THESIS

EFFECTS OF CYANOBACTERIAL FERTILIZERS COMPARED TO COMMONLY-USED ORGANIC FERTILIZERS ON NITROGEN AVAILABILITY, LETTUCE GROWTH, AND NITROGEN USE EFFICIENCY ON DIFFERENT SOIL TEXTURES

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ABSTRACT

EFFECTS OF CYANOBACTERIAL FERTILIZERS COMPARED TO COMMONLY-USED ORGANIC FERTILIZERS ON NITROGEN AVAILABILITY, LETTUCE GROWTH, AND NITROGEN USE EFFICIENCY ON DIFFERENT SOIL TEXTURES

Nitrogen plays a crucial role in synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis, and yield development of lettuce. Generally, organic farmers use composted manure, legume cover crops, and off-farm fertilizers such as fish emulsion to meet the nitrogen (N) demand of crops. However, the nutrient composition of off-farm fertilizers such as composted manure and fish emulsion varies widely depending on animal species and often have higher transportation costs. Therefore, an evaluation of the application of cyanobacteria in comparison to the commonly-used organic fertilizers was conducted as an alternative potential N biofertilizer. The laboratory soil incubation and greenhouse studies were conducted to evaluate the effect of N availability from potentially mineralizable N on different types of soil textures. Then, a greenhouse study was conducted to assess the effect of N availability from cyanobacterial fertilizers compared to the commonly-used organic fertilizers on lettuce growth, fertilizer recovery and lettuce root response on N use efficiency. Lettuce (*Lactuca sativa*) is a shallow-rooted crop and requires an extensive amount of N fertilizer to produce yield.

The aims of the soil incubation study were to determine the rates of mineralization for different organic fertilizers, influence of soil texture on N mineralization, and to evaluate changes in soil microbial biomass from fertilizer application to sandy and clayey soils. In this study, N mineralization potential of cyanobacterial ferilizers were compared with traditional organic fertilizers in two soils with contrasting textures in a laboratory incubation study at constant temperature (25^{o}C) and moisture content (60% water-filled pore space) for 140 days. Soils were destructively sampled over the course of 140 days and analyzed for NH_4^+-N, and NO_3^--N, soil microbial biomass C, soil organic C, and soil total C and N. In

both soils, soil NH_4^+-N was the highest at day 56 and decreased from day 56 to 140 due to its conversion to soil NO_3^--N. Compost treatment significantly increased soil microbial biomass C (207.5 mg C kg^{-1} soil) compared to fish emulsion (115.42 mg C kg^{-1} soil) in sandy soil. The N availability was 9% greater from fish emulsion than liquid cyanobacteria, and 6% greater from solid cyanobacteria than compost in sandy soil. The fish emulsion treatment showed 5% higher N availability compared to the solid and liquid cyanobacterial fertilizers.

In the greenhouse study, percentage fertilizer recovery (PFR) was quantified to assess the efficiency of N uptake by lettuce to produce yield. A greenhouse study was conducted for 63 days to evaluate cyanobacterial and traditional organic fertilizers application on lettuce N response. Total leaf area, fresh yield, leaf dry weight, and leaf total N content were measured at the end of the greenhouse study. Total N uptake in lettuce tissue and PFR were calculated based on the analyses results. Soil applied fish emulsion recorded significantly higher fresh yield at 112 kg N ha^{-1} (147 g) compared to 56 kg N ha^{-1} (117 g) in clayey soil relative to sandy soil. Soil-applied liquid cyanobacteria recorded significantly higher yield compared to composted manure by 58%. Solid cyanobacteria recorded significantly higher total N uptake at 56 kg N ha^{-1} compared to 112 kg N ha^{-1} in clayey soil. In conclusion, soil applied fish emulsion treatment recorded higher PFR (99%) than soil applied composted manure (44%) at 56 kg N ha^{-1} on clayey soil. Soil applied fish emulsion has significantly higher PFR (57%) compared to the combination soil and foliar fertilizer (FFCom and FLScyb) at 56 kg N ha^{-1} in sandy soil.

Nitrogen is also acquired from the soil by the plant roots. In the greenhouse study, root response to N fertilization was assessed to determine the efficiency of N uptake by lettuce to produce yield. A greenhouse study was conducted for 63 days to evaluate cyanobacterial and traditional organic fertilizers application on lettuce root response. Root: shoot ratio, root dry weight, root surface area, and root length density were measured at the end of the greenhouse study. Nitrogen use efficiency (NUE) was calculated based on the analyses results. There was no significant difference observed in root dry weight. The composted manure (Com) treatment recorded significantly higher root: shoot ratio at 56 kg N ha^{-1} while foliar and soil applied liquid cyanobacteria (FLScyb) treatment recorded lower root: shoot ratio at 112 kg

N ha^{-1}. The foliar applied fish emulsion and soil applied composted manure (FFCom) treatment recorded the highest root surface area compared to other treatments at 112 kg N ha^{-1} on clayey soil . The FLScyb treatment recorded higher root surface area compared to the Com treatment at 112 kg N ha^{-1} on sandy soil. The fish emulsion (Fish) treatment recorded higher root length density at 112 kg N ha^{-1} on clayey soil while FLSCyb recorded higher root length density on sandy soil at 112 kg N ha^{-1} compared to the Fish and solid cyanobacteria (Scyb) treatments. In conclusion, the Fish treatment recorded 35 % higher NUE at 56 kg N ha^{-1} on clayey soil while Scyb treatment has significantly 24% higher NUE compared to Com treatment at 56 kg N ha^{-1} in sandy soil.

Overall, the soil applied fish emulsion treatment recorded higher percentage fertilizer recovery and NUE compared to the solid and liquid cyanobacterial fertilizers at 56 kg N ha^{-1} on clayey soil. However, the combined soil and foliar cyanobacterial fertilizer and soil applied solid and liquid cyanobacterial ferilizers recorded higher percentage fertilizer recovery and NUE at 56 kg N ha^{-1} compared to the composted manure which correspond to lettuce yield component which was higher in fish emulsion compared to the composted manure.

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OVERALL INTRODUCTION

Nitrogen (N) is generally the most difficult nutrient to manage for organic crop production, and N uptake is dependent on the amount of plant-available N supplied by the soil. Before N in organic matter (OM) can be taken up by plant roots, the organic matter must be broken down so that N is in the form of NH_4^+ or NO_3^- ions. Nutrient management in organic systems should be approached from an ecosystem perspective that, acknowledges the importance of plants, soil organic matter (SOM), soil organisms in regulating N availability (Watson et al., 2002). In organic systems, the most important limiting factor appears to be N availability since N in soil commonly exists in organic forms that are not available to plants (Clark et al., 1999). In managing plant nutrients over continuing crop cycles, farmers face the challenge of estimating the amount of the soil organic N resource that is made available for plant uptake overtime (Deenik, 2006).

Nitrogen mineralization (N_{min}) is the process that controls N availability to plants (Aber and Melillo, 2001). It is important to estimate how much net N_{min} may vary between organic fertilizers applied to different soil textures. In most soils, the NH_4^+ form is quickly converted to the NO_3^- form. This NO_3^- form is not held on soil particles and is soluble in soil water. Consequently, in sandy soils, NO_3^- can move with the water to depths below the root zone. Even in clay soils with excessive rain, NO_3^- can be leached below the root zone or converted to a gaseous form and lost to the atmosphere. Nitrogen can be lost from farmers' fields through soil erosion, runoff, or leaching of NO_3^- or dissolved forms of organic N or through gaseous emissions to the atmosphere in the forms of ammonia (NH_3), nitrogen oxides (NO_x),and nitrous oxide (N_2O) (Goulding, 2000). Understanding the mechanisms that enable some organically managed cropping systems to achieve high yields while reducing N losses will contribute to improving the management of organic N fertilizers (Drinkwater et al., 1998).

There are many factors that affect the process of N availability such as temperature, oxygen supply, soil moisture content, soil texture, C/N ratio, and types of organic N as well as management factors (Stolze et al., 2000). Griffin et al. (2002) found that under conditions that are optimal for mineralization and nitrification (20°C to 30°C) and soil water content (50% to 65% water-filled pore space), textural differences influenced organic N availability. Previous work on C and N mineralization by Sorensen and Jensen (1995) on different soil textures proved that soil type influences the N availability from organic fertilizers. In sandy soil, Thomsen and Olsen (2000) found that increased sand content generally led to increased N availability from manure, presumably due to both increased aeration in sandy soils and increased physical protection of C and N substrates as the soil clay content increased. The influence of soil texture on N availability is related to clay content (Breland and Hansen, 1996).

The types and rate of organic N applied are critical to the N_{min} and N availability processes. Organic vegetable growers commonly use supplementary fertilizers such as fish emulsions and composted manure (Emino, 1981). The efficiency of these fertilizers in crop production appears to be dependent on a number of factors including the type of crops, rate of application, and the composition of the product. Increasing cost of fertilizer has made it imperative to find other source of fertilizers to produce foods in a sustainable way. Biofertilizers are environmentally safe, cheaper and could satisfy the nutrient demands of crops (Badawy et al., 1996). Cyanobacteria can be a potential biofertilizer since it has the unique ability to fix N from the atmosphere using solar energy through photosynthesis. Cyanobacteria have been reported to benefit plants by producing growth promotion substances (gibberelin and auxin), vitamins, amino acids, polypeptides, antibacterial and antifungal substances that improve plant growth and productivity (Zaccaro et al., 2001).

In organic vegetable production, optimizing fertilizer recovery is important to protect the environment, and to secure the N supply for crops (Thorup-Kristensen, 2001). Fertilizer recovery is defined as the amount of nutrient in the plant as a ratio of the amount applied (Moll et al., 1982). Fertilizer application needs to be given attention for the purpose of maximizing the efficiency of fertilizer use and to minimize leaching losses. Fertilizer cost savings could be achieved by making informed choices about fertilizer type, application rates, application methods and the timing of applications to meet crop demand (Phillips et al., 2009). Fertilizer recovery is also closely related to N use efficiency. In organic vegetable production, optimizing NUE is important to protect the environment, and to secure the N supply for the crops (Thorup-Kristensen 2001). It is important to know the rooting patterns of vegetable crops when trying to optimize NUE in vegetable production since root characteristics are important indicators of potential uptake of water and nutrients.

Many studies have been conducted on N availability of organic fertilizers but none of them have compared cyanobacterial ferilizers with commonly-used organic fertilizers. Therefore, a soil incubation study was conducted to evaluate the effect of N availability from potentially mineralizable N on different types of soil textures. Then, a greenhouse study was conducted to assess the effect of N availability from cyanobacterial fertilizers compared to the commonly-used organic fertilizers on lettuce growth, fertilizer recovery and N use efficiency.

NITROGEN AVAILABILITY FROM CYANOBACTERIAL FERTILIZERS COMPARED TO COMMONLY-USED ORGANIC FERTILIZERS APPLIED TO DIFFERENT SOIL TEXTURES

Preface

Generally, organic farmers use composted manure, legume cover crops, and off-farm fertilizers such as fish emulsion to meet the nitrogen (N) demand of crops. However, the nutrient composition of off-farm fertilizers such as composted manure and fish emulsion varies widely depending on animal species and often have higher transportation costs. Therefore, an evaluation of the application of cyanobacteria on soils was conducted as an alternative potential N biofertilizer. The aims of the study were to determine the rates of mineralization for different organic fertilizers, influence of soil texture on N mineralization, and to evaluate changes in soil microbial biomass from fertilizer application to sandy and clayey soils. In this study, N mineralization potential of cyanobacterial ferilizers were compared with commonly-used organic fertilizers in two soils with contrasting textures in a laboratory incubation study at constant temperature (25^{o}C) and moisture content (60% water-filled pore space) for 140 days. Soils were destructively sampled over the course of 140 days and analyzed for NH_4^+-N, and NO_3^--N, soil microbial biomass C, soil organic C, and soil total C and N. In both soils, soil NH_4^+-N was the highest at day 56 and decreased from day 56 to 140 due to its conversion to soil NO_3^--N. The Compost treatment significantly increased soil microbial biomass C (207.5 mg C kg^{-1} soil) compared to fish emulsion (115.42 mg C kg^{-1} soil) in sandy soil. The N availability was 9% significantly greater from fish emulsion than liquid cyanobacteria, and 6% greater from solid cyanobacteria than the compost treatment on sandy soil. The N availability of the fish emulsion treatment was 6% significantly greater than other fertilizer treatments on clayey soil.

Introduction

Nitrogen (N) is generally the most difficult nutrient to manage for organic crop production, and N uptake is dependent on the amount of plant-available N supplied by the soil. In organic systems, the most important limiting factor appears to be N availability since soil N commonly exists in organic forms that are not available to plants (Clark et al. 1999). In managing plant nutrients over continuing crop cycles, farmers face the challenge of estimating the amount of the soil organic N resource that is made available for plant uptake overtime (Deenik 2006).

Mineralization is the microbial conversion of organic N to inorganic plant-available forms of N (Aber and Melillo 2001). In mineralization, ammonification is the first step and is defined as the biological process by which organic N is transformed to NH_4^+-N. The second step is nitrification, an obligate aerobic process involving oxidation of NH_4^+-N to NO_3^--N (DeBusk et al. 2001). Nitrogen can be lost from farmers' fields through soil erosion, runoff, or leaching of NO_3^- or dissolved forms of organic N, or through gaseous emissions to the atmosphere in the forms of ammonia (NH_3), nitrogen oxides (NO_x),and nitrous oxide (N_2O) (Goulding 2000). Understanding the mechanisms that enable some organically managed cropping systems to achieve high yields while reducing N losses will contribute to improving the management of organic N fertilizers (Drinkwater et al. 1998).

In Colorado, farmers use manure, compost, and fish emulsion as the primary sources of N fertilizer. The potential drawbacks of dependence on these fertilizers include the uncertainty of releasing enough inorganic N at the proper time, high costs, odors, commercial availability of the products and relatively low nutrient contents (Raupp 2005). To date, organic farming is the most rapidly growing segment of the U.S. agricultural economy at 12% annual growth (USDA 2008). Organic farmers in the US spend more than $150,000,000 on fertilizers annually (USDA 2008). Sixty-five percent of organic farmers use manure, and 58% use compost, both of which tend to be low in N content (~1%) and expensive to transport (USDA 2008). The increasing amounts of nutrients from agricultural activities that leach into the environment and the higher price of N fertilizers constitute serious concerns for both the public and farmers (Roberts 2008). The escalating cost of N fertilizers has made it imperative to find alternate sources of N fertilizer. The types of organic N applied are critical to the N mineralization (N_{min}) process. Nitrogen mineralization among organic fertilizers is highly variable and is related to the physical and chemical characteristics of the fertilizer (Stolze et al. 2000). The efficiency of among commonly-used organic fertilizers in crop production appears to be dependent on a number of factors including the type of crops, rate of application, and the composition of the fertilizer (Aung et al. 1983).

Nitrogen in manure is not completely available to growing plants during the first year since much of it may be tied up in organic forms. In general, about 30% to 50% of the organic N becomes available the first year and with time, the manure N mineralization rate slows (Emino 1981). The amount of N mineralized in the first year depends upon the manure source, soil temperature, moisture, and handling. The nutrient composition of farm manure varies widely depending on bedding material, feed ingredients, moisture content, exposure, animal species and conditions of composting (Elliott et al. 2004). The composting process decreases C availability of raw materials using microbial processes to produce stable organic compounds, reducing the volume of the waste, and kills pathogens in the material (Tiquia and Tam 2002). Although composting reduces odors and creates better physical properties, such as stability of both C and N, it reduces the value of the manure as N fertilizer and results in lower amounts of soluble N forms by stabilizing N in organic compounds. Ammonia-N is usually lost as a gas during the composting process (Rosen and Bierman 2005).

Cyanobacteria can be a useful potential biofertilizer whether in solid or liquid forms. Cyanobacteria can both photosynthesize and fix N with great adaptability to various soil types (Mishra and Pabbi 2004). They have the unique ability to fix N from the atmosphere through coupling photosynthesis to N fixation. Biofertilizers are typically environmentally safe, cheaper and could satisfy the nutrient demands of crops (Badawy et al. 1996). El Gaml (2006) explained that biofertilization using cyanobacteria led to increases in the soil microbial community such as soil fungi, actinomycetes, and soil bacteria. Increasing soil microbial activity led to increased organic matter content, dehydrogenase and nitrogenase activities and subsequently improved soil fertility and plant growth performance (Hassan et al. 2008). When inoculated into soils that are then irrigated, dried cyanobacteria will swell up to ten times their dry size, adding to soil organic matter content and increasing soil fertility (Mishra and Pabbi 2004). An extracellular compound secreted by cyanobacteria glues soil particles together in the form of micro-aggregates.

Griffin et al. (2002) found that under conditions that are optimal for mineralization and nitrification of 20°C to 30°C and soil water content of 50% to 65% water-filled pore space, textural differences influenced the availability of applied organic N. Previous work on C and N mineralization by Sorensen and Jensen (1995) on different soil textures proved that soil type influences the net N_{min} from organic fertilizers. In sandy soil, Thomsen and Olsen (2000) found that increased sand content generally led to increased N_{min} from manure, presumably due to both increased aeration in sandy soils and increased physical protection of C and N substrates as the soil clay content increased. Gordillo and Cabrera (1997) applied the same broiler litters to nine soils, and measured net N_{min} over 146 days. Both the rate and extent of mineralization of broiler litter showed a strong positive correlation with sand content of the soil, and a strong negative correlation with silt plus clay content. Castellanos and Pratt (1981) incubated several animal manures in two soils (San Emigdio fine sand and Holtville silty clay) and reported more N mineralized in the fine sand for all types of manures. The influence of soil texture on N mineralization is also related to clay content (Breland and Hansen 1996). In soils with high clay content there is a physical protection from mineralization of organic matter (Stenger et al. 1995). Scott et al. (1996) suggested that small pore spaces protect organic matter from microbial attack whereas large pores facilitate N mineralization due to aeration.

In the past, soil incubation experiments have been done to estimate the potentially mineralizable N in soils (Stanford and Smith 1972). In various studies, the course of mineralization for a number of organic fertilizers has been studied under controlled laboratory conditions (Griffin and Honeycutt 2000). Many studies have been conducted on N mineralization of organic fertilizers but none of them have compared cyanobacterial ferilizers with traditional organic fertilizers. There is no literature that specifically deals with how cyanobacterial ferilizers and commonly-used organic fertilizers could affect

N_{min} rates on different types of soil textures. Therefore, an evaluation of the application of cyanobacteria on soils was conducted as an alternative potential N biofertilizer. The aims of the study were to determine the rates of mineralization for different organic fertilizers, influence of soil texture on N availability, and to evaluate changes in soil microbial biomass, total C and N from fertilizer application to clayey and sandy soils.

Materials and methods

Soil sampling

A clayey soil was collected using shovel and auger from 0-15 cm from the Horticulture Farm, Colorado State University (40°39'12.28"N, 104°59'59.36"W). The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS 1980). A sandy soil was collected using the same method from a certified organic farm in Kersey, CO and was classified as fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aridic Argiustoll of the Altvan series (NRCS 1980). Soils were sieved consecutively with 8.0 mm and 2.0 mm sieves to obtain a uniform particle size.

Soil analyses

The soil physical analyses included bulk density and particle size distribution to determine the percentage of sand, silt and clay. Bulk density was determined using the cylindrical core method (Arshad et al. 1996). Particle size distribution was determined using the hydrometer method based on Stoke's law (Gee and Bauder 1979). Chemical analyses included electrical conductivity and soil pH measured in the supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Organic matter content was determined by loss on ignition method (Blume et al. 1990). The soil chemical and physical analyses result is presented in Table 1.

