ABSTRACT

Title of Thesis:	BLOOM BIOSOLIDS: WHAT IS THEIR MICROBIAL COMMUNITY AND HOW DO THEY AFFECT SOIL AND PLANT HEALTH?
	Eni Baballari Master of Science 2019
Thesis Directed By:	Associate Professor Stephanie Yarwood Environmental Science and Technology

Biosolids are rich in nutrients and organic matter, and are known to improve and maintain productive soils and stimulate plant growth. D.C. Water's new Class A biosolids product, Bloom, was evaluated for its impact on plant and soil health. Using molecular tools, Bloom was examined for the presence of functional genes that would indicate the presence of microbes capable of improving plant growth (i.e.nitrifiers, N-fixers). Using greenhouse and laboratory experiments, we determined Bloom's effect on plant growth, carbon and nitrogen cycling. Bloom has both nitrifying and N-fixing microbes, but their gene numbers vary depending on the stage of production. We show that plants, such as cucumber and tomato, grown in soil amended with Bloom produce more leaves and stems and have higher aboveground biomass, and soybeans produced more bean pods. Lastly, we found that N-mineralization is higher in soil amended with Bloom, even after one growing season, providing increased nutrients.

BLOOM BIOSOLIDS: WHAT IS THEIR MICROBIAL COMMUNITY AND HOW DO THEY AFFECT SOIL AND PLANT HEALTH?

by

Eni Baballari

Thesis submitted to the Faculty of the Graduate School of the
University of Maryland, College Park, in partial fulfillment
of the requirements for the degree of
Master of Science
2019

Advisory Committee:
Professor Stephanie Yarwood, Chair
Associate Professor Gary Felton
Mr. William Brower

Dedication

I dedicate this thesis to my family, and especially my mom, Suzana. She has instilled in me the love for education and learning and the appreciation of hard work. I am proud to be your daughter. Thank you, mama.

Acknowledgements

This work was funded by D.C. Water.

I would first like to thank Dr. Stephanie Yarwood, my thesis advisor, without whom I would not have had the opportunity to work on such a great project. Thank you for your continuous support, patience, motivation, enthusiasm, and guidance throughout the research process and in writing of this thesis. I could not have imagined having a better mentor for my Master's study. I would also like to thank my co-advisors, Dr. Gary Felton and Bill Brower, for offering their encouragement, insightful comments and support along the way. My sincere thanks also go to Trish Steinhilber for her help with my research questions, Mike Dwyer for his help with a truck-full of soil acquisition, and Meghan and Sydney for their help in the greenhouse. This research would not have been possible without the help of many colleagues and friends: Thank you Annie Yarberry, Brian Scott, Christine Maietta, Dani Weissman, Dietrich Epp Schmidt, Henry Wald, Jonathan Moy, Lindsay Wood, Logan Kline, Martina Gonzalez Mateu, Marya Anderson, Taniya Roy Chowdhury, Tori Montsaint-Queeney, and everyone else in the ENST department. Finally, I'd like to thank my parents Suzana and Tomor, my brother Erjon, my partner Michael, and his mom Alison, for their unwavering support and encouragement in the last three years.

Table of Contents

List of Tables

List of Figures

Chapter 1: Introduction

Biosolids: A brief history

Biosolids, the byproduct of domestic sewage sludge, are nutrient-rich organic materials which, can be applied as soil amendments. Sewage sludge processing began close to a century ago when federal legislation was implemented to control water pollution. Wastewater that was originally discharged onto rivers, lakes, and bays, started being treated in newly-built municipal treatment facilities. The Federal Water Pollution Control Act Amendments of 1972 (PL 92-500, 1972) placed further restrictions on the discharge of wastewater to waterways and encouraged other disposal methods such as land applications. Further regulations, such as the Ocean Dumping Ban Act of 1988, and increased cost of incinerating or disposing in landfills made land application an attractive option. In order for sewage sludge to be applied to soils, it has to undergo treatment to reduce pathogens and the attractiveness of disease-carrying vectors. Such treatments include composting, aerobic or anaerobic digestion, lime stabilization, and thermal drying.

In 1991, the Water Environment Federation created the term *Biosolids* to distinguish treated sewage sludge from raw sewage sludge and facilitate land application of processed sewage sludge that would be more acceptable to the public. Biosolids are sewage sludge that have been treated in accordance with federal, state, and local regulations to permit land application. The US Environmental Protection Agency (EPA) defines biosolids as "the nutrient- rich organic materials resulting

from the treatment of sewage sludge" (2011). In 1993, the EPA published the *Standards for the Use or Disposal of Sewage Sludge* (Title 40 of the Code of Federal Regulations (CFR) Part 503). Rule 40 CFR Part 503 establishes standards, which consist of general requirements, pollutant limits, management practices, and operational standards, for the final use or disposal of sewage sludge (EPA, 1994).

Classification and uses

EPA's Part 503 rule establishes requirements for the final use or disposal of biosolids when biosolids are applied to land to condition the soil or fertilize crops or other vegetation grown in the soil; placed on a surface disposal site for final disposal; or fired in a biosolids incinerator. Part 503 requires operational standards to control pollutants, pathogens, and attraction to vectors (e.g. flies, mosquitos, rodents and other potential disease-carrying organisms). In addition, there are general requirements, management practices, and frequency of monitoring, recordkeeping, and reporting requirements that must be met. Biosolids applied to land must fall below the ceiling concentrations for pollutants (**Table 1.1**). Biosolids applied to the land must also meet either pollutant concentration limits, cumulative pollutant loading rate limits, or annual pollutant loading rate limits for the same heavy metals (Part 503, EPA).

***Table 1.1**. Pollutant concentration limits of all biosolids and Exceptional Quality (EQ) biosolids (U.S. EPA, 1994).*

Pollutant	**Ceiling Concentration Limits for All Biosolids Applied to Land (mg/kg d.w.)**	**Pollutant Concentration Limits for EQ Biosolids (mg/kg d.w.)**
Arsenic	75	41
Cadmium	85	39
Chromium	3,000	1,200
Copper	4,300	1,500
Lead	840	300
Mercury	57	17
Molybdenum	75	-------- [a]
Nickel	420	420
Selenium	100	36
Zinc	7,500	2,800
Applies to:	All biosolids that are land applied	Bulk biosolids and bagged biosolids [b]
From Part 503	Table 1, Section 503.13	Table 3, Section 503.13

[a] As a result of the February 25, 1994, Amendment to the rule, the limits for molybdenum were deleted from the Part 503 rule pending EPA reconsideration.

[b] Bagged biosolids are sold or given away in a bag or other container.

EPA's classification as "Class A" or "Class B" biosolids indicates pathogen density. The pathogen reduction alternatives ensure that pathogen levels in biosolids are reduced to levels considered safe for the biosolids to be land applied or surface disposed (Part 503, EPA). If pathogens (*Salmonella* sp. bacteria, enteric viruses, viable helminth ova) are below detectable levels, the biosolids meet Class A designation. Biosolids are designated Class B if pathogens are detectable but have been reduced to levels that do not pose a threat to public health and the environment as long as actions are taken to prevent exposure to the biosolids after their use or disposal. The requirements for Class A biosolids are either the density of fecal

coliform in the biosolids must be less than 1,000 most probable numbers (MPN) per gram total solids (dry-weight basis), or the density of *Salmonella* sp. bacteria in the biosolids must be less than 3 MPN per 4 grams of total solids (dry-weight basis). Class B biosolids contains a higher level of pathogen that requires a maximum of 2 million MPN g^{-1} dry weight of biosolids. There are six alternatives to producing Class A biosolids. These include thermal treatment and high pH-high temperature treatments, as well as composting, heat drying, beta or gamma ray irradiation, and pasteurization. Class A pathogen reduction alternatives make the biosolids virtually pathogen-free after treatment. Class B pathogen reduction alternatives significantly reduce but do not eliminate all pathogens. Land appliers who apply biosolids that are certified by the preparer as Class A have no requirements relative to pathogens, but if the biosolids are Class B, site restrictions must be imposed to allow time for natural processes to further reduce pathogen levels (EPA).

Since vectors such as flies, rodents, and birds can transmit diseases, Part 503 provides guidelines to reduce vector attractiveness and the potential transmission of disease. These options include reducing volatile solids, adding alkali under specified conditions, using aerobic or anaerobic digestion, and injecting biosolids beneath the soil surface.

Exceptional Quality biosolids (EQ) meet low-pollutant and Class A pathogen reduction (virtual absence of pathogens) limits and have a reduced level of degradable compounds that attract vectors. Once the requirements discussed regarding pathogens, metals, and vector attraction are met, EQ biosolids are considered a product that is virtually unregulated for use by federal rules, whether used in bulk or

sold commercially in bags. They are regulated by state rules and guidelines. The biosolids used in this research are EQ-Class A biosolids.

CAMBI process and Bloom

Anaerobic digestion (AD) of biosolids is a common treatment because it reduces volume, stabilizes materials that can be used for soil conditioning, and recovers energy in the form of methane. According to the American Biogas Council, the United States has 1,269 water resource recovery facilities using anaerobic digesters that produce biogas. Approximately 860 of them use the biogas that they produce (2017). Because of space constraints, poor biogas production, high carbon (C) footprint and poor energy efficiency, many wastewater management facilities are implementing advanced treatment of the organic waste. These advanced treatments are prior to digestion. They can maximize energy production, cut energy and operating costs, and remove pathogens from biosolids. Thermal hydrolysis process (THP) is one of the well-studied technologies that is applied in many water resource recovery facilities.

The Blue Plains facility, operated by D.C. Water, has recently installed a thermal hydrolysis facility coupled to an anaerobic digester (THP-AD). Blue Plains is the largest advanced wastewater treatment plant in the world, occupying a 0.6 km^2 site on the bank of the Potomac river, an estuary and the Chesapeake Bay in Washington, D.C., USA. On an average day, the facility treats close to 300 million gallons of wastewater and has peak capacity to treat 1 billion gallons (D.C. Water). DC Water generates a stream of clean water, which is discharged to the Potomac river, and nutrient-rich biosolids.

Blue Plains' treatment of biosolids includes open-air primary sedimentation, activated sludge, and tertiary treatment (nitrification-denitrification, filtration, and disinfection). In the past, 1200 wet tons of lime-stabilized Class B biosolids were produced daily. In an effort to improve to Class A biosolids, in November of 2014, the facility began operating a newly constructed CAMBI thermal hydrolysis pretreatment combined with anaerobic digestion after the thickening process to replace the lime-stabilization addition (D.C. Water). Thermal hydrolysis treats and prepares the sewage solids as a sterile C source for microorganisms in the anaerobic digesters. A high-heat, with 7 bar pressure, process followed by a sudden pressure drop causes the cell walls of the organic matter to burst, making the substrate readily digestible. The methanogens in the digesters convert the organic C to methane. The microbiological conversion of sludge to CH_4 and CO_2 is a slow process and requires high retention time and larger digester volume. The advantages of CAMBI process are the sludge retention time in the anaerobic digester is reduced to about 20 days. Additionally, 50% of the digester volume is saved in comparison with conventional digestion and there is higher biogas production. There is also improved dewaterability (30–40% dry solids content can be achieved), and pasteurized biosolids can be applied to agricultural soils (Camacho et al., 2008). Coupled with anaerobic digesters, THP makes it possible to generate over 10 MW of electricity which is reused by D.C. Water to cut their electricity consumption by one-third. An added benefit, the biosolids produced from CAMBI THP-AD following digester are Class A, Exceptional Quality biosolids. They are trademarked as Bloom.

Nutrients in biosolids

Approximately 55% of biosolids are land applied in the US (NEBRA, 2007). Biosolids contain organic matter and nutrients that are beneficial for soil, crop, and livestock productivity. Typical macronutrients in biosolids include nitrogen (N), phosphorus, sulfur, calcium, magnesium, and potassium. Biosolids also provide plant-essential micronutrients, including copper, boron, molybdenum, zinc, and iron (Sullivan et al., 2015). These micronutrients are not provided by most conventional chemical fertilizers, making biosolids a particularly useful addition in some systems.

The primary nutrients in biosolids are in organic forms, not as soluble as those in chemical fertilizers, and released more slowly. Therefore, biosolids can nourish the plants at a slower rate over a longer period of time with higher use efficiency and a lower likelihood of polluting groundwater, when the appropriate rate is applied (Lu et al., 2012). Nitrogen application is regulated through an agronomic rate approach, requiring an estimate of crop N need and biosolids N availability. In practice, N is typically the factor that controls biosolids application rates. Most of the N in biosolids is in organic form, which is not immediately available to plants. Predicting N availability over the course of the growing season is key to determining appropriate biosolids application rates (Cogger et al. 2006). This is why one aspect of the work presented in here was to determine N-mineralization rates.

Nutrient values of biosolids vary with sources of wastewater and wastewater treatment processes. The composition of nutrients in biosolids is significantly altered by stabilization processes. Similarly, the rate of nutrient release (or mineralization) is also affected by the processes. Mineralization of N from aerobically digested

biosolids (e.g., 32.1%) was reported to be significantly higher than that from anaerobically digested biosolids (e.g., 15.2%) in 26-weeks incubation study (Wang et al., 2003). Soil type, temperature, soil moisture content, aeration, and species and number of soil microorganisms play a role in organic matter mineralization in biosolids. Mineralization rate is also closely related to C:N ratio. The higher the C:N ratio in soil, the lower the N mineralization rate. In some cases, the mineralization process was more influenced by soil type than by rate and type of sludge applied (Lu et al., 2012).

