soil water-holding capacity and microbial biomass and activity, sequestering C, and potentially increasing plant yields. Organic soil amendments have been suggested as an option for supplying nutrients to support agricultural production, while increasing SOC levels (Quilty and Cattle, 2011). Even so, the use of organic amendments in agriculture may have potential environmental disadvantages involving GHG emissions and eutrophication from excess nutrients (Thangarajan et al., 2013).

Approximately 40-50% of the Earth's surface is used for agricultural purposes, which accounts for 10-12% of total GHG emissions (Smith et al., 2007). Despite agriculture being a source of emissions, it also has a technical mitigation potential of 5.5-6.0 Gt CO_2-eq yr^{-1}, with approximately 89% of the mitigation potential due to C sequestration in soil. Of the global anthropogenic GHG emissions, agriculture accounts for about 58% of N_2O emissions. In seven of the ten world regions in 2005, soil N_2O,

mostly associated with N fertilizers and soil applied manure, was the main source of GHG emissions in the agricultural sector (Smith et al., 2007).

Gaseous N losses from soil receiving organic amendments may include NH_3 volatilization and nitric oxide (NO), N_2O, and dinitrogen (N_2) emissions. Nitrous oxide is a greenhouse gas that can be lost from soil by nitrification of NH_4^+ to NO_3^- or denitrification of NO_3^-. In well-drained soil, NO_3^- can be leached and lost from the soil profile, and possibly reach groundwater. The nitrification inhibition effect of LEA applied at 3.0% previously observed (Chapter II) may potentially reduce N losses and increase N use efficiency when applied to organically produced crops and forages.

Carbon dioxide from soil can account for 60-90% of total ecosystem respiration (Longdoz et al., 2008), and thus, is an important C flux to measure in ecosystems, especially for agricultural production systems using an organic amendment that has not been evaluated for its effects on GHG fluxes. Few if any studies have investigated the viability of using LEA residue as a soil amendment and nutrient source and no studies to date have quantified the cumulative loss of CO_2-C, N_2O-N, and NH_3-N from soil amended with LEA. The rate of soil application of LEA required to enhance nutrient availability without simultaneously causing salt toxicity is not known.

Two experiments were conducted, the first with LEA-amended soil left fallow (Exp. I) and the second with LEA-amended soil seeded with a salt-tolerant genotype of ryegrass [*Lolium multiflorum* (Lam.) cv. TXR2011-S] (Exp. II) and comparing LEA to treatments utilizing WS and a positive control (N and P fertilizer) and negative control (no amendment). The objectives of Exp. I were to quantify: a) nutrient availability, b)

cumulative GHG emissions, and c) abundance of bacteria and fungi in LEA-amended soil. Experiment II was aimed at determining the effect of LEA on: a) forage emergence and seedling survival, b) ryegrass HM, c) soil nutrient availability, and d) plant nutrient uptake.

METHODS

The number of experimental units was 24 for each experiment and both experiments utilized a RCBD with four replications. Growth columns (33 cm length) were constructed for each experiment using 10-cm I.D. PVC pipe. The bottoms of the columns were capped to prevent excessive loss of water and nutrients, but drainage holes were drilled in caps to prevent the soil from becoming anaerobic. Weights of both empty and soil-filled columns were measured.

Unamended Parrita soil was added to the bottom half (15 to 30 cm) of all columns and DI H_2O was added to achieve a gravimetric water content of approximately 0.24 g g^{-1}. For each experiment, the remaining upper half of the column was filled with dry soil amended with one of the following on a dry weight basis: 1) positive control (N and P fertilizer), 2) 1.5% LEA, 3) 3.0% LEA, 4) 0.75% LEA + 0.75% WS, 5) 1.5% WS plus N and P fertilizer, and 6) negative control (no added fertilizer). Inorganic N and P (280 kg N ha^{-1} and 112 kg P ha^{-1}) as NH_4NO_3 and $Ca(H_2PO_4)_2 \cdot H_2O$, respectively, were added and incorporated by mixing thoroughly in dry soil in order to prevent N and P limitations and consequently immobilization in treatments 1 and 5. Total C and N and P,

K, Ca, Mg, Na, Zn, Fe, Cu, Mn, and S concentrations of LEA and WS were conducted as previously described.

Experiment I

Fallow columns were used to quantify gas flux rates, determine microbial population changes and quantify N mineralization and nutrient availability and cycling. Immediately after treatment initiation, a mobile Gasmet™ DX4030 FTIR spectrometer equipped with a 10-cm diameter Li-COR survey gas chamber (Li-8100-102) was used to measure CO_2, N_2O, CH_4, and NH_3 gas fluxes from the soil surface. Carbon dioxide fluxes were used as an indicator of microbial respiration and OM mineralization rates. Measurements were made at the same time (midafternoon) from 3 d to 85 d after treatment application approximately every 3 d from 3d to 12 d and 46 d to 56 d, and weekly from 12 d to 24 d; the final measurement was made 85 d after treatment application.

Three soil samples were collected from each fallow column to a depth of approximately 15 cm at 0, 1, 3, 7, 14, 21, 35, 56, and 85 d after treatment initiation using a soil probe with an inner diameter of 1.3 cm. One of the three samples taken from 1 d to 56 d was stored at -80º C until DNA extraction and purification. DNA extractions were performed using a lysozyme-modified version of the manufacturer's protocol (Hollister et al., 2010) of a PowerMax soil DNA extraction kit (Mo Bio Laboratories, Inc., Carlsbad, CA, USA). Following elution, DNA samples were concentrated by an ethanol precipitation and quantified using a NanoDrop ND-1000 spectrophotometer (NanoDrop

Technologies, Wilmington, DE, USA) and a Quant-iT Picogreen dsDNA assay kit (Invitrogen Corp, Carlsbad, CA, USA). Based upon methods and results of Fierer et al. (2005), Boyle et al. (2008), and Hollister et al. (2010), community qPCR assays were used to evaluate relative abundances of bacteria and fungi in LEA treated soil. Assays were performed in triplicate using an Eppendorf Mastercycler® ep Realplex thermal cycler (Eppendorf, Hamburg, Germany). Bacterial primer sets were Eub338 and Eub518 (Fierer et al., 2005) and fungal primer sets included 5.8S and ITS1F (Boyle et al., 2008).

The two remaining soil samples removed at each sampling were weighed and then dried at 65º C to constant weight. Samples were then ground with a flail grinder to pass a 2-mm sieve and analyzed for extractable NH_4^+, NO_3^-, P, K, Ca, Mg, S, and micronutrients by the same methods described previously. Organic C and soil total N were analyzed after further grinding (< 150 µm) in a ring and puck mill. Soil pH and EC was measured using a 1:2 soil to DI H_2O ratio.

Experiment II

Seven days after treatment application, the set of columns not designated for gas flux quantification were planted with salt-tolerant ryegrass (20 seed column^{-1}) to a 2 mm depth by broadcasting onto a wetted soil surface and then covering with dry soil. Constant column weights were maintained to ensure soil water content (0.24 g g^{-1}) was near field capacity for the remainder of the study. Emergence was monitored on a daily basis for 14 d, and plants were allowed to grow for 42 d. At 42 days after planting (DAP), plants were cut to 9-cm stubble height, with additional harvests repeated at 77

and 98 DAP. Days elapsed between the second and third cutting were less than between the first and second because ryegrass reached reproductive stage. Harvested plant material was weighed immediately following harvest and after drying to constant weight at 65º C, ground to pass through a 0.5-mm sieve in a cyclone mill (Udy Cyclone Sample Mill 3010-030; Fort Collins, CO, USA), and analyzed for N, P, K, Ca, Mg, Na, Zn, Fe, Cu, Mn, and S by the same methods previously reported. Nutrient uptake for each harvest was calculated by multiplying the nutrient concentration of the HM by the amount of HM harvested per column.

Since treatments were only applied to the top 15 cm of the column, all columns, including those left fallow were sampled from 0-15 cm after the final ryegrass cutting, dried and processed for nutrient analysis. Soil total N and extractable NH_4^+, NO_3^-, P, K, Ca, Mg, S, Na, Fe, Zn, Cu, and Mn and pH and EC measurements were conducted as previously described.

Statistical Analyses

Nonlinear regression was used to depict the relationship between cumulative CO_2-C and time. Lipid-extracted algae-C and WS-C mineralized to CO_2-C were calculated as a percentage of the added amendment-C. Lipid-extracted algae-N and WS-N mineralized to inorganic N (N_{inorg}) was calculated as a percentage of added amendment-N; $N_{inorg} = NH_4^+$-N + NO_3^--N.

Statistical analyses were conducted using SAS version 9.3. Effects were analyzed using a linear mixed analysis of variance (ANOVA) procedure at a significance level of $P < 0.05$. Means of significant effects were separated using Fisher's protected LSD.

RESULTS

Experiment I

Carbon Dioxide Losses and Soil Organic Carbon

Cumulative CO_2-C lost from the positive control (38.0 g m^{-2}) was not significantly different from the negative control (49.8 g m^{-2}) (Fig. 4.1). Carbon dioxide-C evolution in organically amended soil was greater than either control at each d following treatment initiation. Lipid-extracted algae applied at a 3.0% rate resulted in greater CO_2-C loss than any other treatment and LEA applied at a 1.5% rate resulted in greater CO_2-C emissions than either control. The 0.75% LEA + 0.75% WS treatment resulted in less CO_2-C than 1.5% WS (+N,+P) but more CO_2-C than 1.5% LEA.

At 85 d, mineralized C from 1.5% WS (+N,+P) and 0.75% LEA + 0.75% WS treatments was 15.3% and 13.4% of total C added, respectively (Fig. 4.2). Lipid-extracted algae-derived C mineralized from the 1.5% and 3.0% LEA treatments was significantly less than the other treatments with 9.2% and 9.9% of total C lost as CO_2, respectively. No difference existed between the percentages of LEA-C mineralized from 1.5% and 3.0% LEA treatments at 85 d post treatment application.

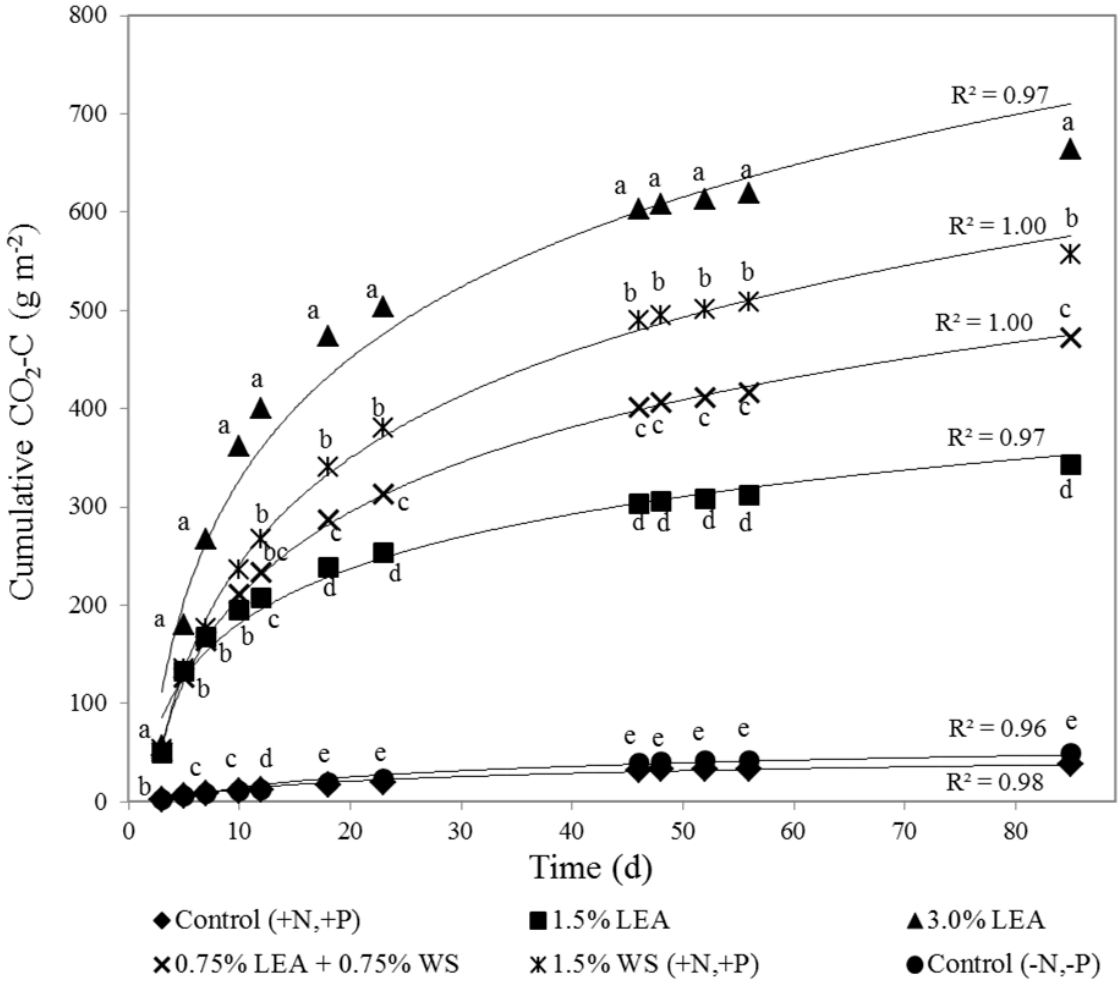

Fig. 4.1. Cumulative CO_2-C emission over 85 d. Mean values within day followed by the same letter are not different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

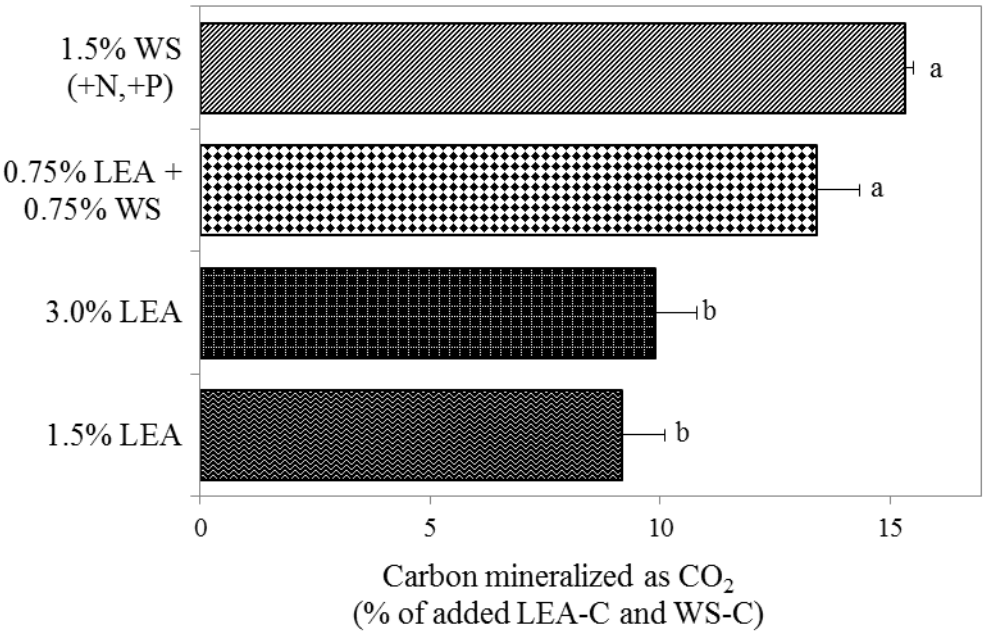

Fig. 4.2. The percentage of LEA-C and WS-C mineralized in fallow soil 85 d after treatment application. Mean percentages are not different at $P < 0.05$ by Fisher's protected LSD when followed by the same letter. Lipid-extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

Soil organic C 85 d after treatment application was greatest with the 3.0% LEA treatment (1.4 %) compared to all other treatments (Fig. 4.3). Furthermore, the 3.0% LEA treatment increased SOC by 0.4% and 0.5% C compared to the positive and negative controls, respectively. The 1.5% WS (+N,+P) treatment resulted in significantly greater SOC than the 1.5% LEA and 0.75% LEA + 0.75% WS treatments and controls, while the 0.75% LEA + 0.75% WS treatment also resulted in greater SOC than the 1.5% LEA treatment or the controls. There was no difference in SOC between the 1.5% LEA treatment and the controls.

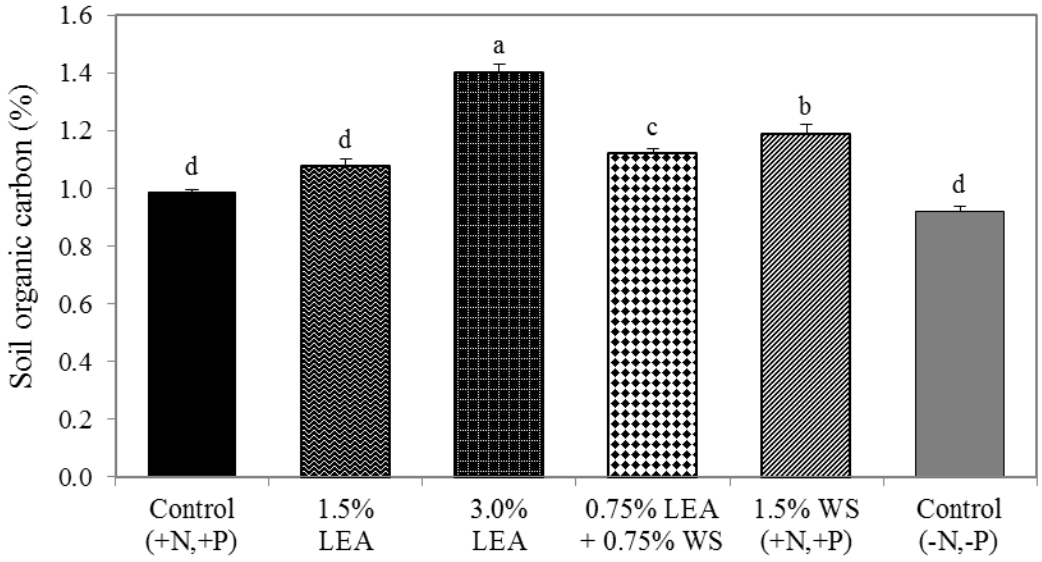

Fig. 4.3. Soil organic C in fallow soil measured 85 d after treatment application. Mean percentages followed by the same letter are not different at $P < 0.05$ by Fisher's protected LSD. Lipid-extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

Nitrogen Transformations, Ammonia Volatilization, and Nitrous Oxide Flux

Ammonium-N at 7 d was greatest for the 3.0% LEA treatment (171.5 mg kg^{-1} dry soil) followed by the 1.5% LEA treatment (100.6 mg kg^{-1} dry soil) with both significantly greater than the controls or other treatments (Fig. 4.4a). At 14 d, NH_4^+-N decreased for both 1.5% and 3.0% LEA treatments, but the 3.0% LEA treatment remained significantly greater than the other treatments or controls. Soil NH_4^+-N increased for 1.5% WS (+N,+P) at this time compared to 7 d. At 35 d, no differences in NH_4^+-N were observed between treatments ($P = 0.78$), but at 56 d, the 0.75% LEA + 0.75% WS resulted in greater soil NH_4^+-N. At 85 d, NH_4^+-N for the positive control was significantly greater than all other treatments, and 1.5% WS (+N,+P) was greater than all treatments, except the positive control.