Fertilizer analyses

The composted manure (Com) was obtained from an organic dairy farm in Gill, CO while the fish emulsion (Fish) was obtained from Daniel's® (3-1-1). The cyanobacteria species used in this study was *Anabaena cylindrica*. Liquid cyanobacterial fertilizer (Lcyb) was obtained from cyanobacteria culture which was cultured for two weeks in an aerated Allen and Arnon media under optimal fluorescence light conditions. Solid cyanobacterial fertilizer (Scyb) was harvested from the cyanobacteria biomass and was sun-dried. The homogenous Scyb was mixed and grounded as powdered form. Total C and N contents of four different types of organic fertilizers were analyzed using a LECO CN analyzer (Leco Corp., St Joseph, MI) to determine the C/N ratio of each fertilizer (Table 2) (Keeney and Nelson, 1982). Both solid and liquid fertilizers were analyzed for NH_4^+-N and NO_3^-N based on a simple automated method for measuring total dissolved N using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX).

Soil incubation

The soil incubation study was conducted in the Natural Resources Ecology Laboratory, Colorado State University in an incubation room where temperature was kept constant at 25^{o}C. Fertilizers were applied at 50 kg N ha^{-1} on sandy and clayey soils except for liquid cyanobacteria. Liquid cyanobacteria was applied to soil based on 25 kg N ha^{-1} to avoid saturating the soil. The treatments were control (no fertilizer), liquid cyanobacteria, solid cyanobacteria, liquid fish emulsion, and composted manure. Fifty gram sub-samples of air-dried soil were mixed with fertilizer according to the treatment assigned in 1 Liter Mason jars. Water content was adjusted to 60% Water-Filled Pore Space (WFPS) with distilled water and mixed well (Griffin et al., 2002). Sandy and clay soils treated with five fertilizer treatments and four replicates were arranged in blocks to represent each sampling date. The total experimental units were 280 jars. The soil was incubated for 140 days at a constant room temperature of 25^{o}C.

Sampling procedure

Forty experimental units were destructively sampled on each sampling date on seven sampling dates. Initial sampling for the soil inorganic N (NH_4^+-N and NO_3^-N) was determined at t=0.The next sampling dates were 7, 14, 28, 56, 84, 112, 140 days after treatment. Destructive sampling was achieved by taking a single 1.5-cm diameter core from the soil in each jar from the homogenized sample and sub-sampled for analysis.

Determination of total nitrogen and inorganic nitrogen

The C and N contents of soil were determined by heating to a temperature of at least 900 °C in the presence of oxygen gas using the dry combustion method (LECO TruSpec CN, St. Joseph, MI). Inorganic N (NH_4^+-N and NO_3^-N) was determined for each sampling date by extracting a 5 g subsample in 50 mL 2M KCl, shaking for 60 minutes, and filtering to obtain a clear extract (Keeney and Nelson, 1982). Leachates were filtered using Whatman No.42 filter paper. The extract was either analyzed immediately for NH_4^+ and NO_3^- using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) or frozen until analysis at -20^{o}C to prevent further microbial processes until it was ready to be analyzed. Two days before analysis, the frozen samples were thawed by moving them to a refrigerator (4^{o}C) (Bundy and Meisinger 1994).

Determination of soil microbial biomass carbon

Soil microbial biomass carbon (MBC) analysis was determined using the chloroform fumigation extraction method (Beck et al. 1997). The non-fumigated soil sample was placed in a specimen cup with 50 mL 0.05 M K_2SO_4 and was placed on a shaker for an hour at 200 rpm. The supernatant was filtered using Whatman No. 42 filter paper. The filtered extract was transferred to labeled sample vials, and the extracts were frozen prior to analysis. Total dissolved carbon was determined on a LECO analyzer. The difference between C in the fumigated and non-fumigated samples is the chloroform-labile C pool (Cp), and is proportional to microbial biomass C (C):

$$C = Cp/kC \quad \text{(Eqn. 1)}$$

where kC is often estimated as 0.45 (Beck et al. 1997).

Nitrogen mineralization and N availability calculation

Nitrogen mineralization rate was calculated as follows:

$$\text{Net N mineralization} = (NH_4^+\text{-}N_{\text{Treatment}} + NO_3^-\text{-}N_{\text{Treatment}}) - (NH_4^+\text{-}N_{\text{Control}} + NO_3^-\text{-}N_{\text{Control}}) \quad \text{(Eqn. 2)}$$

$$\text{Nitrogen availability \%} = (\text{Net N mineralization/ amount N applied as fertilizer}) \times 100 \quad \text{(Eqn. 3)}$$

Statistical analysis

The experimental units were arranged in a Randomized Complete Block Design (RCBD). Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). The Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data by using the GLM procedure. The Tukey value was calculated from the obtained mean square errors to determine whether main effects or interactions were significant ($P < 0.05$). The relationships between inorganic N, total C, total N and other parameters measured were assessed by linear correlation using the CORR procedure.

Results

Soil inorganic nitrogen (Soil ammonium-N and nitrate-N)

An interaction among treatment and days of incubation in soil NH_4^+-N (P <.0001) and NO_3^--N (P = 0.0003) concentrations was observed. Soil NH_4^+-N concentration in Lcyb treatment tended to decrease earlier starting from day 56 onwards in clayey soil while soil NH_4^+-N concentration in other treatments decreased from day 84 to 140 due to the formation of soil NO_3^- -N (Figure 1A). In sandy soil, soil NH_4^+-N concentration in all treatments peaked at 56 days after treatment except for Fish which peaked at 84 days and decreased onwards throughout the end of the study. The Scyb and Fish treatments recorded higher soil NH_4^+-N concentration during the initial incubation, but there was a lag phase between day 7-14 for both treatments. However, soil NH_4^+-N concentration in all treatments started to increase at day 14 (Figure 1B). Soil NO_3^--N concentration in the Fish treatment was significantly higher than the other fertilizers throughout the experiment in the clayey soil (Figure 2A). In sandy soil on days 7-14 of the incubation, soil NO_3^--N concentration in the Fish treatment was significantly higher than other treatments (Figure 2B), and the amount of soil NO_3^--N started leveling off throughout the 140-day incubation study.

Soil nitrogen availability

There was an interaction (P = 0.0017) observed between treatment and days of incubation in N availability. In this 140-day incubation experiment, temporary immobilization occurred between days 56 and 84 in the Fish treatment in the clayey soil (Figure 3A). However, it started to remineralize after day 84 while the immobilization in Scyb occurred between days 56 to 112 and started to remineralize again in clayey soil. At the end of the incubation study, the N availability of the Fish treatment decreased from day 112 to 140 while other treatments increased in clayey soil. The N availability for all treatments started stabilizing at day 84 until the end of the incubation study in the sandy soil (Figure 3B). At the end of the incubation study, the Fish treatment recorded significantly higher N availability compared to other fertilizer treatments on clayey soil while the available N was 9% significantly greater from the Fish treatment than the liquid cyanobacteria treatment and 6% greater from the solid cyanobacteria than the composted manure treatments.

Soil microbial biomass carbon, total carbon, and total nitrogen

Significant differences were observed in soil (P.<0001) and treatment (P=0.0069). There was no significant difference observed in microbial biomass in the clayey soil after the 140-day incubation (Figure 4A). Compost significantly increased soil microbial biomass C (208 mg C kg^{-1} soil) compared to fish emulsion (115 mg C kg^{-1} soil) in sandy soil (Figure 4B). Microbial biomass was significantly lower in sandy soil (95.5 mg C kg^{-1}) compared to clayey soil (186.6 mg C kg^{-1}). In this study, the results showed that soil microbial biomass C was significantly positively correlated with soil OC (r =0.57, P <0.001). Soil microbial biomass was also significantly correlated with soil NO_3-N at 140 days after incubation (r =0.43, P<0.001). Total C, total N, soil OC and soil MBC concentrations were significantly higher in the clayey soil (Table 4).

Discussion

Influence of soil texture from organic fertilizers application on soil inorganic nitrogen

Soil NH_4^+-N concentration under compost treatment on clayey soil was 33% significantly lower than in sandy soils (Table 3). In clayey soil, NH_4^+ can be fixed by soil minerals and may not be totally extractable in KCl solution (Nommik and Vahtraz 1982). In addition, Scherer (1993) claimed that clays which act as media for NH_4^+fixation, can limit N availability. According to Scherer (1993), the effect of clays on N mineralization was due to organic matter trapped in soil aggregates or poor oxygen supply to microorganisms.

Based on a mineralization study by He et al. (2000), the recovered mineral N (NH_4^+-N and NO_3^--N) reached its peak value within 90 days, and then decreased from 90 to 190 days onwards. However, in this study, soil NH_4^+-N concentration increased until day 84 where it reached its peak in both soils and started to decrease onwards. This finding could be attributed to the biosolid incubation study results of He et al. (2000) where NH_4^+-N was the dominant form of mineralized N during the first six months of incubation, but NO_3^--N accounted for more than 50% of the mineral N during the latter part of the incubation. In relation to our finding, Premi and Cornfield (1971) found that mineralized N was accounted for entirely as NO_3^--N after 42 days of incubation. Soil NH_4^+-N concentration decreased until the sixth week and thereafter the NH_4^+-N concentration was close to zero for all treatments (Sanchez et al., 1997, Madrid et al., 2002). In this study, the decrease in soil NH_4^+-N concentration was generally accompanied by a corresponding increase in soil NO_3-N concentration indicating that the NH_4^+-N released from ammonification was nitrified into the NO_3-N form.

Effect of soil texture from organic fertilizers application on soil nitrogen availability

In this study, N availability was higher in sandy soil by 1.6% compared to clayey soil but the value was not significant (Table 3). According to Sorensen and Jensen (1993), the cumulative net mineralization of N from fresh manure was highest in a soil-sand mixture with the lowest clay content (4% clay) after 84 days of incubation. They reported that at 14-28 days after manure application, there was an increase in N availability in the 25% soil and 75% sand treatment, which indicated that part of the microbial biomass that had been stimulated by the manure amendments was being mineralized; however, that was not observed in 100% soil composition or the 50% soil and 50% sand composition. Sorensen and Jensen (1993) claimed that there was a higher N remineralization of immobilized N in the soil-sand mixture with the lowest clay content. In the two soil-mixtures with higher clay contents, the microbial biomass N pool that was stimulated by the manure amendment was stabilized to a higher degree, resulting in a lower N availability in that particular treatment.

In relation to N availability, the Scyb treatment which was significantly higher at day 28 after incubation as compared to composted manure, a study by Mishra and Pabbi (2004) showed that a greater mineralization flush was observed on dried cyanobacteria during the first 10-20 incubation days for air-dried samples. Drying causes changes in soluble organic matter, and solubilized organic compounds may come from the microbial biomass which is killed by drying the samples. On rewetting, the dead biomass is mineralized rapidly. With longer incubations of more than 30 days, Mishra and Pabbi (2004) observed a lower increase in net N mineralization. This is evidently due to the regeneration of microbial biomass and reincorporation of formerly mineralized N in microorganisms (Nordmeyer and Richter, 1985). Sparling and Cheshire (1978) showed that drying as well as prolonged storage decreased microbial populations in soils.

Influence of soil texture on soil microbial biomass carbon, total carbon, and total nitrogen

Microbial biomass C is a measure of the active labile C pool in soil related to the activity of soil microorganisms. Microbial biomass responds more rapidly than organic C to changes in organic matter or to the rate of decomposition (Nannipieri, 1984). There was no significant difference observed in microbial biomass in the clayey soil after the 140-day incubation. This result may be attributed to the finding by Sorensen and Jensen (1993) where they found that there was no significant difference in net N mineralization from manure in 100% soil composition and 50% soil + 50% sand composition on the soil microbial biomass analysis. The reason might be that the “protection capacity” of 50% soil + 50% sand composition was high enough to protect the biomass formed from the applied substrate. The proportion of fresh manure C retained in the soil microbial biomass was expected to be ranked: 100% soil composition > 50% soil and 50% sand composition > 25% soil and 75% sand composition due to the decreasing capacity for protection of soil microbial biomass in the soils having decreasing clay contents (Ladd et al., 1992). Clay has higher water holding capacity which influences soil water content and oxygen content which may affect the microbial processes of mineralization, immobilization and denitrification. Clay content also influences soil structure, which in turn affects the accessibility of organic matter to microbes (Adu and Oades, 1978). Gregorich et al. (1991) reported that increasing amount of clay appeared to increase retention of microbial biomass by increasing the efficiency of soil microorganisms because clay can stabilize SOM by providing a protective coating on soil particles which inhibits microbial decomposition (Bronick and Lal, 2005). However, an increasing amount of clay tended to decrease N mineralization as was observed in this study where N mineralization percentage was higher in sandy soil compared to the clayey soil. In this study, the result showed that there was more labile C pool in clayey soil compared to sandy soil. However, there was no significant difference observed in clayey soil possibly due to the immbolization of C into the inter-layer spaces of the clay. In this study, a direct effect of lower soil OC could be observed where there was a reduced microbial biomass in sandy soil (95.5 mg kg^{-1}) due to a shortage of energy sources for soil microorganisms to trigger the decomposition process as soil OC is the main source of energy and nutrients for soil microorganisms to decompose organic matter (Sikora and Stott, 1996).

In conclusion, soil NH_4^+-N concentration was significantly higher in the Scyb treatment compared to the Compost treatment in clayey soil, but there was no significant difference observed in sandy soil. Soil NH_4^+-N concentration as a function of time was the highest around day 56 and decreased due to the formation of soil NO_3^--N. In this study, Fish treatment recorded significantly higher n availability compared to other treatments which might be due to its higher amount of available N content in the fish emulsion solution. Soil NO_3^--N concentration was significantly higher in clayey soil (57.5 mg NO_3^--N kg^{-1}) than in sandy soil (33.3 mg NO_3-N kg^{-1}) under the Fish treatment compared to other treatments and control. In addition, N availability was 9% greater with Fish than Lcyb, and was 6% greater for Scyb than Compost in sandy soil. Soil MBC, soil organic C, total C and N were significantly higher in clayey soil compared to sandy soil due to the protection capacity of organic N in the clayey soil that appeared to increase the retention of microbial biomass. From this soil incubation study, we conclude that fish emulsion resulted in significantly higher N availability compared to liquid cyanobacteria while solid cyanobacteria was mineralized more rapidly than composted manure. The N availability was higher in sandy soil compared to clayey soil on the fish emulsion and solid cyanobacteria treatments.

TABLES

Table 1 Soil chemical and physical analyses on clayey and sandy soils.

	Chemical Analyses							
	pH	EC	CEC	Total C	Total N	OM		
Soil		dS m^{-1}	cmol kg^{-1}	---------------%---------------				
Clayey	7.60	1.50	23.10	2.26	0.24	2.90		
Sandy	7.20	1.40	5.30	1.35	0.16	0.70		
	NH_4^+-N[1]	NO_3^--N[1]	P[2]	K[2]	Zn[2]	Fe[2]	Mn[2]	Cu[2]
	------------------mg kg^{-1}------------------							
Clayey	6.90	28.60	24.80	516.00	19.00	7.70	2.20	3.60
Sandy	2.70	23.00	46.80	196.00	3.00	47.90	2.90	1.40
	Physical Analyses							
	Sand	Silt	Clay	Bulk	Texture			
	----------%----------			---g cm^{-3}----				
Clayey	31	33	36	1.20	Clay loam			
Sandy	72	9	19	1.55	Sandy loam			

[1]Soil samples were extracted using 2M KCl

[2]Soil samples were extracted using ammonium-bicarbonate DTPA extraction method

Table 2 Chemical analysis of cyanobacterial and traditional organic fertilizers.

	Fertilizer Analysis					
					Inorganic N	
	pH	Total N	Total C	C/N ratio	NH_4^+-N	NO_3^--N
		------%-----	-----%-----		-----mg kg^{-1}------	
Solid cyanobacteria	7.69	7.67	43.14	5.62	86.22	0.42
Liquid cyanobacteria	7.56	0.10	0.562	5.62	0.24	0.02
Fish emulsion	5.89	2.73	5.20	1.90	2,003	16,412
Composted manure	8.71	0.48	5.73	11.94	2.18	1.70

Table 3 Soil NH_4^+-N, Soil NO_3^--N, and net N Mineralization of clayey and sandy soils after 140 days of incubation. Parameter with a common letter between soil types are not significantly different from each other ($p<0.05$) according to Tukey's test for mean separation.

Clayey soil	Treatment	Soil NH_4^+-N ---mg kg^{-1}---	Soil NO_3^--N ---mg kg^{-1}---	N availability ------------%------------
	Ctrl	5.0 ab	39.2 c	-
	Fish	4.1 ab	57.5 a	11.2 a
	Lcyb	5.1 ab	47.7 b	5.5 b
	Compost	3.9 b	44.8 b	2.8 b
	Scyb	6.0 a	45.8 b	5.4 b
		Soil NH_4^+-N	Soil NO_3^--N	N availability
Sandy soil	Treatment	---mg kg^{-1}---	---mg kg^{-1}---	------------%------------
	Ctrl	4.8 a	19.3 d	-
	Fish	4.9 a	33.3 a	12.8 a
	Lcyb	5.7 a	21.6 cd	3.5 b
	Compost	5.8 a	22.6 c	2.7 b
	Scyb	5.9 a	27.3 b	9.1 a

Table 4 Total C, total N, soil organic C, and soil microbial biomass C of clayey and sandy soils after 140 days of incubation. Parameter with a common letter between soil types are not significantly different from each other ($p<0.05$) according to Tukey's test for mean separation.

	Parameters			
	Total C	Total N	Soil OC	Soil MBC
Soil	----------%----------		----------mg kg^{-1}----------	
Clayey	52.2a	0.5a	89.9a	186.6a
Sandy	2.2b	0.3b	3.8b	95.5b

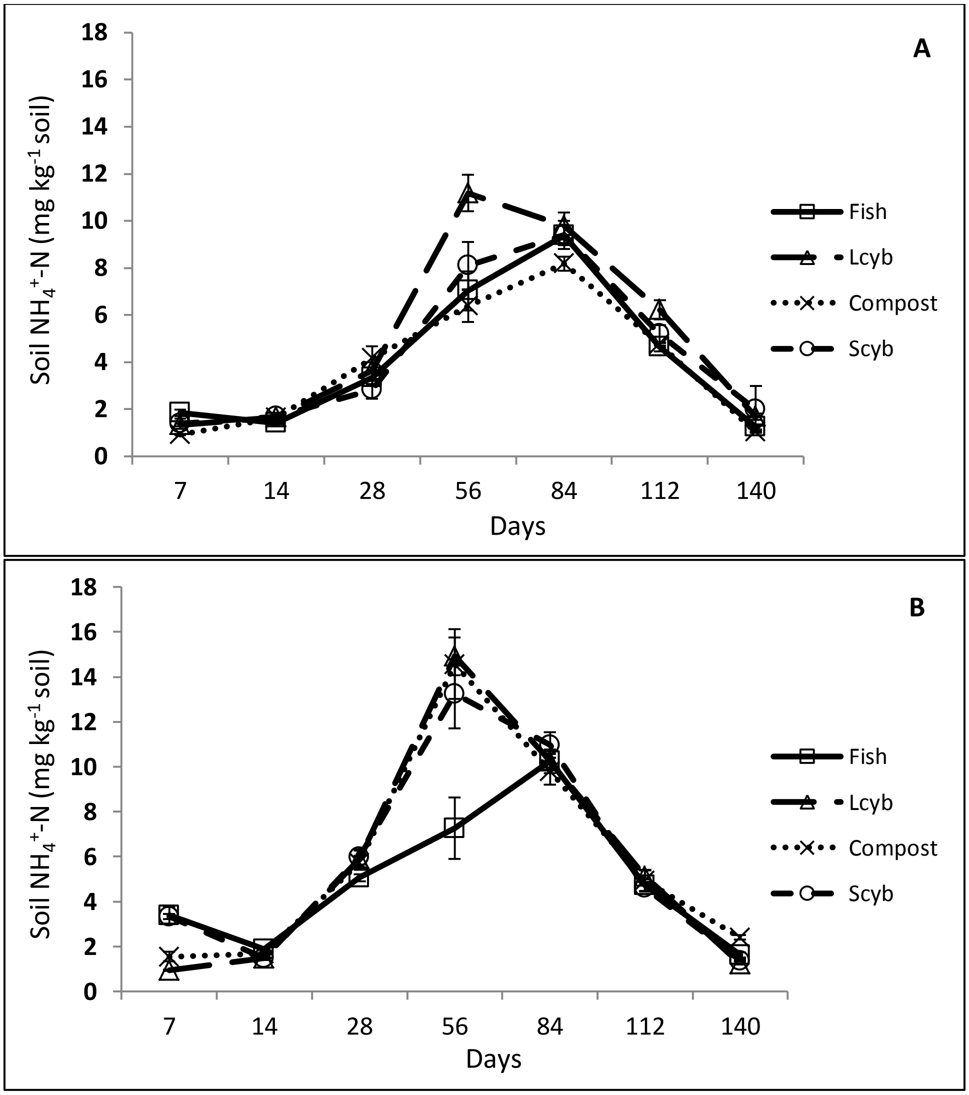

Figure 1 Soil NH_4^+-N concentration as a function of time in (A) clayey soil and (B) sandy soil during the 140-day incubation experiment. Bars represent standard errors of mean. 'Ctrl' = Control, 'Fish' = Fish emulsion, 'Lcyb' = Liquid cyanobateria, 'Compost' = Co Composted manure, and 'Scyb'= Solid cyanobacteria.