Soil microbial community

Microbial communities in soils are very important because, among other functions, they control decomposition and nutrient cycling in soils (Coleman et al., 2017). Biosolids additions, like other organic amendments, can change the soil microbial community. For example, adding Bloom may add or enhance the population of microbes that can carry out denitrification, nitrification, and nitrogen fixation. As a C source, organic amendments have great influence on the heterotrophic microbial communities. The quantitative effect of organic amendments on microbial activity can be illustrated by measurement of soil microbial biomass C, which almost invariably shows an increase following organic amendment addition to degraded soils (Ros et al. 2003; Mabuhay et al. 2006; Belyaeva and Haynes 2009). Long term effects of biosolid additions have also been reported. García-Gil et al. (2004) reported increased microbial biomass, basal respiration, metabolic quotient, and enzymatic activities in semi-arid soil, amended 9 or 36 months previously, with biosolids at approximately 36 Mg ha^{-1}. Barbarick et al. (2004) examined microbial

responses to biosolids in semi-arid grassland (0 and 30 Mg ha^{-1}) and shrubland (0 and 40 Mg ha^{-1}) sites 6 years after biosolids surface application. They found that CO_2 evolution and actively metabolizing microbial biomass were greater in plots 6 years following biosolids application than in control (non-amended) plots. Similarly, Pascual et al. (1999) examined microbiological and biochemical parameters of semi-arid soils 8 years after biosolids amendment (65 and 260 Mg ha^{-1}). Compared to control plots, the amended plots exhibited greater total organic C, microbial biomass, basal respiration rate, and enzymatic activity even 8 years after biosolids incorporation. Microbial activity is very influenced by moisture content (Liang et al., 2003). Sullivan et al. (2006) reported that land application of biosolids to a semi-arid grassland soil enhanced microbial mineralization activities and plant productivity when soil moisture levels were adequate.

Soil amendments, soil and plant health

Soil health can be measured using physical, chemical, or biological properties. A soil amendment is any material added to a soil to improve its properties, such as water retention, permeability, drainage, pH, nutrient availability, and microbial activity. The goal is to provide a better environment for roots. Organic amendments such as peat, wood chips, grass clippings, straw, compost, manure, biosolids, and sawdust, increase soil organic matter (SOM) content and offer many benefits. Over time, the organic matter improves soil aeration, water infiltration, and both water- and nutrient-holding capacity. The long-term effects of organic amendments are summarized well in the review by Diacono and Montemurro (2011). Improvements include: soil biological functions, soil organic C content, aggregate stability, lower

bulk density, and increased crop yields. Short term studies also show increased plant yields or biomass with organic amendment additions (Naeini and Cook, 2000, Adegbidi, 2003, Fernández et al. 2009, Latare et al, 2014, Koutroubas et al, 2014).

Study objectives

The goal of this work was to determine whether DC water's newly produced product, Bloom, can improve soil and plant health. Soil health parameters measured in the study were C, N, pH, SOM, microbial communities and biomass, and soil respiration. Plant health parameters were above- and belowground biomass, leaf and stem counts, plant height, and yield.

The first objective of this thesis was to determine the quantity of functional genes in Bloom at different stages of production that serve as markers for beneficial rhizosphere microbes. This was done by quantifying the genes coding for archaeal ammonia monooxygenase (AOA), bacterial ammonia monooxygenase (AOB), and nitrogenase (nifH) with quantitative PCR. I hypothesized that these genes would be present in Bloom at all stages. I further hypothesized that Cured Bloom would have greater gene copy numbers of these functional genes compared to Fresh Bloom. After anaerobic digestion the material is exposed to air, therefore favoring the growth of nitrifying archaea and bacteria that can only carry out ammonia oxidation when oxygen is present. Although nitrogen fixation is an anaerobic process, many free-living nitrogen fixers also require a partially aerobic environment in order to generate the energy required to break the N_2 triple bond. Although gene copy numbers are not a direct measure of N cycling activity, the presence of the genes should be considered

as a measure of the potential of the material to increase these functions when applied to soil.

The second objective was to determine N mineralization rates in soil amended with Bloom. During a one-month period, N-mineralization incubations were established for soil mixed with Cured and Dried Bloom. The Cured Bloom was left in piles outside following the dewatering stage. In contrast, the Dried Bloom was sent to an off-site facility for drying for 30 minutes at 90ºC. At weekly intervals microcosms were destructively sampled to measure ammonia- and nitrate-N, soil C:N, pH, SOM, microbial biomass, and soil respiration. I hypothesized that additions of Bloom would increase N-mineralization rates compared to soils with no addition. Secondly, Cured Bloom would have higher N-mineralization compared to Dried Bloom, because the drying process was likely to decrease the quantity of the microbial community.

The third objective was to determine the effects of Bloom addition on plant growth. For this objective a greenhouse experiment was established where soil with fertilizer, Bloom, or Leafgro, was used to grow tomatoes, cucumbers, and soybeans. The plants were measured regularly for height and weight, as well as leaf, stem, flower, and fruit counts. Later nutrient analysis was performed on the tomato and soybean plants. The soils from the greenhouse were also used to determine N-mineralization rate, soil C:N, pH, SOM, microbial biomass, soil respiration. I hypothesized that plants treated with Bloom would have higher growth parameters than those treated with fertilizer alone or Leafgro. The chemical analysis of Bloom that DC water conducted, showed high amounts of N and some other micronutrients. These are likely to improve plant growth over a product such as Leafgro that does not

have a great deal of N present. Bloom also increases the amount of organic matter likely leading to improved moisture holding capacity and lower bulk density, physical parameters that improve soil health. Lastly, I hypothesized that following one growing season, Bloom amended soils would still release N during a second N-mineralization incubation. Given water quality concerns in the mid-Atlantic region and also a drive to more efficiently apply N-containing material in agriculture, this last experiment provides important information on the amount of residual N and could be built on to provide guidance for continued application of Bloom by home gardeners and commercial producers.

Chapter 2: Materials and methods

Microbial analysis of Bloom

Biosolids were sampled at the resource recovery facility at Blue Plains, Washington, DC in September 2016 and January 2017. Samples were taken from 4 stages of production: at dewatering, after dewatering (fresh cake), 1-month cured, and 3-months cured. Each stage was sampled 5 times. The material was collected using sterile 50-mL centrifuge tubes, it was transported in a cooler with ice, and stored in 4ºC refrigerator for 1 day. The samples were then centrifuged in order to discard most water content.

DNA was extracted from 2g of moist sample using MoBio PowerFecal DNA isolation kit, according to the manufacturer's protocol (MoBio Laboratories, Carlsbad, CA). DNA was quantified using a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY) and diluted to a final concentration of 2.5 ng μl^{-1} with autoclaved water. Extracts were stored at -80ºC until further analysis. About 5 g of sample was used to determine the water content by drying in 105ºC oven for 24 hours.

Gene abundances were determined using real-time PCR (qPCR). Three functional genes were analyzed: ammonia oxidizing archaea (AOA), ammonia oxidizing bacteria (AOB), and nitrogen fixation (nifH) (**Table 2.1**). Plasmid standards were constructed by amplifying functional genes from pure culture or environmental samples (**Table 2.1**). Target genes of interest were amplified using a 20 µl PCR reaction with the following reagent concentrations: 1X GoTaq® Colorless

Flexi Buffer (Promega Corporation, Madison, WI), 1.75 mM $MgCl_2$, 0.20 mM dNTPs, 0.50 µM forward primer, 0.5 reverse primer, 0.064% bovine serum albumin (BSA), and 0.025 U μl^{-1} GoTaq® Hot Start Polymerase (Promega Corporation, Madison, WI). Amplified functional gene fragments were subsequently cloned using the Topo TA cloning™ kit (Invitrogen, Carlsbad, CA) according to manufacturer's instructions. Plasmid standards were linearized using EcoRV (Thermo Scientific, Waltham, MA) and purified using the Ultra Clean PCR clean-up kit (Mo Bio Laboratories, Carlsbad, CA). Standard plasmid concentrations were quantified using a Qubit 2.0 Fluorometer (Life Technologies, Grand Island, NY) and subsequently adjusted to 2.5 ng μl^{-1}; this stock solution was then serially diluted 10-fold to 2.5 x 10^{-6} ng μl^{-1}.

Table 2.1: *Conditions for quantifying functional genes via quantitative PCR (qPCR).*

Gene	**Function**	**Pure Culture**	**Thermocycler Conditions** (Acquisition Step Bolded)	**Number of Cycles**	**Plasmid standard and soil correction efficiency (%) (r^2 values)**	**Reference**
AOB	Ammonia oxidation (bacterial)	*Nitrosomonas europaea*	95°C for 5 min 95°C for 30 s / 56°C for 30 s / **72°C for 1 min**	1 40	95-98% soil = 92% All $r^2 > 0.96$%	Aoi et al., 2004
AOA	Ammonia oxidation (archaeal)	Environmental clone	94°C for 15 min 94°C for 15 s / 52°C for 45 s / 72°C for 30 s / **78°C for 10 s**	1 45	94-105% soil = 95% All $r^2 > 0.99$%	Mincer et al., 2007
nifH	Nitrogen reduction	*Sinorhizobium meliloti*	95°C for 5 min 95°C for 5 s/ 53°C for 30 s/ 72°C for 10 s/ **83°C for 10 s**	1 40	95-103% soil = 93% All $r^2 > 0.99$%	Rösche et al., 2002

Gene copy numbers were corrected to account for PCR inhibition (Hargreaves et al., 2013). Briefly, all DNA extracts at equal concentrations were pooled. The pooled samples were serially diluted from 2.5 ng μl^{-1} to 10-fold to 2.5 x 10^{-6} ng μl^{-1}. Biosolids DNA extracts, plasmid standards, and pooled standards were run in triplicate 20 µl reactions with 10.0 µl of KiCqStart® SYBR® Green qPCR ReadyMix™ with ROX (Sigma, St. Louis, MO), 1.0 µl of 0.5 µM final concentration of each the forward and reverse primer, 6.0 µl nanopure water and 2 µl 2.5 ng template DNA. All reactions were run on the StepOne Plus RealTime PCR instrument (Applied Biosystems, Foster City, CA). The primers, qPCR conditions, and the range of efficiencies for each gene are found in **Tables 2.1** and **2.2**. The efficiency and intercept of the pooled samples were calculated and used to correct for relative copy number differences between the plasmid standard curve and the pooled soil sample standard curve. Final gene abundance values (genes g^{-1} dry biosolids) were log-normally distributed prior to statistical analysis.

Table 2.2: *Primer sets for functional gene quantification with qPCR.*

Gene	Primer sets (F/R)	Reference
AOB	F - 5' - GGGGTTTCTACTGGTGGT R - 5' - CCCCTCKGSAAAGCCTTCTTC	Aoi et al., 2004
AOA	F - 5′- GCARGTMGGWAARTTCTAYAA R - 5′- AAGCGGCCATCCATCTGTA	Mincer et al., 2007
nifH	F - 5' - AAAGGGGWATCGGYAARTCCACCAC R - 5' - TTGTTSGCSGCRTACATSGCCATCAT	Rösche et al., 2002

Nitrogen mineralization of Cured and Dried Bloom

A one-month N-mineralization experiment was set up in jar mesocosms using soil, soil and cured Bloom, soil and dried Bloom. Cured Bloom are biosolids product that is cured outdoors for 2-3 months. Dried Bloom are biosolids product that is dried for 30 minutes using a commercial drying oven at 90°C. The mesocosms consisted of 100 g soil mixed with 6 g of Cured or Dried Bloom. The dry equivalents were 82.5 g soil, 3.7 g Cured Bloom, and 3.5 g Dried Bloom. This rate was based on greenhouse application rate, later used in greenhouse study. The soil mixes were wetted with distilled water at 60% water holding capacity and maintained at 25°C and moisture at 60%. Treatments were replicated three times. The jars were opened regularly to maintain aerobic conditions. Destructive sampling of the mesocosms was done weekly to measure NH_4^+ and NO_3^- N, total soil respiration, microbial biomass, pH, and soil organic matter (SOM).

Ammonium-N (NH_4^+ -N) and Nitrate-N (NO_3^- -N) were analyzed on a Lachat analyzer (Lachat Instruments, Loveland, CO) using QuickChem® Method 12-107-04-1-A and 10-107-06-2-A and a similar protocol as Pastor et al. (1987). NH_4^+ and NO^-_3 were extracted by weighing 2 g of soil into centrifuge tubes, adding 25 mL of 0.5 M KCl, and shaking for 1 hour. The extracts were filtered using Whatman no. 42 paper, collected into clean centrifuge tubes and frozen until analysis (4 weeks).

Soil respiration was monitored by getting a representative 0.5 mL gas sample every day for the first week, and every other day for the last three weeks, from the headspace of the mesocosm jars using a Valco® Precision Sampling syringe (series A-2) with a removable point style 2 bevel tip (Sigma-Aldrich Co. LLC, St. 141 Louis,

MO). After gas was sampled silicone was applied to the punctured gas port before placing mesocosms back in the incubator. The 0.5 mL gas sample was analyzed using gas chromatography (Agilent Technologies, Inc. Shanghai China; model 7890A). Before each sample run, a 90%:20% N_2: CO_2 standard gas mixture was used to produce a standard calibration curve; calibrations were acceptable if the R^2 value exceeded 99.9%.

A TOC/TN Analyzer (Shimadzu Corporation, Kyoto, Japan) was used to quantify dissolved organic content (DOC). Dextrose was used to generate DOC standards curves (0.1 – 100 mg L^{-1}), and nanopure water served as a blank. Microbial biomass was determined by chloroform fumigation (Vance et al., 1987), and measuring DOC for fumigated and unfumigated samples. Microbial biomass C and N were calculated by taking the difference between unfumigated and fumigated subsamples of the same soil. Briefly, 5 g of each soil sample was weighed into small beakers. To fumigate, the subsamples and a container of chloroform were arranged into a desiccator under vacuum for 48 hours. Microbial C and N were extracted by weighing 2 g of the fumigated and unfumigated samples into centrifuge tubes, adding 25mL of 0.5 M K_2SO_4, and shaking for 1 hour. The extracts were filtered using Whatman no. 42 paper, collected into clean centrifuge tubes and frozen until analysis (4 weeks).