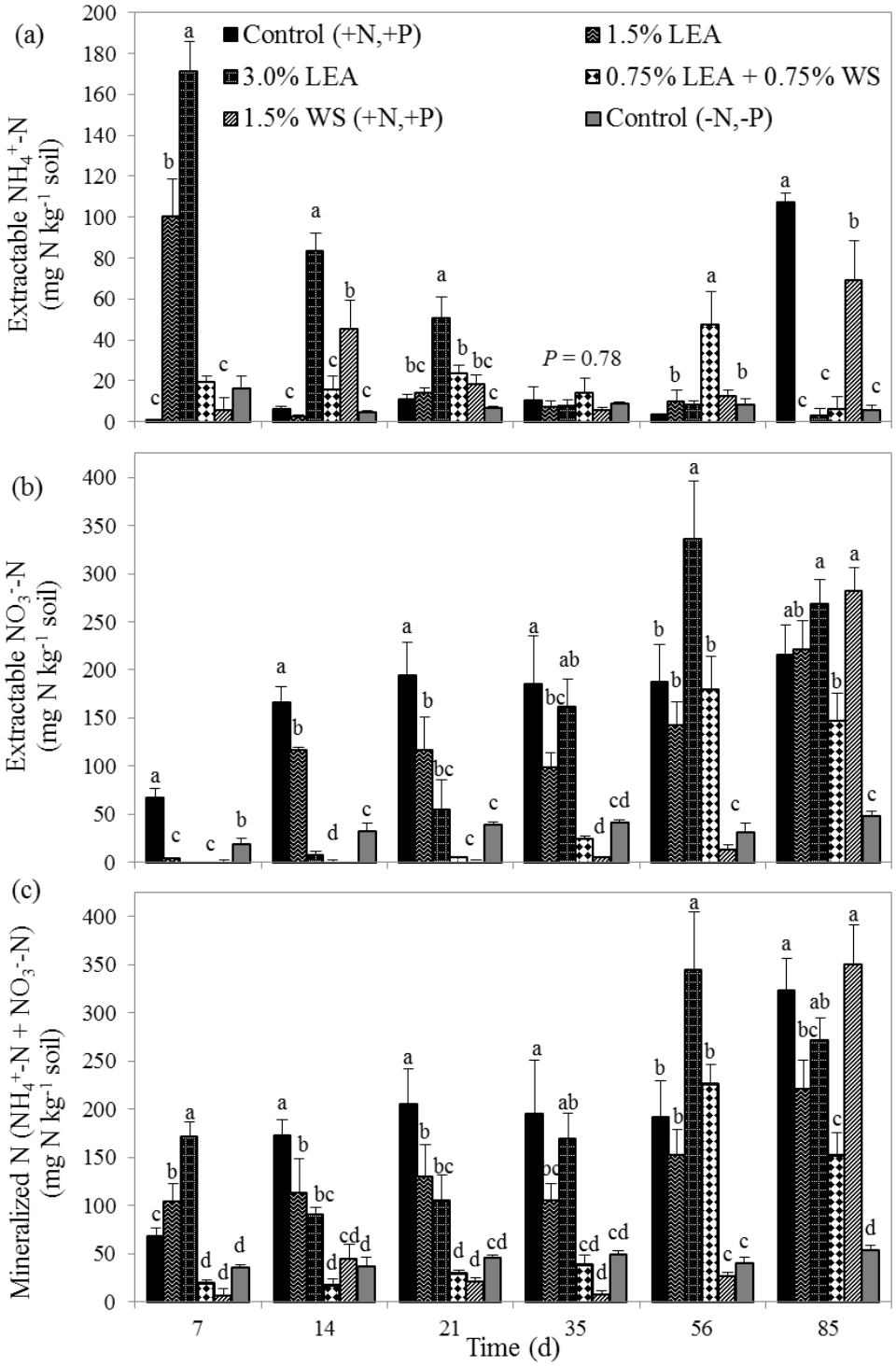

Fig. 4.4. Soil extractable N as (a) extractable NH_4^+-N, (b) extractable NO_3^--N, and (c) mineralized N. Mean values followed by the same letter within N form and measurement day are not different at $P < 0.05$ by Fisher's protected LSD. Lipid-extracted algae and wheat straw amendments are denoted as LEA and WS, respectively.

As NH_4^+-N in soil amended with 1.5% LEA decreased from 7 to 14 d, there was a subsequent increase in extractable NO_3^--N (Fig. 4.4b). Fourteen-d after treatment application, NO_3^--N was significantly greater for 1.5% LEA (116.6 mg N kg^{-1}) than any other treatment except the positive control (166.7 mg N kg^{-1}). Eventhough there was an accumulation of NH_4^+-N for the 3.0% LEA treatment at this time, nitrification was delayed with this treatment compared to the 1.5% LEA treatment until at least 21 d after treatment application. Extractable NO_3^--N increased for the 3.0% LEA treatment (54.9 mg N kg^{-1} at 21 d to 336.3 mg N kg^{-1} at 56 d) at which point it was significantly greater than the other treatment. No difference in NO_3^--N measured at 85 d was determined between the positive control, the 1.5% and 3.0% LEA treatments, or the 1.5% WS (+N,+P) treatment.

The percentage of added N from LEA and WS residue mineralized was different among organic treatments within each measurement day, except 85 d (Fig. 4.5). The 1.5% WS (+N,+P) treatment was not included in analysis because of the addition of inorganic N and P. The percentage of LEA-N mineralized for the 1.5% and 3.0% LEA treatments was not different, except at 14 d. The percentage of N mineralized in the 0.75% LEA + 0.75% WS treatment was negative, possibly as a result of immobilization, until 56 d, at which point this treatment had a significantly greater percentage of added N mineralized than other treatments. By 85 d, however, all treatments exhibited similar percentages of N mineralized.

Mineralized N reported as extractable NH_4^+-N plus NO_3^--N was greatest at 7 d for the 3.0% LEA treatment followed by the 1.5% LEA treatment and the positive

control (Fig. 4.4c). The greater portion of mineralized N at 7 d for both 1.5% and 3.0% LEA treatments was in the form of NH_4^+. However, at 14 d, mineralized N for the 1.5% LEA treatment was comprised of much more NO_3^--N than NH_4^+-N. This was not the case for the 3.0% LEA treatment until 21 to 35 d after treatment application.

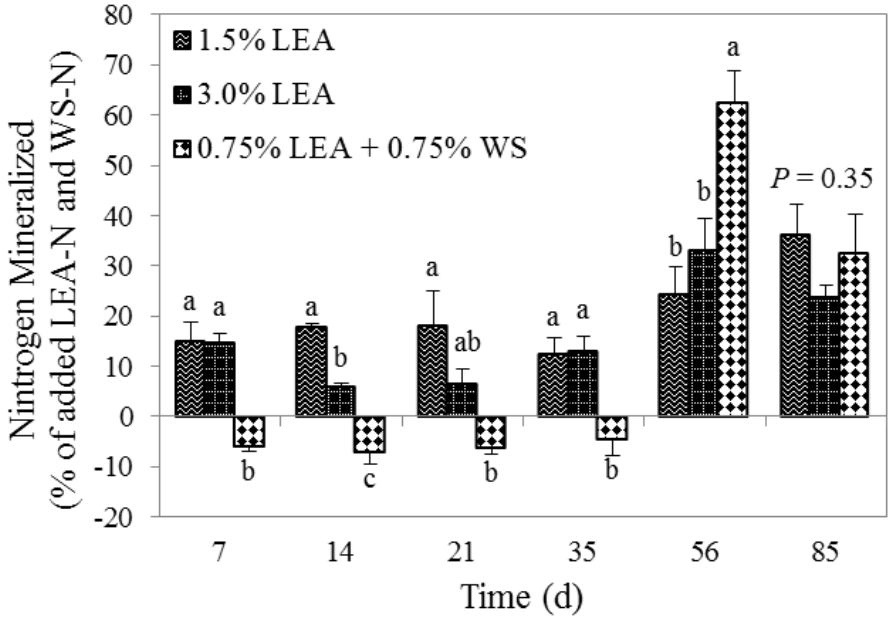

Fig. 4.5. Nitrogen mineralized (NH_4^+-N plus NO_3^--N) in fallow soil over 85 d as a percentage of added N as lipid-extracted algae (LEA) and wheat straw (WS). Mean values followed by the same letter within measurement day are not different at $P < 0.05$ by Fisher's protected LSD.

Prior to 7 d after treatment application, no significant differences were observed for cumulative NH_3-N loss between amendment treatments and controls, but after 7 d, 3% LEA-treated soil resulted in greater quantities of NH_3-N generated than other treatments (Fig. 4.6). The flux of NH_3 from the 3.0% LEA treatment increased nearly ten fold from 7 to 18 d, but then plateaued by 23 d. Ammonia emissions were detected from 1.5% and 3.0% LEA-treated soil possibly as a result of high soil extractable NH_4^+ (Fig. 4.4a) with these treatments and the alkalinity of LEA (pH = 9.9) (Table 2.1). All other treatments showed no or very minmal NH_3 loss.

From treatment application to 12 d, N_2O-N lost from 0.75% LEA + 0.75% WS-amended soil was greater than any other treatment or the controls (Fig. 4.7). Cumulative N_2O loss with 1.5% WS (+N,+P) was greater than other treatments, except 0.75% LEA + 0.75% WS, until 7 d. The flux rate of N_2O for 1.5% and 3.0% LEA treatments increased dramatically starting at 7 d and decreased at 21 d, but to a greater degree with 1.5% LEA than the 3.0% LEA treatment. Cumulative N_2O-N lost from 3.0% LEA-amended soil did not increase from 45 to 85 d; nonetheless, cumulative N_2O-N lost at 85 d was greatest for this treatment compared to any other treatment or the controls.

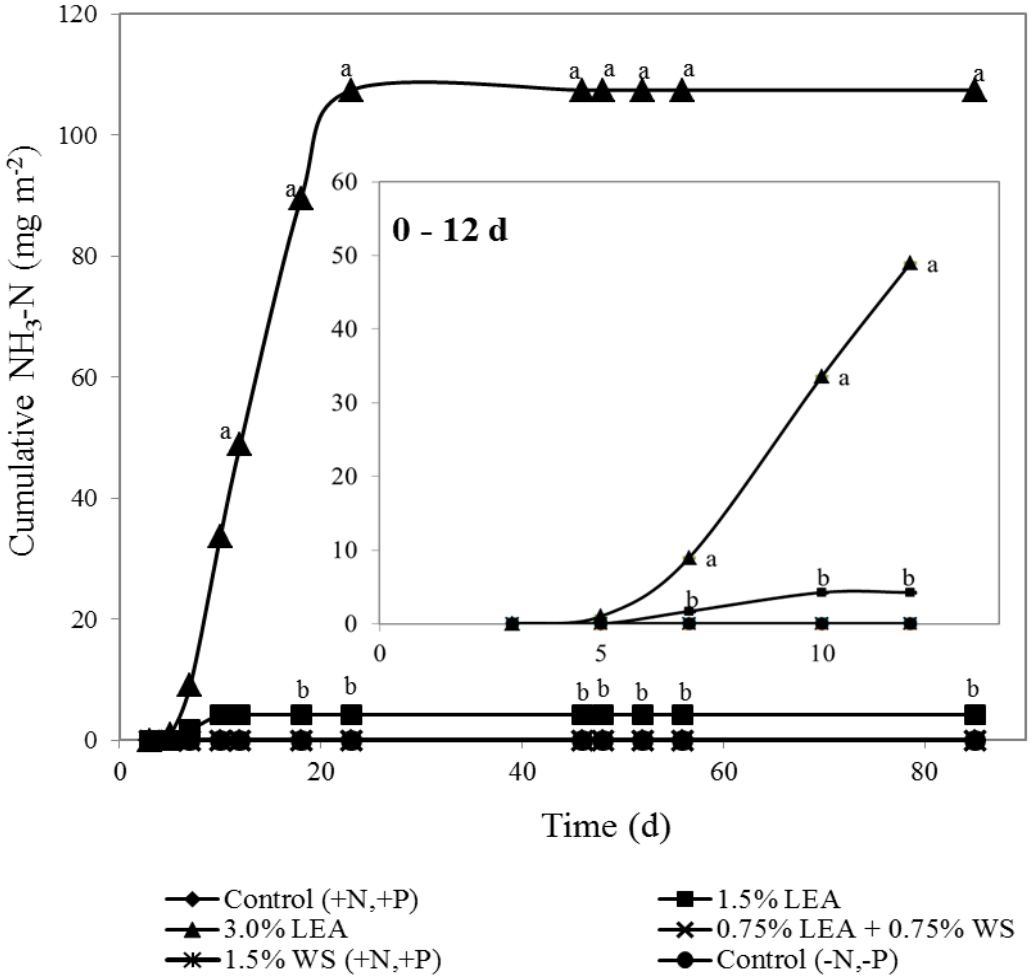

Fig. 4.6. Cumulative NH_3-N volatilized over 85-d in fallow soil. Insert is a magnified view of 0 to 12 d after treatment application. Mean values followed by the same letter within measurement day are not different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

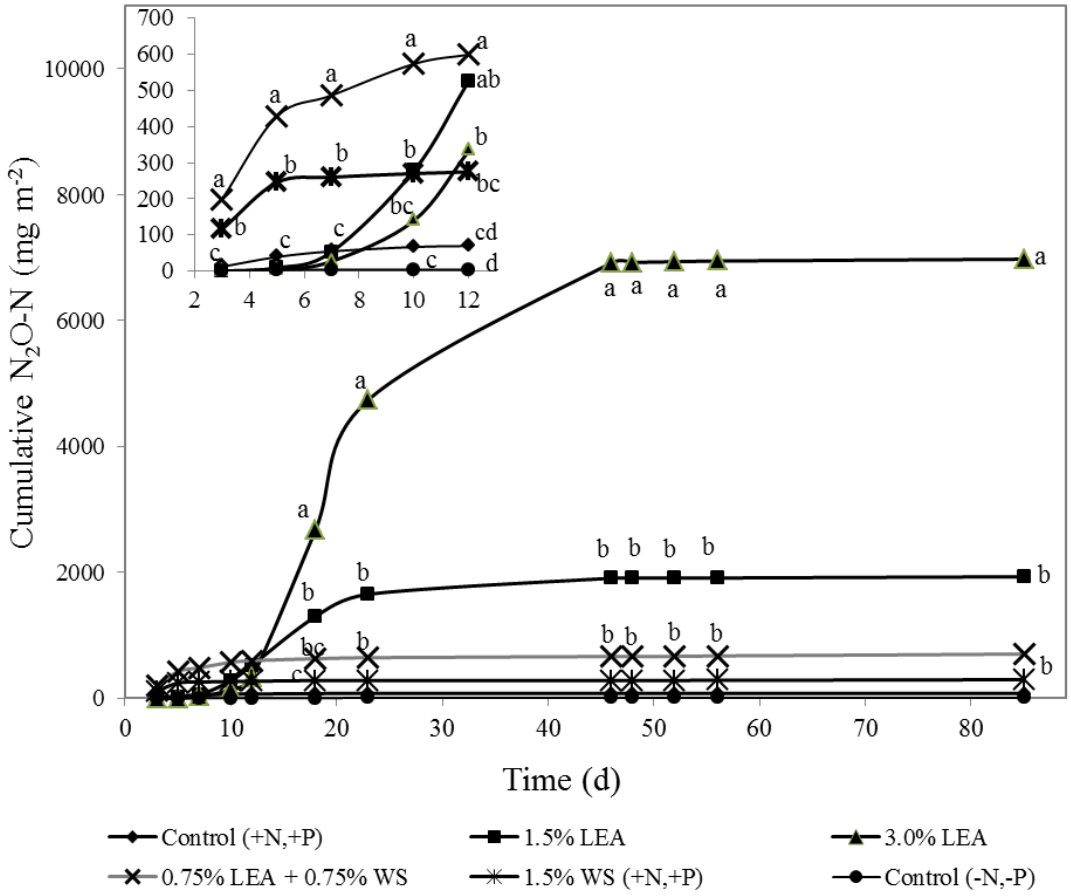

Fig. 4.7. Cumulative N_2O-N lost over 85-d from fallow soil. Insert is a magnified view of 0 to 12 d after treatment application. Mean values followed by the same letter within measurement day are not different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

Nutrient Availability and Soil pH and EC

Treatment differences were observed for plant available NO_3^--N, macronutrients, and micronutrients, except for Fe, 85 d after treatment application in fallow soil (Table 4.1). No differences were observed for extractable NO_3^--N among the positive control, 1.5% and 3.0% LEA, and 1.5% WS (+N,+P) treatments. The 0.75% LEA + 0.75% WS treatment resulted in significantly less NO_3^--N compared to the 3.0% LEA or 1.5% WS (+N,+P) treatments, but not the 1.5% LEA treatment or positive control. Nitrate-N for the negative control was significantly less than all other treatments.

Extractable P was significantly greater for the positive control and 1.5% WS (+N,+P) (both added 112 kg P ha-15 cm^{-1} to the soil). Phosphorus was not different between the 1.5% and 3.0% LEA treatments (78 and 116 mg P kg^{-1} dry soil, respectively). Less than other soil treatments, 0.75% LEA + 0.75% WS and the negative control were not different from each other in extractable P.

Extractable K was greatest for 1.5% WS (+N,+P) (435 mg K kg^{-1} dry soil) followed by 3.0% LEA, 0.75% LEA + 0.75% WS, and the 1.5% LEA (334, 318, and 294 mg K kg^{-1} soil, respectively). Extractable K was similar for the negative control and 1.5% LEA treatments. Plant available Ca was greater for 1.5% and 3.0% LEA compared to all other treatments, but was approximately 500 mg kg^{-1} greater for 3.0% than 1.5% LEA.

Table 4.1. Nutrient availability, pH, and electrical conductivity (EC) in fallow soil 85 d after treatment application.

Soil Treatment	pH	EC	NO_3^--N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	------	$dS\ m^{-1}$	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------	----------
							$mg\ kg^{-1}$						
Control (+N,+P)	6.6 c[†]	1.3 c	216 ab	162 a	259 e	3303 b	343 c	13 c	93 d	5.8	0.32 c	74.1 a	0.50 b
Control (-N,-P)	7.2 ab	0.3 d	48 c	38 d	276 de	3307 b	329 cd	13 c	106 d	4.0	0.31 c	59.3 b	0.41 c
0.75% LEA[‡]+0.75% WS[§]	7.3 a	1.6 c	147 b	48 d	318 bc	3069 b	291 d	34 c	532 c	4.1	0.64 ab	43.9 c	0.53 b
1.5% LEA	7.5 a	2.8 b	222 ab	78 c	294 cd	4167 a	436 b	78 b	1371 b	3.7	0.43 bc	30.2 d	0.58 b
3.0% LEA	7.4 a	3.4 a	269 a	116 bc	334 b	4628 a	502 a	126 a	2180 a	5.2	0.74 a	32.5 d	0.72 a
1.5% WS (+N,+P)	6.9 bc	1.4 c	282 a	158 a	435 a	3236 b	320 cd	13 c	119 d	5.9	0.48 bc	76.9 a	0.52 b
p-value	0.0002	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.054	0.0052	<0.0001	<0.0001

[†]Means followed by the same letter are not significantly different at $P < 0.05$.
[‡]LEA denotes lipid-extracted algae.
[§]WS denotes wheat straw.

Extractable Mg, S, Na, and Cu were all significantly greater for the 3.0% LEA treatment compared to any other treatment. Sodium was approximately 1.5, 4, 18, 21, and 23 times greater for 3.0% LEA compared to 1.5% LEA, 0.75% LEA + 0.75% WS, and 1.5% WS (+N,+P) or the negative and positive controls, respectively. The availability of Mn was significantly less in LEA-amended soil compared with the 1.5% WS (+N,+P) treatment and both controls possibly as a result of increased soil pH with LEA amendments (Table 4.1).

Lipid-extracted algae increased soil pH compared to the 1.5% WS (+N,+P) treatment and positive control, but not the negative control. Soil pH 85 d after treatment application for LEA treatments ranged from 7.3 to 7.5, while soil pH for the positive control was 6.6. Electrical conductivity in soil treated with 3.0% LEA residue was significantly greater than all other treatments. The 1.5% LEA treatment resulted in greater EC (2.8 dS m^{-1}) than the positive or negative control (1.3 and 0.3 dS m^{-1}, respectively) and other treatments, except for 3.0% LEA (3.4 dS m^{-1}). However, no differences existed between the positive control and the 0.75% LEA + 0.75% WS or 1.5% WS (+N,+P) treatments (1.6 and 1.4 dS m^{-1}, respectively) 85 d after treatment application.

Soil Bacterial and Fungal Quantitative PCR

Bacterial and fungal copy numbers (g^{-1} soil) were significantly different at each measurement date except at 35 d, and additionally for fungal copy numbers at 56 d (Fig. 4.8). Soil amended with 3.0% LEA had the greatest bacterial copy numbers ($3x10^{10}$ g^{-1} soil) compared to other treatments at 3 and 7 d after treatment application, but decreased significantly by 14 d. At 7 d, 1.5% LEA was not different from the positive and negative controls or 1.5% WS (+N,+P) and 0.75% LEA + 0.75% WS treatments. Between 14 and 21 d, bacterial copies increased for 1.5% LEA and 1.5% WS (+N,+P) treatments but decreased for 0.75% LEA + 0.75% WS. At 56 d, the 0.75% LEA + 0.75% WS treatment exhibited less than $1x10^{10}$ bacterial copies, but was significantly greater than other treatments, except 1.5% WS (+N,+P).