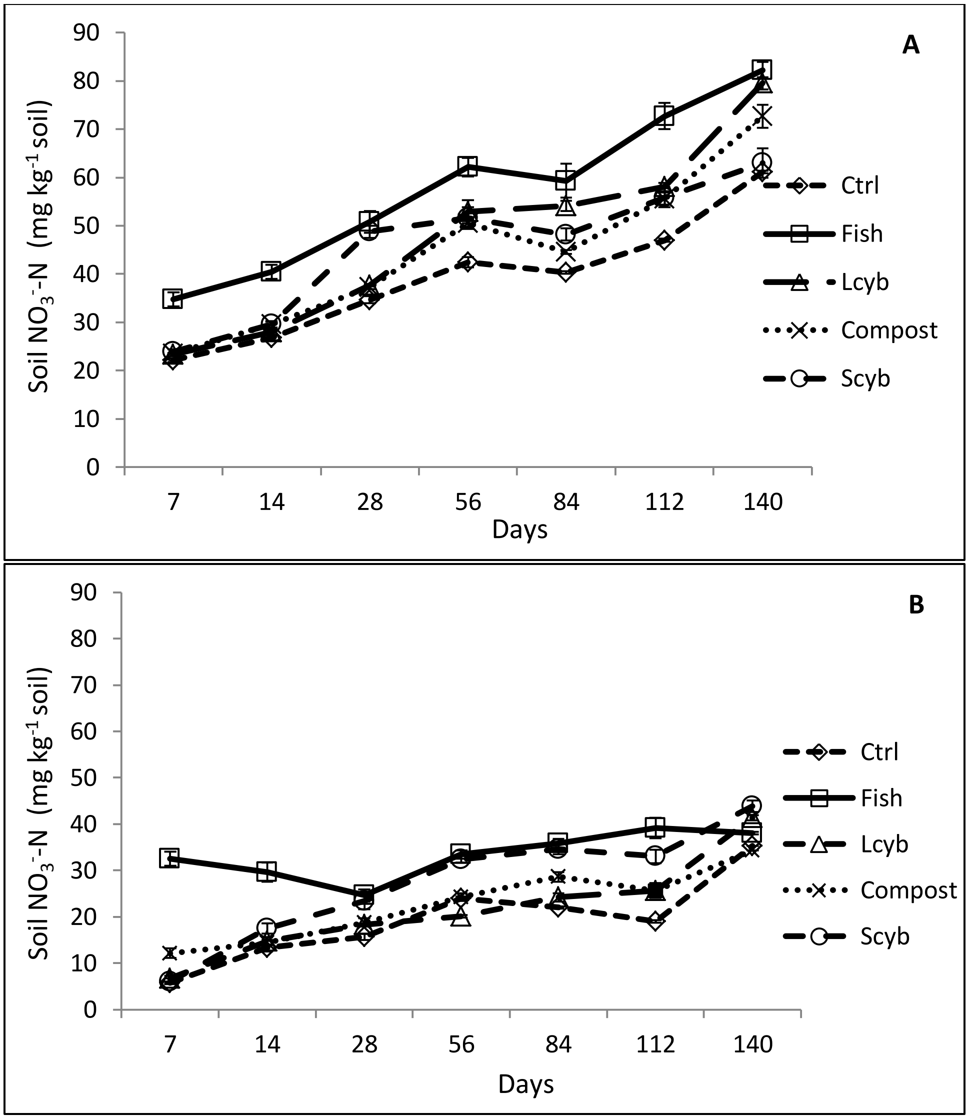

Figure 2 Soil NO_3^--N concentration as a function of time in (A) clayey soil and (B) sandy soil during the 140-day incubation experiment. Bars represent standard errors of mean. 'Ctrl' = Control, 'Fish' = Fish emulsion, 'Lcyb' = Liquid cyanobateria, 'Compost' = Composted manure, and 'Scyb'= Solid cyanobacteria.

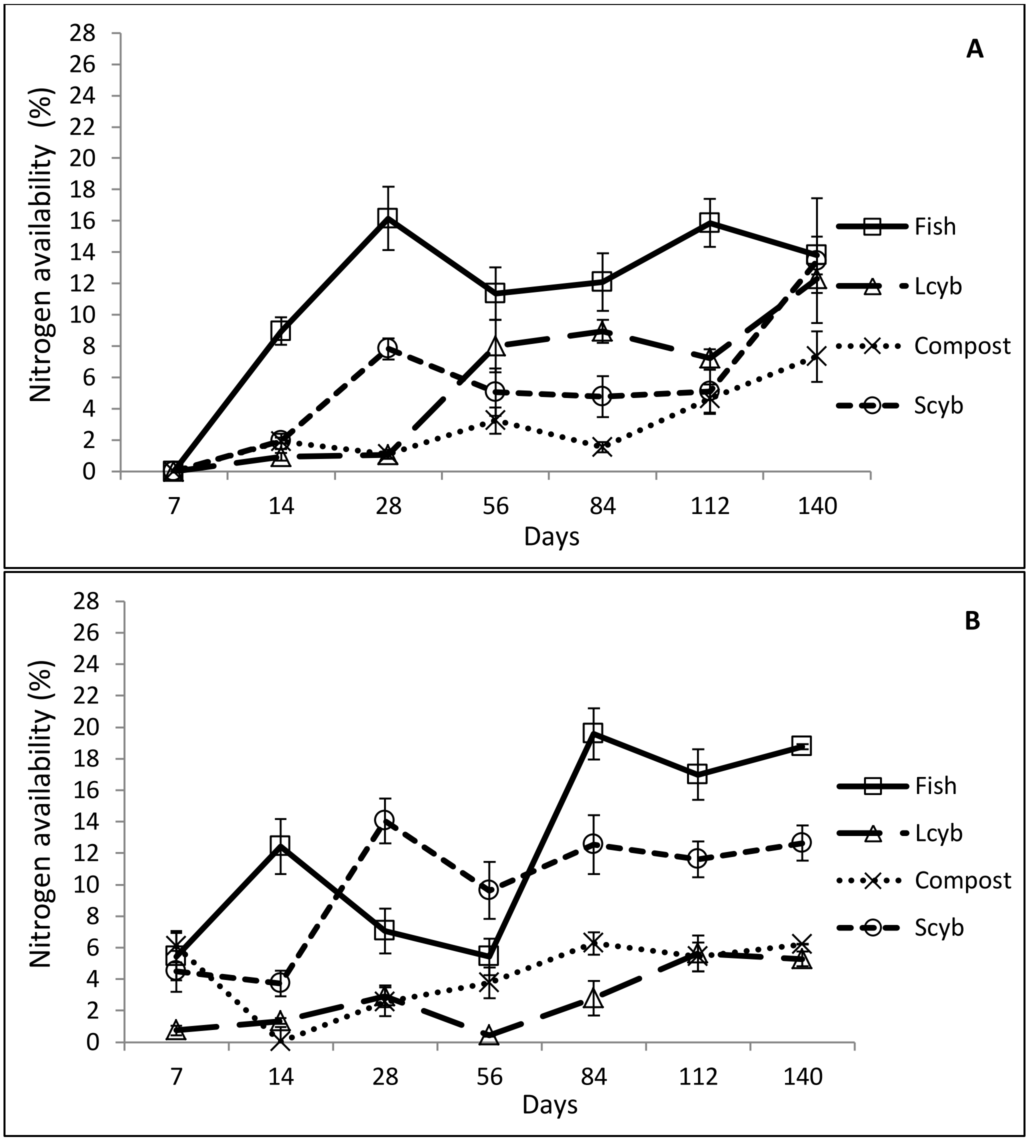

Figure 3 Nitrogen availability as a function of time in (A) clayey soil and (B) sandy soil during the 140-day incubation experiment. Bars represent standard errors of mean. 'Ctrl' = Control, 'Fish' = Fish emulsion, 'Lcyb' = Liquid cyanobateria, 'Compost' = Composted manure, and 'Scyb'= Solid cyanobacteria.

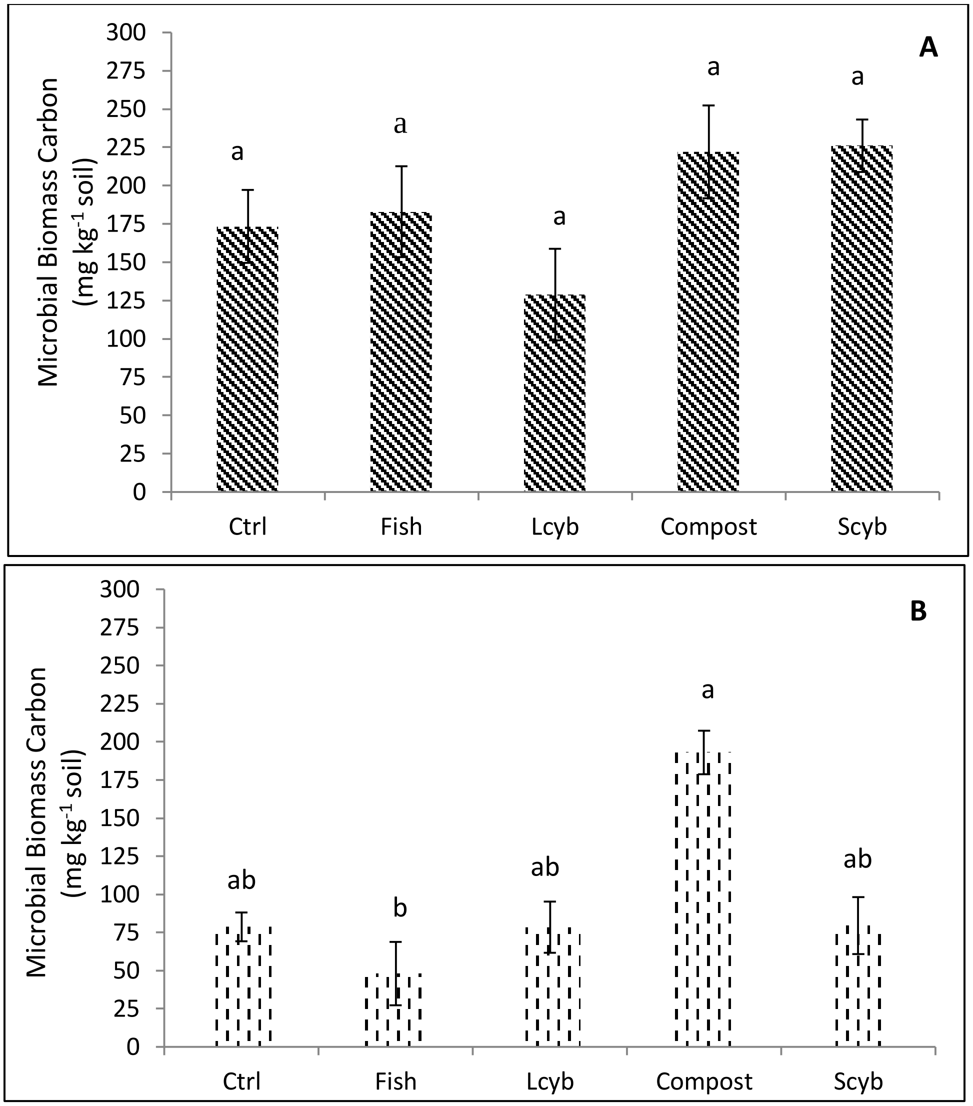

Figure 4 Soil microbial biomass C concentration following a 140-day incubation experiment in clayey (A) and sandy (B) soils. Bars represent standard errors of mean. Fertilizers with a common letter within soil type are not significantly different from each other ($p<0.05$) according to Tukey's test for mean separation. 'Ctrl' = Control, 'Fish' = Fish emulsion, 'Lcyb' = Liquid cyanobateria, 'Compost' = Composted manure, and 'Scyb'= Solid cyanobacteria.

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APPENDIX I

Table 1a. The F-value from Analysis of Variance (ANOVA) of soil ammonium-N, soil nitrate-N, and N availability from soil incubation from cyanobacterial and commonly-used organic fertilizers treatments on clayey and sandy soils after 140 days of incubation.

	Soil ammonium-N	Soil nitrate-N	N availability
Source	------------------------------F-value	--------------------------	
soil	1.51 ns	1300.00*	1.23 ns
treatment	3.91*	74.55*	30.60*
soil*treatment	2.29 ns	222.18 ns	4.27 ns
days	65.12*	4.96*	3.45*
soil*days	6.20 ns	22.26 ns	7.34 ns
treatment*days	3.23*	2.65*	1.70*
soil*treatment*days	3.30 ns	2.48 ns	2.40ns

[*]Significantly difference in comparison with p-value ($p<0.05$) according to Tukey's test of mean separation
[ns]No significant difference in comparison with p-value ($p>0.05$) according to Tukey's test of mean separation

GROWTH, YIELD, AND FERTILIZER RECOVERY OF GREENHOUSE LETTUCE AS RESPONSE TO SOIL AND FOLIAR APPLIED CYANOBACTERIAL AND COMMONLY-USED ORGANIC FERTILIZERS APPLIED TO DIFFERENT SOIL TEXTURES

Preface

Lettuce (*Lactuca sativa*) is a shallow-rooted crop and requires an extensive amount of N fertilizer to produce yield. Nitrogen plays a crucial role in synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis, and yield development of lettuce. In this study, percentage fertilizer recovery (PFR) was quantified to assess the efficiency of N uptake by lettuce to produce yield. A greenhouse study was conducted for 63 days to evaluate cyanobacterial and traditional organic fertilizers application on lettuce N response. Total leaf area, fresh yield, leaf dry weight, and leaf total N content were measured at the end of the greenhouse study. Total N uptake in lettuce tissue and PFR were calculated based on the analyses results. Soil applied fish emulsion recorded significantly higher fresh yield at 112 kg N ha^{-1} (147 g pot^{-1}) compared to 56 kg N ha^{-1} (117 g pot^{-1}) in clayey soil relative to sandy soil. Soil-applied liquid cyanobacteria recorded significantly higher yield compared to composted manure by 58%. Solid cyanobacteria recorded significantly higher total N uptake at 56 kg N ha^{-1} compared to 112 kg N ha^{-1} in clayey soil. In conclusion, soil applied fish emulsion treatment recorded higher PFR (99%) than soil applied composted manure (44%) at 56 kg N ha^{-1} on clayey soil. Soil applied fish emulsion has significantly higher PFR (57%) compared to the combination soil and foliar fertilizer (FFCom and FLScyb) at 56 kg N ha^{-1} in sandy soil.

Introduction

Lettuce is a cool-season salad crop commercialized internationally based on its consumption rate and economic importance throughout the world, and it ranks second among all vegetables produced for consumption in the United States (Coelho et al., 2005). The harvested part of lettuce is the photosynthetic leaf area. Thus, it is especially important to maintain optimal growth through the application of N (Gallardo et al., 1996). Nitrogen plays a crucial role in synthesis of amino acids and proteins, plant growth, chlorophyll formation, leaf photosynthesis, and yield development (Havlin *et al.,* 1999). Total N fertilizer recommendation for lettuce varies from 50 to 200 kg ha^{-1}(Smith et al., 2011). Sanchez (2000) demonstrated that lettuce yield increased in response to N fertilization applied at 84, 134, 253, 372, and 423 kg ha^{-1}. Shahbazie (2005) reported that by increasing the N level from 0 to 200 kg N ha^{-1}, lettuce yield increased, but among 100, 150, 200 kg N ha^{-1} applications no significant differences were observed. Burns (1996) showed that lettuce plants have reduced growth during the whole growth period if they are exposed to N deficiency in early growth while higher supply of N has several effects such as delayed senescence and higher shoot to root ratio (Masson et al., 1991).

The mineralization of organic N sources contributes to N availability to plants. The N nutritional status of plants may greatly affect the uptake, transport and translocation of N by affecting the activity of any of the proteins and nitrogenous species involved. Based on the previous study on N uptake, Sanchez (2000) found that 88% and 77% of the applied N at 32 kg ha^{-1} and 61 kg ha^{-1} was not recovered in the above ground plant tissue indicating the potential for NO_3^--N leaching in the coarse-textured soil (95% sand).

Percentage Fertilizer Recovery (PFR) is defined as the amount of nutrient in the plant as a ratio of the amount applied (Moll et al., 1982). In organic vegetable production, optimizing PFR is important to protect the environment, and to secure the N supply for crops (Thorup-Kristensen, 2001). Fertilizer application needs to be given attention for the purpose of maximizing the efficiency of fertilizer use and to minimize leaching losses. Fertilizer cost savings could be achieved by making informed choices about fertilizer type, application rates, application methods and the timing of applications to meet crop demand (Phillips et al., 2009). Several studies have been conducted on the release of N from organic amendments, but there is less accurate information on the comparison of soil and foliar organic fertilizers to supply mineralized N for organic lettuce production.

According to the USDA National Agriculture Statistics Service, organic farmers in the U.S. spend more than $150,000,000 on fertilizers annually. Sixty-five percent of organic farmers use manure, and 58% use compost; both are low in N content (~1%) and expensive to transport (USDA, 2008). The potential drawbacks of traditional organic fertilizers are relatively low nutrient contents, the uncertainty of releasing enough nutrients at the proper time, high costs, odors, and commercial availability of the products (Raupp, 2005). The increasing amount of nutrients from agricultural activities that leach into the environment and the higher price of N fertilizers constitute serious concerns for the farmers and has made it imperative to find alternate sources of N fertilizer (Roberts, 2008).

Cyanobacteria, such as *Nostoc* and *Anabaena* can be a useful potential biofertilizer since it can both photosynthesize and fix N with great adaptability to various soil types (Mishra and Pabbi, 2004). El-Gaml (2006) explained that biofertilization using cyanobacteria led to increased microbial diversity community in soil through increased organic matter, microbial activity, and in turn, increased dehydrogenase and nitrogenase activities and subsequently improved soil fertility.

Many studies have been conducted on N availability response of organic fertilizers, but none of them compared cyanobacterial bio-fertilizer and traditional soil and foliar applied fertilizers on organic lettuce. There is also no literature dealing specifically with how soil and foliar applied organic fertilizers affect N response in lettuce applied on different soil textures. The objectives of this study were i) to assess N response from soil and foliar applied organic fertilizers applied to clayey and sandy soils, ii) to determine the influence of different soil textures on growth, yield, and PFR of lettuce due to soil and foliar organic fertilizer applications.

Materials and Methods

Site description. The pot experiment was carried out from May to August 2012 in a greenhouse at Colorado State University. Romaine lettuce (*Lactuca sativa*) variety 'Summer Crisp' was grown in a greenhouse equipped with an evaporative cooling system under natural daylight at the following geographic coordinates: 40°34’ 17.56”N, 105°04’49.44”W.

Soil sampling. Two soil samples were collected from different areas that represent two soil textures sandy and clayey soils. Clayey soil was collected using shovel from 0-15 cm from the Horticulture Farm, Colorado State University (40°39'12.28"N, 104°59'59.36"W). The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS, 1980). The sandy soil was collected using the same method from the Hediger Farm, Nunn, Colorado (40°20'9.90"N, 104°34'24.67"W) and was classified as fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aridic Argiustoll of the Altvan series (NRCS, 1980). Soils were sieved consecutively with 8.0 mm and 2.0 mm sieve to obtain a uniform particle size.

