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Soil carbon and nitrogen dynamics as affected by lipid-extracted algae application

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ABSTRACT

Algae are being intensively researched as a potential bioenergy feedstock. Although algae are more productive per area of cultivation compared to first-generation biofuel feedstocks, its production may not be economically sustainable without high-value coproducts. One of many possible coproducts is algal residue following lipid extraction that might be used as a soil amendment for agricultural production. This experiment was aimed at determining, under laboratory conditions, the effects of lipid-extracted algae (LEA) (*Nannochloropsis salina*) amendment on soil C and N mineralization, soil microbial biomass, and soil pH and salinity over time. Soil organic C measured 392-d after amending soil with 1.5% or 3.0% LEA (dry weight basis) was increased by approximately 0.2% and 0.3% organic C (OC), respectively, compared to the control. Approximately 50% of added LEA-C was mineralized compared with 65% of added wheat (*Triticum aestivum* L.) straw-C. Lipid-extracted algae application may be one means of increasing OC; however, problems with excess soil salinity, sodicity, and nitrate-N may occur at high (3.0% or greater) addition rates.

1. Introduction

The amount of existing U.S. cropland required to meet 50% of all U.S. transport fuel needs will be 130 times less using algae feedstock compared to soybean [*Glycine max* (L.) Merr.] (Christi, 2007). However, algae production currently is not economical without high-value coproducts. If algae are cultivated and harvested in sufficient quantity to provide significant biodiesel, then large quantities of lipid-extracted algae (LEA) residue will be available to use for animal feed or soil amendment.

Organic soil amendments (e.g., biosolids, manure, and compost) have been suggested as alternative nutrient sources to synthetic inorganic fertilizers (Eghball, 2001), while at the same time possibly increasing soil C accumulation and storage (Quilty and Cattle, 2011). Organic amendments contain plant nutrients within organic molecular structures, such as proteins and other cellular components, and thus, are not immediately available for plant use. Heterotrophic soil microorganisms degrade macromolecules of organic amendments into their component monomers, and under favorable environmental conditions, these monomers will then be mineralized releasing CO_2 (microbial respiration) and inorganic plant available nutrients, such as N, P, and S. Microbial respiration was one of the earliest and continues to be one of the most commonly used indices for assessing microbial activity in soil (Waksman and Starkey, 1924; Franzluebbers et al., 1995). Potential C mineralization should be highly related to soil microbial biomass C (SMBC) because in agricultural soils microbial growth and activity are often limited by substrate availability. However, the strength of the relationship is dependent on the method used for measuring SMBC [i.e., chloroform fumigation incubation (CFI) or chloroform fumigation extraction (CFE)]. Research has demonstrated potential C mineralization to be strongly related to CFI measured SMBC, but only weakly related to CFE (Haney et al., 2001; Franzluebbers et al., 1999). Organic amendments from different sources and with varying chemical compositions may result in different microbial growth and activity (Ng et al., 2014); thus, it is essential to determine the decomposability of previously unreported organic amendments, such as LEA.

The chemical composition of organic amendments, especially C:N ratio and lignin content (or other resistant macromolecules), is important for determining how quickly decomposition proceeds (Vanlauwe et al., 2005). As decomposition of organic amendments progresses and the less stable components are degraded, the relative proportion of more recalcitrant materials, such as aliphatic macromolecules, increases (Augris et al., 1998; Poirier et al., 2000), which will consequently cause the mineralization rate to decrease. Hydrocarbon molecules of aliphatic nature including algaenans, cutans, and suberans, are insoluble in aqueous media (nonhydrolyzable) and are more resistant to biological and chemical degradation than macromolecular components derived from proteins and polysaccharides (Gelin et al., 1999; Poirier et al., 2000).

Cutans and suberans are widely distributed in the plant kingdom, but algaenans are localized in the cell walls of unrelated groups of

Abbreviations: LEA, lipid-extracted algae; WS, wheat straw.

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microalgae. These materials are widely preserved in fossils, and although there is no definite evidence of their contribution to the composition of soil organic matter (SOM), it is inevitable that cutans, suberans, and algaenans are present (Gelin et al., 1999), especially if plant residue or LEA is added to soil. By adding a potentially stable C source to soil, such as that from LEA, it may be possible to reduce the net flux of CO_2 to the atmosphere by sequestering C in soil in the form of recalcitrant materials.

Besides the mineralization rate of organic amendments like LEA, timing and rate of application impacts the synchronization of nutrients to plants. It is, therefore, necessary to determine the effect of LEA on microbial activity and mineralization/immobilization to better understand nutrient availability from LEA. The primary objective of this experiment was to determine C and N mineralization over a 392-d period from LEA and wheat straw (WS) applications and changes in soil NH_4^+ and NO_3^-, pH, and electrical conductivity (EC) in microcosms. It was hypothesized that LEA would rapidly mineralize with a lesser percentage of added LEA-C remaining in soil compared to added WS-C, with concurrent greater N availability in LEA-amended soil.