Fungal copies at 3 d were significantly greater for 3.0% LEA than other treatments, except 1.5% LEA (Fig. 4.8b). By 7 d, fungal copies for 3.0% LEA were greater than those for other treatments, continued to increase until 14 d and then declined significantly. Fungal copy numbers also generally increased from 3 to 14 d for treatments receiving WS. No differences in fungal copies among treatments occurred at 14 and 21 d

Lipid-extracted algae applied at 3.0% increased fungal copies from 7 to 14 d, whereas bacterial copies decreased during this interval (Fig. 4.8). Moreover, a bacterial:fungal ratios less than 1.0 at 14 and 21 d after treatment application were observed with the 3.0% LEA, 0.75% LEA + 0.75% WS, and 1.5% WS (+N,+P) treatments (Table 4.2). A similar effect was also seen for the negative control at 14 d.

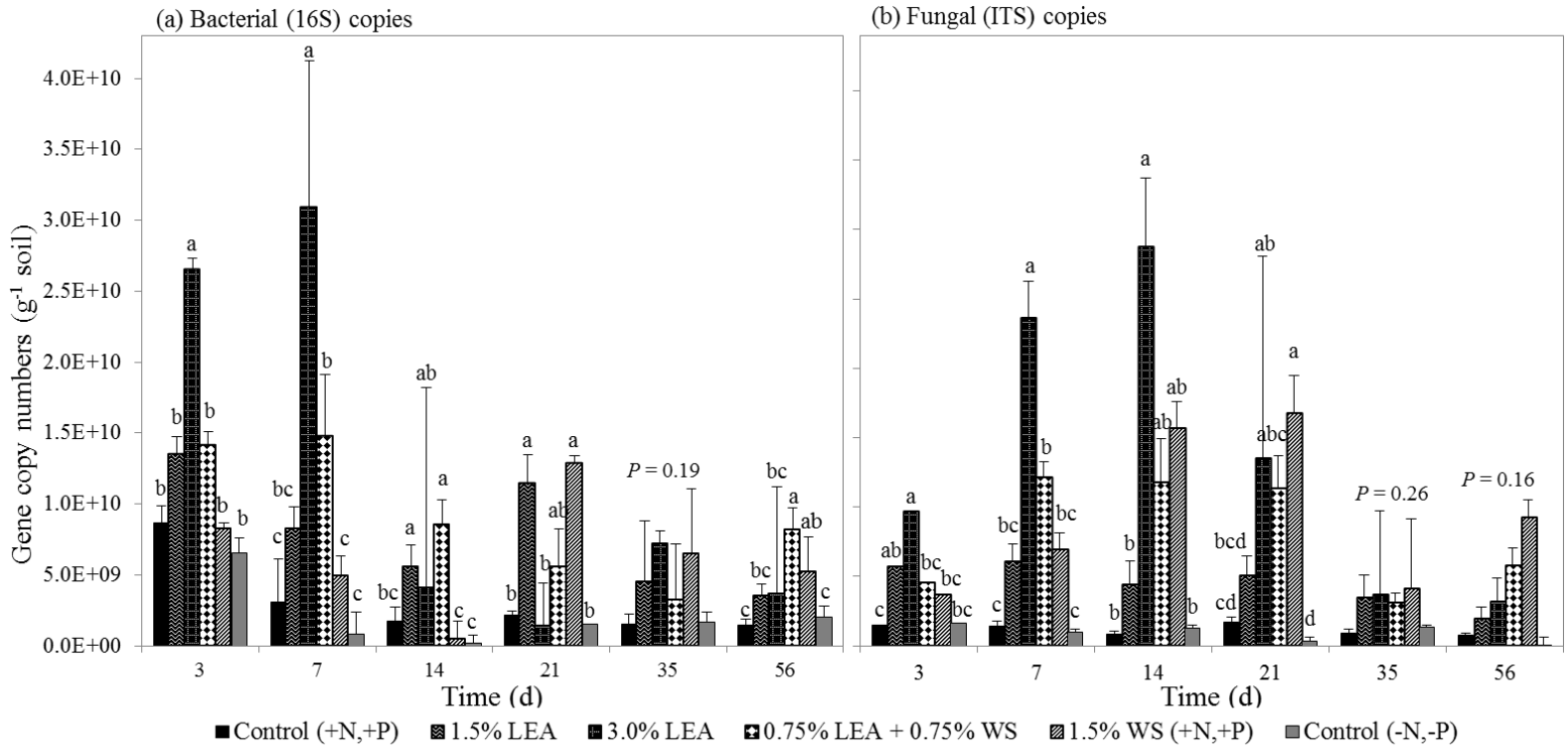

Fig. 4.8. (a) Bacterial and (b) fungal gene copies g^{-1} soil (dry weight basis) quantified over 56-d period in fallow soil after treatment application. Mean values followed by the same letter within measurement day are not different at $P < 0.05$ by Fisher's protected LSD. Bars above columns represent standard error of the mean. LEA and WS denote lipid extracted algae and wheat straw, respectively.

No treatment differences were observed for bacterial:fungal ratios at 3, 7, and 35 d after treatment application, but 14 d post application, the ratio was significantly greater for the positive control compared to all treatments, except 1.5% LEA (Table 4.2). At 21 and 56 d, the negative control had a bacterial:fungal ratio greater than any other treatment. Of the LEA-amendment treatments, 1.5% LEA was the only one to have a bacterial:fungal ratio greater than 1.0 at each measurement day.

Table 4.2. Bacterial to fungal copy ratio over a 56-d period in fallow soil after treatment application. Mean values within measurement day followed by the same letter are not different at $P < 0.05$ by Fisher's protected LSD.

	Bacterial to fungal ratio over time (d)					
	3	7	14	21	35	56
Controls						
Positive (+N,+P)	5.4	2.2	1.8a[†]	1.2bc	1.6	1.7b
Negative (-N,-P)	4.0	1.2	0.2cd	4.8a	1.7	24a
Treatments						
1.5% LEA[‡]	2.7	1.5	1.3ab	2.2b	1.4	1.6b
3.0% LEA	4.1	1.3	0.3cd	0.1c	1.9	1.3b
0.75% LEA + 0.75% WS[§]	3.4	1.6	0.8bc	0.5c	1.4	1.6b
1.5% WS (+N,+P)	2.9	0.7	0.1d	0.7c	1.5	3.3b
p-value	0.39	0.19	0.0001	<0.0001	0.89	<0.0001

[†]Within columns, mean values followed by the same letter are not different.
[‡]LEA denotes lipid-extracted algae.
[§]WS denotes wheat straw.

Experiment II

Ryegrass Seedling Emergence

Although seedling emergence of salt tolerant ryegrass measured 12 d after planting was significantly greater in soil treated with 1.5% LEA rather than 3.0% LEA, emergence was inhibited by both 1.5 and 3.0% LEA compared to the other treatments (Fig. 4.9). No statistical differences were determined for seedling emergence between the other treatments.

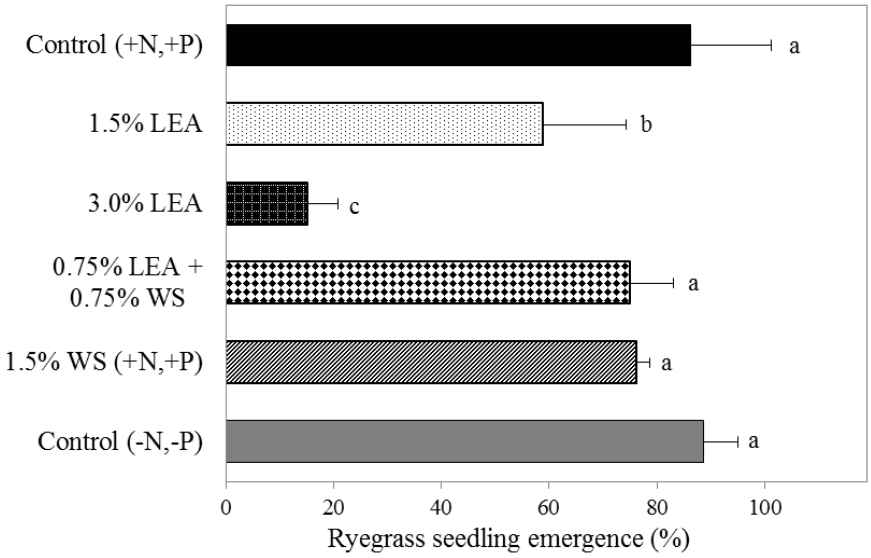

Fig. 4.9. Percent ryegrass seedling emergence measured for each treatment 12 DAP. Means followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. Lipid extracted algae and wheat straw are denoted by LEA and WS, respectively.

Ryegrass Herbage Mass and Nutrient Uptake

Even though seedling emergence was less for the 1.5 and 3.0% LEA treatments, no significant differences were observed for HM between the 1.5 and 3.0% LEA, 1.5%

WS (+N,+P), or the negative control at the first harvest (Fig. 4.10). Herbage mass at the first harvest for the positive control was not statistically different from the 0.75% LEA + 0.75% WS treatment, but at the second and third harvest, the positive control produced greater yield than any other treatment. At the second and third harvests, HM was greater for 1.5% LEA compared to the negative control. At the third harvest, no differences existed between 1.5% LEA, 0.75% LEA + 0.75% WS, or 1.5% WS (+N,+P). The 3.0% LEA treatment was replanted after the first harvest; however, it produced no HM for the second or third harvests. Total HM followed the order positive control > 0.75% LEA + 0.75% WS, 1.5% WS (+N,+P), 1.5% LEA > negative control > 3.0% LEA.

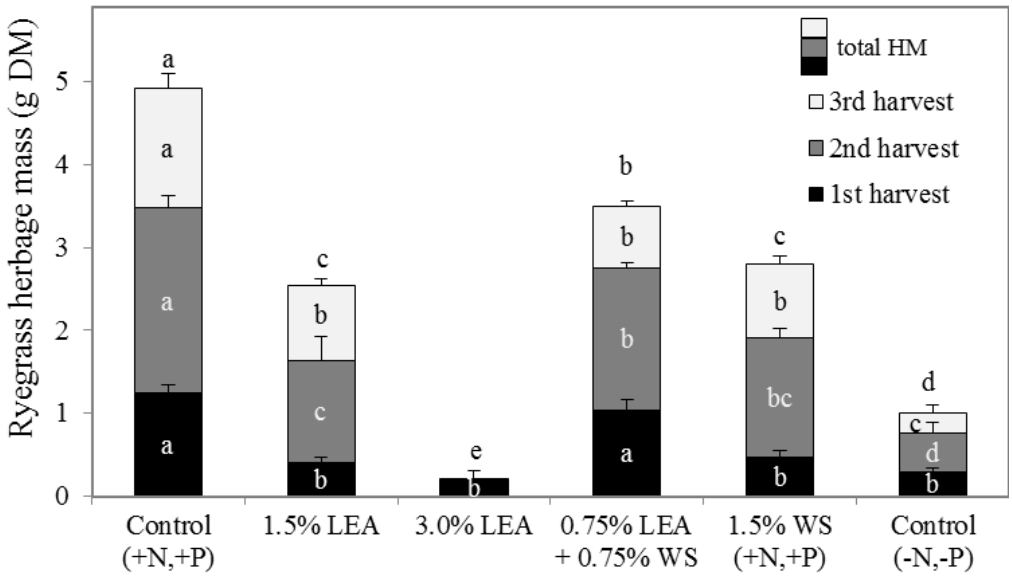

Fig. 4.10. Ryegrass herbage mass (HM) from three harvests. Total yield was calculated as the sum of each harvest and represented by the height of treatment columns. Means within harvest or total harvest followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

Nutrient uptake was affected by treatment for each nutrient for all harvests, except for Mg, Zn and S in the third harvest (Table 4.3). Ryegrass nutrient uptake followed the same trend as HM. The greatest uptake generally occurred with the positive control, while the least was observed with the 3.0% LEA treatment.

Total N uptake in the first harvest was greatest for the positive control followed by the 0.75% LEA + 0.75% WS treatment, which was significantly greater than any other treatment or negative control (Table 4.3). At the third harvest, TN uptake was not different among 0.75% LEA + 0.75% WS, 1.5% LEA, or 1.5% WS (+N,+P) treatments; however, these treatments demonstrated greater N uptake compared to the negative control. Greater P uptake occurred with addition of inorganic P (positive control and 1.5% WS (+N,+P)); although, it was not until the final harvest that these two treatments were similar and greater than other treatments. Potassium uptake was generally greatest for each harvest for the positive control and 0.75% LEA + 0.75% WS and least for 3.0% LEA.

Table 4.3. Nutrient uptake by ryegrass at the first, second, and third harvests.

Treatment	TN	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn	S
	mg column^{-1}										
	1st Harvest										
Control (+N,+P)	66 a[†]	5 a	64 a	15 a	5 a	3 c	0.04 a	0.16 ab	0.10 a	0.12 a	3.66 a
Control (-N,-P)	14 c	1 c	13 b	4 c	1 c	1 d	0.01 c	0.03 c	0.01 b	0.01 d	0.91 b
0.75% LEA[‡]+0.75% WS[§]	50 b	3 b	47 a	10 b	4 b	6 a	0.03 ab	0.12 bc	0.03 b	0.08 b	3.11 a
1.5% LEA	20 c	2 bc	17 b	4 c	1 c	4 bc	0.01 c	0.04 c	0.01 b	0.05 c	1.34 b
3.0% LEA	16 c	2 bc	12 b	3 c	1 c	5 ab	0.01 c	0.24 a	0.02 b	0.04 cd	0.89 b
1.5% WS (+N,+P)	24 c	2 bc	23 b	5 c	2 c	1 d	0.02 bc	0.06 c	0.02 b	0.03 cd	1.38 b
p -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0007	0.0055	0.042	<0.0001	<0.0001
	2nd Harvest										
Control (+N,+P)	114 a	8 a	86 a	23 a	7 a	5 b	0.10 a	0.28 a	0.05 a	0.18 a	6.19 a
Control (-N,-P)	19 c	1 d	18 d	6 c	2 c	1 c	0.02 c	0.06 d	0.01 c	0.02 c	1.50 c
0.75% LEA+0.75% WS	69 b	4 bc	68 ab	16 b	6 ab	10 a	0.09 ab	0.21 b	0.04 ab	0.15 ab	6.14 a
1.5% LEA	52 b	4 c	44 c	15 b	4 b	12 a	0.06 b	0.14 c	0.03 b	0.18 a	4.38 b
3.0% LEA	0 c	0 d	0 d	0 c	0 c	0 c	0 c	0 d	0 c	0 c	0 c
1.5% WS (+N,+P)	65 b	6 b	61 bc	16 b	5 b	2 c	0.07 ab	0.17 bc	0.03 b	0.10 bc	3.88 b
p -value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0010	<0.0001
	3rd Harvest										
Control (+N,+P)	70 a	7 a	52 a	19 a	5	4 b	0.17	0.35 a	0.04 a	0.15 a	3.66
Control (-N,-P)	10 c	2 b	12 b	5 c	3	1 c	0.03	0.04 b	0.00 c	0.05 c	2.13
0.75% LEA+0.75% WS	26 b	2 b	29 ab	6 bc	3	5 b	0.05	0.15 b	0.02 b	0.07 b	2.58
1.5% LEA	40 b	3 b	32 ab	9 ab	3	8 a	0.06	0.17 b	0.02 b	0.10 ab	3.10
3.0% LEA	0 c	0 b	0 b	0 c	0	0 c	0	0 b	0 c	0 c	0
1.5% WS (+N,+P)	40 b	7 a	56 a	17 ab	11	2 bc	0.12	0.15 b	0.02 b	0.09 b	3.12
p -value	<0.0001	0.0066	0.017	0.044	0.47	0.0010	0.14	0.0097	<0.0001	0.0022	0.082

[†]Means within harvest and column followed by the same letter are not significantly different at $P < 0.05$.
[‡]LEA and TN denote lipid-extracted algae and total N, respectively.
[§]WS denotes wheat straw.

Calcium and Mg uptake generally followed similar trends, and was usually greatest for the positive control and least for the negative control and 3.0% LEA. Sodium uptake by ryegrass tended to be greater for treatments receiving LEA, except at the 3.0% rate, and the negative control (Table 4.3). The 3.0% LEA treatment resulted in lowest uptake for harvests two and three because of no yield. Iron, Mn, Zn, and Cu uptake generally followed similar trends, being greatest for the positive control and lowest for the negative control and 3.0% LEA, especially in the second and third harvests. Sulfur uptake was greatest for 0.75% LEA + 0.75% WS and positive control and least for the negative control and 3.0% LEA treatments.

Residual and extractable soil concentrations of all nutrients except Zn after the final ryegrass harvest were significantly affected by treatment (Table 4.4). Possibly a result of increased soil pH (Table 4.1), extractable Fe and Mn concentrations were the only two nutrients not greatest for the 3.0% LEA treatment. Comparing the 1.5% LEA and 0.75% LEA + 0.75% WS treatments to the positive and negative controls, residual extractable K, Ca, Mg, and S were greater in soil amended with LEA. Magnesium was greater for the 1.5% LEA treatment compared to the 0.75% LEA + 0.75% WS treatment. Residual NO_3^--N, P, S, and especially Na were greatest for 3.0% LEA.

Table 4.4. Extractable soil nutrients remaining after the final ryegrass harvest at 0-15 cm depth.

Soil Treatment	NO_3^--N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	mg kg^{-1}										
Control (+N,+P)	14 c[†]	106 b	155 d	2858 d	285 e	9 c	60 d	5.8 a	0.26	67.0 a	0.43 bc
Control (-N,-P)	16 c	35 e	213 c	3037 c	315 d	11 c	88 d	4.2 b	0.37	51.6 b	0.36 c
0.75% LEA[‡]+0.75% WS[§]	7 c	45 e	241 b	3450 b	336 c	21 bc	536 c	2.7 c	0.99	35.5 c	0.46 ab
1.5% LEA	41 bc	70 d	228 bc	3616 b	376 b	28 b	728 b	3.6 bc	0.46	28.8 d	0.42 bc
3.0% LEA	354 a	118 a	327 a	4575 a	513 a	109 a	1956 a	2.8 c	0.70	30.2 d	0.51 a
1.5% WS (+N,+P)	59 b	89 c	333 a	2942 cd	306 d	10 c	77 d	3.9 b	0.44	64.1 a	0.40 bc
p-value	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	<0.0001	0.0002	0.29	<0.0001	0.013

[†]Means within nutrient followed by the same letter are not significantly different at $P < 0.05$.
[‡]LEA denotes lipid-extracted algae.
[§]WS denotes wheat straw.

DISCUSSION

Experiment I

Lipid-extracted algae applied at a 3.0% rate resulted in greater CO_2-C loss than any other treatment, whereas, 1.5% LEA-amended soil resulted in CO_2-C emissions greater than only the controls. Soil amended with 0.75% LEA + 0.75% WS resulted in less CO_2-C than 3.0% LEA and 1.5% WS (+N,+P) but more CO_2-C than 1.5% LEA. Eighty-five d after treatment application, C mineralized for 1.5% WS (+N,+P) or 0.75% LEA + 0.75% WS treatments ranged from 13 to 15% of total added C, whereas mineralized LEA- C ranged from 9 to 10% of C added for 1.5% and 3.0% LEA treatments. Thus, greater C was sequestered with LEA treatments compared to the WS and LEA plus WS treatments (Figs. 4.2 and 4.3).

As previously discussed in Chapter II, the C:N ratios of LEA and WS (10.8 and 50.5, respectively) (Table 2.1) are indicative of potential rapid C mineralization with LEA application and a reduced rate with WS. Applications of LEA were predicted to rapidly decompose, sequestering less SOC than WS; however, possibly due to its molecular makeup rather than C:N ratio, LEA applications mineralized a lesser percentage of added C, and thus, sequestered greater SOC than WS. Nonhydrolyzable macromolecules, or algaenans, which have been identified in the marine microalgae, *N. salina* (Gelin et al., 1999), may be a major contributor to LEA's greater resistance to decay compared to WS.

Mineralized NH_4^+-N concentrations in soil ranged from 100 to 175 mg kg^{-1} 7 d after treatment application for 1.5 and 3.0% LEA, respectively. As extractable NH_4^+-N decreased from 7 to 14 d in 1.5% LEA-amended soil from, extractable soil NO_3^--N increased. Even though NH_4^+-N also decreased from 7 to 14 d with the 3.0% LEA treatment, a proportional NO_3^--N increase was not observed. As reported in Chapter II, it was postulated that an increase in soil salinity with 3.0% LEA may be responsible for nitrification inhibition, which was demonstrated by the positive and negative relationships of NH_4^+-N ($R^2 = 0.81$) and NO_3^--N ($R^2 = 0.85$), respectively, with EC (Fig. 4.11). Previous research has reported a similar nitrification inhibition observed by NH_4^+-N accumulation with increasing rates of NH_4Cl to soil (Wichern et al., 2006; Megda et al., 2014). Delayed nitrification and consequent NH_4^+ accumulation in soil, may increase plant available N by decreasing NO_3^--N losses from soil. Moreover, environmental concerns pertaining to NO_3^- leaching and groundwater contamination may be reduced.