Soil analyses. The soil physical analyses included bulk density and particle size distribution to determine the percentage of sand, silt and clay. Bulk density was determined using the cylindrical core method (Arshad et al. 1996). Particle size distribution was determined using the hydrometer method based on Stoke’s law (Gee and Bauder 1979). Chemical analyses including electrical conductivity and soil pH were measured in the supernatant suspension of 1:1 soil to water using a Mettler Toledo pH meter (Thermo Fischer Scientific, Waltham, MA). Organic matter content was determined by loss on ignition method (Blume et al. 1990). The soil chemical and physical analyses results are presented in Table 5.

Fertilizer analyses. The composted manure was obtained from an organic dairy farm while the fish emulsion was obtained from Daniels® (3-1-1) (Daniels Agro Sciences LLC, East Greenwich, RI). The cyanobacteria species used in this study was *Anabaena cylindrica*. Liquid cyanobacterial fertilizer was obtained from cyanobacteria culture which was cultured for two weeks in an aerated Allen and Arnon media with optimal lighting condition. Solid cyanobacterial fertilizer was filtered from the liquid cyanobacteria and was sun-dried. The homogenous solid cyanobacteria was mixed and ground to obtain a homogenous powdered form of solid cyanobacterial fertilizer. Total C and N contents of the different types of organic fertilizers were analyzed using a LECO CN analyzer (Leco Corp., St Joseph, MI) to determine the C/N ratio of each fertilizer (Keeney and Nelson, 1982) (Table 6). Both solid and liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) for inorganic N analyses (NH_4^+ and NO_3^-) using colorimetric method.

Experimental design. Treatments consisted of two soil textures (clayey and sandy soils), three nitrogen rates (0, 56, and 112 kg N ha^{-1}) and six types of organic fertilizers (liquid cyanobacteria, solid cyanobacteria, fish emulsion, composted manure, combination fertilizer of foliar applied liquid cyanobacteria with soil applied solid cyanobacteria, and combination fertilizer of foliar applied fish emulsion with composted manure). The treatments were arranged in a Randomized Complete Block Design (RCBD) with factorial combinations of soil textures, N rates, and types of organic fertilizer with three replications.

Plant material. Seeds of 'Summer Crisp' lettuce (Johnny's Selected Seed, Waterville, ME) were planted in plastic trays containing 3.5 kg of well-mixed growing media with PRO-MIX perlite (Premier Horticulture Inc., Quakertown, PA) in the first week of May 2012. After four weeks of germination, seedlings were transplanted into pots.

Pot experiment. Before planting, the composted manure and solid cyanobacteria fertilizers were homogeneously incorporated into the soil in each pot containing 2.88 kg of soil according to the assigned treatments. When the seedlings were four weeks old, three seedlings were transplanted into each pot, and a few days after seedling emergence, the plants were thinned to one seedling per pot. The pots were watered and maintained at an equal weight throughout the whole experiment.

Methods of fertilizer application. The soil applied liquid fertilizer treatment (liquid cyanobacteria and fish emulsion), was split into four applications to the soil over time (0, 2, 4 and 6 weeks) to mimic fertigation while the soil applied solid fertilizer treatment (solid cyanobacteria and composted manure) was applied all at once prior to planting based on the calculated N rate (56 and 112 kg N ha^{-1}). Nitrogen fertilizers were applied in four split applications every two weeks for the soil and foliar applied treatments (fish emulsion and liquid cyanobacteria). However, due to the adequate level of P and K based on the initial soil analysis, P and K were not applied. The total N fertilizer was split with the ratio of 50:50 into solid and liquid forms for the combination fertilizer treatments (Table 7). Treatments were started immediately after transplanting.

Cultural practices. Weeding was carried out by hand-weeding two weeks after planting. Beneficial insects (*Phytoseiulus persimilis)* were applied on the plant and soil surface one week after transplanting as a form of bran to keep the plants from getting a thrips infestation. To threat aphids and loopers, Entrust® insect control (Dow AgroSciences, Indianapolis, IN) certified by the Organic Materials Review Institute (OMRI) was applied eight weeks after transplanting and at the end of the experiment.

Harvesting. Harvesting was done at 63 days after transplanting. All harvested samples were washed with deionized water and then dried at 70°C for 72 hours. They were weighed to determine their dry matter and then ground for analysis. Sub-samples were taken from each sample for further analysis.

Parameters measured. Total leaf area was measured using the LICOR-3100 leaf area meter (LI-COR, Lincoln, NE) at the end of the study after the harvesting process. The mean weight of three plants from three replications was assumed as the total leaf area. The above-ground part was separated and was weighed separately for fresh yield. Shoot dry matters were determined by cutting, oven-drying at 70^{o}C for 72 hours, and then weighing. The mean weight of three plants from three replications was assumed as the dry matter for shoot. A sample was collected to determine dry matter yield and was dried and ground for determination of total C and N by dry combustion using TruSpec CN Analyzer (Leco Corp., St Joseph, MI). Total plant N uptake was calculated from tissue N concentrations of the shoot dry matter by multiplying the dry weights of the shoot by the concentration in plant tissue.

Calculation of percentage fertilizer recovery (PFR). Percentage fertilizer recovery was calculated by dividing the net total N uptake by lettuce from N fertilized pot by the rate of fertilizer N applied and multiplied by 100 as shown in Eqn. 1 (Dobermann, 2005).

$$PFR = (NF)-(NC) / R * 100 \quad \text{(Eqn. 1)}$$

where,

PFR = Percent Fertilizer Recovery

NF = Total N uptake in lettuce from N fertilized

NC = Total N uptake in lettuce from unfertilized

R = Rate of fertilizer N applied

Statistical analysis. Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). The Univariate and Boxplot procedures were used to evaluate the normality of data distribution. Analysis of variance (ANOVA) was performed on the data by using the GLM procedure. The Tukey value was calculated from the obtained mean square errors to determine whether main effects or interactions were significant ($P < 0.05$). The relationships between leaf chlorophyll content, plant height, total leaf area, fresh yield, total leaf N, and total N uptake and other parameters measured were assessed by linear correlation using the CORR procedure.

Results

Total leaf area

There were significant difference observed in treatment ($P<.0001$) and soil ($P<.0001$) for total leaf area. The Fish treatment (2189 cm^2) recorded significantly higher total leaf area compared to FFCom (1222 cm^2), FLScyb (1086 cm^2), Scyb (1078 cm^2), Compost (819 cm^2) and Ctrl (758 cm^2) treatments when applied at 112 kg N ha^{-1} on clayey soil (Figure 5A). The Fish treatment (1310 cm^2) recorded significantly higher total leaf area compared to FFCom (756 cm^2), Com (655 cm^2), Ctrl (612 cm^2) and Scyb (578 cm^2) treatment in sandy soil when applied with 56 kg N ha^{-1} (Figure 5B).

Fresh yield

There was a significant interaction observed between treatment and N rate for fresh yield ($P=0.0156$). The Fish treatment recorded significantly higher yield (147 g pot^{-1}) at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} (117 g pot^{-1}) on clayey soil (Figure 6A). In cyanobacterial fertilizers, the Lcyb treatment (111 g pot^{-1}) recorded higher yield than other fertilizer treatments except for fish emulsion at 112 kg N ha^{-1} on clayey soil. In sandy soil, the Fish treatment recorded significantly higher yield at 112 kg N ha^{-1} compared to other fertilizer treatments (Figure 6B). The Lcyb treatment recorded significantly higher fresh yield at 56 kg N ha^{-1} (66 g pot^{-1}) compared to 112 kg N ha^{-1} (46 g pot^{-1}).

Leaf dry weight

The Fish treatment recorded significantly higher leaf dry weight compared to the Com treatment (1.67 g) when applied at 112 kg N ha^{-1} in clayey soil (Figure 7A). At 112 kg N ha^{-1}, the Fish treatment recorded significantly higher leaf dry weight compared to the cyanobacterial fertilizers treatment (Scyb, Lcyb, and FLScyb) but there was no significant differences observed between cyanobacterial fertilizers (Scyb, Lcyb, and FLScyb) and the Com treatment on sandy soil at 112 kg N ha^{-1} (Figure 7B).

Total leaf nitrogen

Significant interaction was observed in total leaf N between treatment and N rate (P <.0001). In clayey soil, at 112 kg N ha^{-1}, the Fish treatment recorded significantly higher total leaf N (4.4%) while Lcyb (3.4 %) recorded significantly higher total leaf N compared to Scyb (3.4 %) when applied at 56 kg N ha^{-1} (Figure 8A). The total leaf N at 56 kg N ha^{-1} on clayey soil was higher than sandy soil, and Scyb recorded significantly higher total leaf N than Lcyb in clayey soil at 56 kg N ha^{-1}. The combined soil and foliar fertilizer (FLScyb) treatment recorded significantly higher total leaf N than the Scyb (2.8%) and Com (2.6%) treatments at 56 kg N ha^{-1} on sandy soil (Figure 8B).

Total nitrogen uptake

There was a significant interaction between treatment and N rate (P=0.0448) in total N uptake in lettuce tissue. Clayey soil recorded significantly higher total N uptake compared to sandy soil at 112 kg N ha^{-1} (Figure 9A). At 56 kg N ha^{-1}, FLScyb, Scyb, and Com treatments recorded significantly higher total N uptake in lettuce tissue (93, 87, 74 mg N respectively) on clayey soil while at 112 kg N ha^{-1}, the Fish treatment (193 mg N) recorded significantly higher total N uptake followed by Lcyb (119 mg N) and FFCom (105 mg N). The Fish treatment recorded significantly higher total N uptake on sandy soil (Figure 9B) compared to other fertilizer treatments at both N rates while the Lcyb treatment (68 mg N) recorded significantly higher total N uptake compared to the Com treatment (28 mg N) at 56 kg N ha^{-1} on sandy soil.

Percentage Fertilizer Recovery

There was a significant difference observed in percentage fertilizer recovery (PFR) between soils ($P < .0001$) where clayey soil recorded significantly higher PFR compared to sandy soil. The Fish treatment recorded significantly higher PFR compared to the Com treatment at 56 and 112 kg N ha^{-1} on both soils (Fig. 10). In addition, the combination of soil and foliar applied fertilizers; FFCom and FLScyb recorded significantly higher PFR compared to the Com treatment at 112 kg N ha^{-1} on sandy soil (Fig. 10B). The PFR results showed a similar pattern as total N uptake and NUE, where the Fish treatment recorded significantly higher total N uptake and NUE, followed by soil and foliar applied fertilizers (FFCom and FLScyb). The Com and Scyb treatments were consistently the lowest in total N uptake.

Discussion

Nitrogen response on lettuce growth

Total leaf area plays a significant role in the photosynthesis process to absorb nutrients for N assimilation in biomass production. Total leaf area was found to be correlated with total N accumulation in lettuce tissue ($r=0.77$, $P<.0001$), and PFR ($r=0.55$, $P<.0001$). Based on a study conducted on romaine lettuce by Boroujerdnia and Ansari (2007), 120 kg N ha^{-1} enhanced leaf growth and photosynthesis, thus increasing total leaf area. An increasing total leaf area in lettuce in response to N fertilizer results in higher photo-assimilates and increased dry matter accumulation. The Fish treatment recorded significantly higher total leaf area compared to other treatments could be explained by the chemical properties of fish emulsion as described by El-Tarabily et al. (2003) based on his study on radish. He reported that fish emulsions contain many essential amino acids, proteins, lipids, vitamins, and a combination of bacteria, actinomycetes, and plant growth regulators which might explain the positive effects of fish emulsions in radish production. The high concentrations of plant growth regulators were suggested as a major reason why the radishes had equal growth to those with inorganic fertilizer despite lower plant nutrient concentrations (El-Tarabily et al., 2003). Based on this study, the primary reason for the higher total leaf area in the Fish treatment is probably due to the high inorganic N content in the fish emulsion according to the fertilizer analysis (Table 6).

Nitrogen response on lettuce yield

The increase in yield due to increasing fertilizer rate in the Fish treatment (147 g pot^{-1}) at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} (117 g pot^{-1}) on clayey soil could be explained by the chemical properties of fish emulsion which contains high concentration of available-N which can be taken up directly by plants in a short time span. Horticultural crops have a high demand for N over a short period and it is important to ensure adequate N supply during the crop growth stage. Emino (1981) found that the ability of fish emulsion to produce larger plants despite lower N-P-K values is due to its complex composition. Increases in yield could also be as a result of N effects on photosynthesis, dry matter partitioning, and the amount of assimilates that are produced by the plant (Dordas and Sioulas, 2008). In other study, Shahbazie (2005) reported that by increasing the N level from 0 kg N ha^{-1}, lettuce yield increased, but among 100, 150, 200 kg N ha^{-1} applications no significant differences were observed when supplied as ammonium nitrate. However, in a study by Tourte et al. (2000), there was no increase in yield compared to control when fish emulsion was used as a foliar spray on field-grown tomatoes.

Higher yield was recorded also in the Lcyb treatment compared to the Com treatment at 112 kg N ha^{-1} on clayey soil might be due to the effect of cyanobacterial fertilizer led to increase in soil microbial activity in enhancing the decomposition of soil organic matter (El-Gaml, 2006). Cyanobacteria have positive effects on plant growth and can produce phytohormones such as auxin which could increase plant growth performance (Long et al., 2003). Mishra and Pabbi (2004) claimed that an extracellular compound secreted by cyanobacteria glues soil particles together in the form of micro-aggregates and hence improves soil nutrient availability. It has been shown that laboratory studies indicated that laboratory studies indicated that cyanobacteria excrete indole-3-acetic acid (IAA) and amino acids which can stimulate the growth of soil microbial populations (Karthikeyan et al., 2007).

Nitrogen response on dry matter production and nitrogen uptake

In this study, the Com treatment recorded significantly lower leaf dry weight compared to the Fish treatment. The reason might be because of the reduced efficiency of N uptake in the Com treatment to be assimilated in microbial biomass (Sukor et al., 2013). Composted manure has higher amounts of recalcitrant C fractions and is more resistant to microbial degradation (Ribeiro et al., 2010). The reduced amount of labile C decreases the soil microbial activity needed to mineralize organic N. The form of organic N fertilizer incorporated in the soil and the amount of inorganic N availability influences the N uptake in plant tissue. Abu-Rayyan et al. (2004) reported that NH_4^+-N proved to be the optimum N form for lettuce, leading to the highest dry matter content. A study conducted by Tusun and Ustun (2004) found that N forms significantly affected lettuce plant diameter and the number of total marketable leaves.

The Fish treatment recorded significantly higher total leaf N (4.4%) compared to other treatments. The properties of fish emulsion which contain higher inorganic N (NH_4^+-N and NO_3^--N) compared to other fertilizer tested (Table 2) also recorded higher leaf total N content at on clayey soil when applied at 112 kg N ha^{-1}. Noctor and Foyer (1998) reported that NO_3^--N treated plants had more efficient photosynthetic electron flow due to the role of cytokinin which enhanced stomatal opening via stimulation of chlorophyll synthesis in some species which help to boost higher yield. However, there was no significant difference observed in leaf chlorophyll content or soil inorganic N (NH_4^+-N and NO_3-N) in this study. In fact, the photosynthesis rate, and inorganic N (NH_4^+-N and NO_3^--N) in plant tissue were not measured, which might help to understand the details. As a comparison between the cyanobacterial fertilizers, at 56 kg N ha^{-1}, the Scyb treatment recorded higher total leaf N compared to the Lcyb treatment. However, at 112 kg N ha^{-1}, higher total leaf N was recorded in the Lcyb treatment compared to the Scyb the which might correspond to the biweekly application of liquid cyanobacteria to the soil to simulate fertigation. Although the initial total N of liquid cyanobacteria was low (0.10 % N), but the biweekly application of liquid cyanobacteria to soil and an increased in N application rates by two-fold might increase plant N uptake efficiency according to the growth stage as compared to one dose application of solid cyanobacterial fertilizer.

In this study, leaf total N was significantly correlated with total leaf area, fresh yield and leaf dry weight which might explain the effect of N fertilization on plant physiological processes such as photosynthesis and cell division. Czerpak and Piotrowska (2003) found that cell division and plant growth were influenced by cytokinin content which could enhance plant biomass as the effect of higher total leaf area intercepting with solar radiation in the photosynthesis process. Sakibara et al. (2006) found that cytokinin is related to the acquisition of macronutrients for the efficient acquisition and use of N under variable N supply conditions. They explained that plants have the ability to sense internal and external N status, and to adapt to changing N conditions by modifying enzyme activities. In their study, they reported that cytokinin determines the status of N supply to regulate the N uptake by the presence of NO_3^--N. The finding by Sakibara et al. (2006) might elucidate the reason on the higher leaf total N content in the Fish treatment since the amount of inorganic N in fish emulsion was higher compared to other fertilizers tested although the cytokinin content in lettuce tissue was not measured in this study.

The Fish treatment recorded significantly higher total N uptake compared to the Com treatment which might affect the N availability in soil which influenced the N uptake in plant tissue. In this study, an increase in the total N accumulation in plant tissue gave a significantly higher correlation in the fresh yield of lettuce than other parameters investigated. Fresh yield was highly correlated with total N uptake ($r=0.92$, $P<.0001$), followed by leaf dry weight ($r=0.89$, $P<.0001$), total leaf area ($r=0.84$, $P<.0001$), and total leaf N ($r=0.74$, $P<.0001$). Hermanson et al. (2000) reported that the amount of N accumulated by a crop is affected by the amount of N supplied by the soil or N added as fertilizer. Lawlor (2002) reported that total N concentrations in plant tissue are related to the plant physiological requirements such as photosynthesis, and assimilate partitioning in determining yield.

Effect of soil and foliar applied fertilizers on fertilizer recovery

In this study, PFR was significantly higher at 56 kg N ha^{-1} compared to 112 kg N ha^{-1} in clayey and sandy soils as reported by the study done by Montemurro and Maiorana (2007) that lettuce had a better ability and higher economic yield at low N application rates, although it could produces higher yield at 112 kg N ha^{-1}. According to a study of N fertilizer application on spinach, Zarehie (1995) reported that by increasing the N fertilizer rate to 200 kg N ha^{-1} increased yield, but the corresponding increase in yield was not economical. Schenk (2004) reported that NUE influence fertilizer recovery because it depends on the ability of roots to absorb nutrients from the soil and affects the yield production per unit of absorbed nutrient.

The higher PFR observed in the clayey soil compared to the sandy soil might be due to the soil texture. Clayey soil has higher CEC (23 cmol kg^{-1}) compared to the sandy soil (5 cmol kg^{-1}). Soil with higher CEC has higher nutrient retention rate which can become available for plant uptake. In this study, PFR was also found to be correlated with total leaf area ($r=0.74$, $P<.0001$). Total leaf area plays a significant role in the photosynthesis process to absorb nutrients for N assimilation in biomass production. In addition, significant correlations were also found for leaf dry weight with PFR ($r=0.87$, $P<.0001$). Fresh yield was highly correlated with total N uptake ($r=0.92$, $P<.0001$), followed by leaf dry weight ($r=0.89$, $P<.0001$), and PFR ($r=0.85$, $P<.0001$). An increase in the total N uptake in plant tissue gave a significantly higher correlation in the fresh yield of lettuce than other parameters investigated.

In this study, other parameters that could be related to PFR are total leaf area and plant dry weight. The Fish treatment recorded significantly higher total leaf area compared to FFCom, FLScyb, and Compost treatments similarly to the response for PFR at 56 and 112 kg N ha^{-1} on both soils. Based on a study conducted on romaine lettuce by Boroujerdnia and Ansari (2007), 120 kg N ha^{-1} enhanced leaf growth and photosynthesis, thus increasing total leaf area. An increasing total leaf area in lettuce in response to N fertilizer results in higher photo-assimilates and increased dry matter accumulation. According to Squire et al. (1987), the main effect of N fertilizer was to increase the rate of leaf expansion which leads to increased interception of daily solar radiation leading to higher PFR. Gourley et al. (1994) reported that plant dry weight and total leaf area may provide the best estimate of fertilizer efficiency response. To evaluate N fertilizer response, the measured parameters must be closely correlated with plant productivity (Lynch, 1998). In this study, indeed leaf area is related to PFR based on the significant positive correlation coefficients of total leaf area with PFR, fresh yield, and total N uptake in plant tissue.