The plant available nitrogen (PAN) for Cured and Dried Bloom was calculated using the following formula:

$$PAN = NO_3\text{-}N + K_{vol}\ (NH_4\text{-}N) + K_{min}\ (Org\text{-}N)$$

where, K_{vol} = volatilization factor, K_{min} = mineralization factor (Evanylo, 2006).

Soil pH was determined by mixing 1-part field-moist soil with 2 parts 0.01 M calcium chloride ($CaCl_2$), or roughly 10 g soil mixed with 20 mL $CaCl_2$. Soil slurries were mixed with a metal spatula for 30 seconds and then left undisturbed for 7.5 minutes. After 7.5 minutes, a double-junction combination pH electrode probe (Fisher Scientific™, Waltham, MA) was submerged into the upper aqueous layer. Soil pH values were recorded after readings stabilized or the total settling time reached 10 minutes. This soil pH procedure was selected because it provided stable and reproducible results (Maietta, 2017). Gravimetric soil moisture content was determined by drying ~10 g of field-moist soil to a constant mass at 105°C for 36 h and calculating moisture content by taking the difference in weight before and after drying and dividing by the dry weight. Soil organic matter was calculated using loss-on-ignition, adapted from Storer (1984) (500°C for 2 hr). Total C and N for soil samples were determined by combusting 200 ± 5 mg of finely ground soil at 950°C on a LECO CHN-2000 analyzer (LECO Corp, St. Joseph, MI).

The percent C lost was calculated by dividing total C respired in 1 month by total C content in either Cured or Dried Bloom.

Greenhouse experiment

Soil from the University of Maryland's Central Maryland Research & Education Center in Clarksville, MD was collected from the top 15cm of an area growing turf. The soil is a Typic Hapludult clay loam belonging predominantly to the Glenelg series (**Figure 2.1**).

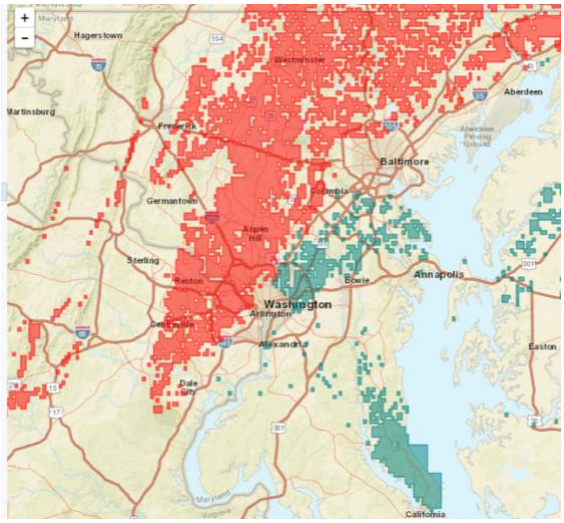


Figure 2.1 *Map of Glenelg soil series (in red) in Maryland, USA.*

The soil was sieved with a 2mm sieve and homogenized. The soil was mixed with amendments and placed into 2-gal pots in the greenhouse. Each pot received one of the four soil treatments: (1) soil (control), (2) soil and fertilizer (Osmocote), (3) soil and fertilizer and Bloom, (4) soil and fertilizer and Leafgro® (**Table 2.3**). The rate of application was determined following label application rates for each product. Leafgro® (Maryland Environmental Service) and Osmocote fertilizer were purchased at local HomeDepot. Bloom was sourced at Blue Plains Facility. N:P:K ratios are written for values of N, P, K, and not of N, P_2O_5, K_2O (**Table 2.3**).

Table 2.3. *Common practitioner use rates for fertilizer and amendments as recommended by product labels.*

Amendment	Rate of application	N:P:K	Amounts added to greenhouse mixes
Osmocote Smart Release 14:14:14	3 tbsp./ 4 ft^2	14-6.1-11.6	10 gr per pot mixed to top 3 inches of soil
Cured Bloom	1/2 inch on top of soil	4.2-3.3-0.1	426g Bloom + 7.5 kg soil
Leafgro	1:1 volume ratio with soil	1.3-0.3-0.9	1 kg Leafgro + 5.25 kg soil

The plants selected were common garden vegetables: tomato (F1 Hybrid, Plant Hart's Seeds), cucumber (Dwarf Bush Type, Plant Hart's Seeds), and soybean (Edamame Soybean, Lake Valley Seed). The plants were started from seed in potting mix, then transferred into treatment pots as seedlings (2-3 weeks after germination). Established plants were transplanted into treatment pots and set in the greenhouse in a randomized complete block design. A total of 65 pots (3 plants, 4 treatments, 5 replicates) were established, along with an additional soybean treatment containing soil and Bloom only. The pots' moisture was maintained daily with drip irrigation. Plant growth parameters such as plant height, number of leaves, stems, flowers, and fruit, were measured weekly throughout the growing season (12-14 weeks). At the end of the experiment, above and belowground biomass and plant nutrients were measured. Nodules were measured on the soybeans at the sampling date.

Nitrogen mineralization of greenhouse soils

A one-month N-mineralization experiment was set up in jar mesocosms using the soils from the greenhouse study after the tomato plants had been grown. The mesocosms were incubated at 25º Celsius under aerobic conditions and soil moisture was maintained at 60%. Destructive sampling of a portion of the mesocosms was done weekly to measure ammonia (NH_3) and nitrate (NO_3^-) N, total soil respiration, microbial biomass, pH, and soil organic matter. The methods are listed in *Nitrogen mineralization of Cured and Dried Bloom* above.

Statistical analyses

Samples for microbial analysis were taken for 4 treatments, on 2 sample dates, and had 5 replicates. Statistically significant differences among gene abundances at different time points were determined using one-way analysis of variance (ANOVA) model with the aov() function within the R 'stats' package (R Core Team, 2019) and post-hoc Tukey's HSD with the HSD.test() function within the R 'agricolae' package (de Mendiburu, 2019) (ANOVA, $p < 0.05$, and Tukey's test, $p < 0.05$).

The greenhouse study was a randomized complete block design, with 3 plants, 4 (for tomato and cucumber) and 5 (for soybean) treatments, and 5 blocks. All growth measurements, below and aboveground biomass, and yield data were analyzed to determine significant main effects of treatments at peak production. Data were analyzed using one-way ANOVA and post-hoc Tukey's HSD.

N-mineralization of Cured and Dried Bloom was a randomized design study with 3 treatments, and 3 replicates. N-mineralization of greenhouse soil mixes was a randomized design study with 4 treatments, and 3 replicates. Measurements were taken at time intervals, and were analyzed using one-way ANOVA at each time point and post-hoc Tukey's HSD.

Data for all experiments were checked for normality and homogeneity. Results were considered significant at the $\alpha = 0.05$ level.

Chapter 3: Results

Microbial analysis

Genes coding for archaeal ammonia monooxygenase (AOA), bacterial ammonia monooxygenase (AOB), and nitrogenase (nifH) were present at different stages of Bloom biosolids production. Gene copy number for nifH varies between the two sampling dates, while AOA and AOB gene copy number are similar. From the September 2016 sampling, AOA and nifH gene copy numbers were higher in the influent of filter press (6.6×10^6 and 2.95×10^8 gene copies g^{-1} of dry solids, respectively) and the dewatered cake (4.0×10^6 and 1.5×10^8 gene copies g^{-1} of dry solids) than in the cured Bloom ($p<0.01$) (**Table 3.1**). The gene copy numbers of AOB were higher than AOA by at least two orders of magnitude. There were no significant differences for the AOB gene copy numbers in the September sampling due to high variability in the replicates (**Table 3.1**), however AOB gene copy numbers are two orders of magnitude higher in 2 month Cured than in other stages (**Table 3.2**). In both sampling dates, AOA and nifH gene copy numbers were lower in the cured for 2 months Bloom (4.6×10^5 and 2.6×10^8 gene copies g^{-1} of dry solids, respectively) than all other stages (**Table 3.2**). We compared the two sampling times, but no significant differences were observed (data not shown).

Table 3.1. Gene abundances (gene copy number g^{-1} dry solids) in different treatment stages of Bloom biosolids sampled in Sep 28, 2016 (n=5, mean ± SEM). A one-way analysis of variance (ANOVA) was used to test mean differences between stages of biosolids production for each gene.

Genes	Treatment stages				Statistic model
	Influent of belt filter press	Dewatered cake	Cured 1 month	Cured 3 months	
	---------- gene copy number g^{-1} dry solids----------				
AOA	6.57 ± 1.05 x 10^6 a	4.01 ± 1.11 x 10^6 a	1.49 ± 0.51 x 10^5 b	3.39 ± 0.68 x 10^5 b	$F(3, 16) = 16.41$, $p < 0.001$***
AOB	6.60 ± 1.11 x 10^8 a	3.11 ± 0.54 x 10^8 a	7.03 ± 3.27 x 10^9 a	3.51 ± 1.96 x 10^{10} a	$F(3, 16) = 2.748$, $p = 0.077$
nifH	2.95 ± 0.75 x 10^8 a	1.53 ± 0.18 x 10^8 ab	7.47 ± 2.90 x 10^6 b	5.35 ± 0.65 x 10^7 b	$F(3, 16) = 10.74$, $p < 0.001$***

Treatment stages with the same letter are not significantly different.

Table 3.2. Gene abundances (gene copy number g^{-1} dry solids) in different treatment stages of Bloom biosolids sampled in Jan 3, 2017 (n=5, mean ± SEM). A one-way analysis of variance (ANOVA) was used to test mean differences between stages of biosolids production for each gene.

Genes	Treatment stages				Statistic model
	Influent of belt filter press	Dewatered cake	Cured 1.5 months	Cured 2 months	
	---------- gene copy number g^{-1} dry solids----------				
AOA	4.34 ± 0.29 x 10^6 a	2.99 ± 0.28 x 10^6 b	1.95 ± 0.51 x 10^6 b	4.58 ± 1.42 x 10^5 c	$F(3, 16) = 24.32$, $p < 0.001$***
AOB	2.51 ± 0.38 x 10^8 b	1.48 ± 0.09 x 10^8 b	2.97 ± 0.18 x 10^8 b	3.48 ± 1.07 x 10^{10} a	$F(3, 16) = 10.47$, $p < 0.001$***
nifH	1.09 ± 0.08 x 10^9 a	6.80 ± 0.33 x 10^8 b	8.57 ± 0.70 x 10^8 ab	2.61 ± 0.77 x 10^8 c	$F(3, 16) = 26.83$, $p < 0.001$***

Treatment stages with the same letter are not significantly different.

N-mineralization of Cured and Dried Bloom

The first nitrogen mineralization experiment was established with three treatments: Soil, Soil + Cured Bloom, and Soil + Dried Bloom. The average mineral nitrogen was significantly higher in Dried Bloom (534 µg N g^{-1} dry soil) than in cured Bloom (242 µg N g^{-1} dry soil), and higher in Cured Bloom than in soil alone (21 µg N g^{-1} dry soil) ($p<0.01$) (**Table 3.3**). N-mineralization rate was calculated at 1-week time intervals for 1 month. The N-mineralization rate was higher in Dried Bloom during the first week compared to soil or Cured Bloom (**Table 3.4**). After the first week, the rate decreased for Cured Bloom, but there were no significant differences between rates. The total N-mineralization rate was calculated for 28 days; Dried Bloom had a significantly higher total rate (9.3 µg N g^{-1} dry soil day^{-1}) than the other treatments ($F=7.332$, $p= 0.0245$). PAN for first year was 36.53 % in Cured Bloom, and 48.16% in Dried Bloom.

The C/N ratio was higher in soil alone than in soil with Cured or Dried Bloom ($p<0.01$). The C:N was higher in Cured Bloom than in Dried Bloom but the difference between the two was not statistically significantly (**Figure 3.1**) C:N ratio for soil alone was significantly lower than soil with Cured or Dried Bloom ($p<0.001$).

There are no differences in microbial biomass carbon (MBC) content between treatments. However, the average microbial biomass nitrogen was lower in Dried Bloom (4.7 µg N g^{-1} dry soil) than in Cured Bloom (9.3 µg N g^{-1} dry soil) and soil alone (5.5 µg N g^{-1} dry soil) (**Table 3.5**). There are no differences for microbial biomass C:N ratio between treatments.

Total CO_2 respired was 1.5-fold higher in soil with Cured Bloom (296.8 µg C g^{-1} dry soil) than in soil alone (176.7 µg C g^{-1} dry soil) and 8-fold higher in soil with Dried Bloom (1436.3 µg C g^{-1} dry soil) (**Figure 3.2**). The total rate of respiration, as calculated by dividing the measured concentration by time passed, was also higher in the soil with Dried Bloom (51.3 µg C g^{-1} dry soil day^{-1}) than with Cured Bloom (10.6 µg C g^{-1} dry soil day^{-1}) or soil alone (6.3 µg C g^{-1} dry soil day^{-1}) ($F=2077$, $p<0.001$). The percent C lost in 1 month was higher in Dried Bloom (10.36%) than in Cured Bloom (1.61%). However, carbon use efficiency, calculated by dividing the respired CO_2-C by the MBC, showed that the organisms in soil with Dried Bloom had much greater C use efficiency (**Table 3.6**).