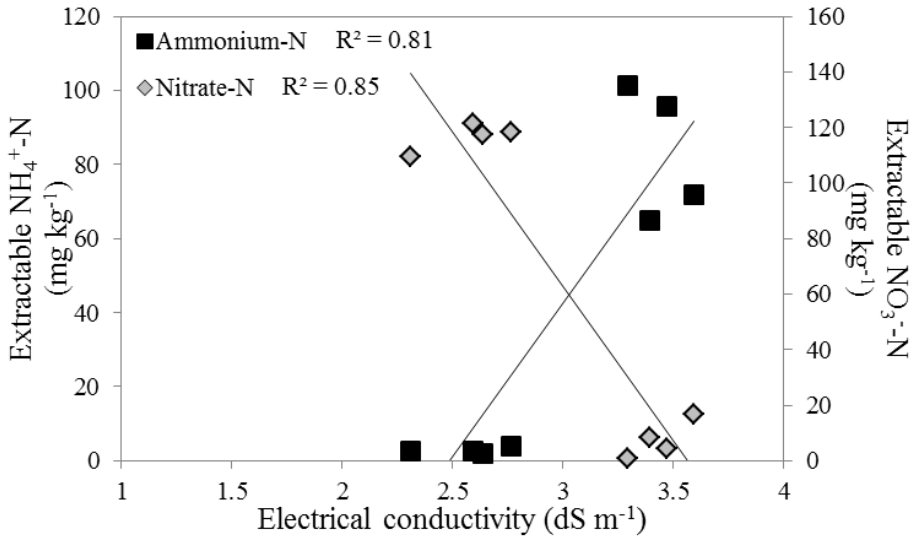

Fig. 4.11. Relationship between extractable NH_4^+-N and NO_3^--N with electrical conductivity of soil treated with 1.5% and 3.0% LEA.

Gaseous N losses from soil amended with 1.5 and 3.0% LEA included NH_3 volatilization and N_2O emissions. The N_2O-N lost from 3.0% LEA-amended soil was equivalent to 70 kg N on a hectare basis and accounted for 3.9% of the added LEA-N. However, NH_3 volatilization from soil amended with 3.0% LEA accounted for only a minor loss of LEA-N (0.6%) compared to N_2O-N losses. Nitrous oxide emissions may be attributed to nitrification of NH_4^+-N to NO_3^--N, denitrification of NO_3^--N, or both (Stevens et al., 1997). In this experiment, the increase of N_2O-N followed the increase of extractable NO_3^--N for the 3.0% LEA treatment; therefore, it was hypothesized that nitrification was the major source of N_2O emissions. In agreement with our findings, other studies have reported nitrification as the primary source of N_2O under aerobic conditions in agricultural soil, with denitrification contributing to a small percentage of total N_2O emissions (Stevens et al., 1997; Li and Lang, 2014). Anaerobic microsites

within macro- and microaggregates could provide conditions enabling denitrification; however, denitrification was ruled out as the source of N_2O because CH_4 fluxes were low to non-detectable (data not shown). Methane production occurs in anaerobic conditions.

The availability of macro and micronutrients in fallow soil 85 d after treatment application of 1.5 and 3.0% LEA (Table 4.1) demonstrated the potential to support multiple crop growth cycles during a single growing seasons (e.g. multiple cuttings) or possibly multiple growing seasons (e.g. summer and winter grasses). A significant increase in secondary nutrient concentrations in 3.0% LEA-amended soil was observed compared to the negative control. Calcium, Mg, and S increased by approximately 1320, 170, and 110 mg kg^{-1}, respectively, in the 3.0% LEA treatment vs. the negative control. Increases were also noted for NO_3^--N, P, K, Na, Zn, and Cu.

As a result of 3.0% LEA applications to soil, a shift of bacterial and fungal populations was observed along with an increase in copy numbers compared to both the positive and negative controls (Fig. 4.8). Both bacterial and fungal copy numbers per g soil increased in soil treated with 3.0% LEA from 3 to 7 d, but between 7 and 14 d, bacterial copy numbers decreased and fungal copies increased. Bacteria:fungi ratios were less than 1.0 at both 14 and 21 d after treatment application of 3.0% LEA. Similar results were not observed for the 1.5% LEA treatment, which did not result in any notable microbial community fluctuations. It was speculated that both recalcitrance and salinity associated with LEA applied at a high rate (3.0%) influenced the observed shift. Even though fungi are thought to have a greater sensitivity to salinity compared to

bacteria (Pankhurst et al., 2001; Sardinha et al., 2003; Chowdhury et al., 2011), our results along with the results of Wichern et al. (2006) suggest a greater fungal tolerance to salinity.

Experiment II

Compared to other treatments, ryegrass seedling emergence was most inhibited in soil amended with 3.0% LEA. An inhibitory effect on seedling emergence was also observed for the 1.5% LEA treatment, but emergence was not as reduced compared to 3.0% LEA. However, when WS was added to soil with LEA (0.75% LEA + 0.75% WS), the inhibitory effect of LEA on seedling emergence was not demonstrated.

Poor seedling emergence in soil amended with 3.0% LEA may be the result of high salinity. Compared to other treatments, 3.0% LEA significantly increased soil EC (5.6 dS m^{-1}). Not only does past research indicate that salinity may inhibit seedling emergence (Marcar, 1987; Mueller and Bowman, 1989), but also that seedling emergence may be reduced by excessive soil NH_3 (Qin et al., 2014). Soil amended with 3.0% LEA produced greater soil salinity and NH_3 compared to the controls and all other treatments. Thus, both soil salinity and excess NH_3 may have contributed to reduced seedling emergence with this treatment.

Herbage mass from the first harvest for 0.75% LEA + 0.75% WS-amended soil was similar to the positive control and greater than the other LEA treatments; thus, applying LEA and WS together (0.75% LEA + 0.75% WS) may have reduced the negative effects associated with LEA, such as salinity, on seedling emergence and plant

growth. At the second and third harvests, HM from the 0.75% LEA + 0.75% WS treatment was less than the positive control; possibly as a result of WS-associated N immobilization and consequently, less plant available N. Even though 230 kg N ha^{-1} was added to soil for both the positive control and 1.5% WS (+N,+P) treatment, N uptake for the latter was less than the positive control at all harvests, which also possibly points to N immobilization with the addition of WS. Soil amended with 1.5% LEA and 0.75% LEA + 0.75% WS resulted in greater total HM compared to the negative control; therefore, the enhanced nutrient availability with these LEA treatments may outweigh potential negative plant growth effects associated with LEA amendments.

Greater Na uptake by ryegrass at the first harvest for the 3.0% LEA treatment may have reduced K and Ca uptake compared to the controls or 1.5% WS (+N,+P). A study by Hu and Schmidhalter (2005) reported nutrient deficiencies or imbalances in saline soil due to the competition of Na^+ and Cl^- with nutrients such as K^+, Ca^{2+}, and NO_3^-. Since low HM was produced by the 3.0% LEA treatment, little nutrient uptake occurred and residual extractable soil nutrient concentrations, except Fe and Mn, were greater than for all other treatments. Increased soil pH in 3.0% LEA-treated soil may have decreased Fe and Mn availability (Soliman et al., 1992).

After the final harvest, soil K, Ca, Mg, S, and Na for treatments of 1.5% LEA and 0.75% LEA + 0.75% WS were greater than or similar to the controls, demonstrating the potential of LEA as a source of K, Ca, Mg, and S for plant growth. However, high soil Na or salt concentrations associated with LEA may be detrimental to soil physical

and chemical properties, microbiological processes, or plant growth (Pathak and Rao, 1998).

CHAPTER V

EFFECTS OF LIPID-EXTRACTED ALGAE ON SOIL PHYSICOCHEMICAL DYNAMICS AND RYEGRASS GROWTH IN THE FIELD

INTRODUCTION

The largest pool within the terrestrial C cycle is SOC and its storage is the net effect of OM inputs to soil and losses through decomposition (Schlesinger, 1997; Amundson, 2001). Improved soil physical and chemical properties as a result of organic amendments may include increased water holding capacity, greater cation exchange capacity, enhanced retention of nutrients in the root zone, greater buffering capacity against pH change, improved ability to chelate and form complex ions, and more stable soil structure as a result of aggregate formation (Degens et al., 2000). All these attributes may reduce soil degradation, erosion and compaction, and increase nutrient availability to plants and microorganisms and the capacity for C storage in long-term cropping systems (Karami et al., 2012). Long-term SOC stabilization and short-term nutrient cycling are also influenced by dynamics of aggregate formation and breakdown over time (Plante and McGill, 2002; Six et al., 2002).

Organic amendments can enhance soil aggregate formation by providing active organic materials, such as particulate OM, which act as nucleation sites and binding agents for aggregate formation (Tisdall and Oades, 1982; Chivenge et al., 2011a). Six et al. (1999) suggested that adding crop residues promotes OM stabilization through the binding of primary soil particles and old microaggregates into new macroaggregates.

Depending on the quality or biochemical characteristics, such as C:N ratio and lignin content of organic materials, it may be possible to maintain or improve soil physical and chemical properties as a result of increased SOM, and consequently, enhance microbial activity and aggregate formation. Jastrow et al. (1996) observed an increased quantity of macroaggregates resistant to slaking under long-term pasture grasses compared to corn fields. A monoculture study by Wright and Hons (2005a) showed that aggregation was generally greater for wheat than any of the other crop species (sorghum and soybean). Crops, such as wheat, having low nitrogen (N) contents (high C:N ratio) will usually decompose at much slower rates than residues with higher N contents (Ghidey and Alberts 1993; Franzluebbers et al., 1995). Slower decomposition will lead to increases in SOM and aggregate formation and stability.

Aggregates can contain SOM of various origins, composition and degree of microbial degradation, and thus, add to the difficulty of studying the role of organic amendments in aggregate formation and the ensuing effect of aggregate turnover on soil C stabilization. However, natural differences between the stable, nonradioactive ^{13}C isotopic composition of soil and organic material can provide a useful approach to determine the primary C source sequestered in aggregates and SOM fractions (Balesdent and Mariotti, 1987).

Stable C isotopes (^{12}C and ^{13}C) are useful tracers for studying the dynamics involved in C cycling in SOM pools of both agricultural and natural ecosystems (Tieszen and Boutton, 1989). Carbon in nature is comprised of 98.89% ^{12}C and 1.11% ^{13}C (Boutton, 1996). The uneven distribution of isotopes among and within compounds can

potentially reveal information about the physical, chemical, and metabolic processes involved in C cycling dynamics (Farquhar et al., 1989). According to Wada et al. (1995) isotope changes have been continuously documented at all-time aspects and scales in the biosphere, which allows for OM samples to be analyzed using natural abundance isotope techniques. The ^{13}C content of SOC relates closely to the ^{13}C content of the plant or microbial material it originated from (Gerzabek et al., 1997).

Cool-season (C_3) and warm-season (C_4) plant species discriminate against $^{13}CO_2$ during photosynthesis to different degrees. Carbon-3 species discriminate against $^{13}CO_2$ to a greater extent (O'Leary, 1981), making it possible to determine the relative contribution of C_3 and C_4 plant vegetation to SOM. Stable isotopic C studies can be used to differentiate sources of OM and its distribution in soils (Solomon et al., 2002). Growing C_4 plants on soil that has previously been under C_3 vegetation, or vice versa, can be used as an *in situ* labeling of the incorporated SOM (Balesdent et al., 1987). Carbon isotope tracers allow for the quantification of the rate of C losses from the original vegetation and the simultaneous accumulation of new C from the current vegetation or recent organic material addition.

Isotopic abundance analysis is typically conducted in conjunction with soil particle fractionation to identify sources of SOC and determine where OC is stored relative to aggregate structures. Authors have measured total OC in bulk soil and then partitioned OC into particle and aggregate size fractions. Christensen (1996) and Desjardins et al. (1994) have confirmed that OC concentrations increase with decreasing particle size; silt > clay > fine clay > fine sand > coarse sand. Gerzabek et al. (2001)

reported that the silt-size fraction acted as a medium-term sink for introduced OC. According to Buyanovsky et al. (1994), OC in clay-sized particles is of high stability and slow turnover rates.

Physical size-fractionation of soil aggregates in conjunction with isotopic analyses ($\delta^{13}C$ and $\delta^{15}N$) of those fractions have been used to (a) determine where organic C is stored relative to aggregate structure, (b) identify sources of SOC, (c) quantify turnover rates of SOC in specific soil fractions, and (d) evaluate OM quality (Liao et al., 2006). A study by Jastrow et al. (1996) showed that newly introduced OM is found mostly in larger soil aggregates, making it more susceptible to decomposition because macroaggregates are more likely to be destroyed by agricultural practices compared to microaggregates (Tisdall and Oades, 1982), but in perennial pasture systems this may not be the case.

The objective of this study was to determine the effects of LEA, an organic soil amendment, on soil quality in a field environment by: a) isolating and quantifying SOC pools associated with macroaggregates (>250 µm), free microaggregates (53-250 µm), and free silt and clay (<53 µm), b) investigating the influence of LEA incorporation on aggregate formation, c) determining SOC and total N storage within fractions, d) evaluating the distribution and C sources in aggregate fractions by utilizing the natural abundances of the stable isotope $\delta^{13}C$ of Parrita soil ($\delta^{13}C$ = -16.3 ‰) and LEA ($\delta^{13}C$ = -27.6‰) and WS material ($\delta^{13}C$ = -28.9‰).

METHODS

Study Area

The study was conducted at the Texas A&M Agrilife Research Station near Beeville, TX (28º27'30", 97º 42'21.78", 75.9 m). The average temperature and precipitation for this semi-arid environment was reported to be 21ºC and 81 cm, respectively, by the U.S. Climate Data service. Soil at this location was characterized as a Parrita series, and is as a sandy clay loam with a pH of 6.9 (loamy, mixed, superactive, hyperthermic, shallow Petrocalcic Paleustolls). It consists of shallow, well drained soils that formed in loamy sediments derived from calcareous sandstone of the Goliad Formation of Pliocene age (USDA – NRCS, 2006).

Treatment Preparation

The study was designed as a split-plot and arranged in a randomized complete block design with sampling time as the main plot and soil amendment as the split plot. *In situ* field incubations were conducted in PVC columns measuring 10 cm (i.d.) x 33 cm. The bottoms of columns were capped to prevent excessive loss of water and nutrients, but drainage holes were drilled in caps to prevent the soil from becoming anaerobic. Weights of both empty columns and soil-filled columns were measured.

Soil columns were removed at different times (24 hr and 3, 6, 9, and 12 months) after treatment application throughout the study and destructively sampled. Study treatments included: 1) 1.5% LEA, 2) 3.0% LEA, and 3) 1.5% LEA + 1.5% WS, and 4) control plus inorganic N (140 kg ha^{-1} NH_4NO_3) and P [112 kg ha^{-1} $Ca(H_2PO_4)_2 \cdot H_2O$].

There were four replications per time (6) x amendment (4) combination, totaling 96 columns. Treatments were prepared by mixing the designated rate of inorganic fertilizer, LEA or WS with sieved (< 2 mm) Parrita soil on a dry weight basis (g g^{-1}). The bottom 15 cm of each column was filled with unamended soil and the top 15cm with amended soil so that the soil bulk density was ~0.8 g cm^{-2}. Each column was then placed and securely packed within holes measuring 11 cm wide and 30 cm deep that were carefully excavated with a post-hole digger at a field site near the soil collection location at the Beeville Research Station.

Soil Sampling and Analyses

Columns were removed at 24 hr (reported as 0 month), and 3, 6, 9, and 12 months. Soil was sampled from three depth increments (0-5, 5-15, and 15-30 cm) for determining aggregate formation, isotopic analyses, SOC, total N, pH and electrical conductivity (EC) and sampled from 0-15 cm for SOC, total N and extractable NH_4^+, NO_3^-, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, and Cu. Wet and dry weights were measured prior to and after oven drying (65ºC) to constant weight. Soil samples for aggregate-size fractionation were gently crushed and sieved (< 4 mm) prior to separating 50-g aliquots into three aggregate sizes [>250 μm (macroaggregates), 250 – 53 μm (microaggregates) , and <53 μm (silt and clay)] using a rotary sieve-based dry sieving methods (Chepil and Bisal, 1943; Kemper and Chepil, 1965). Mean weight diameter (MWD) was calculated as a weighted average of the soil size fraction percentages. Separated size fractions were weighed and ground using a ring and puck mill (< 150 μm) prior to isotopic analyses.

Sub-samples of soil collected at 0, 3, 6, and 12 months at 0-15 cm depth were ground with a flail grinder (< 2 mm) and analyzed for extractable NH_4^+, NO_3^-, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, and Cu by the same methods previously described. Soil pH and EC were measured using a 1:2 soil to DI H_2O ratio. Sub-samples for soil total C and N were further ground (< 150 μm) using a ring and puck mill prior to weighing and combustion analysis described previously. Lipid-extracted algae- and WS-N mineralized to inorganic N (N_{inorg}) was calculated as the percent of total N added (N_{inorg} is equal to NH_4^+-N plus NO_3^--N).

Soil aliquots of 24 or 30 mg, depending on C concentration, of samples of the three size fractions from 0-5, 5-15, and 15-30 cm depths were weighed for elemental and isotopic analysis of SOC, soil total N, $\delta^{13}C$, and $\delta^{15}N$, which were performed in the Stable Isotopes for Biosphere Science (SIBS) Laboratory, Texas A&M University, College Station, TX. Organic matter inputs of LEA and WS were also measured for SOC, soil total N, $\delta^{13}C$, and $\delta^{15}N$. A Costech Elemental Combustion System interfaced with a Thermo Scientific Delta V Advantage mass spectrometer operating in continuous flow (He) mode was used to determine isotope ratios relative to the Vienna Pee Dee Belemnite (V-PDB) standard for C (Coplen, 1996) and atmospheric N_2 for N (Hoefs, 1997). Carbon and N isotope ratios are expressed in per mil (‰) using the standard delta notation (δ):

$$\delta = (R_{SAMPLE} - R_{STD})/R_{STD}) \times 10^3, \quad (1)$$

where R_{SAMPLE} is the $^{13}C/^{12}C$ or $^{15}N/^{14}N$ ratio of the sample and R_{STD} is the $^{13}C/^{12}C$ ratio of the V-PDB standard or $^{15}N/^{14}N$ ratio of atmospheric N_2. Quality control was

performed using calibration curves, which were derived using standards of USGS glutamic acid-40 ($\delta^{13}C$ = –26.39‰, $\delta^{15}N$ = –4.52‰) and USGS glutamic acid-41 ($\delta^{13}C$ = 37.63‰, $\delta^{15}N$ = 47.57‰). Plant material of corn ($\delta^{13}C$ = –12.78‰, $\delta^{15}N$ = 5.40‰) and ecen ($\delta^{13}C$ = –39.88‰, $\delta^{15}N$ = 29.88‰) were analyzed as internal standards to determine the accuracy and precision of isotopic analysis.

The relative proportions of SOC derived from the LEA (F_C) vs. the native soil C were estimated by mass balance:

$$F_C = (\delta_{SOIL} - \delta_{SAMPLE})/(\delta_{SOIL} - \delta_{LEA}), \quad (2)$$

where δ_{SOIL} was the $\delta^{13}C$ value of native soil C at the start of the experiment, δ_{SAMPLE} was the $\delta^{13}C$ value within a soil size fraction at sampling, and δ_{LEA} was the $\delta^{13}C$ value of LEA. The percentage of SOC derived from LEA (LEA − C, %) within soil size fractions was calculated as:

$$LEA - C, \% = F_C \times 100. \quad (3)$$

Ryegrass Planting and Harvesting

One of the six main plots designated as time of each block was seeded (15 Nov 2012) with salt-tolerant ryegrass [*Lolium multiflorum* (Lam). cv. TXR2011-S] by broadcasting. Ryegrass was cut to ground level 140 DAP (4 April 2013). Harvested herbage was weighed prior to and after drying to constant weight at 65º C, ground to pass through a 0.5-mm sieve in a cyclone mill (Udy Cyclone Sample Mill 3010-030; Fort Collins, CO, USA), and analyzed for total N, P, K, Ca, Mg, S Na, Zn, Fe, Cu, and Mn by the same methods previously reported. Nutrient uptake for each harvest was calculated by multiplying the mineral concentration of the HM by the amount of HM harvested per column. Columns were removed and sampled at 0-15 cm 12 months after treatment application. Soil was dried (65ºC), ground (< 2 mm) and analyzed for extractable NH_4^+, NO_3^-, P, K, Ca, Mg, S, Na, Fe, Zn, Mn, and Cu, and pH and EC as previously described.