In this study, it was observed that the Fish treatment recorded significantly higher PFR at 56 and 112 kg N ha^{-1} on both soils compared to the Com treatment. The reason might be due to the properties of fish emulsion which influence the availability of N in both soils to be taken up by the plants. El Tarabily et al. (2003) reported that fish emulsions contain many essential amino acids, proteins, lipids, vitamins and a combination of bacteria, actinomycetes, and plant growth regulators which might trigger the activity of soil microorganism to increase available N content of soil.

Based on the soil microbial biomass C which was measured in the soil incubation mineralization study, the amount of soil microbial biomass was significantly higher in clayey soil (187 mg kg^{-1}) compared to the sandy soil (96 mg kg^{-1}) (Sukor et al., 2013a). The higher soil microbial biomass results in a higher rate of organic matter decomposition and interaction between the soil microbial activity and nutrient uptake in clayey soil. The fertilizer analysis conducted before the study showed that fish emulsion has 2,003 mg kg^{-1} NH_4^+-N and 16, 412 mg kg^{-1} (NO_3^--N) compared to other fertilizers tested (Table 2). The Com treatment recorded significantly lower PFR at 56 and 112 kg N ha^{-1} on sandy and clayey soils because the total N uptake of fertilizer was not efficient to accumulate N in plant tissue. At 56 kg N ha^{-1}, the total N uptake was the lowest compared to all other treatments on sandy soil which might be the explanation on the negative value of the Compost treatment at 56 kg N ha^{-1} on sandy soil.

Conclusion

Overall, PFR of the Fish treatment was consistently highest, followed by the combination fertilizers (FFCom and FLScyb), Lcyb, Scyb, and composted manure. The Fish treatment recorded higher PFR

(99%) compared to composted manure (44%) at 56 kg N ha^{-1} on clayey soil. In sandy soil, the Fish treatment also recorded significantly higher PFR (57%) compared composted manure (-10%) at 56 kg N ha^{-1}. Although lettuce yield was reported the highest in fish emulsion treatment at 112 kg N ha^{-1} on clayey soil, lettuce could be produced economically at 56 kg N ha^{-1}. In summary, cyanobacterial fertilizers could appear as an alternative source of biofertilizer to composted manure since it provides an amount of available N and recorded higher lettuce yield compared to composted manure. Additional field research is required to quantify N response on lettuce production to help understand the effect of environmental variables in relation to N response from different types of organic fertilizers to optimize fertilizer recovery. Fertilizer recovery optimization in crop production is important to protect the environment, save fertilizer cost and to secure the N supply for the crop production.

TABLES

Table 5 Physicochemical properties of clayey and sandy soils used in the greenhouse study.

Soil	pH	EC	OM	NH_4-N[1]	NO_3-N[1]	P[2]	K[2]	Zn[2]	Fe[2]	Mn[2]	Cu[2]
	Chemical Analyses										
		dS m^{-1}	%	------------------mg kg^{-1} ------------------							
Clayey	6.3	0.3	2.4	3.5	29.3	22.0	1006	2.6	4.9	4.0	3.6
Sandy	7.5	0.3	1.0	1.1	20.9	16.0	511	2.6	4.3	1.0	0.8
	Physical Analyses										
	Sand	Silt	Clay	Texture							
	----------% ----------										
Clayey	62	17	21	Sandy Clay Loam							
Sandy	73	11	16	Sandy Loam							

[1]Soil samples were extracted using 2M KCl
[2]Soil samples were extracted using ammonium-bicarbonate DTPA extraction method

Table 6 Chemical composition of cyanobacterial fertilizers and traditional organic fertilizers as soil-applied and foliar-applied fertilizers in the greenhouse study.

	pH	Total N	Total C	C/N ratio	NH_4^+-N	NO_3^--N
		---%---	---%---		---------mg kg^{-1}---------	
Solid cyanobacteria	7.65	7.67	43.14	5.62	86.22	0.42
Liquid cyanobacteria	7.56	0.10	0.56	5.62	0.24	0.02
Fish emulsion	5.82	2.70	5.20	1.90	2,003.00	16,412.00
Composted manure	8.65	0.45	5.73	11.94	2.18	1.70

Table 7 Six treatments with a combination of two types of fertilizer application methods applied on clayey and sandy soils at 56 and 112 kg ha-1on lettuce under greenhouse conditions.

Treatment	Details	Application Method	Fertilizer Timing
FLScyb	50:50 treatment (Liquid + solid cyanobacteria)	Foliar	0, 2, 4, and 6 weeks after planting
		Soil	Pre-plant
FFCom	50: 50 treatment (Fish emulsion + composted manure)	Foliar	0, 2, 4, and 6 weeks after planting
		Soil	Pre-plant
Lcyb	Liquid cyanobacteria	Soil	0, 2, 4, and 6 weeks after planting
Scyb	Solid cyanobacteria	Soil	Pre-plant
Fish	Fish emulsion	Soil	0, 2, 4, and 6 weeks after planting
Com	Composted manure	Soil	Pre-plant

FIGURES

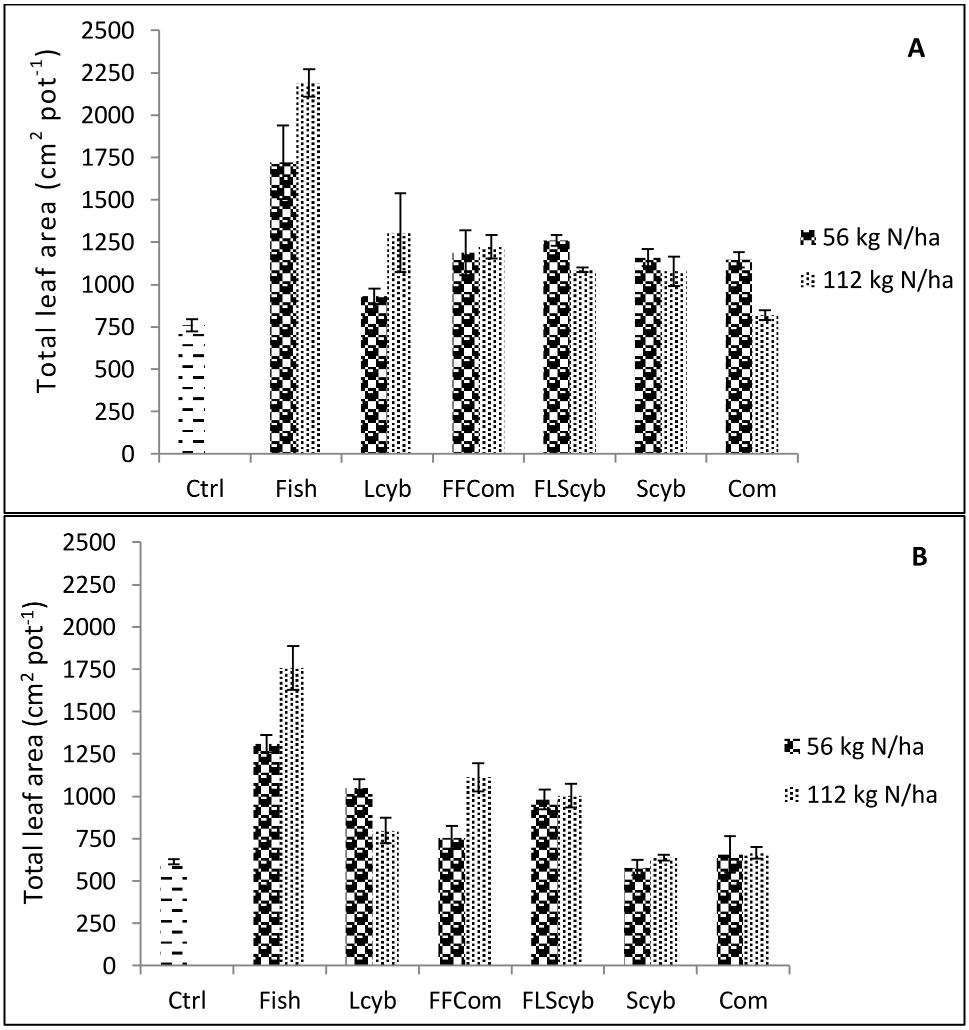

Figure 5 Total leaf area of lettuce from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Scyb'= Soil applied solid cyanobacteria. Bars represent standard errors of mean.

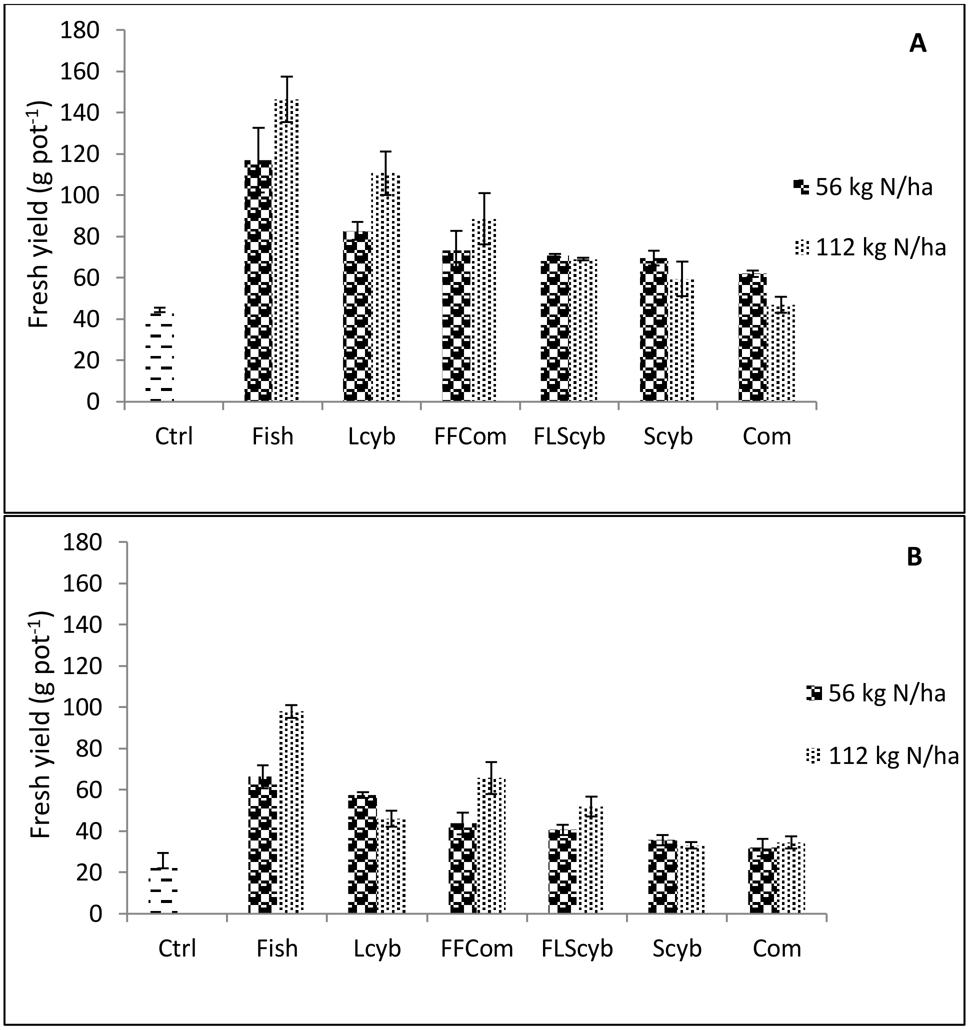

Figure 6 Fresh yield of lettuce from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Scyb'= Soil applied solid cyanobacteria. Bars represent standard errors of mean.

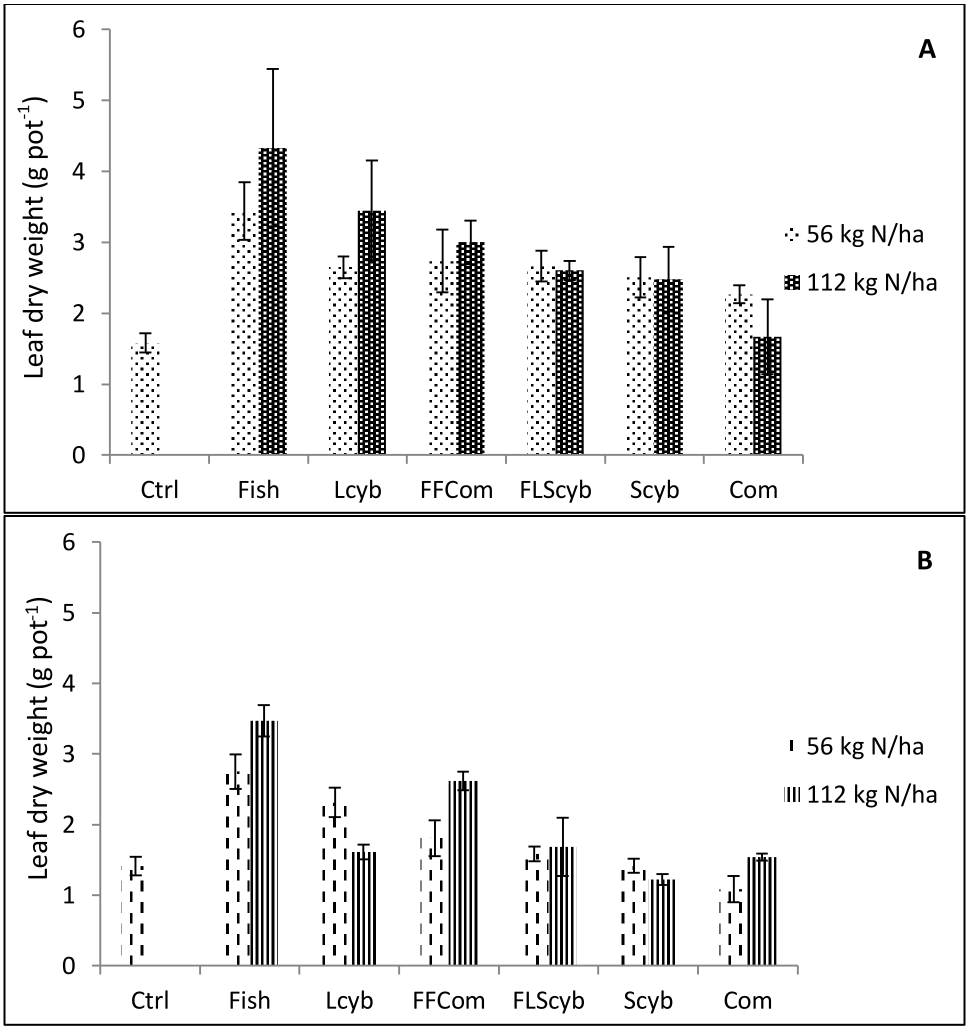

Figure 7 Leaf dry weight of lettuce tissue from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Scyb'= Soil applied solid cyanobacteria. Bars represent standard errors of mean.

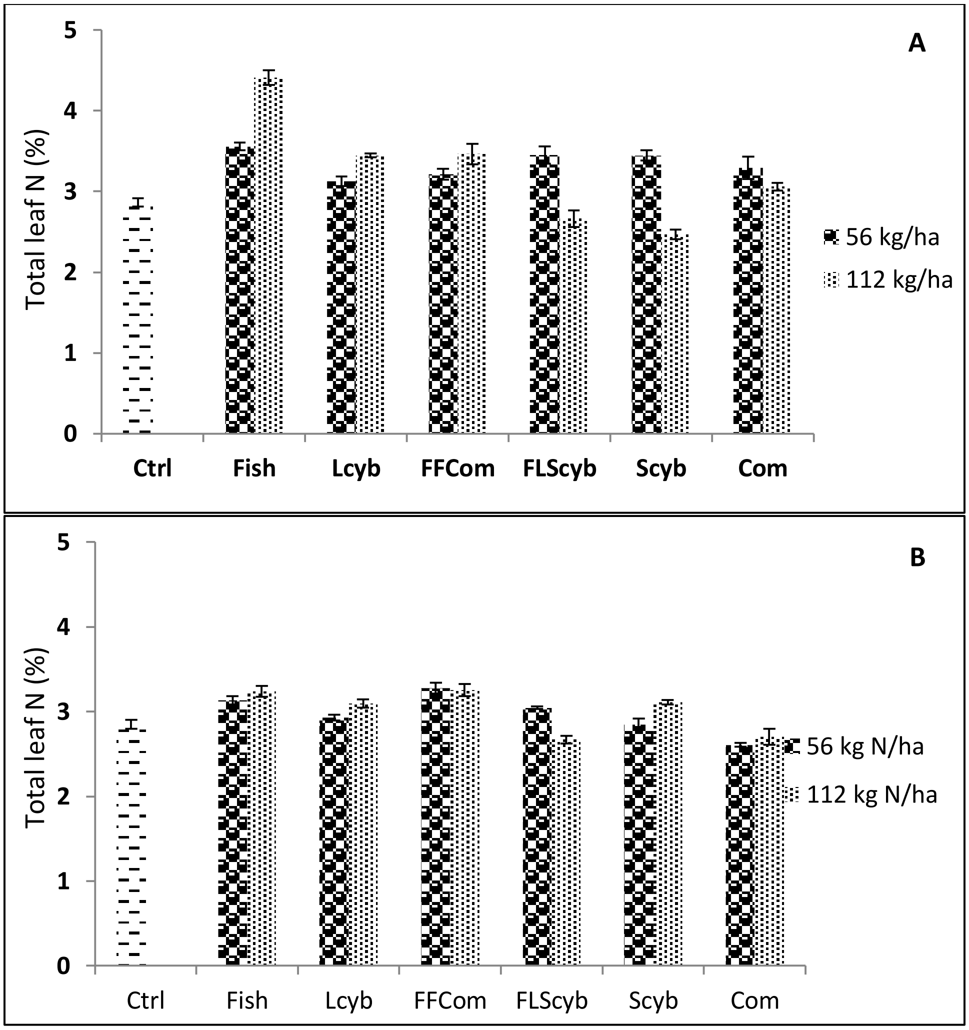

Figure 8 Total leaf N in lettuce tissue from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Scyb'= Soil applied solid cyanobacteria. Bars represent standard errors of mean.