2. Materials and methods

2.1. Soil and lipid-extracted algae characterization

The soil used for this study was collected from the Texas A&M Agrilife Research Station near Beeville, TX (28°27′30″, 97° 42′21.78″, 75.9 m) and was characterized as Weesatche soil. The average temperature and precipitation for this semi-arid environment was reported to be 21 °C and 81 cm, respectively, by the U.S. Climate Data service. The Weesatche series is described as a sandy clay loam (fine-loamy, mixed, superactive, hyperthermic Typic Argiustolls) with a pH of 6.1 (USDA — NRCS). This site was previously planted to Kleingrass [*Panicum coloratum* (L.)] and grazed. Soil was air dried for approximately 28 d, thoroughly mixed and stored until further use. The LEA source for all studies was *Nannochloropsis salina*, a microalgae cultivated in open ponds near Pecos, TX.

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

2.2. Fiber analysis: lipid-extracted algae and wheat straw

Lipid-extracted algae and WS samples were weighed and dried at 105 °C for 24 h for dry matter (DM) determination. Concentrations of neutral detergent fiber (NDF) and acid detergent fiber (ADF) were measured sequentially using an ANKOM 200 Fiber Analyzer (Ankom Technologies, Macedon, NY) with the method based on Van Soest et al. (1991) and AOAC (1990; method 973.18). Heat stable α-amylase was used for NDF analysis. Lignin, or the most stable C fraction, was sequentially measured following ADF using the Ankom (2013) method by incubating the Ankom bags in 72% sulfuric acid in order to solubilize cellulose (Van Soest, 1967). All chemical constituents were reported on a DM basis.

2.3. Aerobic incubation

Microcosms in the laboratory arranged as a RCBD were utilized to measure C respiration and the quantity of N mineralized or immobilized by oxidation of various organic matter (OM) additions to soil. Weesatche soil was amended with two types of OM, LEA and lignocellulosic WS, and then wetted to 60% water-filled pore space. Lipid-extracted algae were applied at 1.5% and 3.0% on a dry weight basis ($g\ g^{-1}$) and WS was applied at 1.5% ($g\ g^{-1}$). The control soil was without OM addition. Each treatment was replicated four times totaling 16 microcosms per destructive sample set. The total weight of each dry soil/OM mixture equaled 45 g soil plus the added residue (0.66 and 1.31 g with 1.5% and 3.0% LEA treatments, respectively). The soil water content was maintained throughout the experiment by weighing sample containers and adding deionized (DI) H_2O to a constant weight. Samples were placed in 1-liter glass containers along with 10 ml DI H_2O, tightly sealed, and incubated at 30 °C in the dark. Aerobic conditions were maintained by venting; microcosm lids were removed for five minutes at least once every seven days. There were five sets of 16 microcosms with one of the sets destructively sampled at 4, 14, 28, 224 or 392 d following treatment application and wetting.

The fifth set, which was not destructively sampled until the final incubation day (392-d) was used to measure cumulative CO_2 evolution after 1, 4, 7, 14, 28, 56, 112, 168, 224, 280, 336, and 392 d, and therefore, to determine the rate of C mineralization as well as the percentage of added LEA-C or WS-C mineralized at each time point. Carbon dioxide trapped in 25 ml 1 M KOH was back titrated with 0.5 M HCl after adding $BaCl_2$ to precipitate the trapped CO_2 as $BaCO_3$. Soil organic C and total N and extractable NH_4^+-N and NO_3^--N concentrations and soil pH and EC of destructive samples were measured throughout the incubation by methods described above.

2.4. Soil microbial biomass

Chloroform fumigation incubation (CFI) was used to estimate SMBC with some modifications to the original method proposed by Jenkinson and Powlson (1976). The hypothesized pH difference between the control soil and LEA-treated soil influenced the decision to use CFI rather than CFE. Haney et al. (2001) demonstrated that the CFE extractant molarity and soil pH are important variables that interact to produce vastly different SMBC estimates using the CFE method.

The same treatments used for the aerobic incubation detailed above were also used for CFI. Smaller quantities of soil (15 g) were moistened to 50% water-filled pore space, placed into 1-liter glass containers in the presence of 10 ml deionized H_2O, and sealed tightly. Soil was incubated at 30 °C for a period of 14 d in order to establish a steady state of microbial activity prior to fumigation (Franzluebbers et al., 1999). After fumigating samples with ethanol-free chloroform for 24 h and then removing the fumigant by vacuum, the flush of CO_2-C over a 10-d incubation period was quantified by titration of 1 M KOH alkali traps with 0.5 M HCl in order to quantify the response of soil microbiota to LEA and WS soil amendments.

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