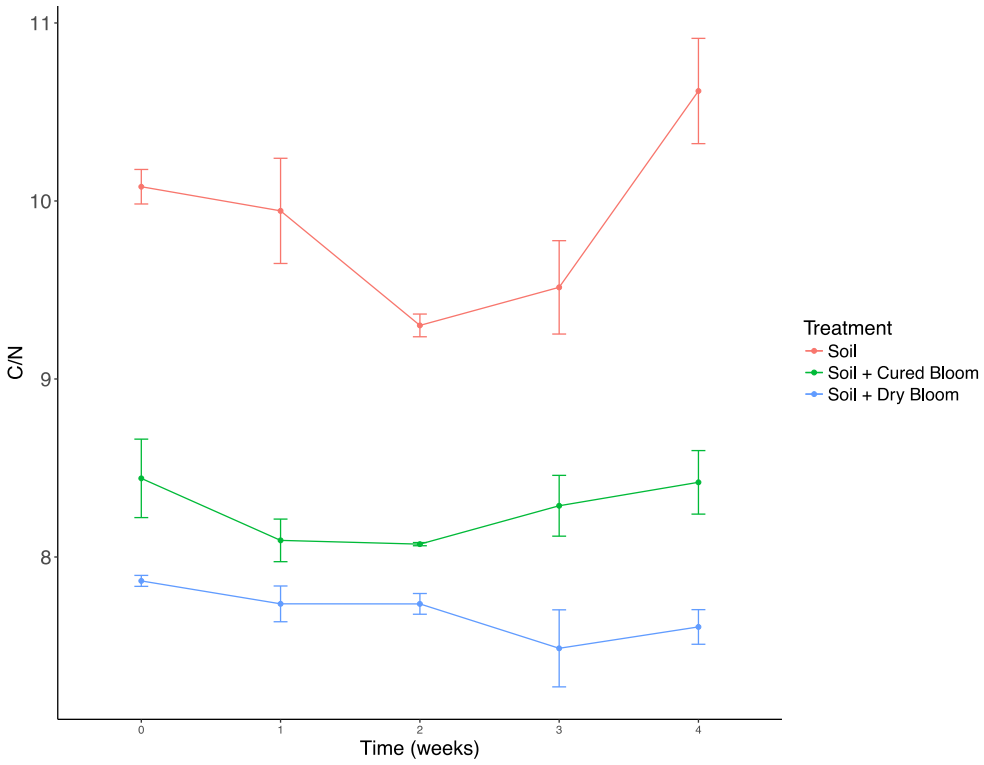


Figure 3.1 *The C:N ratio for treatments during 1-month period. Each point is the mean of 3 replicate samples.*

Table 3.3. Mineral N (NH_4-N + NO_3-N) for soils amended with Bloom biosolids during a 1-month period (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to compare mean differences between treatments at separate time points.

	Treatment			Summary statistic
Time	Soil	Soil + Cured Bloom	Soil + Dried Bloom	Model
	ug N g^{-1} dry amended soil			
Initial	16.57 ± 3.45 c	202.99 ± 5.47 b	375.71 ± 18.69 a	$F(2, 6) = 247.5$, $p < 0.001$ ***
Week 1	11.62 ± 4.25 c	270.55 ± 31.51 b	506.44 ± 14.43 a	$F(2, 6) = 150.7$, $p < 0.001$ ***
Week 2	30.92 ± 1.92 c	258.96 ± 8.92 b	566.88 ± 14.82 a	$F(2, 6) = 716.8$, $p < 0.001$ ***
Week 3	35.91 ± 4.12 c	254.90 ± 6.13 b	584.76 ± 53.60 a	$F(2, 6) = 78.22$, $p < 0.001$ ***
Week 4	12.27 ± 1.83 c	221.59 ± 52.36 b	636.22 ± 58.19 a	$F(2, 6) = 49.34$, $p < 0.001$ ***

Treatments with the same letter are not significantly different.

Table 3.4. N-mineralization rate for soils amended with Bloom biosolids during a 1-month period (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to compare mean differences among treatments at separate time intervals.

	Treatment			Summary statistic
Time interval	Soil	Soil + Cured Bloom	Soil + Dried Bloom	Model
	ug N g^{-1} dry soil day^{-1}			
T1	-0.71 ± 0.94 b	9.65 ± 4.54 ab	18.68 ± 4.72 a	$F(2, 6) = 6.441$, $p = 0.0321$ *
T2	2.76 ± 0.70 a	-1.66 ± 5.12 a	8.63 ± 2.32 a	$F(2, 6) = 2.49$, $p = 0.163$
T3	0.71 ± 0.31 a	-0.58 ± 1.03 a	2.55 ± 8.12 a	$F(2, 6) = 0.111$, $p = 0.897$
T4	-3.38 ± 0.48 a	-4.76 ± 7.85 a	7.35 ± 15.67 a	$F(2, 6) = 0.429$, $p = 0.67$
	ug N g^{-1} dry soil $28days^{-1}$			
28 Day Rate	-0.15 ± 0.12 b	0.66 ± 2.03 b	9.30 ± 2.67 a	$F(2, 6) = 7.332$, $p = 0.0245$ *

Treatments with the same letter are not significantly different.

Table 3.5. Microbial biomass nitrogen of soils amended with Bloom® biosolids during a 1-month period (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments at separate time periods.

Time	Treatment			Summary statistic
	Soil	Soil + Cured Bloom®	Soil + Dried Bloom®	Model
	MBN (ug N g^{-1} dry soil)			
0	7.29 ± 0.52 a	16.66 ± 2.92 a	33.21 ± 16.67 a	F(2, 6) = 1.802 , p = 0.244
Week 1	6.87 ± 0.40 b	17.15 ± 3.36 a	11.38 ± 2.17 ab	F(2, 6) = 4.929 , p = 0.0542
Week 2	6.00 ± 0.63 a	8.88 ± 1.73 a	-3.69 ± 1.53 b	F(2, 6) = 22.72 , p = 0.01 **
Week 3	2.46 ± 1.73 a	1.00 ± 0.56 a	-9.88 ± 0.57 b	F(2, 6) = 37.73 , p < 0.001 ***
Week 4	4.86 ± 0.41 a	2.72 ± 0.40 a	-7.65 ± 2.84 b	F(2, 6) = 16.01 , p < 0.01 **

Treatments with the same letter are not significantly different.

Table 3.6. Carbon use efficiency (microbial respiration/microbial biomass carbon) of soil amended with Cured or Dried Bloom® (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments at separate time points.

Time	Treatment			Summary statistic
	Soil	Soil + Cured Bloom®	Soil + Dried Bloom	Model
	CO_2/MBC			
Week 1	1.33 ± 0.10 b	1.88 ± 0.06 b	11.01 ± 1.49 a	F(2, 6) = 39.76 , p < 0.001 ***
Week 2	0.85 ± 0.03 b	1.19 ± 0.19 b	4.99 ± 0.34 a	F(2, 6) = 103 , p < 0.001 ***
Week 3	0.75 ± 0.03 b	1.13 ± 0.13 b	4.04 ± 0.24 a	F(2, 6) = 126.8 , p < 0.001 ***
Week 4	0.68 ± 0.08 b	0.86 ± 0.07 b	3.99 ± 0.72 a	F(2, 6) = 19.53 , p < 0.01 **
	Cumulative CO_2/average MBC			
	3.53 ± 0.17 b	4.96 ± 0.43 b	22.41 ± 1.15 a	F(2, 6) = 217.3 , p < 0.001 ***

Treatments with the same letter are not significantly different.

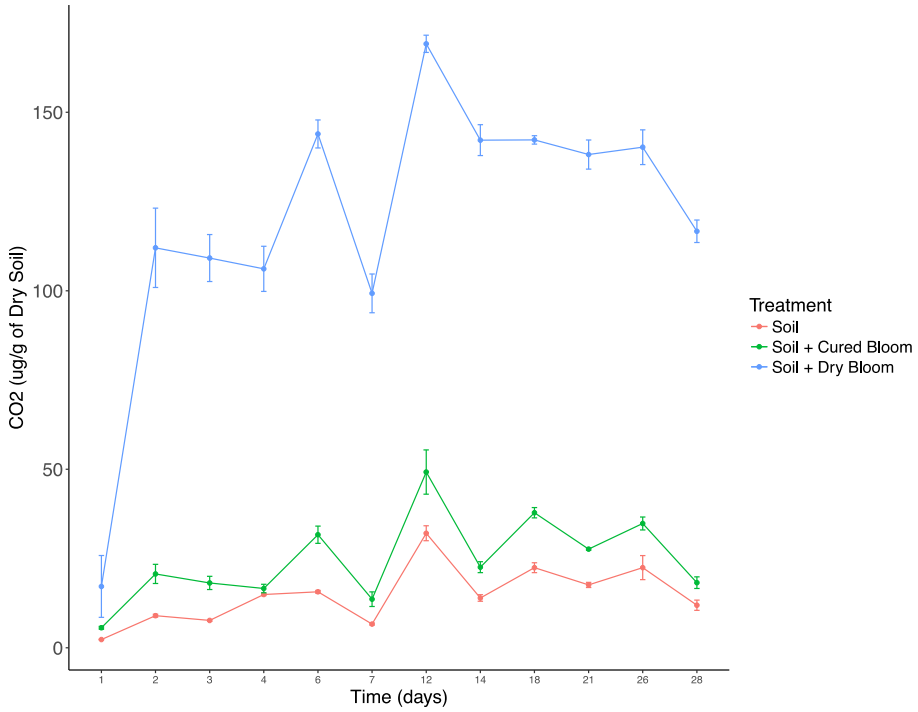


Figure 3.2. *CO2 concentration (µg C g-1 dry soil) during a 1-month period. Each point is the mean of 3 replicate samples.*

Greenhouse experiment

Tomato

Tomato plants grown in fertilizer + Bloom had significantly more leaves (550), more stems (25), and higher belowground biomass (2.31 g) than those treated with fertilizer only (331, 19, 1.8 g, respectively) ($p < 0.01$), and more stems than those treated with fertilizer + Leafgro (20) ($p<0.01$) (**Figure 3.3**). Plants treated with fertilizer, + Bloom, and + Leafgro had significantly more aboveground biomass than non-amended soil ($p<0.01$) (F**igure 3.3**). Plants treated with fertilizer + Leafgro produced more flowers (5) than the other treatments (S=0.8, SF=2.6, SFB=3.2)

(p=0.0216), but were no significant differences in plant height and fruit production for any treatments. Fruit production was negatively affected by high temperatures in the greenhouse.

Tomato plants treated with fertilizer + Bloom appeared to be a deeper green color than other treatments. This led us to do a plant tissue analysis (**Table 3.7**). Total N was significantly higher in fertilizer + Bloom treatments compared to the other treatments. The same plants also had a lower Mg concentration (**Table 3.7**).

Cucumber

Cucumber plants treated with fertilizer + Bloom had significantly more leaves (46), more stems (61), and higher aboveground biomass (16.5 g) than those treated with fertilizer only (26, 33, 13 g, respectively) ($p < 0.01$) (**Figure 3.3**). Plants treated with fertilizer + Leafgro also had higher leaf counts (32) and aboveground biomass (17.9 g) than plants treated with fertilizer only ($p<0.01$). Cucumber plants treated with fertilizer + Bloom had more leaves then those treated with fertilizer + Leafgro ($p<0.01$) (**Figure 3.3**). Plants treated with fertilizer, + Bloom, and + Leafgro had more fruit production than non-amended soil (p=0.0298). However, plants with amended soil had low fruit production, averaging 1 cucumber per plant, due to high temperatures in the greenhouse. There are no statistical differences in belowground biomass and plant height between any treatments. Tissue analysis was not performed on cucumbers because the vines had senesced by the end of the experiment.

Soybean

Soybean plants treated with fertilizer + Bloom had significantly more leaves (61), stems (23), fruit (23), and higher belowground biomass (2.1 g) than those treated with fertilizer + Leafgro or fertilizer only ($p < 0.01$) (**Figure 3.3**). Soybean plants grew taller with fertilizer + Leafgro treatment (117 cm) than in fertilizer + Bloom (96 cm) or Bloom alone (97 cm) treatment ($p = 0.015$) (**Figure 3.4**). Soybean plants treated with Bloom only produced more leaves (57) than those treated with fertilizer only (42) ($p<0.01$). Plants treated with fertilizer + Bloom (23) or with Bloom (21) produced more soybean pods than those with fertilizer alone (16) (**Figure 3.4**). Plants treated with any fertilizer or amendment tended to have less nodules in their roots than plants in soil only, but the difference was not significant. There are no statistical differences in flower production for any of the treatments or in soybean aboveground biomass.

The percent total N did not differ in soybean plant tissue as it had in tomatoes (**Table 3.8**). In treatments where Bloom was present, there were higher concentrations of Ca, Mg, and Zn in the soybean tissue (**Table 3.8**).