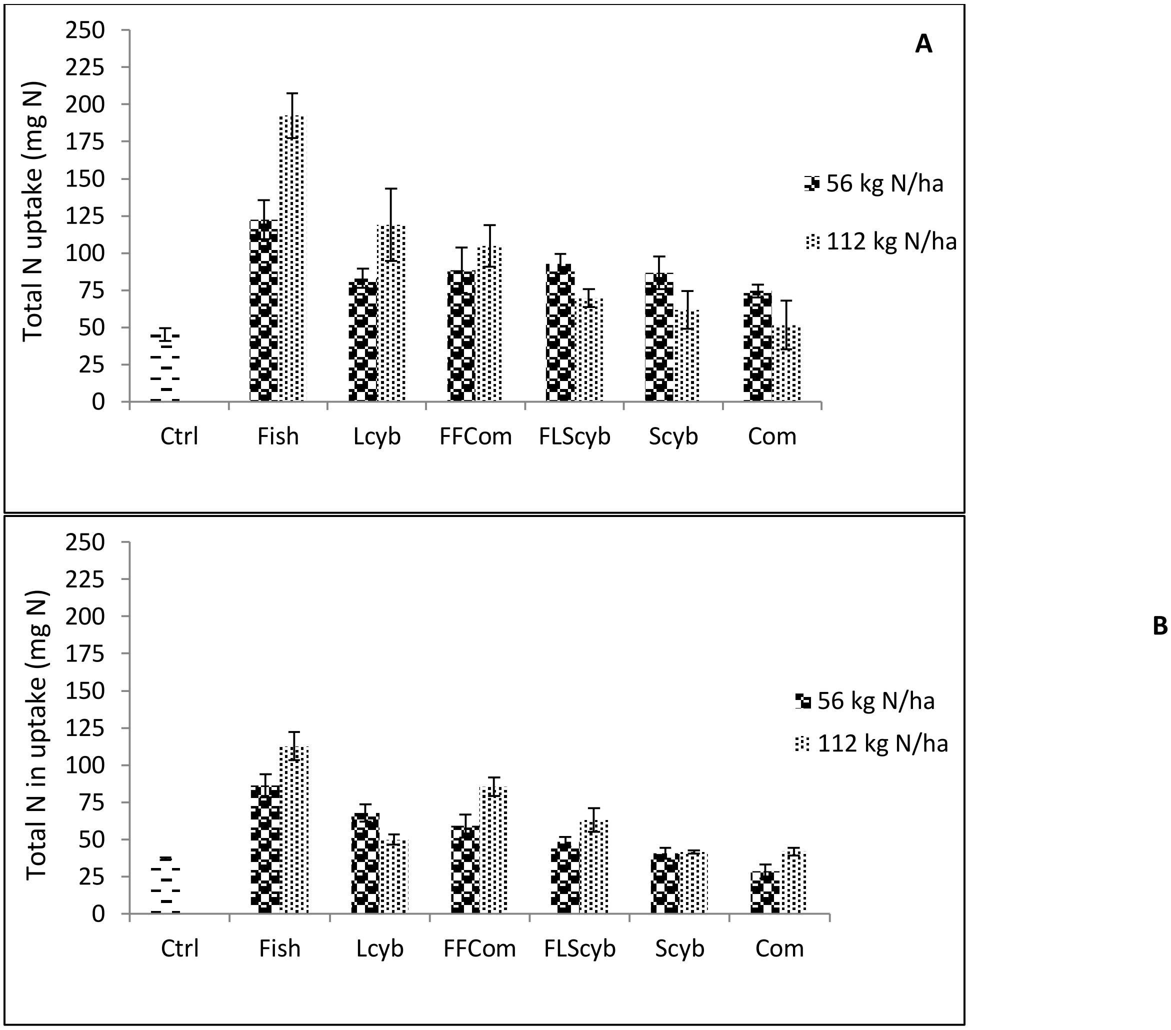

Figure 9 Total N uptake in lettuce tissue from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Scyb'= Soil applied solid cyanobacteria. Bars represent standard errors of mean.

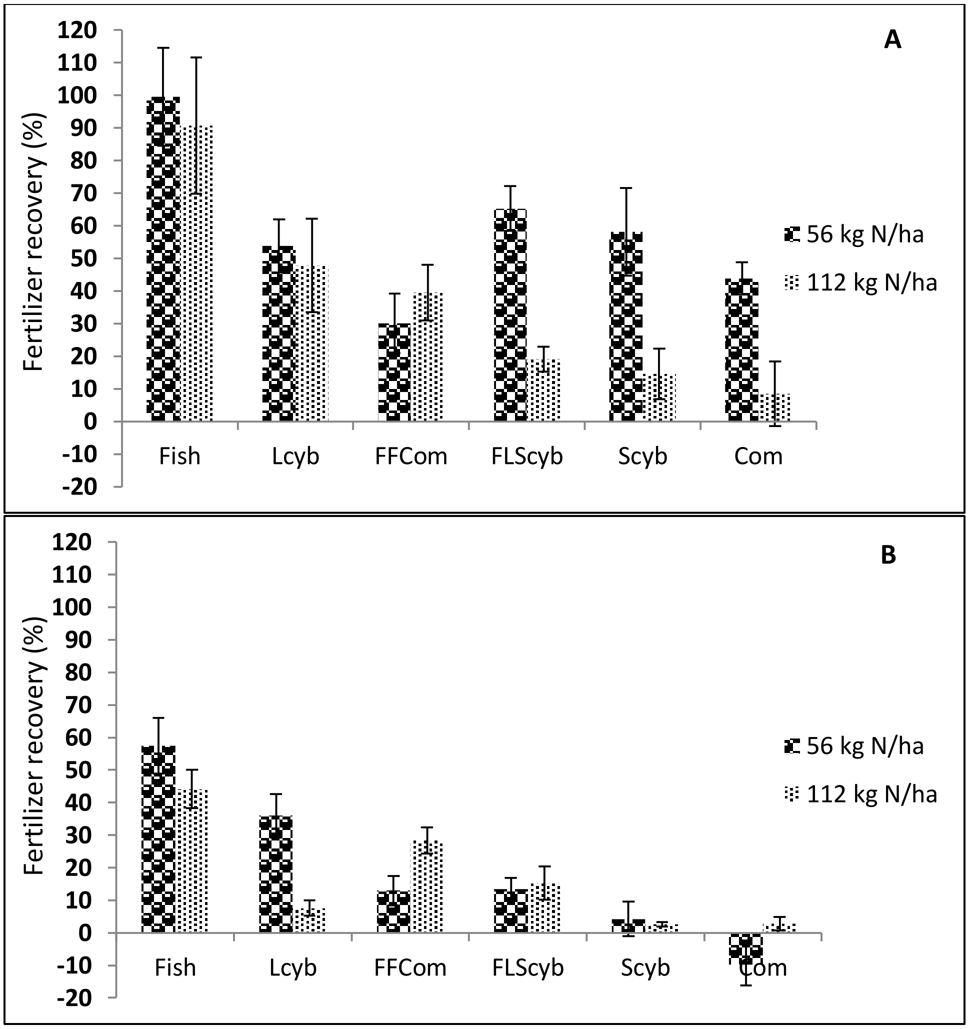

Figure 10 Percentage fertilizer recovery in lettuce tissue from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Scyb'= Soil applied solid cyanobacteria. Bars represent standard errors of mean.

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APPENDIX II

Table 2a. The F-value from Analysis of Variance (ANOVA) of total leaf area, fresh yield, leaf dry weight, total leaf N, total N uptake and percentage fertilizer recovery of lettuce from soil and foliar applied organic fertilizer treatments on clayey and sandy soils at 56 and 112 kg N ha^{-1}.

	Total leaf area	Fresh yield	Leaf dry weight	Total leaf N	Total N uptake	Percentage fertilizer recovery
Source			---F-value---			
soil	18.41*	118.90*	24.76*	279.48*	23.87*	50.09*
treatment	19.13*	55.92*	14.08*	220.77*	16.40*	19.67*
N rate	1.58 ns	12.15*	2.09 ns	75.84*	1.38 ns	1.48 ns
soil*treatment	0.72 ns	3.33 ns	0.42 ns	5.03 ns	5.42 ns	6.32 ns
soil*N rate	0.22 ns	0.22 ns	0.01 ns	24.62 ns	0.17 ns	2.99 ns
treatment *N rate	2.12 ns	5.03*	1.17 ns	130.86*	2.77*	2.44 ns
soil*treatment*N rate	1.40 ns	3.13 ns	1.55 ns	98.36 ns	2.64 ns	2.87 ns

*Significantly difference in comparison with p-value ($p<0.05$) according to Tukey's test of mean separation
nsNo significant difference in comparison with p-value ($p>0.05$) according to Tukey's test of mean separation

NITROGEN USE EFFICIENCY AND LETTUCE ROOT RESPONSE TO SOIL AND FOLIAR APPLIED CYANOBACTERIAL AND COMMONLY-USED ORGANIC FERTILIZERS APPLIED TO DIFFERENT SOIL TEXTURES

Preface

Lettuce (*Lactuca sativa*) is a shallow-rooted crop and requires an extensive amount of nitrogen (N) fertilizer to produce yield. Nitrogen is the main mineral element in plant tissues and N is acquired from the soil by the roots. In this study, root response to N fertilization was assessed to determine the efficiency of N uptake by lettuce to produce yield. A greenhouse study was conducted for 63 days to evaluate cyanobacterial and traditional organic fertilizers application on lettuce root response. Root: shoot ratio, root dry weight, root surface area, and root length density were measured at the end of the greenhouse study. Nitrogen use efficiency (NUE) was calculated based on the analyses results. There was no significant difference observed in root dry weight. The composted manure (Com) treatment recorded significantly higher root: shoot ratio at 56 kg N ha^{-1} while foliar and soil applied liquid cyanobacteria (FLScyb) treatment recorded lower root: shoot ratio at 112 kg N ha^{-1}. The foliar applied fish emulsion and soil applied composted manure (FFCom) treatment recorded the highest root surface area compared to other treatments at 112 kg N ha^{-1} on clayey soil . The FLScyb treatment recorded higher root surface area compared to the Com treatment at 112 kg N ha^{-1} on sandy soil. The fish emulsion (Fish) treatment recorded higher root length density at 112 kg N ha^{-1} on clayey soil while FLSCyb recorded higher root length density on sandy soil at 112 kg N ha^{-1} compared to the Fish and solid cyanobacteria (Scyb) treatments. In conclusion, the Fish treatment recorded 35 % higher NUE at 56 kg N ha^{-1} on clayey soil while Scyb treatment has significantly 24% higher NUE compared to Com treatment at 56 kg N ha^{-1} in sandy soil.

Introduction

Lettuce is a shallow-rooted crop commercialized internationally based on its consumption rate and economic importance throughout the world, and it ranks second among all vegetables produced for consumption in the United States (Coelho et al. 2005). Nitrogen (N) is generally the most difficult nutrient to manage for organic crop production, and N uptake is dependent on the amount of plant-available N supplied. Before N in organic matter (OM) can be taken up by plant roots, the organic matter must be broken down so that N is in the form of ammonium (NH_4^+) or nitrate (NO_3^-) ions. Nitrogen taken up as NH_4^+ is incorporated into organic compounds in the roots while NO_3^- can be stored in vacuoles of the roots and shoots. Both ions (NH_4^+ and NO_3^-) affect the anion-cation balance in both the plant and the rhizosphere. In the soil, NO_3^- moves more easily than NH_4^+ and is often more available to plants (Marschner 1995). The beginning of rapid growth and increased N uptake by roots occurs after emergence to efficiently take up N to develop the root system and produce high yield (Masson et al. 1991). Burns (1996) showed that lettuce plants have reduced growth if exposed to N deficiency in early growth while higher N supply has several effects such as smaller roots and higher shoot to root ratio at the expense of root growth. The growth and development of a root system is sensitive to modification by extrinsic factors such as the supply and distribution of nutrients in the soil.

Nitrogen use efficiency (NUE) is affected by the amount of N available which can be quantified with measurements of fertilizer N recovery. In organic vegetable production, optimizing NUE is important to protect the environment, and to secure the N supply for the crops (Thorup-Kristensen 2001). It is important to know the rooting patterns of vegetable crops when trying to optimize the NUE in vegetable production since root length and surface area are important indicators of potential uptake of water and nutrients. Information on root growth can also be used to design crop rotations with low N leaching losses and high overall NUE (Thorup-Kristensen and Grevsen 1999). In simulation models, Greenwood et al. (1996) reported that rooting depth development must be included as rooting depth determines how much of the soil inorganic N is actually available for the plants. Lack of synchrony between N mineralized and crop N uptake is a challenge for fertility management in organic systems (Gaskell et al. 2010).

Knowledge regarding nutrient release from organic N fertilizers is important to estimate nutrient availability to crops, to determine optimum application rates, timing of fertilizer application and potential leaching of nutrients (Hadas and Portnoy 1997). Fertilizer cost savings could be achieved by making the right choice about fertilizer type, application rates, application methods, and the timing of applications to meet N crop demand (Phillips et al. 2009). For soil-applied fertilizers, timing is critical because single-application soil-applied fertilizers should coincide with root development to optimize nutrient uptake (Conradie 1980) while foliar-applied fertilizers enhanced the root growth of cucumber plants and effectively increased the total plant dry biomass (Nelson and Van Staden 1984). Use of foliar-applied seaweed products on greenhouse-grown tomatoes also increased root growth, which resulted in increased nutrient uptake (Vavrina et al. 2004).

According to the USDA National Agriculture Statistics Service, organic farmers in the U.S. spend more than $150,000,000 on fertilizers annually. Sixty-five percent of organic farmers use manure, and 58% use compost; both are low in N content (~1%) and expensive to transport (USDA 2008). The potential drawbacks of traditional organic fertilizers are relatively low nutrient contents, high transportation costs, odors, and the uncertainty of releasing enough nutrients at the proper time (Raupp 2005). The increasing amounts of nutrients from agricultural activities that leach into the environment and the higher price of N fertilizers constitute serious concerns for both the public and farmers (Roberts 2008). The escalating cost of N fertilizers has made it imperative to find alternate sources of N fertilizer.

Cyanobacteria can be a useful potential biofertilizer since it can both photosynthesize and fix N and have great adaptability to various soil types (Mishra and Pabbi 2004). Biologically active compounds may be liberated from cyanobacteria growing on the surface of moist soils and liquid culture. Growth-simulating compounds may be released either actively by living algal cells or following cell death and lysis (Rodgers et al. 1979). The compounds can be assimilated by plants and enhance their growth. Positive effects on plants from cyanobacterial application to the soil can be observed through the secretion of plant growth regulators such as auxins, gibberellins, and cytokinins that stimulate metabolic activities in the roots (Cocking 2003). Nitrogen-fixing bacteria are able to enter roots from the rhizosphere, particularly at the base of emerging lateral roots, between epidermal cells and through root hairs (Linkohr et al. 2002). Roots act as a source of organic carbon as a food source to bacteria to initiate the process of N mineralization. El-Gaml (2006) explained that biofertilization using cyanobacteria led to increased microbial diversity in soil through increased organic matter, microbial activity, increased dehydrogenase and nitrogenase activities and subsequently improved soil fertility. Microorganisms that colonize the rhizosphere help plants acquire N from soils via effects on root morphology and physiology. Plant root systems can respond to nutrient availability and distribution by changing their root system architecture. Changes in NO_3^- availability were found to have contrasting effects on primary root length and lateral root density (Linkohr et al. 2002).

Many studies have been conducted on N response of plants to organic fertilizers, but none of them compared cyanobacterial bio-fertilizer and traditional soil and foliar applied organic fertilizers on lettuce root response. There is also no literature dealing specifically with how soil and foliar applied organic fertilizers affect N response on lettuce applied to different soil textures. The objectives of this study were i) to assess N response of lettuce roots to soil and foliar applied organic fertilizers applied to clayey and sandy soils, and ii) to determine the influence of different soil textures on root characteristics and NUE of lettuce due to soil and foliar organic fertilizer applications.

Materials and methods

Site description

The pot experiment was carried out from May to August 2012 for 63 days in a greenhouse at Colorado State University. Romaine lettuce (*Lactuca sativa*) variety ‘Summer Crisp’ was grown in a greenhouse equipped with an evaporative cooling system under natural daylight at the following geographic coordinates: 40°34’ 17.56”N, 105°04’49.44”W.

Soil sampling

Two soil samples were collected from different areas that represent two soil textures, sandy and clayey soils. Clayey soil was collected using shovel from 0-15 cm from the Horticulture Farm, Colorado State University (40°39'12.28"N, 104°59'59.36"W). The soil was classified as a fine, smectitic, mesic Aridic Argiustoll of the Nunn series (NRCS 1980). The sandy soil was collected using the same method from a certified organic farmer in Nunn, Colorado (40°20'9.90"N, 104°34'24.67"W) and was classified as fine-loamy over sandy or sandy-skeletal, mixed, superactive, mesic Aridic Argiustoll of the Altvan series (NRCS 1980). Soils were sieved consecutively with 8.0 mm and 2.0 mm sieve to obtain a uniform particle size.

Soil analyses

The soil physical analyses included bulk density and particle size distribution to determine the percentage of sand, silt and clay. Bulk density was determined using the cylindrical core method (Arshad et al. 1996). Particle size distribution was determined using the hydrometer method based on Stoke’s law (Gee and Bauder 1979). Chemical analyses including electrical conductivity and soil pH were measured in the supernatant suspension of 1:1 soil to water using a Mettler Toledo pH/EC meter (Thermo Fischer Scientific, Waltham, MA). Organic matter content was determined by loss on ignition method (Blume et al. 1990). The soil chemical and physical analyses results are presented in Table 8.

Fertilizer analyses

The composted manure was obtained from an organic dairy farm in Gill, CO while the fish emulsion was obtained from Daniels® (3-1-1) (Daniels Agro Sciences LLC, East Greenwich, RI). The cyanobacteria species used in this study was *Anabaena cylindrica*. Liquid cyanobacterial fertilizer was obtained from cyanobacteria culture which was cultured for two weeks in Allen and Arnon media with optimal light conditions. Solid cyanobacterial fertilizer was filtered from the liquid cyanobacteria and was sun-dried. The solid cyanobacteria was mixed and ground to obtain a homogeneous powdered form of dried cyanobacterial fertilizer. Total C and N contents of the different types of organic fertilizers were analyzed using a LECO CN analyzer (Leco Corp., St Joseph, MI) to determine the C/N ratio of each fertilizer (Keeney and Nelson, 1982) (Table 9). Both solid and liquid fertilizers were analyzed using an Alpkem Flow Solution IV Auto Analyzer (OI Analytical, College Station, TX) for inorganic N analyses (NH_4^+ and NO_3^-).

Experimental design

Treatments consisted of two soil textures (clayey and sandy soils), three nitrogen rates (0, 56, and 112 kg N ha^{-1}) and six types of organic fertilizers (liquid cyanobacteria, solid cyanobacteria, fish emulsion, composted manure, combination fertilizer of foliar applied liquid cyanobacteria with soil applied solid cyanobacteria, and combination fertilizer of foliar applied fish emulsion with soil-applied composted manure). The treatments were arranged in a Randomized Complete Block Design (RCBD) with factorial combinations of soil textures, N rates, and types of organic fertilizer with three replications.

Plant material

Seeds of 'Summer Crisp' lettuce (Johnny's Selected Seed, Waterville, ME) were planted in plastic trays containing 3.5 kg of well-mixed growing media with PRO-MIX perlite (Premier Horticulture Inc., Quakertown, PA) in the first week of May 2012. After four weeks of germination, seedlings were transplanted into pots.

Pot experiment

Before planting, the composted manure and solid cyanobacteria fertilizers were homogeneously incorporated into the soil in each pot containing 2.88 kg of soil according to the assigned treatments (Table 10). When the seedlings were four weeks old, three seedlings were transplanted into each pot, and a few days after seedling emergence, the plants were thinned to one seedling per pot. The pots were watered and maintained at an equal weight throughout the whole experiment.

Methods of fertilizer application

The soil applied liquid fertilizer treatments (liquid cyanobacteria and fish emulsion) were split into four applications to the soil over time (0, 2, 4 and 6 weeks) to mimic fertigation, while the soil applied solid fertilizer treatment (solid cyanobacteria and composted manure) was applied all at once prior to planting based on the calculated N rate (56 and 112 kg N ha^{-1}). Liquid fertilizers were applied in four split applications every two weeks for the soil and foliar applied treatments (fish emulsion and liquid cyanobacteria). However, due to the adequate level of P and K based on the initial soil analysis, P and K were not applied (Table 1). The total N fertilizer was split with the ratio of 50:50 into solid and liquid forms for the combination fertilizer treatments (Table 10). Treatments were started immediately after transplanting.

Cultural practices

Weeding was carried out by hand two weeks after planting. Beneficial insects (*Phytoseiulus persimilis)* were applied on the plant and soil surface one week after transplanting as a form of bran to keep the plants from getting a thrips infestation. To threat aphids and loopers, Entrust® SC insect control (Dow AgroSciences, Indianapolis, IN) certified by the Organic Materials Review Institute (OMRI) was applied eight weeks after transplanting and at the end of the experiment.

Root measurement analyses

Before root measurement analysis was conducted, any loose plant residue on the soil surface was first brushed away from the sampling site after harvesting. Roots were washed using the root washing protocol developed by the Natural Resources Ecology Lab Soil Preparation Laborarory (Jorin 2010) at 63 days after transplanting. Roots were separated and weighed separately for fresh root mass. For the measurement of root surface area and root length density, each sample was spread on a clear plastic tray (25 cm x 40 cm x 5 cm). The tray was filled with distilled water to a level above the thickest root to minimize images with many meniscuses between the surface of the root and the surface of the fluid. The root sample was uniformly spread across the entire surface of the tray. The tray was covered with a clear plastic lid and then covered with a light box for scanning. Then, root samples were scanned using WinRHIZO (Regent Instruments Inc., Quebec) desktop optical root scanner to determine the root surface area, and root length density. All harvested samples were then dried at 70°C for 72 hours and weighed.