Statistical Analyses

Statistical analyses were conducted using SAS version 9.3. Effects were analyzed using a linear mixed analysis of variance (ANOVA) procedure at a significance level of $P < 0.05$. Means of significant effects were separated using Fisher's protected LSD at $P < 0.05$.

RESULTS

Soil pH and Electrical Conductivity

At 0 months regardless of depth, LEA-amendments resulted in similar soil pH values that were greater than the control (+N,+P). By 12 months, pH had a tendency to decrease for LEA-treatments and the control (+N,+P) (Table 5.1). No differences in pH were observed between treatments at 12 months at 0-5 cm depth ($P = 0.25$). However, soil pH at 12 months was similar for all LEA-amendments within the 5-15 and 15-30 cm depths, with LEA amended soil having pH values (mean pH = 6.9) greater than the control (+N,+P) (mean pH = 6.6).

At 0 and 12 months and for all soil depths, EC was greatest for soil amended with 3.0% LEA (Table 5.2). As depth increased, the magnitude of the difference in EC between all treatments decreased. At 0-5 cm and 12 months, the 3.0% LEA treatment resulted in an EC of 4.3 dS m^{-1}. Regardless of depth at 12 months, soil EC was similar for the control (+N,+P), 1.5% LEA and 1.5% LEA + 1.5% WS treatments.

Table 5.1. Soil pH measured at 0 and 12 months after treatment application at 0-5, 5-15, and 15-30 cm depths.

	Time (months)	
Treatment	0	12
	depth: 0-5 cm	
Control (+N,+P)	7.3b[†]	6.7
1.5% LEA[‡]	8.2a	6.8
3.0% LEA	8.3a	6.6
1.5% LEA+1.5% WS[§]	8.1a	6.9
p -value	<0.0001	0.25
	depth: 5-15 cm	
Control (+N,+P)	7.0b	6.6b
1.5% LEA	8.1a	6.9a
3.0% LEA	8.3a	6.9a
1.5% LEA+1.5% WS	8.2a	7.0a
p -value	<0.0001	0.013
	depth: 15-30 cm	
Control (+N,+P)	7.2b	6.5b
1.5% LEA	8.2a	6.8a
3.0% LEA	8.4a	7.0a
1.5% LEA+1.5% WS	8.0a	7.0a
p -value	0.0004	0.0042

[†]Within time and depth, means followed by the same letter are not significantly different at $P < 0.05$.
[‡]LEA denotes lipid-extracted algae.
[§]WS denotes wheat straw.

Table 5.2. Soil electrical conductivity measured at 0 and 12 months after treatment application at 0-5, 5-15, and 15-30 cm depths.

	Time (months)	
Treatment	0	12
	depth: 0-5 cm	
Control (+N,+P)	0.3c[†]	0.3b
1.5% LEA[‡]	1.1b	1.9b
3.0% LEA	2.0a	4.3a
1.5% LEA+1.5% WS[§]	1.0b	1.0b
p -value	<0.0001	0.0026
	depth: 5-15 cm	
Control (+N,+P)	0.4c	0.2b
1.5% LEA	1.2b	0.5b
3.0% LEA	1.9a	1.3a
1.5% LEA+1.5% WS	1.1b	0.5b
p -value	<0.0001	0.0002
	depth: 15-30 cm	
Control (+N,+P)	0.3d	0.2b
1.5% LEA	0.8b	0.5b
3.0% LEA	1.2a	1.2a
1.5% LEA+1.5% WS	0.5c	0.5b
p -value	<0.0001	0.0008

[†]Within time and depth, means followed by the same letter are not significantly different at $P < 0.05$.
[‡]LEA denotes lipid-extracted algae.
[§]WS denotes wheat straw.

Carbon and Nitrogen Dynamics

Aggregate Formation

No treatment differences for aggregate MWD were noted within 0-5 and 15-30 cm depths and any sampling time, except at 0 month and 0-5 cm; however, differences were observed at 0, 6, and 12 months at the 5-15 cm depth (Table 5.3).

Table 5.3. ANOVA results for treatment effect on aggregate mean weight diameter (MWD) within time and soil depth.

	Time (months)				
	0	3	6	9	12
Depth (cm)	ANOVA (p-value < 0.05)				
0-5	0.03	0.43	0.08	0.29	0.62
5-15	0.01	0.23	0.04	0.65	0.05
15-30	0.63	0.50	0.28	0.38	0.21

The control (+N,+P) resulted in greater MWD at 5-15 cm depth compared to 1.5% and 3.0% LEA-treated soil immediately following treatment application (24 hr) (Fig. 5.1). Six months after treatment application, MWD of the control (+N,+P) was similar to that of soil amended with 3.0% LEA, but greater than the other two treatments. Twelve months after treatment application, the 1.5% and 3.0% LEA treatments resulted in greater MWD than 1.5% LEA + 1.5% WS but not the control.

Although an overall increase of MWD was observed over the 12-month study for the control (+N,+P) as well as organic amendments, 1.5 and 3.0% LEA-amended soil resulted in the greatest percentage increase in MWD (54% and 56%, respectively) compared to the control (+N,+P) and 1.5% LEA + 1.5% WS treatment, which increased by 22% and 15%, respectively.

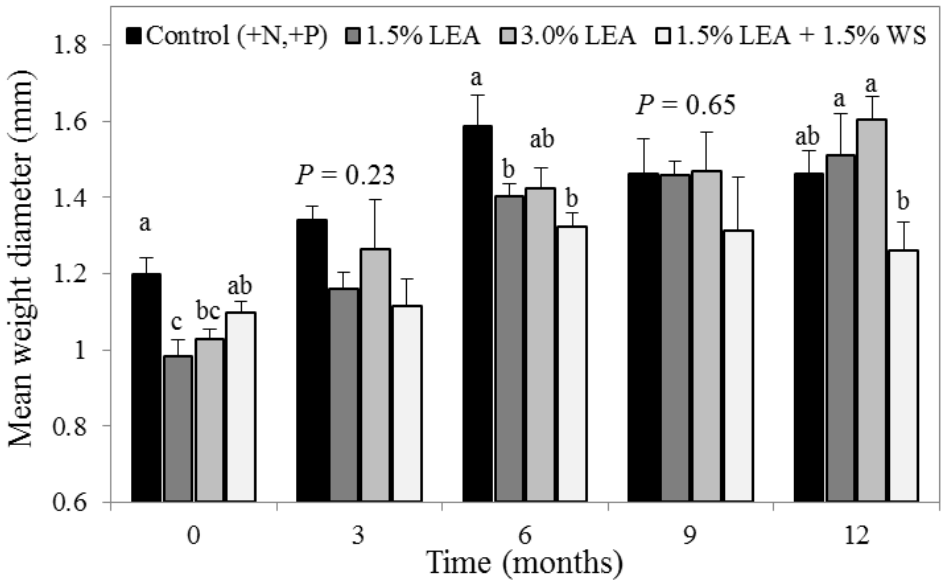

Fig. 5.1. Aggregate formation represented as mean weight diameter over time within the 5-15 cm depth. Means followed by the same letter within time are not significantly different at $P < 0.05$ by Fisher's protected LSD.

Soil Organic Carbon Dynamics of Fallow Soil

At 0-5 cm depth, significant differences in $\delta^{13}C$ were observed between the control (+N,+P) and other treatments for macroaggregates, microaggregates, and silt and clay fractions at each measurement time, except at 3 months for the silt and clay fraction ($P = 0.48$) (Fig. 5.2a,b,c). Twenty-four hours after treatment application (0 month) at

this depth, macroaggregate $\delta^{13}C$ values for soil amended with 1.5% LEA + 1.5% WS or 3.0% LEA were more depleted in ^{13}C compared to the control (+N,+P) (Fig 5.2a). At 3, 6, and 12 months, treatments with LEA were all more depleted in ^{13}C than the control (+N,+P) for macroaggregates at 0-5 cm, and by 12 months the 3.0% LEA treatment was most depleted in ^{13}C (-18.3‰).

Although microaggregate $\delta^{13}C$ signature trends over time at 0-5 cm were similar to those of macroaggregates, microaggregate signatures were more ^{13}C depleted than the control (+N,+P) at each measurement time (Fig. 5.2b). Also different from macroaggregates, the $\delta^{13}C$ signatures of microaggregates at 12 months for 3.0% LEA and 1.5% LEA + 1.5% WS were similar, but more negative and more like LEA's signature ($\delta^{13}C$ = -27.6‰) than the control (+N,+P) or 1.5% LEA treatment. At 0, 6, and 12 months at 0-5 cm, the silt and clay fraction had more negative $\delta^{13}C$ signatures for LEA-amended treatments than the control (+N,+P) (Fig. 5.2c). Over time, $\delta^{13}C$ values in all aggregate fractions for soil amended with LEA exhibited decreasing negative signatures. Comparing LEA-amendments among size fractions at 0-5 cm, the silt and clay fraction tended to have the most negative signatures for LEA treatments at 0 months and the least negative at 12 months.

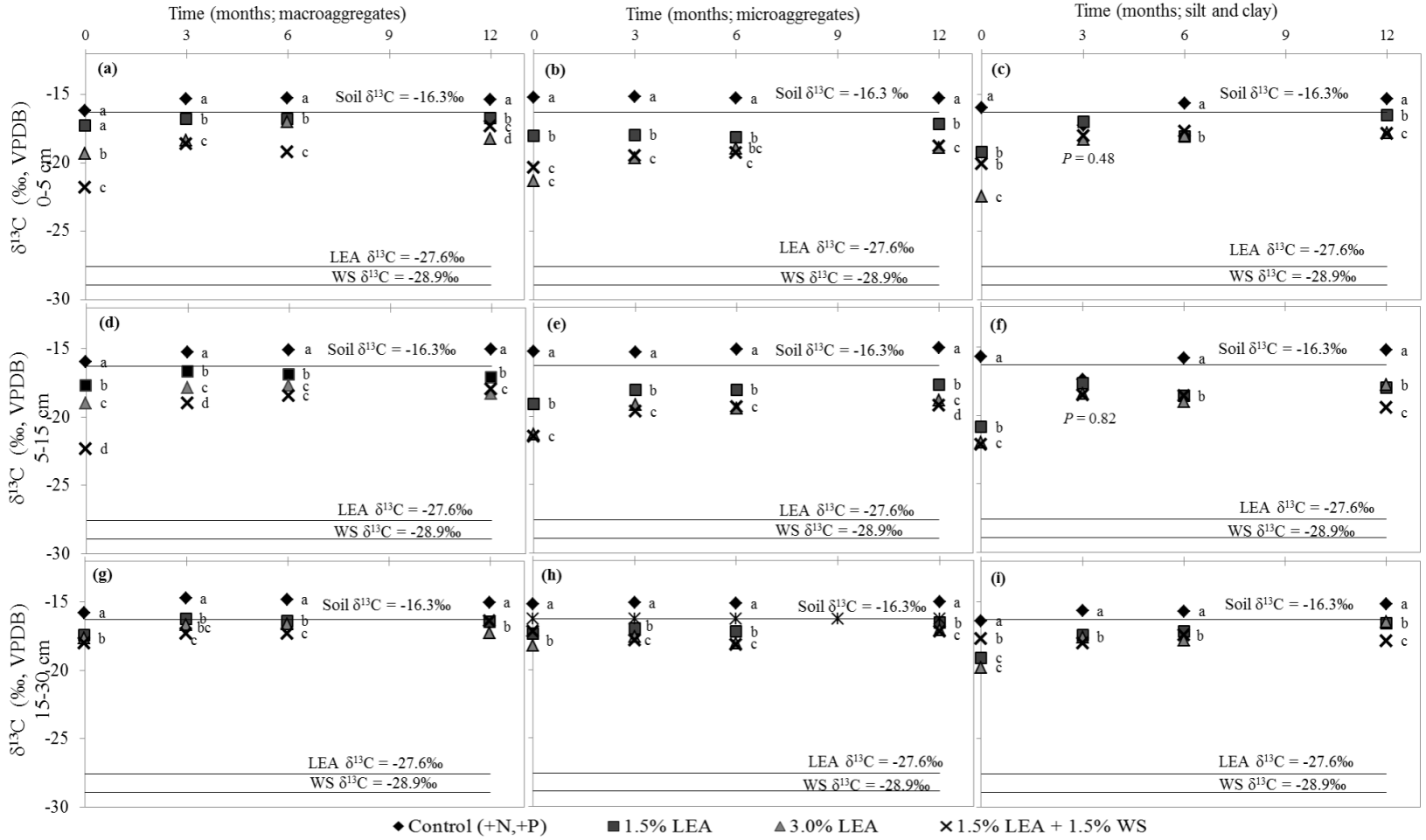

Fig. 5.2. Fallow soil $\delta^{13}C$ (‰) measured over 12-month field incubation at depths (rows) of 0-5 cm (a-c), 5-15 cm (d-f), and 15-30 cm (g-i) and size fractions (columns) of macroaggregates (a,d,g), microaggregates (b,e,h), and silt and clay (c,f,i). Means followed by the same letter within depth, size fraction, and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars represent standard error of the mean.

Similar to the 0-5 cm depth, differences in $\delta^{13}C$ signatures occurred at 5-15 cm for macroaggregates, microaggregates, and the silt and clay fraction at all sampling times, except at 3 months for the silt and clay fraction (Fig. 5.2d,e,f). Macroaggregates at 5-15 cm for LEA-amendments were more depleted in ^{13}C at each measurement time compared to the control (+N,+P) (Fig. 5.2d). Values for treatments of 3.0% LEA and 1.5% LEA + 1.5% WS were also more depleted than 1.5% LEA. $\delta^{13}C$ signatures of microaggregates from soil amended with 3.0% LEA and 1.5% LEA + 1.5% WS were most negative and similar at 0, 3, and 6 months, but not at 12 months where 1.5% LEA + 1.5% WS was most depleted (Fig. 5.2e). Microaggregates from all LEA treatments were more depleted in $\delta^{13}C$ compared to the control (+N,+P) at all sampling times. For the 3.0% LEA treatments at 5-15 cm depth, $\delta^{13}C$ values of microaggregates, tended to be more depleted (-18.8‰) compared to macroaggregates (-18.3‰) (Fig. 5.2d,e). Silt and clay $\delta^{13}C$ signatures over time at 5-15 cm generally followed similar trends to signatures at 0-5 cm (Fig. 5.2c,f).

While differences in $\delta^{13}C$ were noted between the control (+N,+P) and LEA-amendments at 15-30 cm for all size fractions and measurement times, signatures tended to be less depleted in ^{13}C at this depth compared to 0-5 and 5-15 cm regardless of LEA-treatment (Fig. 5.2g,h,i). At 12 months at 15-30 cm depth, $\delta^{13}C$ values of macroaggregates and free silt and clay fractions for 1.5% LEA and 3.0% LEA were similar; however, free microaggregate signatures for 3.0% LEA and 1.5% LEA + 1.5% WS treatments were most depleted in ^{13}C at this time (Fig. 5.2h). Unlike signatures at 0-5 and 5-15 cm of 3.0% LEA-amended soil, $\delta^{13}C$ signatures at 15-30 cm tended to be

more depleted for macroaggregates (-17.3‰) rather than free microaggregates (-17.1‰) or silt and clay (-16.4‰) (Fig. 5.2).

Over the 12-month sampling period and regardless of aggregate size fraction, SOC measured at 0-5 cm for LEA-amended soil tended to decrease (Fig. 5.3). Soil organic C concentration of macroaggregates at 0-5 cm was significantly different between the control (+N,+P) and at least one LEA treatment at 0, 3, and 6 months (Fig. 5.3a). By 12 months, however, no differences were observed. Soil amended with 1.5% LEA + 1.5% WS resulted in greater SOC at 0, 3, and 6 months compared to 1.5 and 3.0% LEA treatments or the control (+N,+P). Soil organic C of macroaggregates measured at 3 and 6 months was similar amongst the control (+N,+P) and 1.5% and 3.0% LEA treatments (mean SOC = 1.4%).

Treatment differences in SOC for microaggregates were observed at 0 and 12 months after treatment application but only at 12 months for the silt and clay fraction (Fig. 5.3b,c). Microaggregate SOC at 12 months was similar for 3.0% LEA (1.3%) and 1.5% LEA + 1.5% WS (1.2%), with both being greater than SOC for the control (+N,+P) (1.0%) or 1.5% LEA treatment (1.1%) (Fig. 5.3b). Soil organic C at 12 months for the silt and clay fraction was greater for the 1.5% LEA + 1.5% WS treatment (2.4%) compared to the control (+N,+P) (2.0%) or 1.5% LEA treatment (2.2%) but not greater than the 3.0% LEA treatment (2.3%).

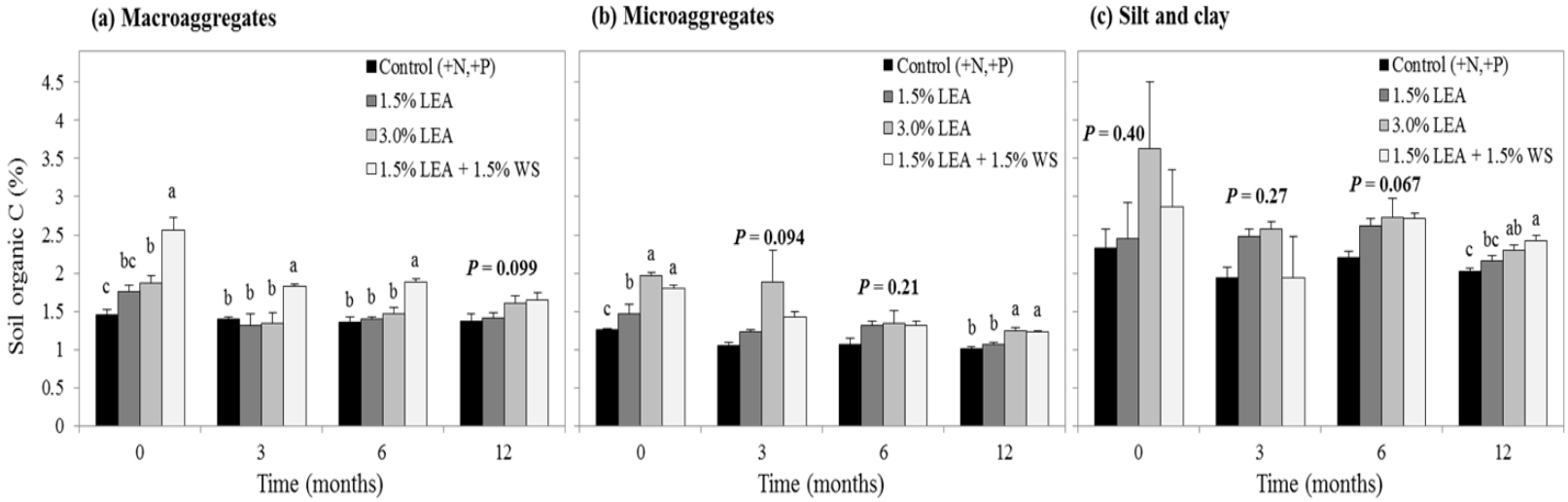

Fig. 5.3. Soil organic C (%) of fallow soil measured *in situ* throughout a12-month field incubation at 0-5 cm depth for size fractions of a) macroaggregates, b) microaggregates, and c) silt and clay. Means followed by the same letter within size fraction and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars represent standard error of the mean.

At 5-15 cm depth, SOC differences occurred for both macro- and microaggregates at each measurement time (Fig. 5.4a,b); however, no differences were observed for the silt and clay fraction at 0 and 3 months ($P = 0.75$ and $P = 0.21$, respectively) (Fig. 5.4c). Macro- and microaggregate-associated SOC at 5-15 cm depth at 12 months was greater for 1.5% LEA (1.4 and 1.2%, respectively), 3.0% LEA (1.6 and 1.4%, respectively), and 1.5% LEA + 1.5% WS (1.5 and 1.4%, respectively) compared to the control (+N,+P) (1.2 and 1.1%, respectively) (Fig. 5.4a,b). Soil organic C of the silt and clay fraction at this depth at 12 months was greater in soil amended with 3.0% LEA (2.8%) and 1.5% LEA + 1.5% WS (3.0%) compared to the control (+N,+P) (2.4%) but not to the 1.5% LEA treatment (2.7%) (Fig. 5.4c). Soil organic C in both macro- and microaggregates tended to decrease with time, but remained fairly stable in the silt and clay fraction.