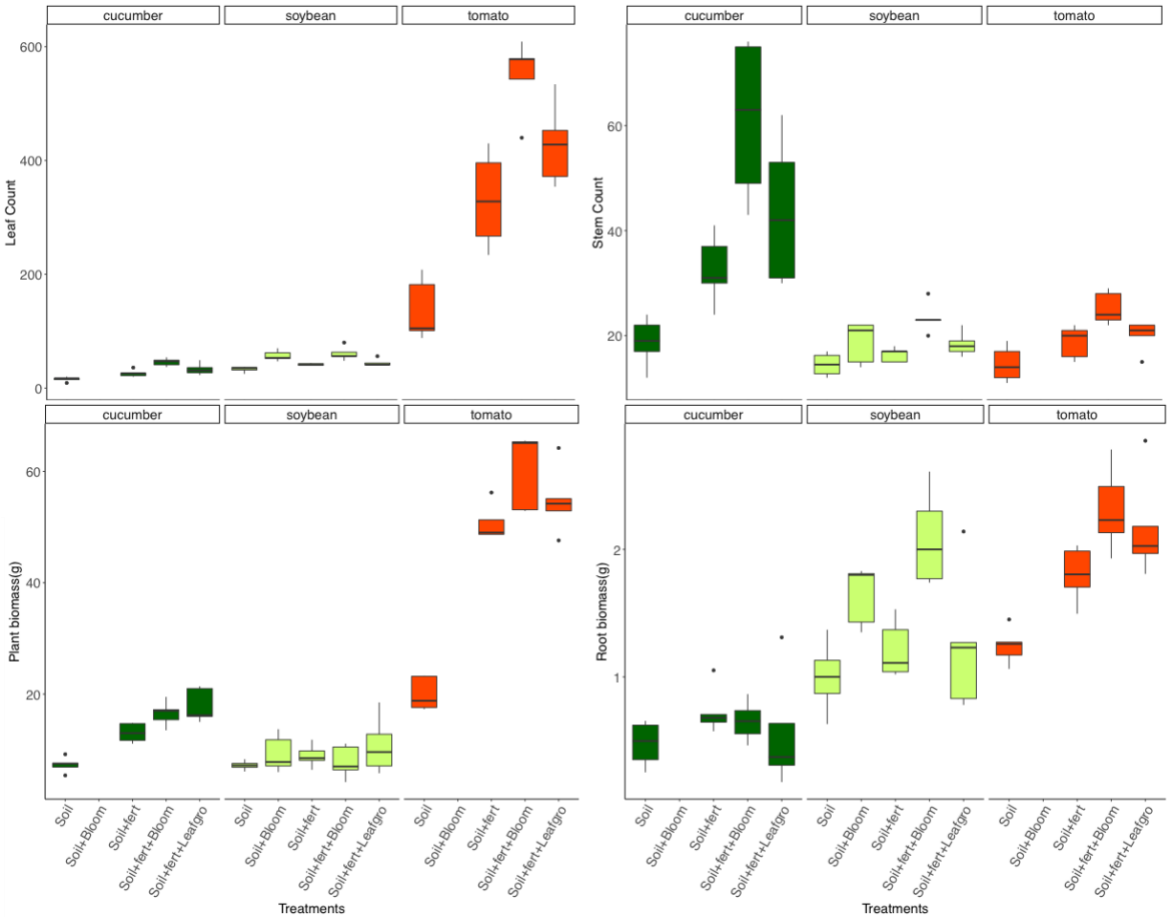


Figure 3.3. *The mean number of leaves (upper left) and stems (upper right), and above- (lower left) and belowground (lower right) biomass for cucumber, soybean, and tomato plants. n=5.*

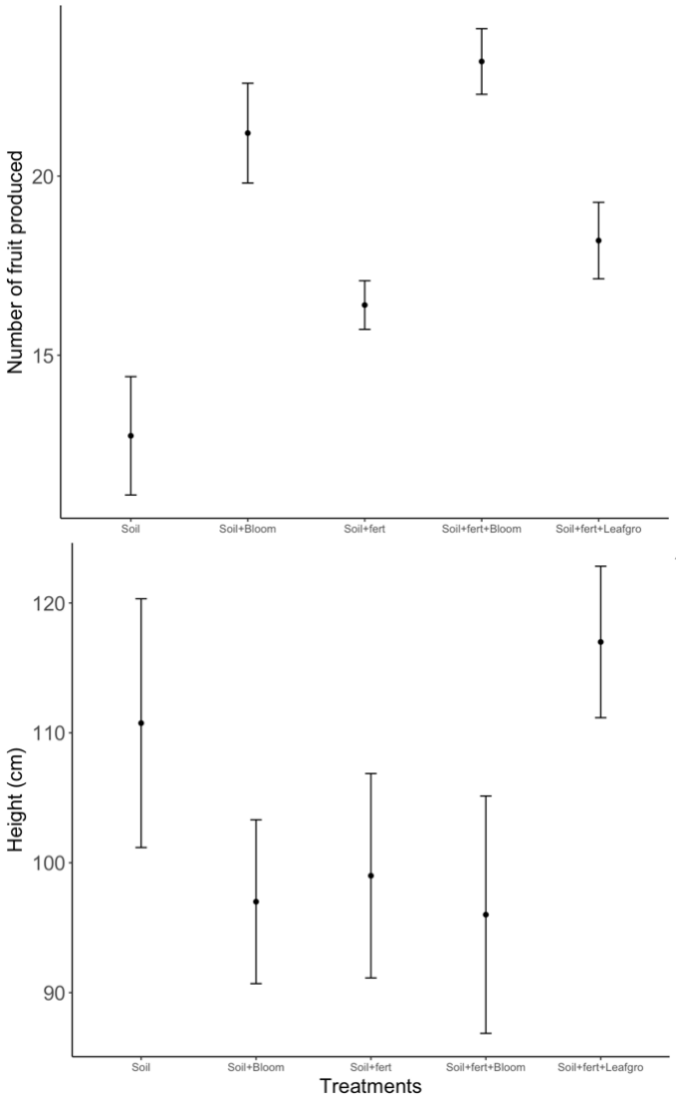


Figure 3.4. *Fruit count (upper) and height (lower) for soybean plants. n=5.*

Table 3.7. Summary of leaf tissue analysis for tomato plants (n=5, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments.

	Treatment				Summary statistic
Nutrient	Soil	Soil + Fert	Soil + Fert + Bloom®	Soil + Fert + Leafgro	Model
	%				
Total C	43.29 ± 0.25 a	43.63 ± 0.16 a	44.26 ± 0.38 a	43.15 ± 0.23 a	$F(3, 12) = 2.928$, $p = 0.077$
Total N	3.28 ± 0.21 b	3.22 ± 0.17 b	4.55 ± 0.18 a	3.04 ± 0.10 b	$F(3, 12) = 18.179$, $p < 0.001$ ***
C:N	13.42 ± 0.83 a	13.69 ± 0.65 a	9.79 ± 0.35 b	14.25 ± 0.44 a	$F(3, 12) = 12.211$, $p < 0.001$ ***
P	0.52 ± 0.07 a	0.41 ± 0.05 a	0.39 ± 0.03 a	0.50 ± 0.05 a	$F(3, 12) = 1.508$, $p = 0.263$
K	3.06 ± 0.26 a	2.39 ± 0.19 b	2.64 ± 0.22 ab	3.15 ± 0.12 a	$F(3, 12) = 5.336$, $p = 0.0144$ *
Ca	1.71 ± 0.11 a	1.70 ± 0.06 a	1.85 ± 0.16 a	1.86 ± 0.16 a	$F(3, 12) = 0.341$, $p = 0.796$
Mg	0.57 ± 0.02 a	0.56 ± 0.01 ab	0.48 ± 0.02 b	0.63 ± 0.02 a	$F(3, 12) = 9.486$, $p < 0.01$ **
S	0.75 ± 0.08 a	0.81 ± 0.04 a	1.01 ± 0.10 a	0.78 ± 0.05 a	$F(3, 12) = 2.448$, $p = 0.114$
	mg kg^{-1}				
Mn	107.68 ± 8.01 b	190.12 ± 28.68 a	187.50 ± 23.45 ab	110.34 ± 17.58 ab	$F(3, 12) = 5.799$, $p = 0.0109$ *
Zn	19.60 ± 3.50 ab	16.88 ± 2.19 ab	28.68 ± 5.68 a	15.06 ± 0.65 b	$F(3, 12) = 3.851$, $p = 0.0384$ *
Cu	16.24 ± 1.72 a	11.94 ± 0.86 a	14.1 ± 1.10 a	13.12 ± 1.39 a	$F(3, 12) = 2.171$, $p = 0.144$
Fe	92.14 ± 11.30 a	81.38 ± 4.84 a	90.84 ± 3.23 a	86.86 ± 2.81 a	$F(3, 12) = 0.519$, $p = 0.677$
B	32.9 ± 3.92 a	18.9 ± 1.53 b	14.94 ± 0.91 b	37 ± 3.26 a	$F(3, 12) = 23.842$, $p < 0.001$ ***
Na	484.68 ± 29.50 b	453.94 ± 44.20 b	1979.72 ± 104.64 a	353.16 ± 34.69 b	$F(3, 12) = 141.895$, $p < 0.001$ ***
Al	18.78 ± 5.77 a	15.52 ± 2.34 a	12.80 ± 3.00 a	11.64 ± 1.02 a	$F(3, 12) = 1.471$, $p = 0.2719$

Treatments with the same letter are not significantly different.

Table 3.8. Summary of leaf tissue analysis for soybean plants (n=5, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments.

Nutrient	Treatment										Summary statistic
	Soil		Soil + Bloom®		Soil + Fert		Soil + Fert + Bloom®		Soil + Fert + Leafgro		Model
	%										
Total C	46.51 ± 0.28	a	44.06 ± 0.57	b	45.78 ± 0.27	ab	44.25 ± 0.63	b	45.61 ± 0.39	ab	F(4, 15) = 6.748 , p < 0.01 **
Total N	2.60 ± 0.15	a	2.44 ± 0.24	a	2.74 ± 0.16	a	2.84 ± 0.16	a	2.80 ± 0.16	a	F(4, 15) = 1.173 , p = 0.3619
C:N	18.06 ± 1.06	a	18.70 ± 1.67	a	16.88 ± 0.86	a	15.72 ± 0.80	a	16.45 ± 0.82	a	F(4, 15) = 1.355 , p = 0.296
P	0.21 ± 0.02	a	0.18 ± 0.01	a	0.19 ± 0.01	a	0.20 ± 0.02	a	0.23 ± 0.02	a	F(4, 15) = 1.992 , p = 0.147
K	1.31 ± 0.07	b	1.00 ± 0.10	b	1.26 ± 0.09	b	1.36 ± 0.07	b	2.04 ± 0.24	a	F(4, 15) = 7.122 , p < 0.01 **
Ca	2.23 ± 0.10	b	3.43 ± 0.36	a	2.41 ± 0.12	b	3.51 ± 0.36	a	2.14 ± 0.14	b	F(4, 15) = 9.057 , p < 0.001 ***
Mg	0.22 ± 0.04	b	0.71 ± 0.07	a	0.27 ± 0.02	b	0.65 ± 0.08	a	0.26 ± 0.02	b	F(4, 15) = 25.699 , p < 0.001 ***
S	0.17 ± 0.01	a	0.15 ± 0.01	a	0.16 ± 0.01	a	0.17 ± 0.02	a	0.17 ± 0.01	a	F(4, 15) = 0.549 , p = 0.703
	mg kg^{-1}										
Mn	156.95 ± 12.14	bc	268.90 ± 30.56	a	220.94 ± 16.08	ab	270.68 ± 25.22	a	140.14 ± 4.60	c	F(4, 15) = 10.700 , p < 0.001 ***
Zn	68.88 ± 9.25	b	156.82 ± 16.72	a	56.08 ± 2.80	b	153.2 ± 9.51	a	68.32 ± 6.78	b	F(4, 15) = 28.239 , p < 0.001 ***
Cu	7.63 ± 0.90	a	5.92 ± 0.18	a	6.06 ± 0.27	a	5.62 ± 0.57	a	6.56 ± 0.66	a	F(4, 15) = 2.290 , p = 0.1077
Fe	71.90 ± 3.27	a	113.48 ± 37.40	a	63.60 ± 2.71	a	71.32 ± 1.50	a	79.40 ± 11.70	a	F(4, 15) = 1.326 , p = 0.305
B	63.20 ± 8.93	ab	67.34 ± 6.85	ab	63.52 ± 2.49	ab	48.98 ± 3.74	b	80.96 ± 8.28	a	F(4, 15) = 4.593 , p = 0.0127 *
Na	30.80 ± 4.52	a	37.20 ± 8.77	a	26.02 ± 1.61	a	30.38 ± 1.58	a	29.12 ± 2.52	a	F(4, 15) = 0.720 , p = 0.592
Al	25.50 ± 3.50	a	48.50 ± 26.15	a	23.00 ± 1.78	a	21.42 ± 1.63	a	20.28 ± 2.43	a	F(4, 15) = 0.911 , p = 0.483

Treatments with the same letter are not significantly different.

N-mineralization of greenhouse soils

The second nitrogen mineralization experiment was established with four treatments: Soil, Soil + fertilizer, Soil + fertilizer + Bloom, and Soil + fertilizer + Leafgro. In this case soils that were used to grow tomatoes were combined and homogenized. Mineral nitrogen was highest in soil + fertilizer + Bloom treatment ($p<0.01$) (**Figure 3.5**). The N-mineralization rate, which was calculated for 1-week intervals, was significantly lower for soil + fertilizer + Bloom for the first week, and then is significantly higher on the 3rd week (**Supplementary Table 1**). The total N-mineralization rate was calculated for 28 days and did not show significant differences between treatments, due to high variation in numbers. The C:N ratio between treatments was significantly different only in the initial measurements and in week 4, where Soil + fertilizer + Bloom had the lowest C:N ratio (9.4 and 8.4, respectively) (**Table 3.9**).

Both microbial biomass C and N were 2-fold higher in soil + fertilizer + Leafgro than in other treatments (**Figure 3.6**). Microbial biomass C:N ratio was higher in soil + fertilizer + Leafgro at the beginning of the incubation (time 0) (13.37) ($p=0.0265$), however, there are no differences between treatments for microbial biomass C:N ratio at later time points.

Total microbial respiration was highest in soil + fertilizer + Leafgro (1468.4 µg C g^{-1} dry soil). Respiration in soil +fertilizer +Bloom was higher (846.8 µg C g^{-1} dry soil) than soil + fertilizer alone (474.9 µg C g^{-1} dry soil) (**Figure 3.7-left**) ($F=170$, $p < 0.001$). The amount of CO_2 respired was statistically different for treatment for each measurement ($p<0.01$) (**Figure 3.7-right**).

The rate of respiration at each time point, as calculated by dividing the measured concentration by time, was also highest in soil + fertilizer + Leafgro. The rate was higher in soil + fertilizer + Bloom than in soil + fertilizer or soil alone. The same is true for the cumulative rate of respiration (**Table 3.10**). Dividing the total CO_2-C respired by MBC showed that the microbial communities of soil + fertilizer + Bloom had a greater C use efficiency (14.63) than other treatments ($F=48.84$, $p<0.001$) (**Table 3.11**).