Calculation of nitrogen use efficiency

Nitrogen use efficiency was calculated by taking the dry weight of lettuce divided by the amount of fertilizer N applied (Dobermann 2005).

Statistical analysis

Data were analyzed using SAS version 9.3 (SAS Institute Inc., Cary, NC). The Univariate and Boxplot procedures were used to evaluate the normality of data distribution. If distribution was normal, then analysis of variance (ANOVA) was performed on the data by using the GLM procedure. The Tukey value was calculated from the obtained mean square errors to determine whether main effects or interactions were significant ($P < 0.05$). The relationships between root: shoot ratio, root surface area, root length density, total N uptake, and NUE were assessed by linear correlation using the CORR procedure.

Results

Root: shoot ratio

Significant interaction was observed among fertilizer treatments and N rate ($P= 0.0185$) on both soils. The FFMan and Com treatments recorded higher root: shoot ratio than other fertilizer treatments at 56 kg N ha^{-1} on clayey soil (Figure 11A). In sandy soil, the Com treatment (0.79) recorded significantly higher root: shoot ratio compared to Scyb (0.25), Ctrl (0.23), Fish (0.23) and Lcyb (0.18) treatments at 56 kg N ha^{-1} (Fig. 1B). At 112 kg N ha^{-1}, FLScyb (0.52) and Lcyb (0.40) recorded significantly higher root: shoot ratios compared to other treatments on sandy soil (Figure 11B). The root: shoot ratio of Lcyb and FLScyb treatments were significantly higher compared to other treatments at 112 kg N ha^{-1} on sandy soil, but not significantly different from each other. At 56 kg N ha^{-1}, the Com treatment was significantly higher than the Fish, Scyb, and Lcyb treatments on sandy soil. In this study, root: shoot ratio was 10% higher in sandy soil than clayey soil in Ctrl. The Com treatment on sandy soil also recorded higher root: shoot ratio in sandy soil compared than clayey soil.

Root surface area

Significant interaction was also observed in root surface area between treatment and N rate (P=0.0126). The FFCom treatment recorded significantly higher root surface area compared to Com and Ctrl at 56 and 112 kg N ha^{-1} on clayey soil (Figure 12A). The Lcyb, FFCom, FLSyb, Scyb, and Com treatments recorded higher root surface area at 112 kg N ha^{-1} compared to 56 kg N ha^{-1}. In sandy soil, FFCom (616 cm^2) recorded significantly higher root surface area compared to Scyb (477 cm^2), FLScyb (461 cm^2), Compost (415 cm^2), and Ctrl (323 cm^2) at 56 kg N ha^{-1} on sandy soil (Figure 12B**)**. At 112 kg N ha^{-1}, the FLScyb (744 cm^2) and Fish (742 cm^2) treatments were significantly different in root surface area compared to Scyb (517 cm^2), FFCom (516 cm^2) and Ctrl (323 cm^2). It was also observed that 10% higher root surface area on sandy soil than clayey soil under Ctrl treatment.

Root length density

Significant interactions were observed in root length density between treatment and N rate (P=0.0030). The Ctrl treatment (2457 cm m^{-3}) recorded significantly lower root length density compared to Scyb, Lcyb, Fish, FFCom, and FLScyb when applied at 56 and 112 kg N ha^{-1} on both soils (Fig. 13). The FLScyb treatment (6601 cm m^{-3}) recorded significantly higher root length density compared to Fish (4884 cm m^{-3}), Scyb (4824 cm m^{-3}), FFCom (4806 cm m^{-3}), and Ctrl (3803 cm m^{-3}) at 112 kg N ha^{-1} on sandy soil (Fig. 13B). In the Ctrl treatment, root length density was almost 39% higher in sandy soil than clayey soil. Higher root length density was also recorded under the FLScyb treatment on sandy soil than clayey soil when applied at both N rates.

Nitrogen use efficiency

Significant differences were also observed for NUE among treatments (P<.0001) and N rates (P <.0001). The Fish treatment (40 g lettuce/g N fertilized) recorded significantly higher NUE compared to the Com treatment (26 g lettuce/g N fertilized) when applied at both N rates on clayey soil (Fig. 14A). The Fish treatment recorded 20% higher NUE on clayey soil compared to sandy soil and the Fish treatment also recorded 35 % higher NUE at 56 kg N ha^{-1} than 112 kg N ha^{-1} on clayey soil. In general, the highest NUE was recorded at 56 kg N ha^{-1} although it was not significantly different from 112 kg N ha^{-1} on both soils. The Lcyb treatment recorded 16% higher NUE compared to the Com treatment on clayey soil, while the Fish treatment recorded 23% higher NUE compared to the Lcyb treatment. In sandy soil, the Fish treatment showed 60% higher NUE compared to the Com treatment while the Lcyb treatment recorded 52% higher NUE compared to the Com treatment (Fig. 14B). The Scyb treatment has significantly higher NUE (24% higher) compared to Com treatment at 56 kg N ha^{-1} in sandy soil.

Discussion

Effect of soil texture and fertilizer treatments on lettuce root response

In this study, root: shoot ratio, root surface area, and root length density was higher in sandy soil than clayey soil under the control treatment. Soil texture significantly affects the amount of root biomass by regulating soil nutrient distribution (Schimel et al. 1985). Plants often respond to changes in nutrient availability by altering the allocation of carbon to root biomass (Cuevas and Medina 1988). Based on the study by Silver et al. (2000), sandy soils had significantly greater root biomass than clayey soils due to greater carbon allocation to roots for nutrient scavenging (Cuevas and Medina 1988) in the Amazon forest system. In this study, significantly higher root: shoot ratio in the Com treatment as compared to Fish, Scyb, and Lcyb fertilizer treatments applied at 56 kg N ha^{-1} to sandy soil was recorded because the shoot yield was lower (Sukor et al. 2013a). The fraction of carbohydrates allocated to root growth generally increases in less favorable conditions due to scavenging for soil nutrients in sandy soil (Soundy et al. 2005). A significant negative correlation was observed between total leaf area and root: shoot ratio (r =-0.23, P <.0001) which is expected due to the lower biomass partitioning into shoots resulting in a higher root: shoot ratio. Plants with higher root: shoot ratio take more carbon away from the shoots, limiting the plant's capacity to fix and store carbon in the harvested yield (Gallais and Coque 2005).

Effect of soil texture on nitrogen use efficiency

In this study, highest lettuce yield was recorded on clayey soil at 112 kg N ha^{-1} (data not shown) while highest NUE was recorded also on clayey soil but at 56 kg N ha^{-1} (Fig. 14A). According to Natake (2012), soil texture determines the surface area in a volume of soil which influences the availability of soil water and nutrients, and root biomass distribution. However, at certain point, root biomass increased with clay content because clay particles create a suitable environment for plant growth and this depends on soil environmental condition such as in the condition of adequate fertilizer and available nutrients to be taken up by roots (Schimel et al., 1985). This could be explained by the higher lettuce yield in clayey soil than sandy soil at 56 and 112 kg N ha^{-1} as clays with a high specific surface area are expected to adsorb more available nutrients than sandy soils (Tate and Theng 1980). As particle size decreases, total surface area per unit volume of soil increases which enhance nutrient absorption and higher cation exchange capacity in clayey soil enhance nutrient retention. Small clay particles have higher water retention, dissolved nutrients, and soil organic matter due to their large surface area per unit volume (Feller and Beare 1997). Newman (1966) evaluated the differences in total root surface area among cultivars of oats and barley and found that yield was correlated with root system parameters. In this study, root surface area was found to be significantly correlated with root dry weight (r=0.33, P<.0031) and fresh yield (r=0.33, P=0.0032) which shows that root growth is important in determining the nutrient supply and biomass assimilation to the shoots which, in turn, affects crop yield. Newman (1966) found that more branched roots increased surface area and eventually increased nutrient uptake, but this is very dependent on soil texture. Soil texture patterns strongly influence nutrient concentrations in the soil. The soil particle size significantly influences soil fertility where larger sand particles have lower retention of nutrients (Chapin et al. 2002). Vitousek and Matson (1988) claimed that less available N concentration was mineralized on sandy soil because it exhibited low potential net NO_3^- production which influence the available nutrients to be taken up by plants (Silver et al. 2000). Clayey soil exhibited higher initial concentrations of NO_3^- and lower NH_4^+ than sandy soil and greater rates of potential net NO_3^- production (Sukor et al. 2013b). In terms of nutrient availability, clay-rich soils tend to have more free cations and exchangeable bases which are necessary nutrients for plants (Chapin et al. 2002).

The amount of N associated with clay particles is mainly affected by soil texture and not by the input of organic N to the soil (Jenkinson, 1988). Clayey soils have higher organic N contents than sandy soils when supplied with similar inputs of organic material (Amato and Ladd 1992). The difference between clayey and sandy soils is assumed to result from greater physical protection of soil organic matter (SOM) in clayey soils. Vitousek and Matson (1988) claimed that less SOM is stabilized in sandy soil than clayey soil. Smaller particles in clayey soil retain water more effectively than larger particles in sandy soil (Hassink 1997) as soil nutrients depend on water movement since 79% of soil N is supplied to the root by mass flow (Barber 1995) which relates to higher yield found in the clayey soil than in the sandy soil when applied at 56 and 112 kg N ha^{-1}. However, sandy soils have better drainage leading to better aeration which increases oxygen concentration in soil for better root proliferation (Silver et al. 2000). This is verified in our study with higher root: shoot ratio, root surface area, and root length density in sandy soil than clayey soil.

Effect of soil applied fertilizers on lettuce root response

The Fish treatment recorded significantly higher root length density and root surface area compared to Control at 112 kg N ha^{-1} on clayey soil while the Lcyb treatment recorded significantly higher root length density compared to Control at 56 kg N ha^{-1}. Greater root length density, which means greater root length per soil volume and greater numbers of smaller diameter roots, can improve nutrient acquisition by increasing root surface area without an increase in carbon allocation to the root (Marschner 1995). Emino (1981) found that the ability of fish emulsion to produce larger plants despite lower N-P-K values is due to its complex composition including plant growth hormones, amino acids, and other vitamins. However, the specific chemical composition of fish emulsion is still unknown because the composition varies according to types of fish used and different ingredients used by different suppliers. Plant roots exude large amounts of complex organic compounds into the soil (Kennedy 1998), and microorganisms in soil have the enzymatic capacity to utilize macromolecular organic N (Paul and Clark 1996). Roots supply soil microbes C, while the microbes access soil organic N and make this available to be taken up by roots (Kinzig and Harte 1998).

Effect of combined soil and foliar applications on lettuce root response

The FLScyb (0.52) treatment recorded a higher root: shoot ratio compared to Lcyb (0.40) and Scyb (0.28) at 112 kg N ha^{-1} on sandy soils. This might be due to different fertilizer application timing influencing the carbohydrate partitioning between roots and shoots which would affect their adaptability to different conditions. Fertilizer placement influenced the root: shoot ratio as the comparison between the FLScyb and Scyb treaments recorded significantly higher root: shoot ratio on the FLScyb treatment. The liquid cyanobacteria in the FLScyb treatment was applied as foliar fertilizer while the liquid cyanobacteria was applied in the Lcyb treatment as soil applied fertilizer every two weeks in comparison to the Scyb treatment which was applied once prior to planting. The FLScyb (0.52) treatment recorded higher significantly higher root: shoot ratio at 112 kg N ha^{-1} compared to the Scyb (0.25) treatment at 56 kg N ha-1 on sandy soil. However, there was no significant observed on the root: shoot ratio between the FLSCyb treatment and the Scyb treatment at both N rates on clayey soil. Root: shoot ratio in the FLScyb and Scyb treatments were lower compared to the Com treatment in clayey soil at 56 kg N ha^{-1}. In soils with high levels of nutrition, root: shoot ratios are generally lower (Marschner 1995). The reduction in root: shoot ratio may reflect a response to available N (Wilson 1988). Laboratory studies have indicated that cyanobacteria excrete indole-3-acetic acid (IAA) and amino acids which can stimulate the growth of microbial populations in soil (Karthikeyan et al. 2007). Cyanobacteria have positive effects on plant growth and can produce phytohormones such as auxin which could increase hormone levels inside the plant (Long et al. 2003). Phytohormones such as auxin could be an important factor contributing to the increasing numbers of roots (Spaepen et al. 2007). Auxin long-distance signal from shoot to root regulates the inhibition of early root development by high rates of NO_3^- supply in *Arabidopsis* seedlings (Forde 2002).

The FLScyb treatment recorded significantly higher root length density compared to Scyb treatment at 112 kg N ha^{-1} on sandy soil. Koltai (2011) claimed that initiation of lateral root and primary root elongation are affected by the concentrations of nutrient and are regulated by phytohormones such as IAA. It has been shown that N is involved in mechanisms to regulate root architecture. Increasing root length density will improve N acquisition in some crops (Kage 1997). Higher root length densities have led to higher NO_3^- uptake capacity and less leaching (Wiesler and Horst 1993). In *Arabidopsis*, high NO_3^- levels inhibit lateral root growth and reduce root length density (Zhang and Forde 1998).

In this study, the foliar applied fish emulsion and soil applied composted manure (FFCom) treatment recorded the highest root surface area at 56 kg N ha^{-1} on clayey and sandy soils (Fig. 12). The FFCom (753 cm^2) treatment recorded significantly higher root surface area at 112 kg N ha^{-1} compared to the Com treatment (354 cm^2) at 56 kg N ha^{-1} on clayey soil. There was no significant difference observed between both of the fertilizers on sandy soil. In the FFCom treatment, fish emulsion was applied as foliar fertilizer, and according to Hoetz and Brown (2004), foliar fertilizer could also be absorbed by roots when excess solution drips into the soil but it is not the primary mechanism which contributes to the highest root surface area. Marschner and Cakmak (1986) reported that foliar application of fertilizers can supply nutrients more rapidly than methods involving root uptake which made the local growers use foliar fertilizers to supplement soil applied nutrients to compensate for decreased root activity. Studies by Alexander (1985) showed that foliar fertilizers promote root nutrient absorption in winter wheat.

In this study, root surface area was found to be significantly correlated with root dry weight ($r=0.33$, $P<.0031$) and fresh yield ($r=0.33$, $P=0.0032$) which shows that root growth is important in determining the nutrient supply to the shoot which in turn affects crop yield. The FLScyb treatment of foliar and soil applied liquid cyanobacteria recorded significantly higher root surface area compared to Scyb, FFCom and Ctrl when applied at 112 kg N ha^{-1} on sandy soil. However, Hegazi et al. (2010) found that the application of cyanobacteria as fertilizer was found to be the best compared to mineral N fertilizer for enhancing plant growth at 50 and 75 kg N ha^{-1} on beans. The reason might be due to the growth-stimulating compounds, such as auxin and amino acids released by living cyanobacteria cells following cell death and lysis coupled with the atmospheric N-fixation (Rodgers et al. 1979).

Effect of nitrogen fertilizer treatments on nitrogen use efficiency

In general, NUE was higher at 56 kg N ha^{-1} compared to 112 kg N ha^{-1} and in clayey soil compared to sandy soil. In this study, higher NUE (40 g lettuce/g N fertilized) was observed under the soil applied fish emulsion treatment at 56 kg N ha^{-1} compared to 112 kg N ha^{-1}. Dobermann (2005) reported that crops rarely utilize more than 40% of applied N, and NUE declines with increasing N-fertilizer use. Nitrogen use efficiency also depends on the timing of fertilizer application according to plant growth demand. In this study, the soil applied fish emulsion and liquid cyanobacteria were applied in a split application at 56 and 112 kg N ha^{-1} on both soils according to the plant growth. According to Epstein and Bloom (2005), efficiency increases with N rate supplied, up to a maximum, and decreases when plants down-regulate their transport mechanisms while only absorbing rates sufficient to meet plant-growth demand. In this study, the higher NUE observed in the clayey soil compared to the sandy soil might be due to their soil properties; for example, the smectitic clayey soil has higher CEC (23 cmol kg^{-1}) compared to the sandy soil (5 cmol kg^{-1}). Soil with higher CEC has higher nutrient retention rate which can become available for plant uptake (Table 8).

The initial NH_4^+ and NO_3^- content in fish emulsion was the highest compared to other fertilizers (Table 2). In soil, NO_3^- stimulates lateral root elongation by increasing rates of cell production in the root tips (Zhang et al. 1999). The accumulation of high tissue concentrations of NO_3^- in the leaf via plant uptake mechanisms is responsible for generating a long-distance signal that regulates root development (Zhang et al. 1999). It has been shown that shoot-derived auxin is important for stimulating root emergence in *Arabidopsis* (Bhalerao et al. 2002). Therefore, it could be predicted that an increase in soil NO_3^- due to fish application would stimulate root development to increase the efficiency of nutrient uptake in lettuce. However, NO_3^- concentration in lettuce leaf was not measured in this study and further research needs to be done to elucidate the relationship between soil-plant nutrient uptake to improve NUE.

In conclusion, the foliar and soil applied liquid cyanobacteria fertilizer recorded higher root surface area compared to composted manure fertilizer at 112 kg N ha^{-1} on sandy soil. The fish emulsion fertilizer recorded 35 % higher NUE compared to other fertilizers at 56 kg N ha^{-1} on clayey soil, while solid cyanobacteria fertilizer had 24% higher NUE compared to composted manure at 56 kg N ha^{-1} in sandy soil.

TABLES

Table 8 Physicochemical properties of clayey and sandy soils used in the greenhouse study.

	Chemical Analyses										
	pH	EC	OM	NH_4-N[1]	NO_3-N[1]	P[2]	K[2]	Zn[2]	Fe[2]	Mn[2]	Cu[2]
Soil		dS m^{-1}	%	----------mg kg^{-1}----------							
Clayey	6.3	0.3	2.4	3.5	29.3	22.0	1006	2.6	4.9	4.0	3.6
Sandy	7.5	0.3	1.0	1.1	20.9	16.0	511	2.6	4.3	1.0	0.8
	Physical Analyses										
	Sand	Silt	Clay	Texture							
	--------------------%-------------------										
Clayey	62	17	21	Sandy Clay Loam							
Sandy	73	11	16	Sandy Loam							

[1]Soil samples were extracted using 2M KCl
[2]Soil samples were extracted using ammonium-bicarbonate DTPA extraction method

Table 9 Chemical composition of cyanobacterial fertilizers and traditional organic fertilizers as soil-applied and foliar-applied fertilizers in the greenhouse study.

	pH	Total N	Total C	C/N ratio	NH_4^+-N	NO_3^--N
		---%---	---%---		---------mg kg^{-1}---------	
Solid cyanobacteria	7.65	7.67	43.14	5.62	86.22	0.42
Liquid cyanobacteria	7.56	0.10	0.56	5.62	0.24	0.02
Fish emulsion	5.82	2.70	5.20	1.90	2,003.00	16,412.00
Composted manure	8.65	0.45	5.73	11.94	2.18	1.70

Table 10 Six treatments with a combination of foliar and soil fertilizer application methods applied on clayey and sandy soils at 56 and 112 kg N ha-1on lettuce under greenhouse conditions.