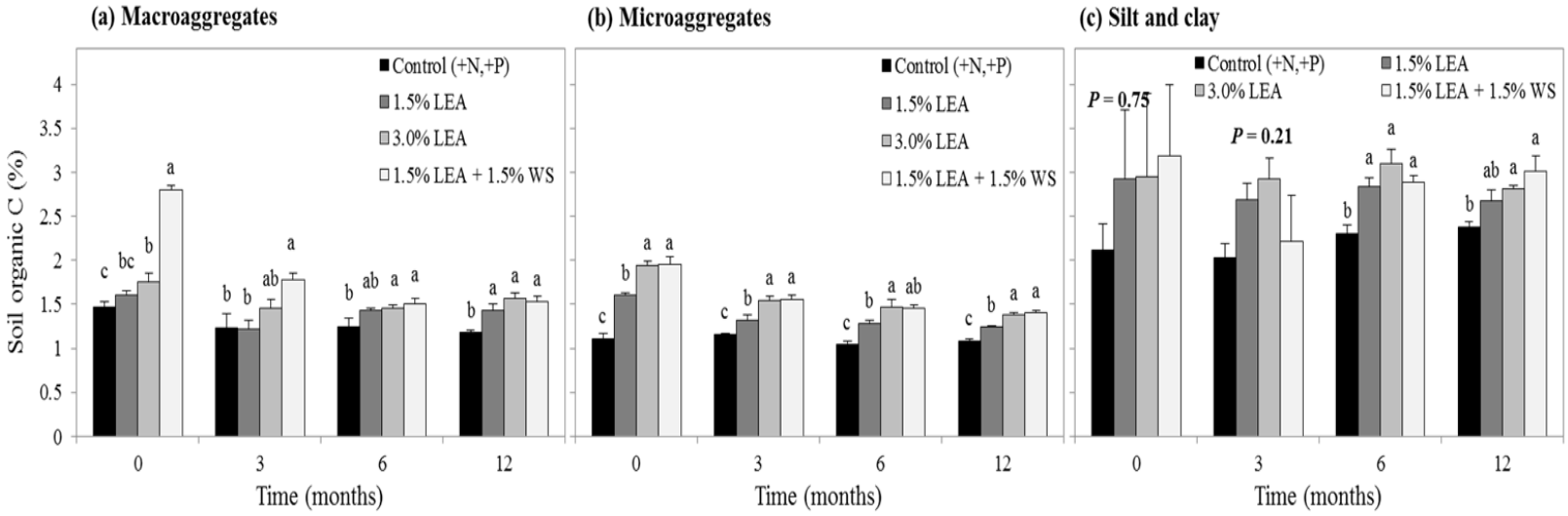

Fig. 5.4. Soil organic C (%) of fallow soil measured in situ throughout 12-month field incubation at 5-15 cm depth for size fractions of a) macroaggregates, b) microaggregates, and c) silt and clay. Means followed by the same letter within size fraction and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars represent standard error of the mean.

No differences were found for SOC within macroaggregates at 15-30 cm depth at 3 months (P = 0.34), or for microaggregates at 6 months (P = 0.083), and silt and clay at 0 and 3 months (P = 0.43 and P = 0.30, respectively) (Fig. 5.5). Macroaggregate SOC at 12 months was greater for soil amended with 3.0% LEA (1.4%), 1.5% LEA + 1.5% WS (1.4%), and 1.5% LEA (1.3%) than the control (+N,+P) (1.2%) (Fig. 5.5a). Soil amended with 1.5% LEA + 1.5% WS resulted in the greatest SOC concentration in microaggregates at 12 months. The 3.0% LEA treatment resulted in greater SOC than the control (+N,+P) but not 1.5% LEA (Fig. 5.5b). Similarly, the 1.5% LEA + 1.5% WS treatment resulted in greater SOC than the control (+N,+P) in the silt and clay fraction, but unlike the microaggregate fraction, the 3.0% LEA treatment was not greater than the control (+N,+P) (Fig. 5.5c). Overall, the general trends observed for SOC in size fractions at 15-30 cm depth were similar to those of the 0-5 and 5-15 cm depths.

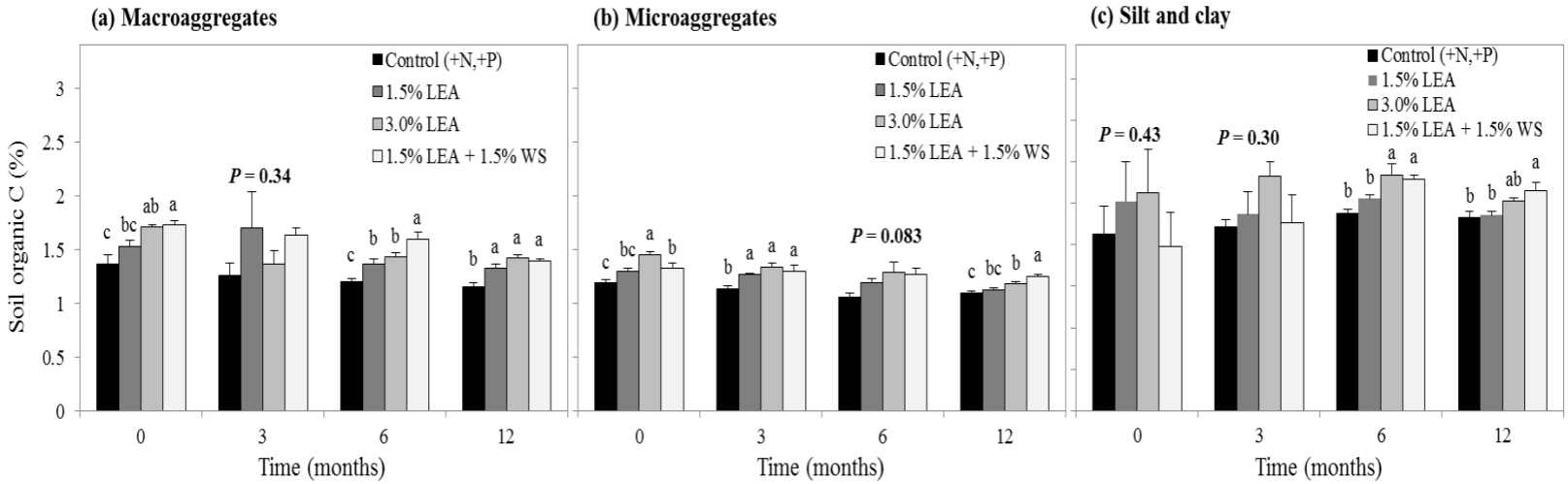

Fig. 5.5. Soil organic C (%) of fallow soil measured in situ throughout 12-month field incubation at 15-30 cm depth for size fractions of a) macroaggregates, b) microaggregates, and c) silt and clay. Means followed by the same letter within size fraction and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars represent standard error of the mean.

Stored LEA-C as a percentage of added C from the 1.5% LEA treatment immediately following treatment application at 0-5 and 5-15 cm was significantly less in the macroaggregate compared to the microaggregate and silt and clay size fractions; no difference was determined between the microaggregate and silt and clay fraction (Fig. 5.6). This trend continued for size fractions at 0-5 cm until 12 months after treatment application, at which time the percentage of C derived from LEA applied at 1.5% was greatest in the microaggregate fraction (Fig. 5.6a); however, at 5-15 cm no differences were seen for the portion of SOC between soil in size fractions at 12 months derived from LEA-C (Fig. 5.6b). Approximately 42% of added LEA-C remained in the 0-15 cm depth 12 months after amending soil with 1.5% LEA.

Differences were detected at all sampling times and depths between the percentages of C within size fractions derived from LEA applied at 3.0% (Fig. 5.7). Three months after treatment application, the greatest percentage of LEA-derived C in the 3.0% treatment at 0-5 cm was found in the microaggregate fraction, with this trend generally occurring throughout the observation period (Fig. 5.7a). At 5-15 cm with this treatment, the portion of SOC derived from LEA in microaggregates and the silt and clay fraction was greater than for macroaggregates during 0 to 6 months, but was only greater for microaggregates at 12 months (Fig. 5.7b). Approximately 66% of LEA-C remained in the 0-15 cm depth at 12 months after adding 3.0% LEA.

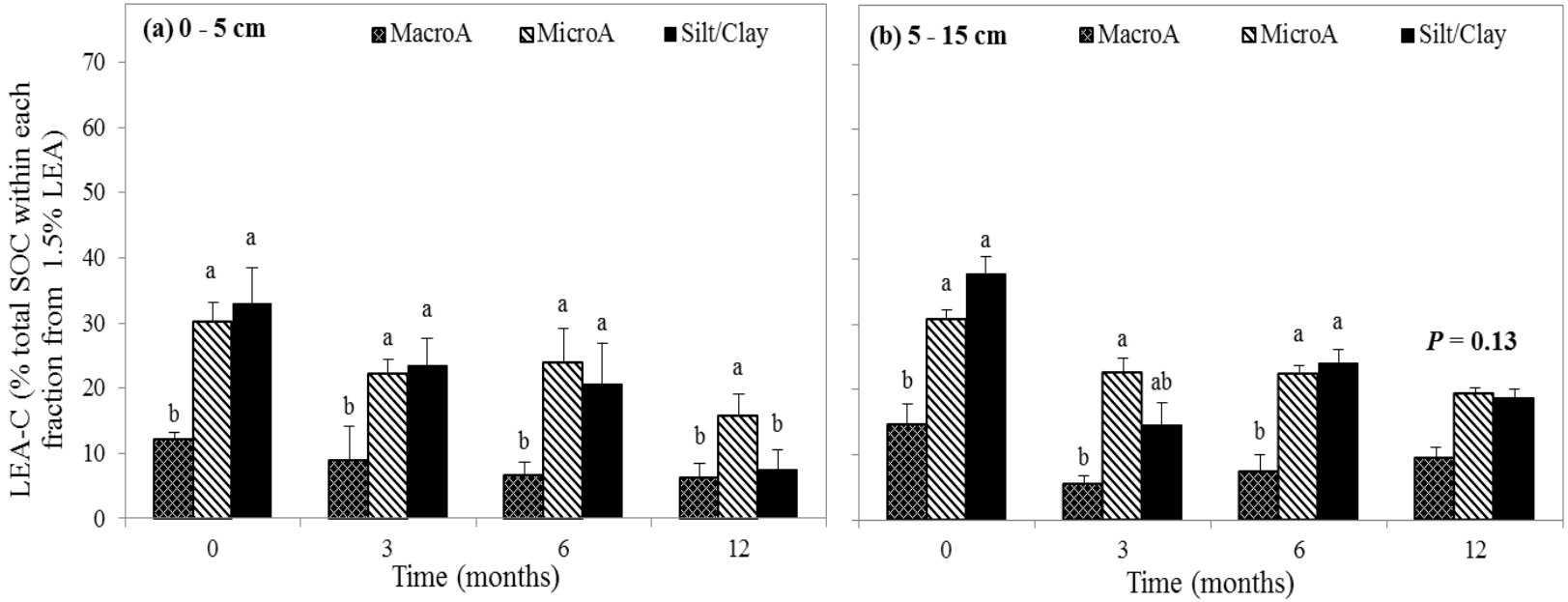

Fig. 5.6. Percentage of organic C within a) 0-5 and b) 5-15 cm depths and size fractions of macroaggregates (MacroA), microaggregates (MicroA), and silt and clay (Silt/Clay) (> 250, 53 - 250, and < 53 µm, respectively) derived from 1.5% LEA. Means within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA denotes lipid-extracted algae. Bars represent standard error of the mean.

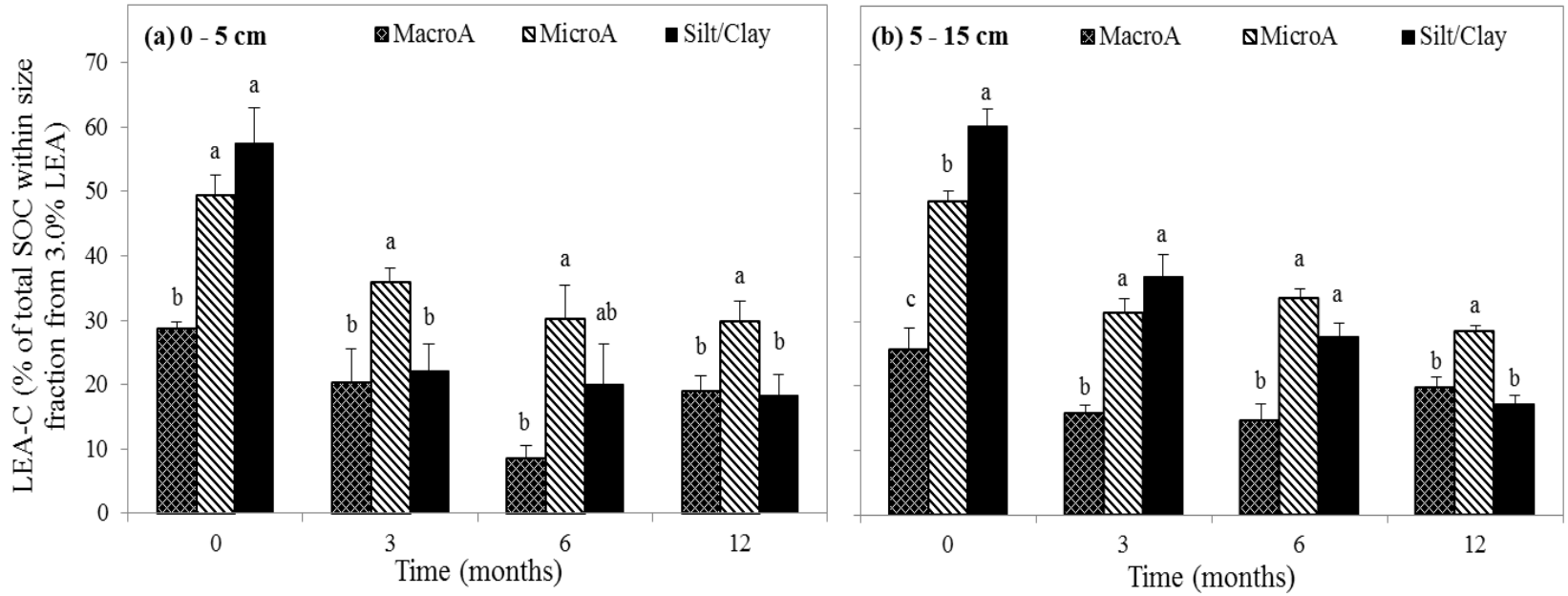

Fig. 5.7. Percentage of organic C within a) 0-5 and b) 5-15 cm depths and size fractions: macroaggregates (MacroA), microaggregates (MicroA), and silt and clay (Silt/Clay) (> 250, 53 - 250, and < 53 µm, respectively) derived from 3.0% LEA. Means within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA denotes lipid-extracted algae. Bars represent standard error of the mean.

Soil organic C within the 0-15 cm depth of bulk soil from the field column study at 0 months after treatment application was greatest with 1.5% LEA + 1.5% WS (1.7%), followed by 3.0% LEA (1.4%) (Fig. 5.8). No difference were detected between the control (+N,+P) and 1.5% LEA at this time. Similar trends were generally observed throughout the remainder of the study. Compared to the control (+N,+P), 3.0% LEA and 1.5% LEA + 1.5% WS increased SOC by 30% and 20%, respectively.

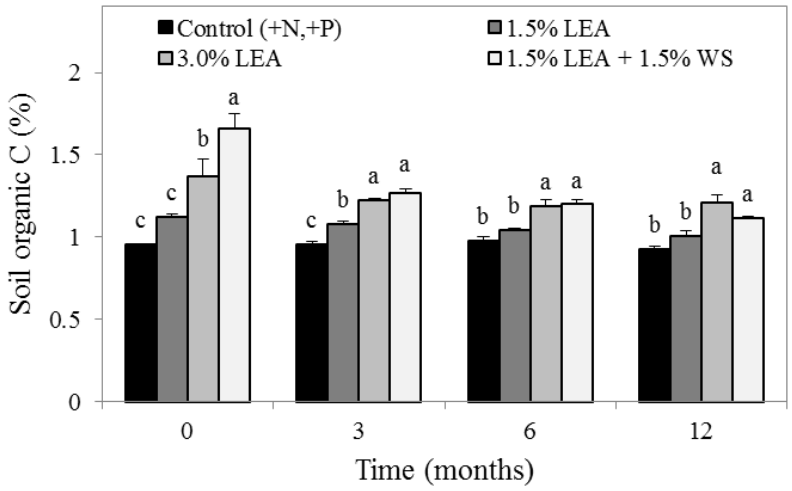

Fig. 5.8. Soil organic C determined in bulk, fallow soil at 0-15 cm depth throughout the 12-month field incubation. Means within time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. Bars above columns represent standard error of the mean.

Nitrogen Dynamics of Fallow Soil

Parrita soil was more enriched in ^{15}N ($\delta^{15}N = 8.3‰$) compared to LEA and WS, which had $\delta^{15}N$ signatures of 1.9 and 3.4‰, respectively. Differences in $\delta^{15}N$ signatures were noted at 0-5 cm depth within time for macroaggregates, microaggregates and silt and clay fractions, except for macroaggregates at 6 months ($P = 0.37$) and free silt and

clay at 3 and 6 months (P = 0.26 and P = 0.10, respectively) (Fig. 5.9a,b,c). Soil from 0-5 cm within columns receiving 3.0% LEA were generally most depleted in ^{15}N throughout the study. Macroaggregate $\delta^{15}N$ from the 0-5 cm depth at 12 months were least enriched in ^{15}N for soil amended with 3.0% LEA (5.5‰) compared to 1.5% LEA (6.6‰) and 1.5% LEA + 1.5% WS (6.6‰) or the control (+N,+P) (7.5‰) (Fig. 5.9a).

Microaggregate $\delta^{15}N$ values for this same depth at 12 months were similar for LEA-amendments ranging from 5.6‰ to 6.5‰, and were less enriched than the control (+N,+P) (7.6‰) (Fig. 5.9b). The $\delta^{15}N$ signature of the silt and clay fraction from this depth was significantly more depleted in ^{15}N for soil amended with 3.0% LEA (6.1‰) compared to other treatments or the control (+N,+P) (7.3‰) at time 0 month (Fig. 5.9c). Regardless of soil size fraction, the 3.0% LEA treatment resulted in less enriched signatures in 0-5 cm samples at 12 months (Fig. 5.9a,b,c). As with ^{13}C, the ^{15}N concentrations of all size fractions tended to become more enriched in ^{15}N over time. Concentrations of ^{15}N in silt and clay also tended to be more depleted than other size fractions.

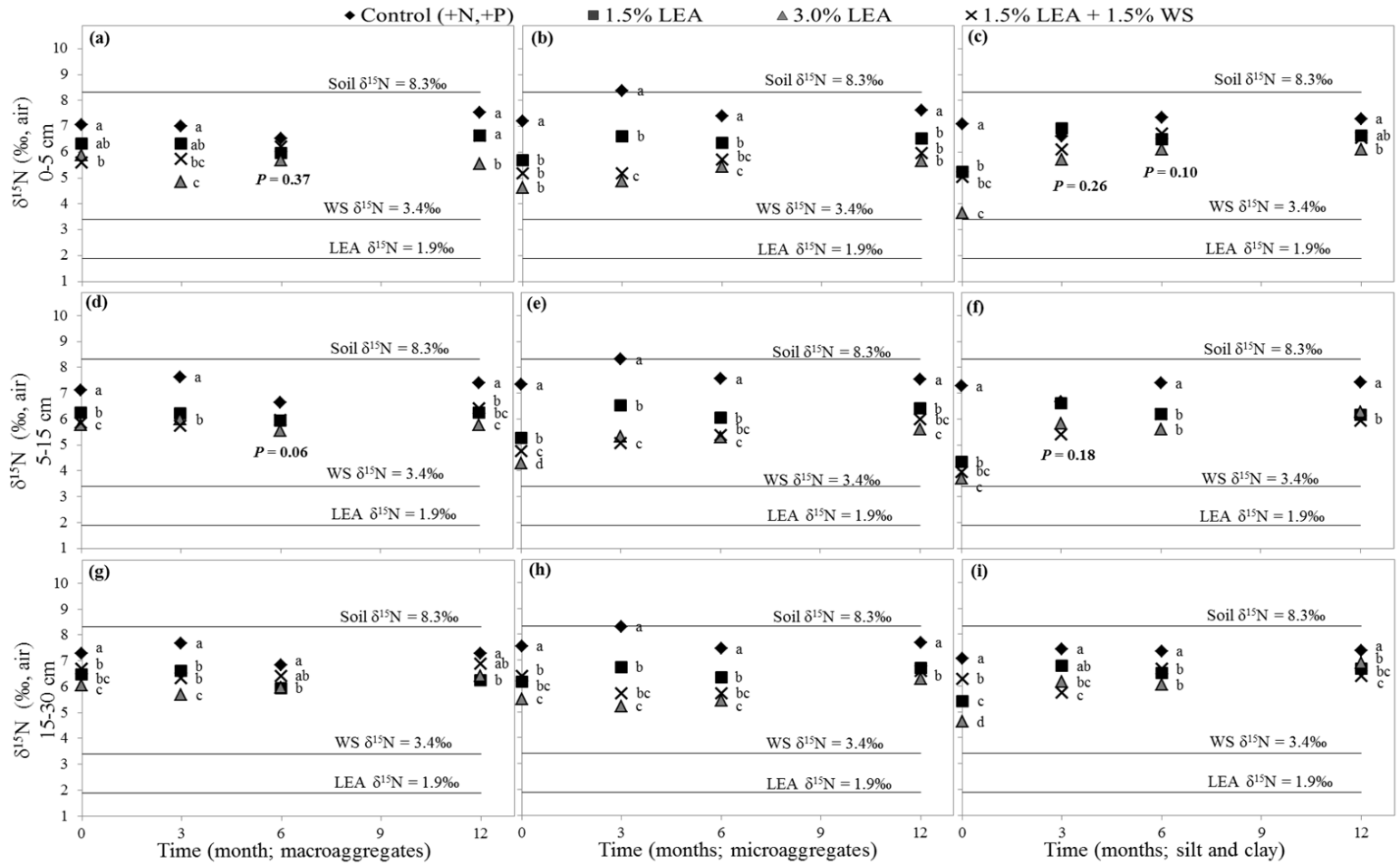

Fig. 5.9. Fallow soil $\delta^{15}N$ (‰) measured over 12-month in situ field incubation at depths (rows) of 0-5 cm (a-c), 5-15 cm (d-f), and 15-30 cm (g-i) and size fractions (columns) of macroaggregates (a,d,g), microaggregates (b,e,h), and silt and clay (c,f,i). Means followed by the same letter within depth, size fraction, and time are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively.