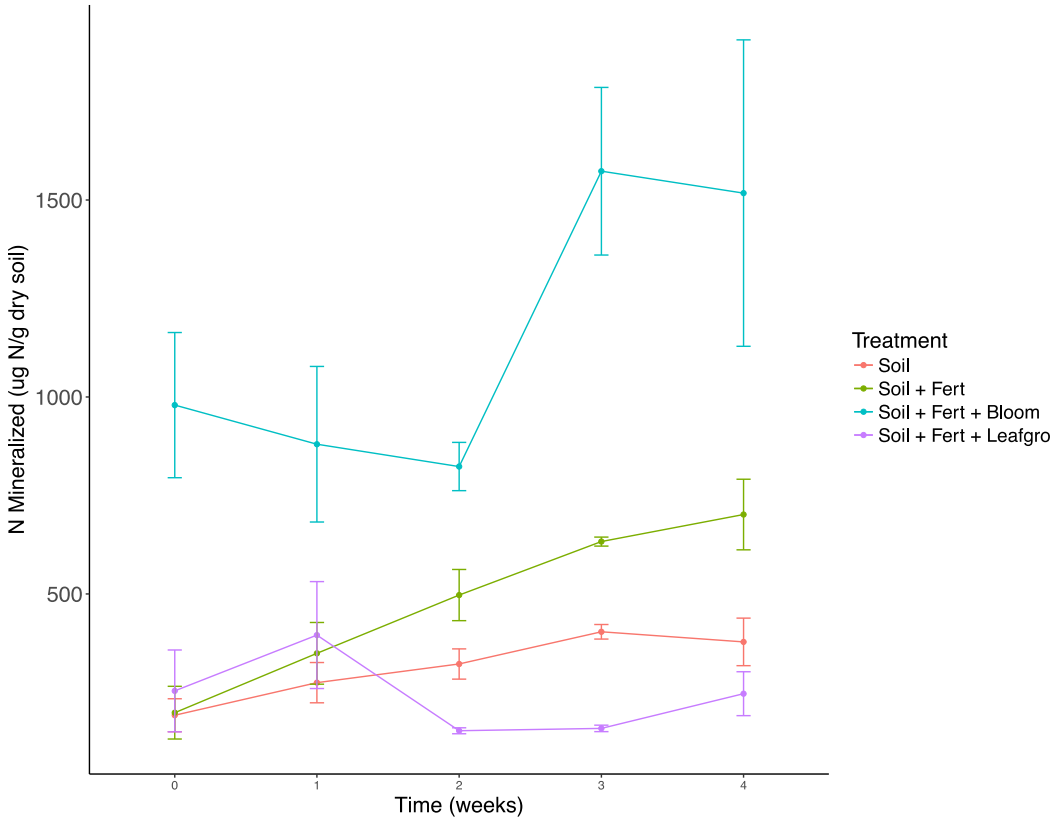


***Figure 3.5**. N-mineralization during a 1-month period for soils that had been used in the greenhouse experiment. Each point is the mean of 3 replicate samples.*

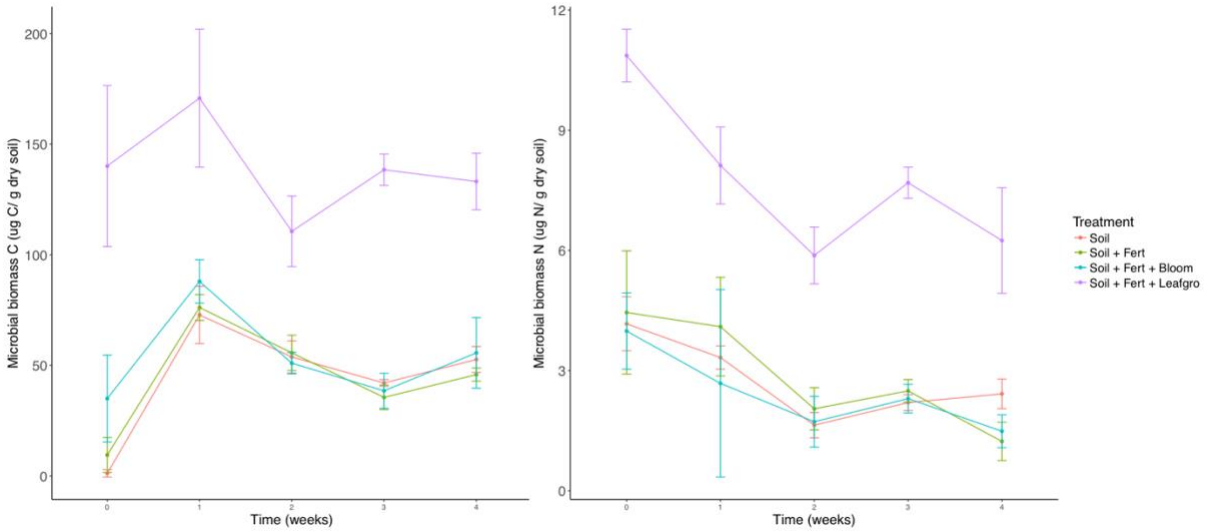


Figure 3.6. *Microbial biomass Carbon (left) and Nitrogen (right) during a 1-month period of soils used in the greenhouse and then incubated for N-mineralization. Each point is the mean of 3 replicate samples.*

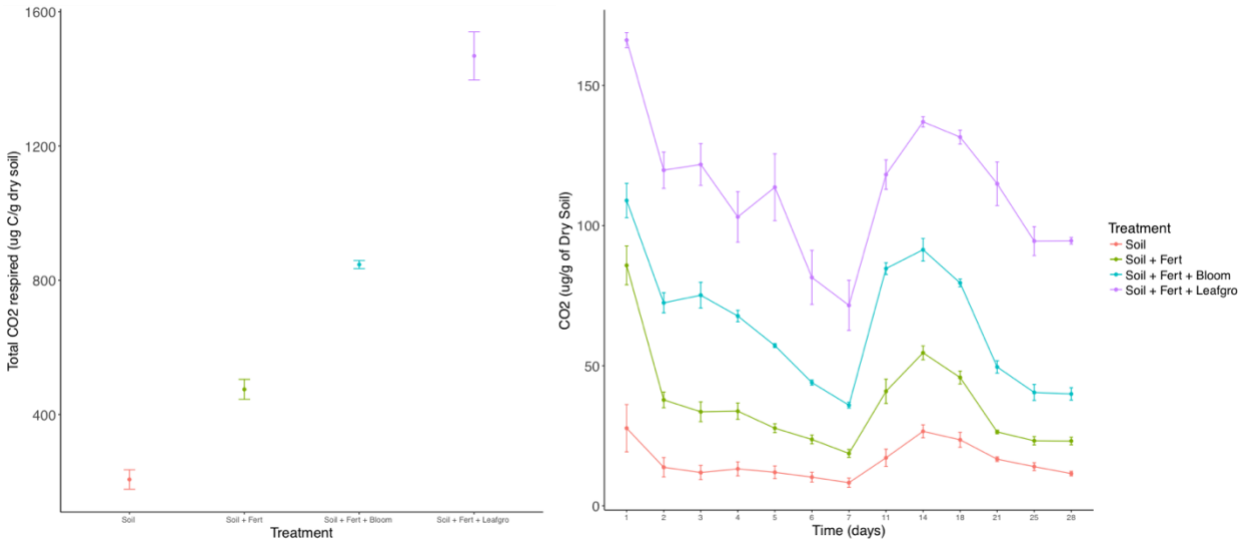


Figure 3.7. *Average total CO2-C respired for greenhouse soils that were then incubated (left). CO2 respiration rates for duration of the incubation study (right).*

Table 3.9. C/N ratios of greenhouse soils during incubation (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to compare mean difference among treatments at separate time points.

	Treatment				Summary statistic
Time	Soil	Soil + Fert	Soil + Fert + Bloom®	Soil + Fert + Leafgro	Model
	---------- C/N ratio ----------				
Week 0	13.67 ± 1.13 ab	13.90 ± 1.47 a	9.39 ± 0.39 b	15.01 ± 0.29 a	$F(3, 8) = 6.667$, $p = 0.0144$ *
Week 1	7.48 ± 7.48 a	7.31 ± 7.31 a	11.89 ± 1.20 a	18.29 ± 0.70 a	$F(3, 8) = 0.954$, $p = 0.46$
Week 2	3.74 ± 2.35 a	11.78 ± 7.77 a	6.32 ± 2.23 a	10.19 ± 2.67 a	$F(3, 8) = 0.684$, $p = 0.586$
Week 3	0.80 ± 0.29 a	0.60 ± 0.18 a	0.88 ± 0.25 a	6.22 ± 3.74 a	$F(3, 8) = 2.111$, $p = 0.177$
Week 4	9.67 ± 0.16 b	10.21 ± 0.27 b	8.42 ± 0.25 c	14.41 ± 0.09 a	$F(3, 8) = 160.8$, $p < 0.001$ *

Treatments with the same letter are not significantly different.

Table 3.10. Rates of CO_2 respiration (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments at separate time periods.

	Treatment				Summary statistic
Time point (days)	Soil	Soil + Fert	Soil + Fert + Bloom®	Soil + Fert + Leafgro	Model
	CO2 (ug C g^{-1} of dry soil day^{-1})				
1	27.69 ± 8.43 c	85.81 ± 6.91 b	108.94 ± 6.14 b	166.19 ± 2.69 a	F(3, 8) = 80.23 , p < 0.0001 ***
2	13.75 ± 3.45 d	37.80 ± 2.81 c	72.44 ± 3.56 b	119.77 ± 6.49 a	F(3, 8) = 113.6 , p < 0.0001 ***
3	11.87 ± 2.53 c	33.55 ± 3.52 c	75.16 ± 4.59 b	121.80 ± 7.45 a	F(3, 8) = 98.82 , p < 0.0001 ***
4	13.17 ± 2.50 c	33.77 ± 2.88 c	67.72 ± 2.04 b	103.10 ± 9.00 a	F(3, 8) = 62.51 , p < 0.0001 ***
5	11.96 ± 2.23 c	27.70 ± 1.57 c	57.18 ± 0.73 b	113.69 ± 11.90 a	F(3, 8) = 53.69 , p < 0.0001 ***
6	10.21 ± 1.75 c	23.67 ± 1.56 bc	43.97 ± 0.91 b	81.49 ± 9.64 a	F(3, 8) = 38.8 , p < 0.0001 ***
7	8.24 ± 1.65 c	18.68 ± 1.42 bc	35.91 ± 1.02 b	71.54 ± 8.95 a	F(3, 8) = 35.87 , p < 0.0001 ***
11	4.28 ± 0.77 d	10.21 ± 1.08 c	21.17 ± 0.52 b	29.55 ± 1.31 a	F(3, 8) = 135.3 , p < 0.0001 ***
14	8.86 ± 0.77 d	18.20 ± 0.82 c	30.46 ± 1.34 b	45.67 ± 0.61 a	F(3, 8) = 295.1 , p < 0.0001 ***
18	5.89 ± 0.67 d	11.44 ± 0.58 c	19.88 ± 0.35 b	32.90 ± 0.62 a	F(3, 8) = 431.9 , p < 0.0001 ***
21	5.55 ± 0.27 c	8.79 ± 0.21 c	16.51 ± 0.74 b	38.31 ± 2.59 a	F(3, 8) = 117.8 , p < 0.0001 ***
25	3.49 ± 0.35 c	5.80 ± 0.37 c	10.11 ± 0.71 b	23.62 ± 1.29 a	F(3, 8) = 132.6 , p < 0.0001 ***
28	3.83 ± 0.27 d	7.71 ± 0.46 c	13.31 ± 0.74 b	31.52 ± 0.40 a	F(3, 8) = 606.5 , p < 0.0001 ***
	CO_2 (ug C g^{-1} of dry soil day^{-1})				
Cumulative rate	7.37 ± 1.04 d	16.96 ± 1.06 c	30.24 ± 0.43 b	52.441939 ± 2.56 a	F(3, 8) = 170.3 , p < 0.0001 ***

Treatments with the same letter are not significantly different.

Table 3.11. Carbon use efficiency (microbial respiration/microbial biomass carbon) of greenhouse soils (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments at separate time periods.

Time	Treatment														Summary statistic
	Soil				Soil + Fert				Soil + Fert + Bloom®				Soil + Fert + Leafgro				Model
	---------- CO_2/MBC ----------																
Week 1	1.50	±	0.47	b	3.48	±	0.41	ab	5.39	±	0.64	a	4.95	±	1.19	a	F(3, 8) = 5.523 , p = 0.0238 *
Week 2	0.82	±	0.05	c	1.80	±	0.29	bc	3.49	±	0.22	a	2.38	±	0.25	b	F(3, 8) = 25.29 , p < 0.001 ***
Week 3	0.96	±	0.11	b	2.11	±	0.25	ab	3.59	±	0.59	a	1.79	±	0.11	b	F(3, 8) = 11.04 , p < 0.01 **
Week 4	0.50	±	0.09	a	1.01	±	0.06	a	1.88	±	0.76	a	1.44	±	0.14	a	F(3, 8) = 2.258 , p = 0.159
	----------Cumulative CO_2/average MBC----------																
	3.85	±	0.75	c	8.89	±	0.33	b	14.63	±	0.93	a	10.61	±	0.32	b	F(3, 8) = 48.84 , p < 0.001 ***

Treatments with the same letter are not significantly different.

Chapter 4: Discussion and Conclusion

Discussion

The primary objective of this study was to determine whether Bloom biosolids can improve soil and plant health. First, we characterized some of the microbial communities of Bloom in order to quantify functional genes that serve as markers for beneficial rhizosphere microbes.

As predicted, genes coding for archaeal ammonia monooxygenase (AOA), bacterial ammonia monooxygenase (AOB), and nitrogenase (nifH) were present in Bloom. Cured Bloom had more AOB genes than the Fresh (**Table 3.2**). However, the number of genes for AOA and nifH are lower in Cured Bloom than in Fresh Bloom (**Table 3.1 and 3.2**). Changes in moisture content between the Fresh and Cured Bloom (Cured being drier) could have contributed to these differences. Liang et al. (2003) reported that moisture content has a significant influence on microbial activity (2003). Although nitrification genes did not, nifH did vary seasonally with more gene copies during the January sampling. It is not clear why we observed these differences, although increased abundance of bacteria and archaea have previously been observed in activated sludge (Ju et al., 2013).