Treatment	**Details**	**Application Method**	**Fertilizer Timing**
FLScyb	50:50 treatment (Liquid + solid cyanobacteria)	Foliar	0, 2, 4, and 6 weeks after planting
		Soil	Pre-plant
FFCom	50: 50 treatment (Fish emulsion + composted manure)	Foliar	0, 2, 4, and 6 weeks after planting
		Soil	Pre-plant
Lcyb	Liquid cyanobacteria	Soil	0, 2, 4, and 6 weeks after planting
Scyb	Solid cyanobacteria	Soil	Pre-plant
Fish	Fish emulsion	Soil	0, 2, 4, and 6 weeks after planting
Com	Composted manure	Soil	Pre-plant

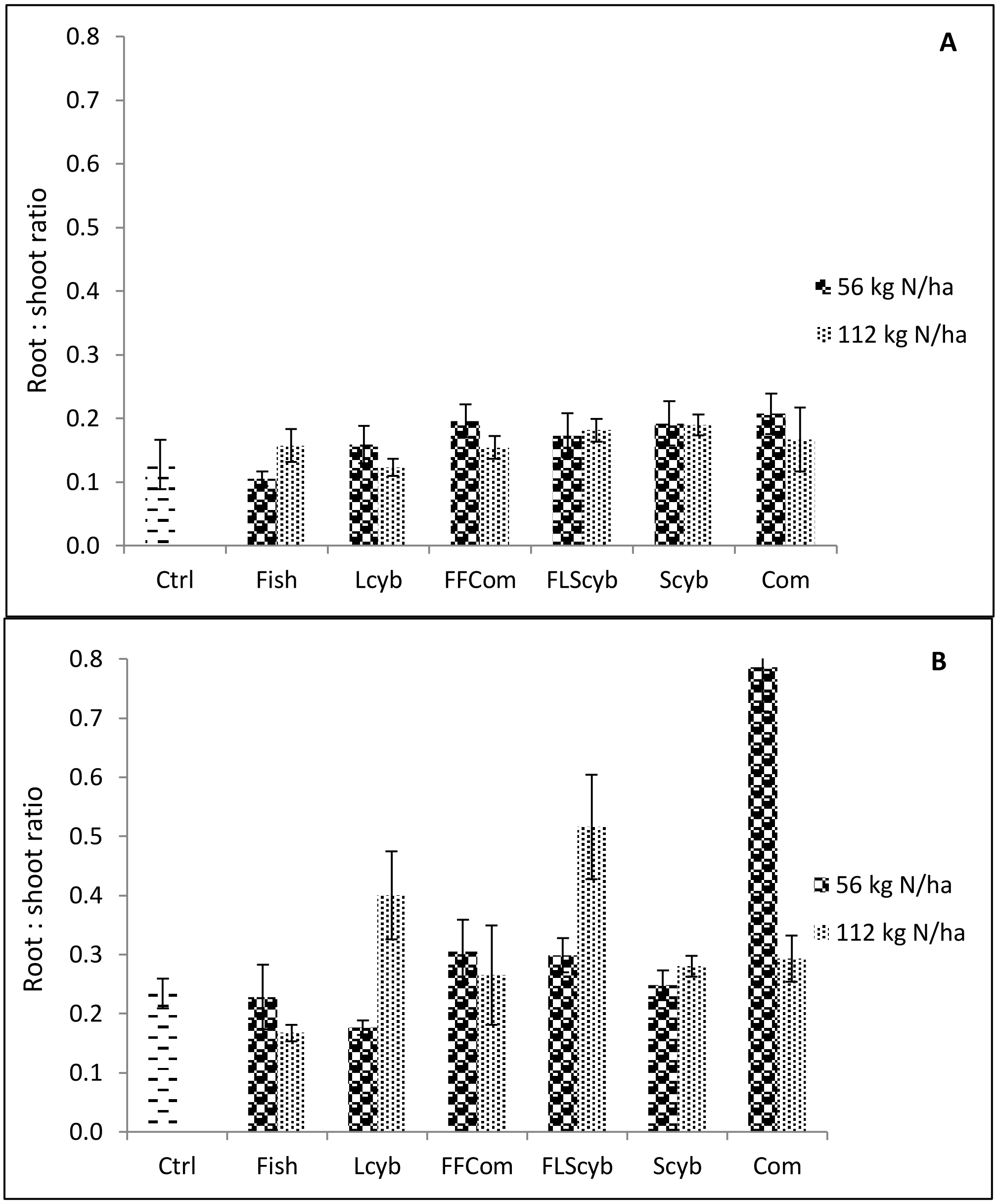

Figure 11 Root: shoot ratio of lettuce from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFMan'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Com'= Soil applied composted manure. Bars represent standard errors of mean.

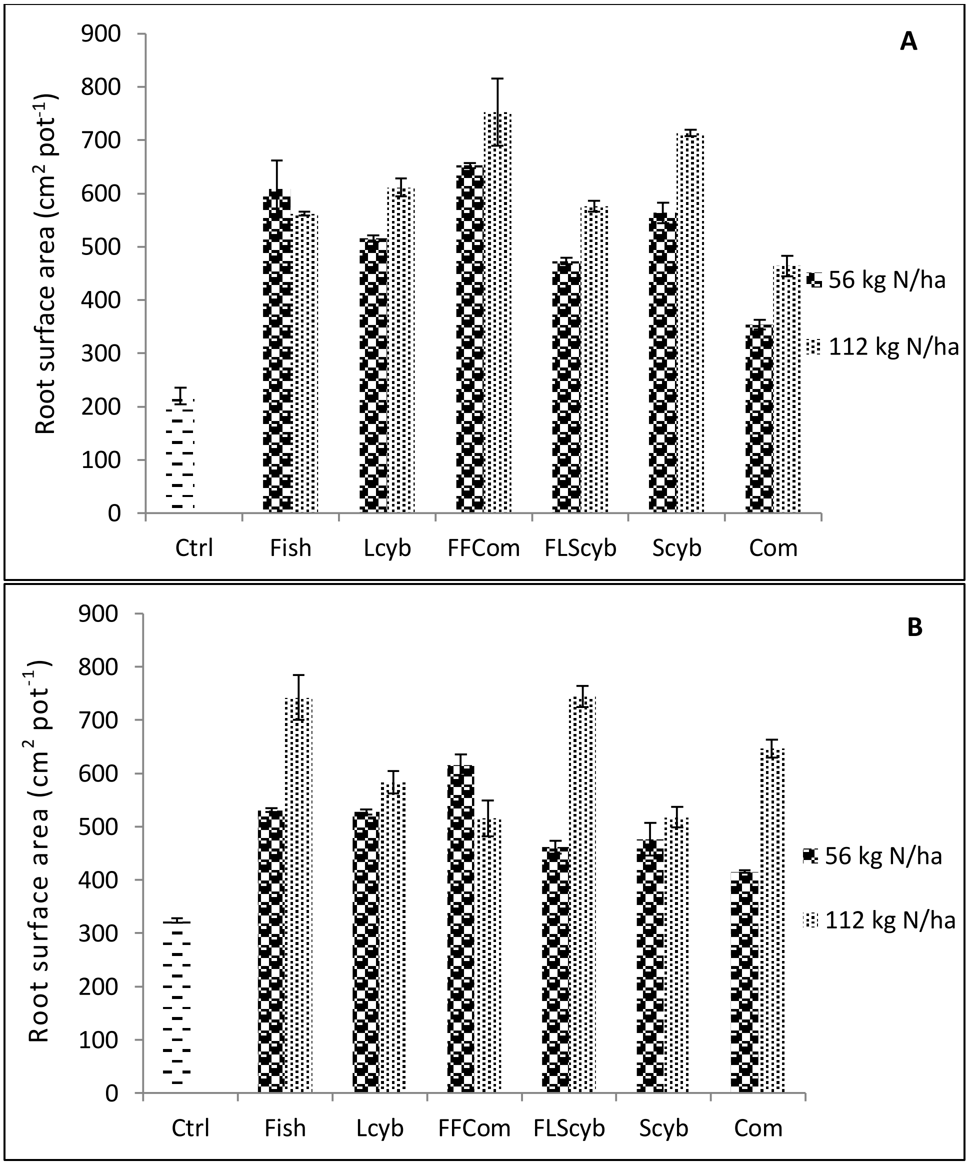

Figure 12 Root surface area of lettuce from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFMan'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Com'= Soil applied composted manure. Bars represent standard errors of mean.

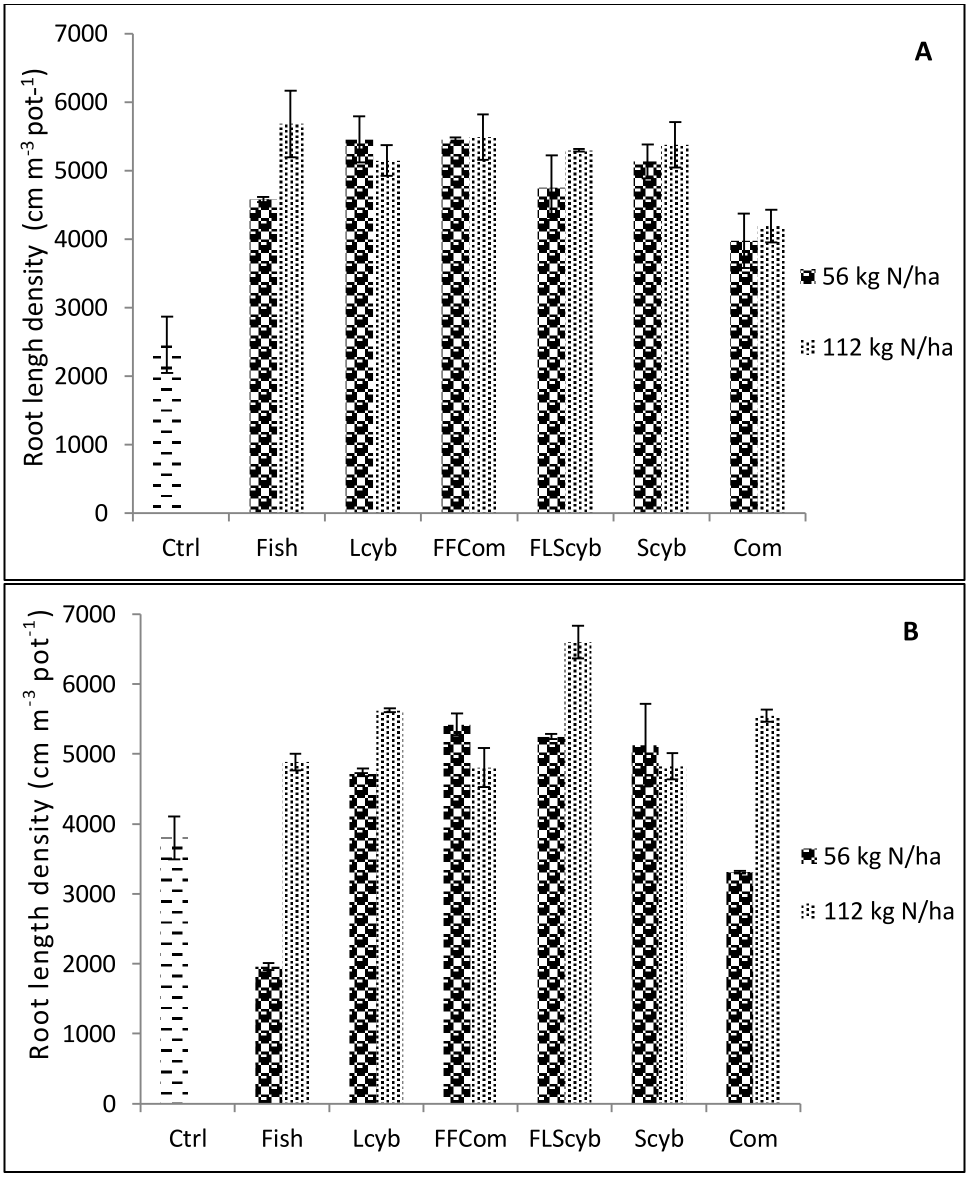

Figure 13 Root length density of lettuce from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFMan'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Com'= Soil applied composted manure. Bars represent standard errors of mean.

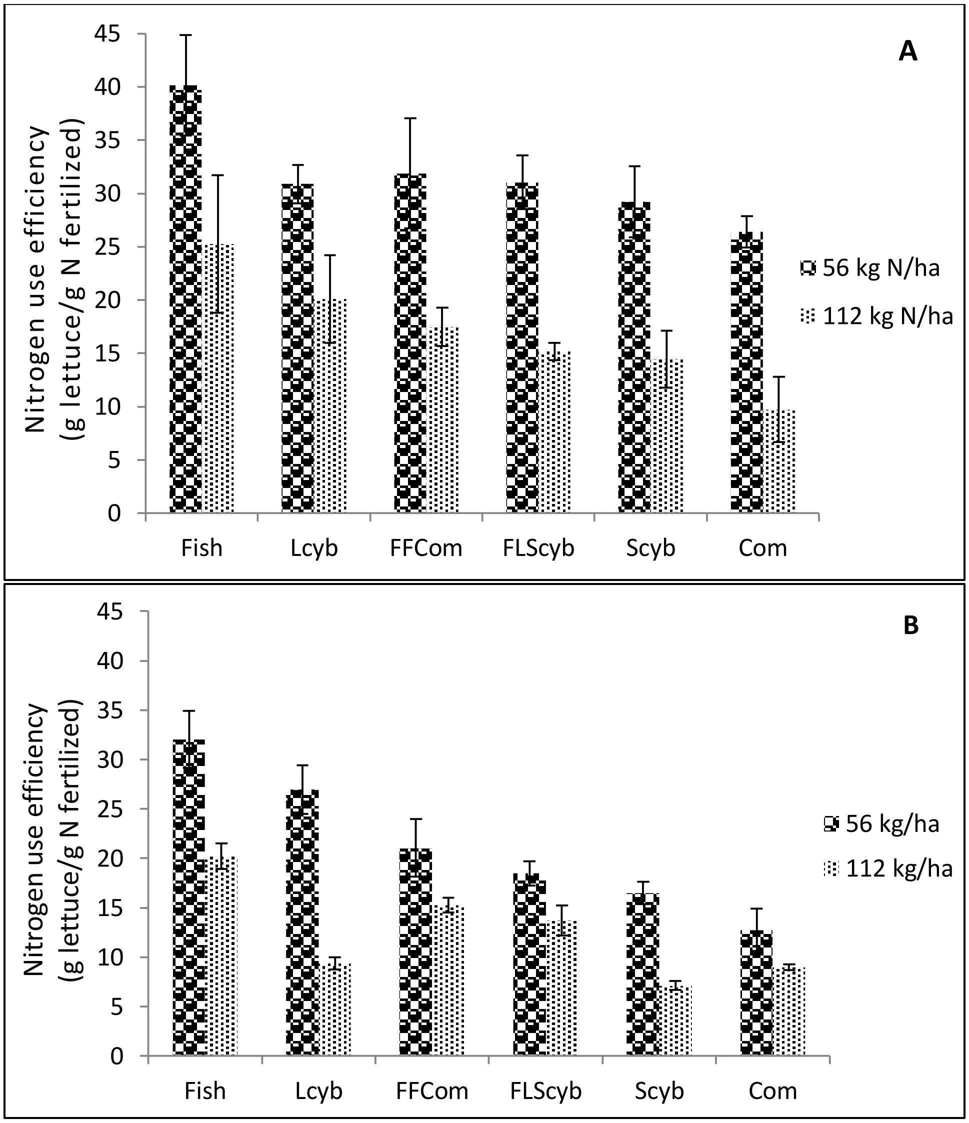

Figure 14 Nitrogen use efficiency of lettuce from soil and foliar applied organic fertilizer treatments on clayey (A) and sandy (B) soils at 56 and 112 kg N ha^{-1}. 'Ctrl' = Control, 'Fish'= Fish emulsion, 'Lcyb'= Liquid cyanobacteria, 'FFCom'= Foliar applied fish emulsion and soil applied composted manure, 'FLScyb' = Foliar applied liquid cyanobacteria and soil applied solid cyanobacteria, 'Scyb' = Soil applied solid cyanobacteria, and 'Com'= Soil applied composted manure. Bars represent standard errors of mean.

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APPENDIX III

Table 3a. The F-value from Analysis of Variance (ANOVA) of lettuce root: shoot ratio, root surface area, root length density, and N use efficiency from soil and foliar applied organic fertilizer treatments on clayey and sandy soils at 56 and 112 kg N ha^{-1}.

	Root: shoot ratio	Root surface area	Root length density	N use efficiency
Source	--F-value---			
soil	1.15 ns	0.92 ns	0.34 ns	34.19*
treatment	3.15*	8.50*	6.41*	12.38*
N rate	0.82 ns	42.15*	16.10*	0.47 ns
soil*treatment	0.47 ns	8.28 ns	4.27 ns	91.07 ns
soil*N rate	1.38 ns	1.23 ns	5.01 ns	3.09 ns
trt*N rate	0.29*	3.26*	4.17*	0.78 ns
soil*treatment*Nrate	3.02 ns	5.33 ns	1.85 ns	1.62 ns

[*]Significantly difference in comparison with p-value (p<0.05) according to Tukey's test of mean separation
[ns]No significant difference in comparison with p-value (p>0.05) according to Tukey's test of mean separation

CONCLUSIONS AND FUTURE RECOMMENDATIONS

Results of the greenhouse study indicate that there was a connection with the soil laboratory incubation study. Based on the soil incubation results, the N availability was 9% greater from fish emulsion than liquid cyanobacteria, and 6% greater from solid cyanobacteria than compost in sandy soil. The fish emulsion treatment showed 5% higher N availability compared to the solid and liquid cyanobacterial fertilizers.

In the greenhouse study, soil applied fish emulsion recorded significantly higher fresh yield at 112 kg N ha^{-1} compared to 56 kg N ha^{-1} in clayey soil relative to sandy soil. Soil-applied liquid cyanobacteria recorded significantly higher yield compared to composted manure by 58%. Solid cyanobacteria recorded significantly higher total N uptake at 56 kg N ha^{-1} compared to 112 kg N ha^{-1} in clayey soil. The soil applied fish emulsion treatment recorded higher PFR (99%) than soil applied composted manure (44%) at 56 kg N ha^{-1} on clayey soil. Soil applied fish emulsion has significantly higher PFR (57%) compared to the combination soil and foliar fertilizer (FFCom and FLScyb) at 56 kg N ha^{-1} in sandy soil. The Fish treatment recorded 35 % higher NUE at 56 kg N ha^{-1} on clayey soil while Scyb treatment has significantly 24% higher NUE compared to Com treatment at 56 kg N ha^{-1} in sandy soil.

Overall, the soil applied fish emulsion treatment recorded PFR and NUE compared to the solid and liquid cyanobacterial fertilizers at 56 kg N ha^{-1} on clayey soil. The foliar and soil applied liquid cyanobacteria fertilizer recorded higher root surface area compared to composted manure fertilizer at 112 kg N ha^{-1} on sandy soil. The combined soil and foliar cyanobacterial fertilizer and soil applied solid and liquid cyanobacterial ferilizers recorded higher PFR and NUE at 56 kg N ha^{-1} compared to the composted manure which corresponds to lettuce yield component which was higher in fish emulsion compared to the composted manure. The fish emulsion fertilizer recorded 35 % higher NUE compared to other fertilizers at 56 kg N ha^{-1} on clayey soil, while solid cyanobacteria fertilizer had 24% higher NUE compared to composted manure at 56 kg N ha^{-1} in sandy soil.

Based on this study, cyanobacterial fertilizer could be a potential N biofertilizer to enhance sustainable crop production, although at the rates tested, it was not as effective as the fish fertilizer. Higher rates of cyanobacterial fertilizer would be necessary to achieve the effect that the fish emulsion fertilizer had. A greater understanding about the regulation of N in the soil environment would elucidate the methods to improve NUE since N is involved in mechanisms to regulate root system. Improving N fertilizer management such as optimizing timing and placement of fertilizer application and using biofertilizer in crop production could minimize N losses to the environment associated with agricultural production.

It is important to understand the mechanism for supplying N to the soil and plant nutrient uptake to enable higher fertilizer use efficiency in order to improve the management of organic N fertilizers. On top of that, root systems play an important role in nutrient absorption, and enhanced root growth can improve the efficiency of N uptake by plants which in turn would produce higher yields. For further research, a field study will be conducted in summer 2013 to optimize cyanobacteria use as bio-fertilizer under field conditions so that a new fertilizer recommendation based on cyanobacterial application to soils could be developed on specific crops which would be beneficial to the local farmers. In future studies, parameter measurements such as photosynthetic rate, phytohormones regulation from N uptake process would be measured to explore the detailed soil-plant mechanisms as influenced by cyanobacterial application to soils.