At 5-15 cm, treatment differences were observed for $\delta^{15}N$ within size fraction and time, except at 3 months for free silt and clay ($P = 0.18$) and at 6 months for macroaggregates ($P = 0.06$) (Fig. 5.9d,e,f). The macroaggregate fraction from the 5-15 cm soil depth at 0, 3, and 12 months for treatments with LEA had lower $\delta^{15}N$ values compared to the control (+N,+P) (Fig. 5.9d). Microaggregate $\delta^{15}N$ values for LEA treatments were similarly less enriched in ^{15}N compared to the control (+N,+P) at each measurement time (Fig. 5.9e). Twelve-months after treatment application, microaggregate $\delta^{15}N$ values for 1.5% LEA, 3.0% LEA, and 1.5% LEA + 1.5% WS decreased by 1.2‰, 1.6‰, and 1.0‰, respectively, compared to the control (+N,+P). At 0 months, $\delta^{15}N$ values of the silt and clay fraction of the 5-15 cm depth tended to be less enriched for LEA-amended soil compared to either macro- or microaggregates (Fig. 5.9d,e,f). However, at 12 months, $\delta^{15}N$ for the silt and clay fraction of LEA-amended soil tended to be more enriched compared to other fractions, yet all treatments were less enriched in ^{15}N compared to the control (+N,+P).

Significant differences within soil fraction and time were also detected for $\delta^{15}N$ at 15-30 cm (Fig. 5.9g,h,i). The $\delta^{15}N$ values of macroaggregates at this depth for LEA-amendments were less than the control (+N,+P) at 0 and 3 months; however, by 6 and 12 months, the 1.5% LEA + 1.5% WS treatment was similar to the control (+N,+P) (Fig. 5.9g). Soil amended with 1.5% and 3.0% LEA resulted in macroaggregate $\delta^{15}N$ signatures that were 1.1 and 0.9‰, respectively, less enriched in ^{15}N than the control (+N,+P) 12 months after treatment application. The microaggregate $\delta^{15}N$ signatures of LEA-amended soil at 15-30 cm were less enriched than the control (+N,+P) for all

sampling times. Moreover, the 3.0% LEA treatment resulted in the least enriched microaggregate $\delta^{15}N$ value (6.3‰) 12 months after treatment application (Fig. 5.9h). At 0, 6, and 12 months, $\delta^{15}N$ values of silt and clay were less for LEA-amended soil compared to the control (+N,+P), ranging from 0.5‰ to 1.0‰ less than the control (+N,+P) at 12 months (Fig. 5.9i). Differences in ^{15}N concentrations tended to be more distinctive with greater treatment separation than observed for ^{13}C (Figs. 5.2 and 5.9).

No differences in extractable soil NH_4^+-N between the control (+N,+P) and LEA treatments were detected in the 0-15 cm depth at 0, 3, and 6 months, after treatment application (Fig. 5.10a). By 12 months, however, the control (+N,+P) resulted in greater NH_4^+-N compared to soil amended with 1.5% LEA and 1.5% LEA + 1.5% WS. At 15-30 cm depth at 0 months, all LEA-amendments resulted in significantly greater NH_4^+-N (46 to 64 mg kg^{-1}) than the control (+N,+P) (22 mg kg^{-1}) (Fig. 5.10b). However, by 3 months, differences were no longer evident.

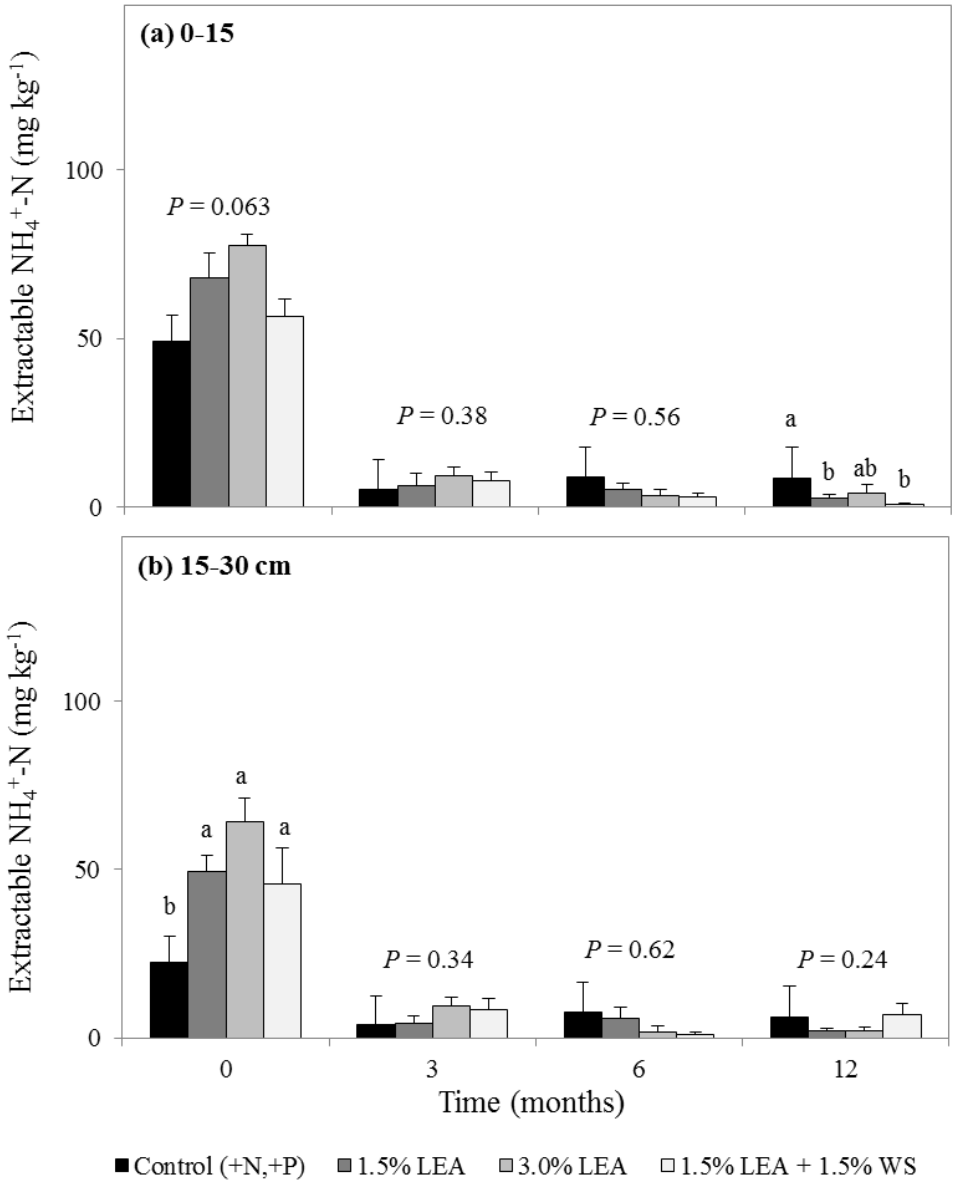

Fig. 5.10. Extractable soil NH_4^+-N at a) 0-15 and b) 15-30 cm depths of fallow soil determined over a 12-month field incubation. Means within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

Extractable soil NO_3^--N showed trends opposite that of NH_4^+, increasing with time instead of decreasing (Fig. 5.11). Extractable soil NO_3^--N was greatest for the

control (+N,+P) compared to amended soil at 0 months in 0-15 cm and 15-30 cm depths of bulk, fallow soil. At 3 months at 0-15 cm depth, 3.0% LEA-amended soil resulted in greater NO_3^--N (313 mg kg^{-1}) compared to the control (+N,+P) (46 mg kg^{-1}) or 1.5% LEA + 1.5% WS treatment (149 mg kg^{-1}) but not 1.5% LEA (222 mg kg^{-1}). Both 1.5% LEA and 1.5% LEA + 1.5% WS treatments exhibited significantly greater NO_3^--N values than the control (+N,+P) at this time (Fig. 5.11a). Nitrate-N at 6 months at this depth was similar for all LEA treatments, ranging from 222 to 322 mg kg^{-1} with all being greater than the control (+N,+P) (21 mg kg^{-1}). Twelve-months after treatment application, extractable NO_3^--N was greatest for the 3.0% LEA-amendment (305 mg kg^{-1}), while 1.5% LEA and 1.5% LEA + 1.5% WS treatments were similar to the control (+N,+P).

The control (+N,+P) at 3 months at 15-30 cm resulted in less extractable NO_3^--N (43 mg kg^{-1}) compared to 1.5% LEA (149 mg kg^{-1}), 3.0% LEA (131 mg kg^{-1}), and 1.5% LEA + 1.5% WS (152 mg kg^{-1}) treatments (Fig. 5.11b). By 6 and 12 months, the 3.0% LEA treatment resulted in significantly greater extractable NO_3^--N compared to the control (+N,+P) or 1.5% LEA and 1.5% LEA + 1.5% WS treatments.

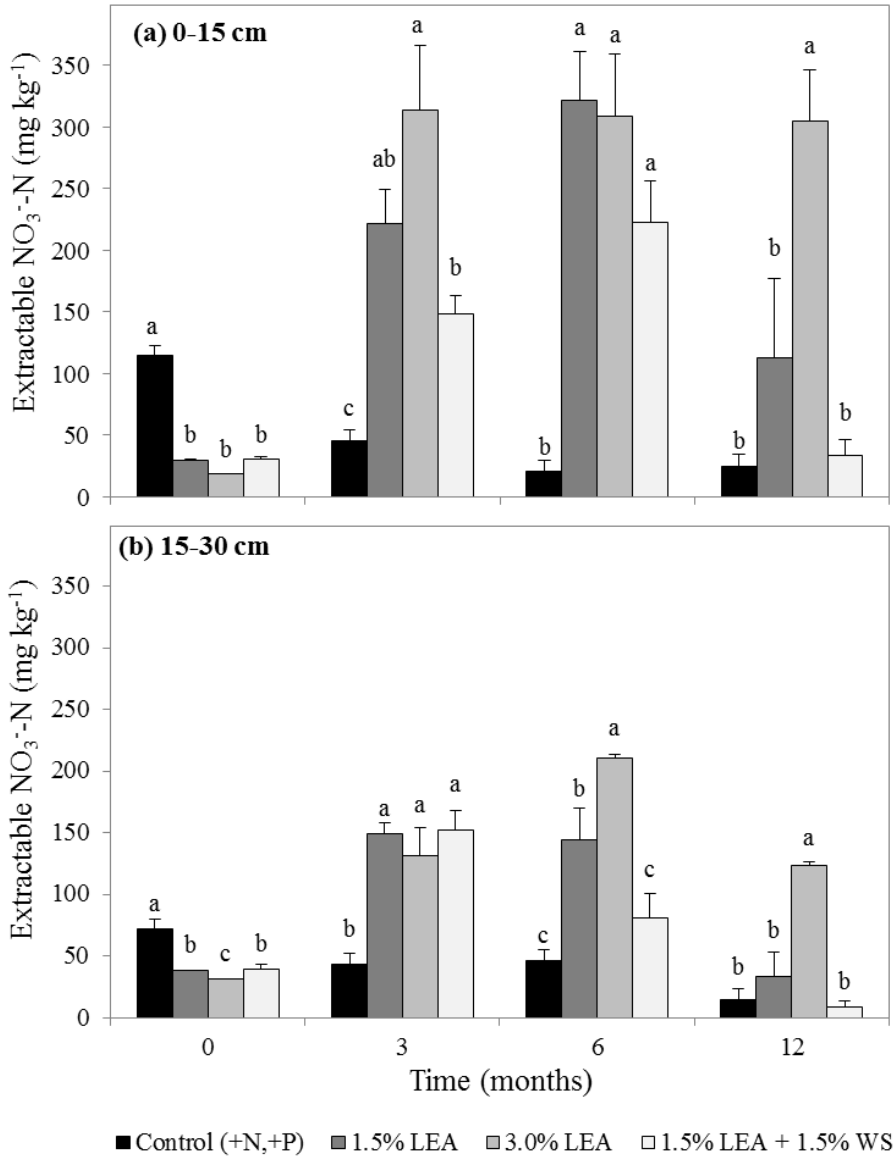

Fig. 5.11. Extractable soil NO_3^--N at a) 0-15 and b) 15-30 cm depths of fallow soil determined over a12-month field incubation. Means within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

At 0-15 cm depth 24-hr after treatment aplication, N mineralized as a percentage of added LEA-N and WS-N was greatest for 1.5% LEA compared to 3.0% LEA and

1.5% LEA + 1.5% WS (Fig. 5.12). At 3 months the percentage of N mineralized and remaining at 0-15 cm was significantly greater with 1.5% LEA (48%) compared to 1.5% LEA + 1.5% WS (26%) but not 3.0% LEA (34%). Soil amended with 1.5% LEA resulted in the greatest percentage of N mineralized 6 months after treatment application (69%). Twelve months after treatment application, the percentage of N mineralized and remaining within 0-15 cm fallow soil was statistically greater with 1.5 and 3.0% LEA (46% and 32%, respectively) compared to 1.5% LEA + 1.5% WS (6%).

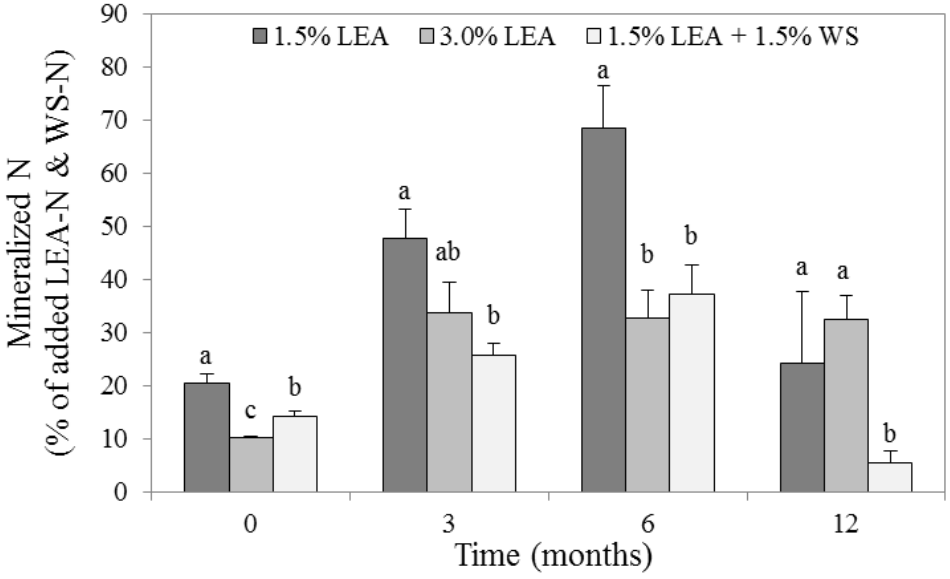

Fig. 5.12. Mineralized N (NH_4^+-N + NO_3^--N) remaining in 0-15 cm of fallow soil as a percentage of added LEA-N and WS-N determined over a 12-month field incubation. Means within time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

Soil total N determined 24 hr after treatment application within 0-15 cm of fallow soil was significantly greater with 3.0% LEA (0.19 %) compared to the control (+N,+P) (0.14%) or 1.5% LEA treatment (0.16%) but not 1.5% LEA + 1.5% WS (0.19

%) (Fig. 5.13a). At 3 months in this soil depth, total N was greatest with the 3.0% LEA (0.21%), with the trend generally continuing with time. Total N of amended soils was greater than the control (+N,+P) at 3 through 12 months. Values for total soil N were much more stable with time compared to SOC (Fig. 5.8). No differences occurred for soil total N at 0 and 3 months after treatment application within the 15-30 cm depth (Fig. 5.13b). By 6 months, however, trends were similar to that observed at 0-15 cm.

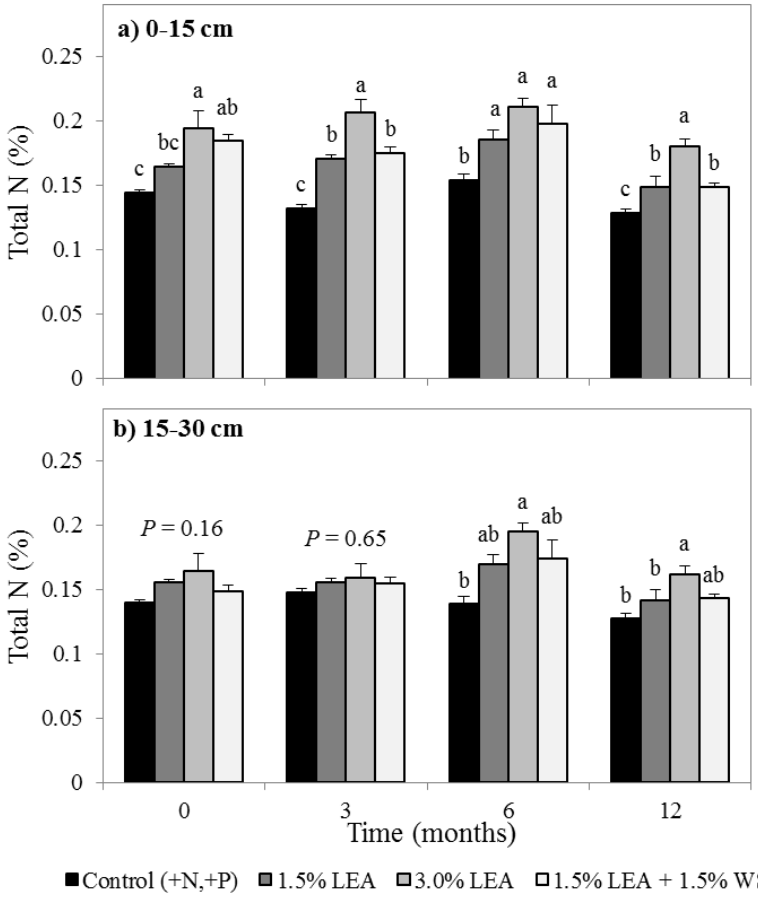

Fig. 5.13. Total N within a) 0-15 and b) 15-30 cm depths of fallow soil over a 12-month field incubation. Mean total N within depth and time followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD. LEA and WS denote lipid-extracted algae and wheat straw, respectively. Bars above columns represent standard error of the mean.

Ryegrass Growth and Nutrient Availability and Uptake

Both pre-plant (3 months after treatment application) and post-harvest, treatment differences were significant for each extractable soil nutrient except NH_4^+-N and Cu (Table 5.4). Pre-plant extractable NO_3^--N, P, Ca, Mg, S, Na, and Zn were greatest for 3.0% LEA, but only P, Ca, S, and Na were greater at post-harvest for this treatment. Pre-plant NO_3^--N at pre-plant was approximately 7 and 5 times greater for 3.0% LEA and 1.5% LEA treatments, respectively, compared to the control (+N,+P). No significant difference occurred for either pre-plant or post-harvest extractable Na between 1.5% LEA and 1.5% LEA + 1.5% WS treatments. Both pre-plant and post-harvest extractable K was greatest for 1.5% LEA + 1.5% WS. Pre-plant extractable Fe and Mn were both greatest in the control (+N,+P) treatment, and this trend continued for post-harvest Mn.