Gene abundances for nifH and AOB tended to be higher than gene copy numbers found in soil. For example, AOA and AOB gene copy numbers on agricultural field were approximately 10^5 and 10^7 (Gubry-Rangin et al., 2010, Yao et

al., 2011, Di et al., 2009), but we observed values similar for AOA and higher for AOB, 10^9. Although nitrification genes are present in Bloom, this does not mean that this addition will enhance activity within the soil. Gubry-Rangin et al. (2010) and Yao et al. (2011) observed a significant positive relationship between nitrification rate and AOA, but not AOB growth, and other research shows a significant relationship between the abundance of ammonia-oxidizing bacteria rather than archaea and the rate of nitrification (Di et al., 2009). Gene abundances for nifH across four agricultural fields was approximately 10^5 (Pereira e Silva et al., 2013), but we observed values from 10^7-10^9 (**Table 3.1 and 3.2**). Although the genes for N-fixation are present in Bloom, this does not mean that this addition will enhance activity within the soil. Wakelin et al. (2010) observed a correlation between nifH gene abundance in soil and N-fixing potential, but other studies have observed marginal (Caton et al., 2018) or no correlations (Brankatschk et al., 2011). Aside from nifH abundance, the composition of the N-fixing community can affect rates (Hsu and Buckley, 2009), but we did not investigate the nifH or AOB and AOA composition. More research would be needed to determine if Bloom addition increases N-fixation or nitrification rates, although the N-mineralization experiment clearly showed that Bloom could change soil N concentrations.

The net N-mineralization rates was 1.5-fold higher in Dried Bloom than in Cured Bloom (**Table 3.4**) and corresponded to difference in the C:N ratios of the two products (**Figure 3.1**). Cured Bloom had a higher C:N ratio than Dried Bloom (**Supplementary Table 2**). Previously, researchers have reported that C:N ratios negatively correlates with N-mineralized (Janssen, 1996; Sollins et al. 1984). Other

research suggests that higher soil moisture correlated to higher N mineralization (Mazzarino, 1991). However, analysis of the material prior to the start of incubations (**Supplementary Table 3**) show that Cured Bloom has higher water content than Dried Bloom. The moisture content remained similarly high in Cured Bloom during the 1-month incubations (**Supplementary Figure 1**). Dried Bloom also has higher plant available nitrogen. Smith and Durham found that found that heat drying increased the rate of N release and mineralizable N of the biosolids (2002). The percent C lost in 1 month was higher in Dried Bloom (10.36%) than in Cured Bloom (1.61%). This might be explained by the wetting effect which is also observed in soils where there is higher CO2 flux in wetting events (Borken et al., 2003, Miller et al., 2005).

Dried Bloom had higher respiration rates than Cured Bloom (**Figure 3.2**). This could help explain the higher N mineralization rate due to a larger microbial community (Bengtsson et al. 2003, Franzluebbers et al. 1996, Zak et al. 1999). Although, there were no differences in microbial biomass C and N between Cured and Dried Bloom. The chloroform fumigation method is able to quantify the total microbial community, but does not distinguish between active and dormant organisms, therefore it is possible that Dried Bloom contained a more active community. Given these observations, it would be advisable for D.C. Water to prepare Bloom for sale by drying. Dried Bloom would provide gardeners with a product that releases more N for plant growth.

The higher N concentration of Bloom likely contributed to the results of the

greenhouse experiment. All three plants grown in soil amended with fertilizer + Bloom produced more leaves and stems than those with fertilizer + Leafgro or fertilizer only (**Figure 3.3**). Tomato and cucumber plants treated with fertilizer + Bloom had significantly higher aboveground biomass than those treated with fertilizer only (**Figure 3.3**). Brown et al. (2003), reported similar response with biosolids amendments. Application of biosolids has shown to have a positive effect on plant growth, increasing yield and nutritional quality as well (Sharma et al. 2017). We show similar results for the soybean plants, those treated with fertilizer + Bloom or with Bloom had higher yields of soybean pods than those with fertilizer alone (**Figure 3.4**). Yield was unexpectedly low in tomato and cucumbers, however. Almost none of the plants produced tomatoes, regardless of treatment. Further research in the literature suggests that high temperatures can result in low fruit set in tomato plants (Charles and Harris, 1972) and that this might be due to the formation of poorer quality pollen at these temperatures (Van Ploeg & Heuvelink, 2005). The plants were grown in summer months of 2017 in a greenhouse where the temperatures inside surpassed the optimal temperature that was set by greenhouse staff. Both water misting and venting were used to decrease temperatures, but they did not seem to help the fruit setting of the tomato plants. There were no treatment differences for cucumber fruit production, but again yield was low. Although cucumbers are a warm season crop they also do not tolerate temperatures consistently above 90^{o}F (Hochmuth, 2015).

In spite of the poor fruit production, tomato plants treated with fertilizer + Bloom appeared to be a deeper green color than other treatments. Plant tissue analysis

revealed that Total N, Zn, and Na were significantly higher in fertilizer + Bloom treatment but Mg was significantly lower compared to the other treatments (**Table 3.7**). Plants respond quickly to increased availability of N, their leaves turning deep green in color (Brady and Weil, 2008). However, Mercado-Luna et al. (2010) reported that the green color they measured in tomato plants had no definite relation with the N concentration in the nutrient solution, and Ward and Miller (1969) report darker green foliage color in tomato plant treated with higher levels of Mg. The Mg content of Leafgro and Osmocote fertilizer is unknown.

After growing the plants, we conducted a second N-mineralization experiment, this time of the soil mixes that were used in the greenhouse. Even though the soils had been used to grow one season of plants, the soil + fertilizer + Bloom mix had higher net N-mineralization rates than other treatments (**Figure 3.5**). The soil + fertilizer + Bloom mix also had higher microbial respiration than soil + fertilizer (Figure 7). However, soil + fertilizer + Leafgro had more microbial biomass C and N and a higher respiration rate than other treatments (**Figure 3.5 and 3.6**). The higher microbial biomass and respiration in Leafgro can be due to differences in microbial communities. It is possible that Leafgro has more fungi than Bloom mix because of difference in the material origin and production. However, our results show that even though soil + fertilizer + Leafgro had higher microbial respiration, soil + fertilizer + Bloom had a greater C use efficiency than other treatments.

Conclusion

The overall goal of this research was to determine if Bloom biosolids can improve soil and plant health. The first experiment evaluated the microbial community of Bloom by quantifying the functional genes that serve as markers for beneficial rhizosphere microbes. Such markers, archaeal ammonia monooxygenase (AOA), bacterial ammonia monooxygenase (AOB), and nitrogenase (nifH), were present in Bloom. Second, mineral N content, N-mineralization rate, and microbial respiration were highest in Dried Bloom then in Cured Bloom. Given these observations, it would be advisable for D.C. Water to prepare Bloom for sale by drying. Purchasing dried Bloom would provide gardeners with a product that released more N for plant growth. Third, we compared the effect of Bloom and Leafgro amendments on plant growth. Bloom has higher mineral N than Leafgro even after one growing season. Leafgro has higher organic matter and water content, microbial biomass C and N, and microbial respiration. However, microbes in Bloom have higher carbon use efficiency. Higher N concentration of Bloom likely contributed to the results of the greenhouse experiment. Plants amended with Bloom produced more leaves and stems than those with Leafgro. Higher root biomass was seen in tomatoes and soybeans, and more pods in soybean plants amended with Bloom then with Leafgro. Differences in plant growth might also be attributed to changes in physical soil properties after the additions of the two amendments. The content of N in Bloom is lower than in commercial fertilizers, such as urea (N-P-K 46-0-0), however, Bloom is a good soil amendment that adds organic matter and beneficial microbial communities that also has some fertilizer value. Future research should be conducted

with different textured soils to analyze differences in bulk density, water holding capacity, aggregate stability, porosity and permeability, after additions of Bloom or Leafgro amendments.

Appendices

Appendix I – Supplementary Figures and Tables

Supplementary Table 1

Table S1. Summary statistics for N-mineralization rate (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments at separate time intervals.

	Treatment				Summary statistic
Time interval	Soil	Soil + Fert	Soil + Fert + Bloom	Soil + Fert + Leafgro	Model
	ug N g-1 dry soil day-1				
T1	11.82 ± 1.63 ab	21.54 ± 10.79 a	-14.18 ± 2.03 b	20.24 ± 6.00 a	F(3, 8) = 6.921 , p = 0.013 *
T2	6.76 ± 4.58 a	21.12 ± 3.71 a	-8.09 ± 22.12 a	-34.70 ± 18.31 a	F(3, 8) = 2.647 , p = 0.12
T3	11.67 ± 4.36 b	19.41 ± 7.66 b	107.12 ± 21.83 a	0.85 ± 0.20 b	F(3, 8) = 17.2 , p < 0.001 *
T4	-3.66 ± 6.42 a	9.81 ± 11.19 a	-7.94 ± 26.01 a	12.59 ± 8.62 a	F(3, 8) = 0.439 , p = 0.731
	ug N g-1 dry soil 28days-1				
28 Day Rate	6.65 ± 1.01 a	17.97 ± 2.03 a	19.23 ± 9.79 a	-0.25 ± 5.48 a	F(3, 8) = 2.662 , p = 0.119

Table 1. Summary statistics for C/N ratios for Cured and Dried Bloom® (n=3, mean ± SEM). A one-way analysis of variance (ANOVA) was used to inspect mean difference among treatments at separate time points.

	Treatment			Summary statistic
Time	Soil	Soil + Cured Bloom	Soil + Dried Bloom	Model
	---------- C/N ratio ----------			
Initial	10.08 ± 0.10 a	8.44 ± 0.22 b	7.87 ± 0.03 b	$F(2, 6) = 67.17$, $p < 0.001$ *
Week 1	9.94 ± 0.30 a	8.09 ± 0.12 b	7.74 ± 0.10 b	$F(2, 6) = 37.73$, $p < 0.001$ *
Week 2	9.30 ± 0.06 a	8.07 ± 0.01 b	7.74 ± 0.06 c	$F(2, 6) = 268.4$, $p < 0.001$ *
Week 3	9.51 ± 0.26 a	8.29 ± 0.17 b	7.49 ± 0.22 b	$F(2, 6) = 21.61$, $p < 0.01$ *
Week 4	10.62 ± 0.30 a	8.42 ± 0.18 b	7.61 ± 0.10 b	$F(2, 6) = 56.53$, $p < 0.001$ *

Treatments with the same letter are not significantly different.

Supplementary Table 3

Table S3*. Water and Organic Matter content of soil, cured Bloom, and dried Bloom.*

	Water Content	Organic Matter
Soil	21.26%	5.10%
Cured Bloom	69.73%	40.50%
Dried Bloom	60.11%	56.74%

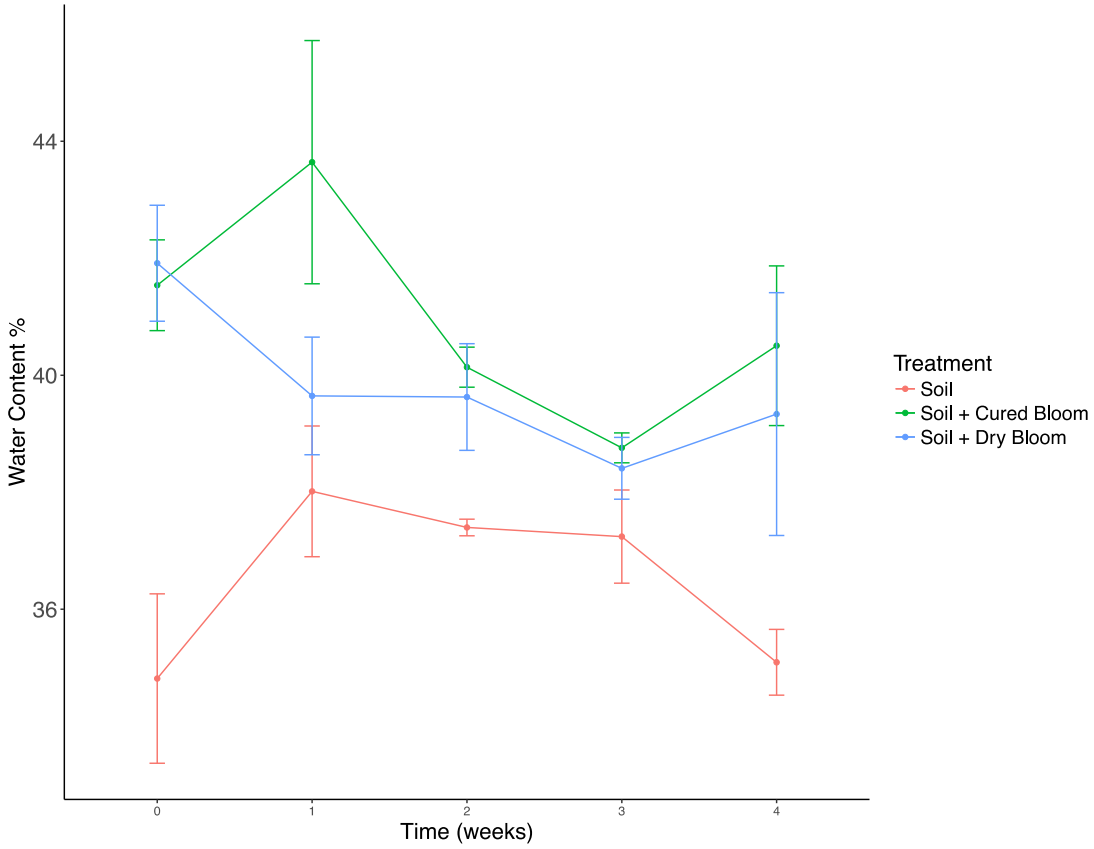


Figure S1*. Gravimetric water content of soils amended with Cured or Dried Bloom during 1-month N-min experiment. Each point shows the mean of 3 replicates.*