Herbage mass was significantly different between treatments ($P = 0.003$), with 1.5% LEA yielding the greatest HM followed by the control (+N,+P), 3.0% LEA, and 1.5% LEA + 1.5% WS (Fig. 5.14). Control (+N,+P) and 3.0% LEA treatments produced similar HM, while, the 1.5% LEA + 1.5% WS resulted in significantly less HM than the control (+N,+P), but similar to 3.0% LEA.

Table 5.4. Nutrient availability in 0-15 cm soil depth as affected by treatment prior to seeding-ryegrass (pre-plant) and after harvest (post-harvest) in the field study.

Treatment	NO_3^--N	NH_4^+-N	P	K	Ca	Mg	S	Na	Fe	Zn	Mn	Cu
	mg kg^{-1}											
Pre-plant												
Control (+N,+P)	46 c[†]	5.3	64 b	252 c	3102 c	329 c	10.7 c	59 c	8.4 a	0.3 d	43.0 a	0.42
1.5% LEA[‡]	222 ab	6.1	66 b	265 c	3662 b	384 b	34.8 b	795 b	4.4 b	0.4 c	20.8 b	0.39
3.0% LEA	313 a	33.9	100 a	303 b	4135 a	457 a	98.8 a	1886 a	4.1 b	0.6 a	21.1 b	0.50
1.5% LEA + 1.5% WS[§]	149 b	7.7	66 b	411 a	3559 b	377 b	35.4 b	749 b	3.8 b	0.5 b	25.2 b	0.42
p -value	0.0004	0.38	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	<0.0001	0.021	<0.0001	<0.0001	0.15
Post-harvest												
Control (+N,+P)	42	9.1	47 c	226 b	2696 d	280 c	8.6 c	170 b	3.8 a	0.3 c	34.1 a	0.41
1.5% LEA	27	8.5	47 c	236 b	3349 c	334 b	10.0 c	222 b	3.1 a	0.3 b	17.4 c	0.43
3.0% LEA	44	13.9	84 a	255 b	3868 a	383 a	25.0 a	814 a	3.1 ab	0.4 a	13.7 d	0.42
1.5% LEA + 1.5% WS	49	11.2	59 b	399 a	3587 b	375 a	14.6 b	299 b	2.1 b	0.4 a	20.8 b	0.36
p -value	0.25	0.063	<0.0001	<0.0001	<0.0001	0.0002	<0.0001	<0.0001	0.023	0.0001	<0.0001	0.26

[†]Within column and time, means followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD.

[‡]LEA denotes lipid-extracted algae.

[§]WS denotes wheat straw.

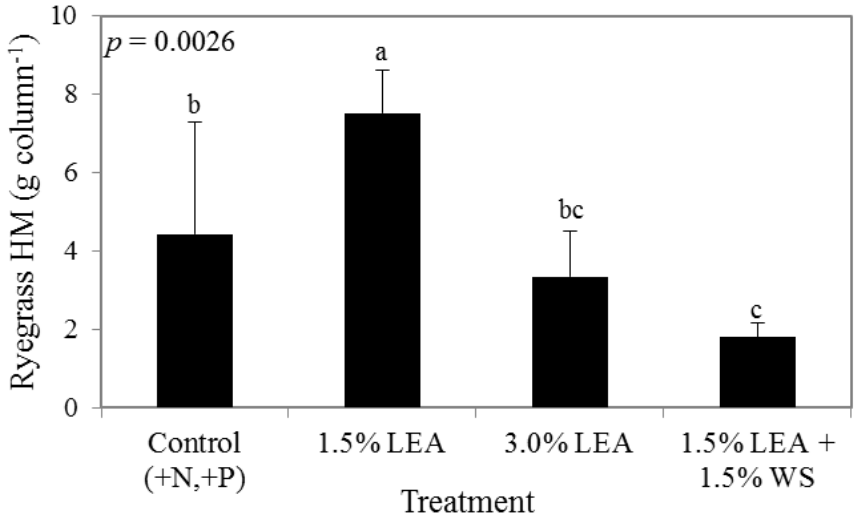

Fig. 5.14. Mean ryegrass herbage mass (HM) collected from one cutting in the field study. Bars represent standard error of means. Means followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD.

Significant treatment differences were observed for plant nutrient concentrations including N, P, Ca, Mg, Na, and Zn but not K, S, Fe, Cu, and Mn (Table 5.5). Nitrogen concentration was significantly greater with 3.0% LEA compared to all other treatments. Treatments of 3.0% LEA and 1.5% LEA + 1.5% WS resulted in greater plant concentrations of P and Na compared to the control (+N,+P). Calcium, Mg, and Zn plant concentrations were significantly greater with the control (+N,+P) compared to LEA treatments.

Table 5.5. Mean plant nutrient concentrations and nutrient uptake by ryegrass in the field study.

Treatment	N	P	K	Ca	Mg	Na	Zn	Fe	Cu	Mn	S
	---% ---	----------	----------	----------	----------	mg kg^{-1}	----------	----------	----------	----------	----------
Plant minerals											
Control (+N,+P)	2.7 b[†]	2509 c	37824	11469 a	4149 a	1740 b	47.0 a	220	28.7	62.6	3213
1.5% LEA[‡]	3.0 b	3872 bc	33561	7614 b	3000 bc	4481 b	22.1 b	513	33.8	74.0	2624
3.0% LEA	4.6 a	4276 b	31162	6816 b	2684 c	16913 a	23.1 b	313	26.2	74.9	2779
1.5% LEA + 1.5% WS[§]	2.6 b	5958 a	41214	7160 b	3319 b	12730 a	28.3 b	273	28.1	85.2	3200
p -value	<0.0001	0.0023	0.098	0.0022	0.0015	<0.0001	0.0009	0.59	0.75	0.44	0.10
	g col^{-1}	----------	----------	----------	----------	mg col^{-1}	----------	----------	----------	----------	----------
Nutrient uptake											
Control (+N,+P)	0.12 bc	11 b	164 ab	50 ab	18 ab	7 b	0.2 a	1.0	0.1 ab	0.3 b	13 ab
1.5% LEA	0.22 a	29 a	254 a	58 a	22 a	34 ab	0.2 ab	4.2	0.3 a	0.6 a	20 a
3.0% LEA	0.15 ab	14 b	103 b	23 bc	9 bc	60 a	0.1 bc	1.1	0.1 b	0.2 b	9 bc
1.5% LEA + 1.5% WS	0.05 c	11 b	73 b	13 c	6 c	23 b	0.1 c	0.5	0.1 b	0.2 b	6 c
p -value	0.008	0.0029	0.0086	0.014	0.011	0.013	0.023	0.25	0.036	0.011	0.0051

[†] Within column and concentration or uptake, means followed by the same letter are not significantly different at $P < 0.05$ by Fisher's protected LSD.

[‡] LEA denotes lipid-extracted algae.

[§] WS denotes wheat straw.

Except for Fe, significant treatment effects were observed for nutrient uptake (Table 5.5). Nitrogen uptake by ryegrass was significantly greater in the 1.5% LEA treatment than the control (+N,+P) and 1.5% LEA + 1.5% WS treatment but not 3.0% LEA. Phosphorus uptake was greatest from soil amended with 1.5% LEA. Plants grown in 1.5% LEA-treated soil also had significantly greater K, Ca , Mg, S, Cu, and Mn uptake than all treatments except for the control (+N,+P), primarily because of greater HM produced. As a result of Na added with the 3.0% LEA treatment significantly increasing ryegrass Na concentration, Na uptake by ryegrass was greatest for this treatment even though HM produced was second to lowest. Zinc uptake was significantly greater for the control (+N,+P) vs. 3.0% LEA and 1.5% LEA + 1.5% WS treatments but not 1.5% LEA.

DISCUSSION

Carbon and Nitrogen Dynamics

Aggregate Formation

Greater aggregate MWD for the control compared to treatments was observed from 0 to 6 months after treatment application, possibly resulting from aggregate disruption when soil was collected, homogenized, and mixed with treatments (Chivenge et al., 2011b), or, high levels of Na^+ in LEA and K^+ in WS may have negated the positive effects of organic amendments on aggregation. Whalen and Chang (2002) also attributed the decrease of macroaggregates after manure application to the breakdown of larger macroaggregates due to dispersion of soil colloids caused by monovalent cations

of Na^+ and K^+ present in animal manure. However, MWD at 12 months for soil amended with 3.0% LEA after treatment application tended to be greater compared to the control or other treatments, and was followed by the 1.5% LEA treatment. Greater MWD in soil amended with 3.0% LEA indicated a greater proportion of macro- and microaggregates, and potentially greater SOC storage over time (Plante and McGill, 2002; Six et al., 2002). Chivenge et al. (2011b) also observed an increase in MWD, which was determined to be the result of a greater proportion of macro- and microaggregates.

Soil treated with 1.5% and 3.0% LEA enhanced aggregate formation compared to 1.5% LEA + 1.5% WS. The addition of organic amendments and plant residues, such as animal manure and WS, respectively, have been demonstrated to improve soil stability by enhancing aggregate formation (Gulde et al., 2008; Six et al., 1999; Whalen and Chang, 2002); however, this study demonstrated that LEA applied with WS had a lesser effect on aggregate formation compared to LEA applied alone. These results may be attributed to the initial rapid mineralization and greater proportion of recalcitrant compounds in LEA compared to WS. Quickly decomposing organic materials with a narrow C:N ratio may produce a rapid but likely only temporary increase in aggregate production, whereas slowly decomposing or more stable organic materials may produce a lesser but more permanent improvement in aggregation (Khaleel et al., 1981; Sun et al., 1995). Thus, the combination of LEA being initially quickly mineralized and comprised of a greater proportion of recalcitrant compounds (algaenans), aggregate formation and stability may both be enhanced with LEA application.

Dynamics of $\delta^{13}C$ and Soil Organic Carbon in Aggregate Size Fractions

The $\delta^{13}C$ isotopic signature of macroaggregates in soil amended with 3.0% LEA was most depleted in ^{13}C 12 months after treatment application, indicating greater macroaggregate LEA-C for this treatment at 0-5 and 5-15 cm soil depths (Fig. 5.2a,d). Regardless of LEA treatment, microaggregates from soils receiving LEA stored more C compared to the control (+N,+P) at 0-5 and 5-15 cm depths as evidenced by more depleted $\delta^{13}C$ values for these treatments (Fig 5.2b). For LEA-treatments, $\delta^{13}C$ signatures at 12 months at 0-5 and 5-15 cm depths tended to indicate greater LEA-C in micro- compared to macroaggregates. However, at 15-30 cm depth, there was a tendency for greater LEA-C in macro- rather than microaggregates. These results demonstrate the transformation of LEA during aggregate formation by $\delta^{13}C$ signatures increasing from 0 to 12 months, but they do not explain aggregate formation *per se*.

Based on $\delta^{13}C$ results, greater LEA-C was associated with the silt and clay fraction immediately following treatment application, but over time, less LEA-C was associated with the silt and clay fraction, while greater LEA-C storage was observed in the macro- and microaggregate fractions, demonstrating that with LEA application aggregate formation was enhanced. Microaggregate associated C may be physically more protected and biochemically more recalcitrant than that of macroaggregates (Jastrow, 1996; Six et al., 2000), and therefore, it may be possible to sequester SOC with LEA.

The change of $\delta^{13}C$ isotope composition from 0 to 12 months may be the result of both: 1) preferential stabilization of substrates with light (lipids, lignin, and phenols)

or heavy (cellulose, amino acids, and hemicellulose) $\delta^{13}C$, and 2) stabilization of organic materials after passing one or more microbial utilization cycle, which releases lighter CO_2-C and leads to heavier $\delta^{13}C$ in remaining OM (Werth and Kuzyakov, 2010). With the above two scenarios occurring together, the change of $\delta^{13}C$ isotopic composition would not be lesser if only one or the other occurred, thus explaining why the difference of $\delta^{13}C$ from 0 to 12 months for 1.5 and 3.0% LEA was greater in the silt and clay fraction compared to macro- and microaggregates. Likely, LEA-C in all sizes was being utilized by microorganisms, therefore increasing $\delta^{13}C$, but it was only in macro- and microaggregates that the more recalcitrant C, which is lighter and more depleted in ^{13}C, was being stored (Chivenge et al., 2011b)

Soil OC tended to be greater in the silt and clay fraction compared to macro- or microaggregates, possibly resulting from aggregate destruction during soil collection and treatment preparation, which consequently, exposes protected OM to decomposers and accelerates SOM decomposition (Cambardella and Elliott, 1993). Soil was homogenized by mixing prior to treatment preparation for the control (+N,+P) and all other treatments in order to reduce experimental error, but consequently may have somewhat disrupted soil structure (Chivenge et al., 2011b). Comparing the 0-5 and 5-15 cm depths 12 months after treatment application, macroaggregate SOC was likely to be greater at 0-5 cm, while microaggregate SOC tended to be greater at 5-15 cm. Soil amended with 1.5% LEA, 3.0% LEA or 1.5% LEA + 1.5% WS resulted in greater SOC at 12 months for all soil size fractions and depths. Thus, LEA addition increased SOC at least in the short-

term. Regardless of organic amendment, Chivenge et al. (2011b) observed greater macro- and microaggregate SOC and N than in the silt and clay fraction.

Dynamics of $\delta^{15}N$ in Aggregate Size Fractions

Adding NH_4NO_3 fertilizer depleted the soil in ^{15}N, with NH_4^+ ($\delta^{15}N = 0‰$) being more depleted than NO_3^- ($\delta^{15}N = 3‰$) (Fry, 2006). Soil amended with 3.0% LEA had more depleted $\delta^{15}N$ values compared to the control (+N,+P), 1.5% LEA and 1.5% LEA + 1.5% WS for macro- and microaggregates at 0-5 cm, thus indicating greater LEA-N in these size fractions with this treatment after 12 months at this depth (Fig. 5.9). At 5-15 and 15-30 cm depths at 12 months, 3.0% LEA also tended tohave greater N in macro- and microaggregates compared to 1.5% LEA, but to a lesser extent in the macroaggregate fraction compared to that observed in the 0-5 cm depth. As organic N is microbiall processed and transformed, such as when manure is composted, lighter ^{15}N is released as N_2O, resulting in heavier and more ^{15}N-enriched residual N (Lynch et al., 2006); this effect may explain the more enriched ^{15}N values in microaggregates and silt and clay for LEA treatments from 0 to 12 months.

Soil Nitrogen Transformations

Although no differences in soil NH_4^+-N concentrations were noted among treatments 24-hr after treatment application (Fig. 5.10a), NO_3^--N at this time was less for the control (+N,+P) than LEA-amendments (Fig. 5.11a), implying that a portion of LEA-N was initially inorganic NO_3^-. Lipid-extracted algae-N mineralized at a relatively rapid

rate, resulting in greater soil NH_4^+-N with the addition of LEA. After 3 months, LEA amendments resulted in greater soil NO_3^--N ranging from approximately 150 to 300 mg kg^{-1}. At these concentrations, plant N deficiencies would not be likely, although environmental issues may arise due to NO_3^- leaching or runoff (Whalen et al., 2001). At 6 months, the concentration of TN was nearly as great at 15-30 cm as at 0-15 cm, implying significant leaching of LEA associated inorganic N and dissolved organic N (DON). With at most 34% of added LEA-N mineralized in the 3.0% LEA treatment, a large percentage of LEA-N was stored and may become available for future plant uptake or loss. Similarly, Lynch et al. (2006) recovered greater than 80% of composted manure-N in the more coarse soil fractions one year after application.

Nutrient Availability and Ryegrass Herbage

As was expected, the availability of most nutrients, except Fe and Mn was greatest for LEA amended soil prior to planting ryegrass (Table 5.4); however, it was not hypothesized that Fe and Mn would not be as great for LEA treatments. The increased soil pH ranging from 8.2 to 8.3 for each of the three LEA treatments (Table 5.1) compared to the control (+N,+P) pH of 7.2 may explain the decreased availability of Fe and Mn (Soliman et al., 1992). Since low nutrient availability was not a concern in soil amended with 3.0% LEA, excess salts associated with Ca, and especially Na, may have been the reason for decreased HM with the 3.0% LEA treatment compared to control (+N,+P) and 1.5% LEA treatments (Marcar, 1987; Mueller and Bowman, 1989). Salinity was likely a major drawback to the use of LEA for ryegrass production. Applying LEA

at 1.5% rather than 3.0% showed that at a lower application rate, salt-tolerant grasses should benefit from increased nutrient availability with LEA-application.

CHAPTER VI

CONCLUSIONS

Lipid-extracted algae residue is mineralizable, but LEA tended to be more resistant to decay than WS and may therefore sequester greater SOC. The recalcitrance of LEA may be associated with nonhydrolyzable macromolecules located in microalgae cell walls termed algaenans that comprised 13.4% (DM basis) of lipid-extracted *Nannochloropsis salina* algae residue. Lipid-extracted algae added at 1.5 and 3.0% by weight lost a smaller percentage of added C as CO_2 compared to WS amendments, including 0.75% LEA + 0.75% WS and 1.5% WS (+N,+P) over an 85-d period. Thus, LEA amendments may have the potential due to greater SOC sequestration to mitigate, or offset, some GHG effects of agricultural production.

Although salinity resulting from 3.0% LEA addition did not appear to delay N mineralization compared to the 1.5% LEA application rate, nitrification of NH_4^+ was inhibited for a period of time with 3.0% LEA. Possibly caused by this delay, proportionally less N mineralization occurred with 3.0 compared to 1.5% LEA. However, the amount of plant available N released from 1.5 and 3.0% LEA additions (336 and 293 mg N kg^{-1}, respectively) by the end of the study would not only be sufficient to support a single crop, but also possibly multiple crops. Fallow soil with excessive NO_3^--N may result in leaching and/or runoff and denitrification losses and consequently, environmental pollution. Grasses, such as pearl millet, ryegrass, and sorghum-sudangrass of moderate or better salt-tolerance may benefit from increased

nutrient availability following LEA application, but at high application, LEA may deter growth due to increased salt concentration. However, growing salt-tolerant grasses or crops on LEA-amended soil may aid in removing excess salts over time.

Germination and emergence of sorghum-sudangrass was greater than that of foxtail or pearl millet, indicating greater seedling-tolerance to LEA. Pearl millet, on the other hand, exhibited greater tolerance at later growth stages as HM was enhanced by 3.0% LEA in the third harvest. Lipid-extracted algae applications of 1.5 or 3.0% produced sorghum-sudangrass HM equivalent to the positive control (+N,+P). Even though this treatment received 561 and 112 kg ha^{-1} of N and P, respectively, increased pearl millet and sorghum-sudangrass HM with 3.0% LEA was likely the result of greater N and P availability with this treatment.

Available soil N, P, Ca, Mg, and S after the final harvest of foxtail millet, pearl millet, and sorghum-sudangrass was greater for 3.0% LEA or 1.5% LEA + 1.5% WS, and sufficient nutrients may have been available to support an additional harvest. Also, it may be possible for LEA to efficiently support a rotation of a warm-season, salt-tolerant grass followed by a cool-season grass. These results demonstrated the potential benefit of LEA over inorganic fertilizer application for moderately salt-tolerant grasses, such as pearl millet, ryegrass, and sorghum-sudangrass.

The short-term nitrification inhibition observed in soil treated with 3.0% LEA may have potential application as an organic nitrification inhibitor when applied to established plants; however, additional work will be required to investigate the mechanism for the observed inhibition. Moreover, delayed nitrification may possibly-

prolong plant available N by decreasing NO_3^- losses from soil, and consequently reducing environmental concerns.

The availability of macro and micronutrients in soil treated with LEA suggested the potential for a single LEA application supporting multiple cuttings of a salt-tolerant grass species or possibly multiple rotations of warm and cool season plants. Most likely a result of specific ion toxicity, LEA application prior to planting salt-tolerant ryegrass reduced seedling emergence and HM. Future studies should focus on effects of extending the time from LEA application to planting, adding LEA post-emergence, or applying at lower rates (< 1.5%).

Soil LEA-application should be a significant source of organic nutrients for microbial transformation and usage and plant uptake, and thus, may reduce inputs of inorganic fertilizer. Addition of LEA may enhance aggregate formation and SOC storage since aggregate MWD and SOC within macro- and microaggregates of LEA treatments increased over time.

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