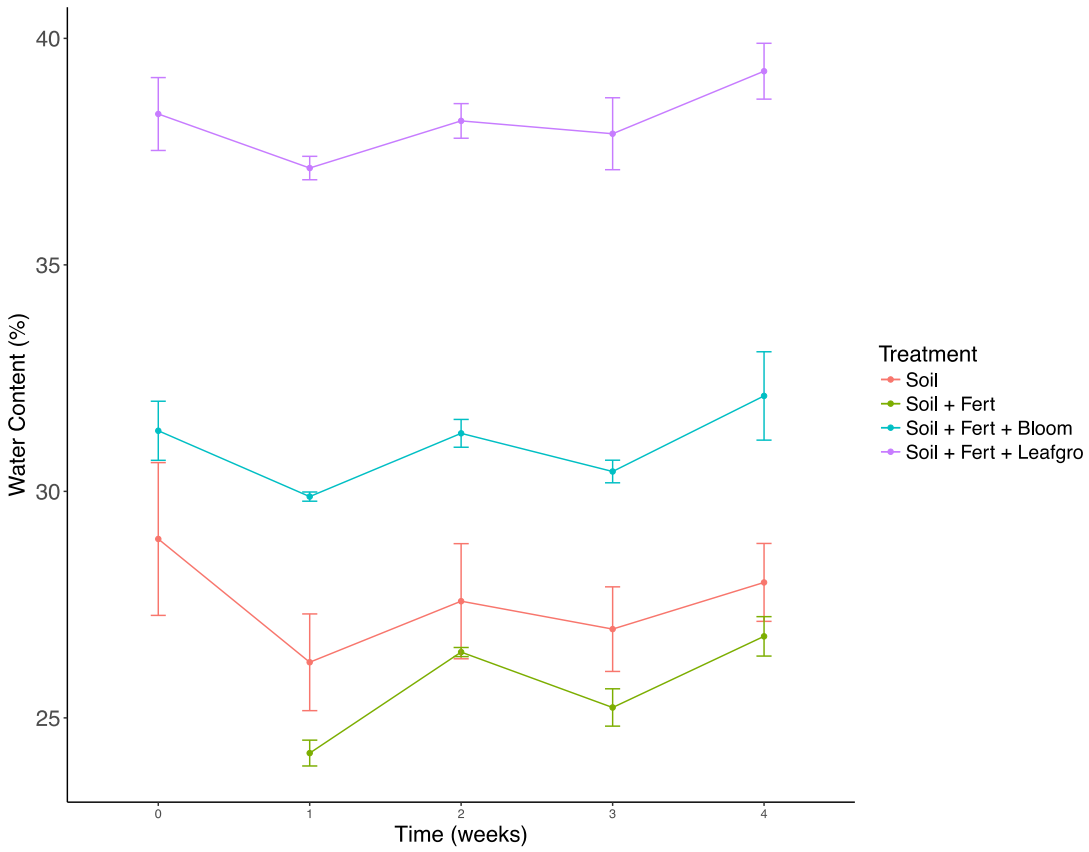


Figure S2. *Water content of greenhouse soil mixes during 1-month incubation. Each point is the mean of 3 replicates.*

Appendix II – Laboratory analysis for Bloom and Leafgro

ANALYTICAL CHEMISTS and BACTERIOLOGISTS Approved by State of California

SOIL CONTROL LAB

42 HANGAR WAY WATSONVILLE CALIFORNIA 95076 USA

TEL: 831-724-5422
FAX: 831-724-3188
www.compostlab.com

Account #: 6040970-3/3-8679
Group: Apr16E #47
Reporting Date: May 10, 2016

DC Water & Sewer Authority
5000 Overlook Avenue, SW
Washington, DC 20032
Attn: Bill Brower

Date Received: 29 Apr. 16
Sample Identification: Bloom Fresh
Sample ID #: 6040970 - 3/3

Nutrients	Dry wt.	As Rcvd.	units
Total Nitrogen:	4.8	1.5	%
Ammonia (NH_4-N):	3600	1100	mg/kg
Nitrate (NO_3-N):	210	64	mg/kg
Org. Nitrogen (Org.-N):	4.4	1.3	%
Phosphorus (as P_2O_5):	6.4	2.0	%
Phosphorus (P):	28000	8600	mg/kg
Potassium (as K_2O):	0.11	0.034	%
Potassium (K):	930	280	mg/kg
Calcium (Ca):	2.3	0.71	%
Magnesium (Mg):	0.35	0.11	%
Sulfate (SO_4-S):	430	130	mg/kg
Boron (Total B):	12	3.6	mg/kg
Moisture:	0	69.5	%
Sodium (Na):	0.043	0.013	%
Chloride (Cl):	0.73	0.22	%
pH Value:	NA	8.47	unit
Bulk Density :	14	46	lb/cu ft
Carbonates ($CaCO_3$):	6.6	2.0	lb/ton
Conductivity (EC5):	7.0	NA	mmhos/cm
Organic Matter:	59.7	18.2	%
Organic Carbon:	33.0	10.0	%
Ash:	40.3	12.3	%
C/N Ratio	7.0	7.0	ratio
AgIndex	> 10	> 10	ratio

Stability Indicator:		Biologically
CO2 Evolution	Respirometery	Available C
mg CO_2-C/g OM/day	6.4	14
mg CO_2-C/g TS/day	3.8	8.2
Stability Rating	*moderately unstable*	*unstable*

Maturity Indicator: Cucumber Bioassay	
Compost:Vermiculite(v:v)	1:2
Emergence (%)	100
Seedling Vigor (%)	93
Description of Plants	*healthy*

Pathogens	Results	Units	Rating
Fecal Coliform	< 7.5	MPN/g	*pass*
Salmonella	< 3	MPN/4g	*pass*

Date Tested: 29 Apr. 16

Inerts	% by weight
Plastic	< 0.5
Glass	< 0.5
Metal	< 0.5
Sharps	ND

Metals	Dry wt.	EPA Limit	units
Aluminum (Al):	7900	-	mg/kg
Arsenic (As):	4.7	41	mg/kg
Cadmium (Cd):	1.0	39	mg/kg
Chromium (Cr):	49	1200	mg/kg
Cobalt (Co)	6.0	-	mg/kg
Copper (Cu):	390	1500	mg/kg
Iron (Fe):	76000	-	mg/kg
Lead (Pb):	27	300	mg/kg
Manganese (Mn):	300	-	mg/kg
Mercury (Hg):	< 1.0	17	mg/kg
Molybdenum (Mo):	13	75	mg/kg
Nickel (Ni):	24	420	mg/kg
Selenium (Se):	4.3	36	mg/kg
Zinc (Zn):	710	2800	mg/kg

Size Distribution	
MM	% by weight
> 50	0.0
25 to 50	0.0
16 to 25	0.0
9.5 to 16	0.0
6.3 to 9.5	0.0
4.0 to 6.3	1.0
2.0 to 4.0	3.8
< 2.0	95.2

Analyst: Assaf Sadeh

*Sample was received and handled in accordance with TMECC procedures.

***Figure S3**. Lab analysis of Fresh Bloom (provided by DC Water).*

ANALYTICAL CHEMISTS and BACTERIOLOGISTS Approved by State of California

SOIL CONTROL LAB

42 HANGAR WAY
WATSONVILLE
CALIFORNIA
95076
USA

TEL: 831-724-5422
FAX: 831-724-3188
www.compostlab.com

Account #: 6030946-1/3-8679
Group: Apr.16 A #29
Reporting Date: April 26, 2016

DC Water & Sewer Authority
5000 Overlook Avenue, SW
Washington, DC 20032
Attn: Bill Brower

Date Received: 31 Mar. 16
Sample Identification: Bloom windrow +30d, post flip
Sample ID #: 6030946 - 1/3

Nutrients	Dry wt.	As Rcvd.	units
Total Nitrogen:	4.2	1.8	%
Ammonia (NH_4-N):	1600	690	mg/kg
Nitrate (NO_3-N):	6.8	2.9	mg/kg
Org. Nitrogen (Org.-N):	4.0	1.7	%
Phosphorus (as P_2O_5):	7.7	3.3	%
Phosphorus (P):	34000	14000	mg/kg
Potassium (as K_2O):	0.11	0.048	%
Potassium (K):	950	400	mg/kg
Calcium (Ca):	2.8	1.2	%
Magnesium (Mg):	0.40	0.17	%
Sulfate (SO_4-S):	2800	1200	mg/kg
Boron (Total B):	<1.0	<1.0	mg/kg
Moisture:	0	57.9	%
Sodium (Na):	0.094	0.040	%
Chloride (Cl):	0.07	0.029	%
pH Value:	NA	6.78	unit
Bulk Density :	20	46	lb/cu ft
Carbonates ($CaCO_3$):	23	9.5	lb/ton
Conductivity (EC5):	5.4	NA	mmhos/cm
Organic Matter:	52.2	22.0	%
Organic Carbon:	29.0	12.0	%
Ash:	47.8	20.1	%
C/N Ratio	6.9	6.9	ratio
AgIndex	> 10	> 10	ratio

Stability Indicator:		Biologically
CO2 Evolution	Respirometery	Available C
mg CO_2-C/g OM/day	2.8	2.9
mg CO_2-C/g TS/day	1.5	1.5
Stability Rating	*stable*	*stable*

Maturity Indicator: Cucumber Bioassay	
Compost:Vermiculite(v:v)	1:2
Emergence (%)	100
Seedling Vigor (%)	111
Description of Plants	*healthy*

Pathogens	Results	Units	Rating
Fecal Coliform	11	MPN/g	*pass*
Salmonella	< 3	MPN/4g	*pass*

Date Tested: 31 Mar. 16

Inerts	% by weight
Plastic	< 0.5
Glass	< 0.5
Metal	< 0.5
Sharps	ND

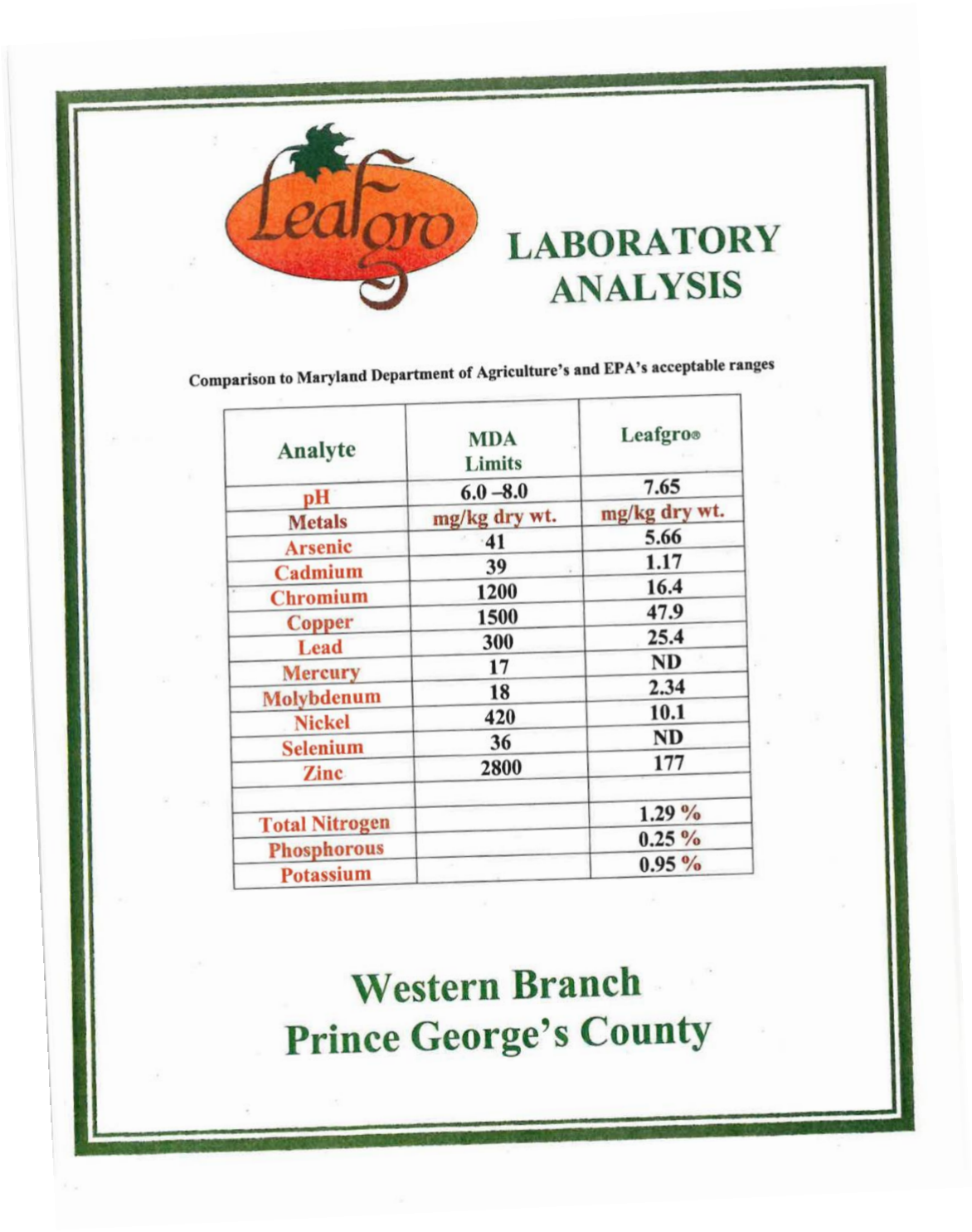
Metals	Dry wt.	EPA Limit	units
Aluminum (Al):	6700	-	mg/kg
Arsenic (As):	11	41	mg/kg
Cadmium (Cd):	3.5	39	mg/kg
Chromium (Cr):	46	1200	mg/kg
Cobalt (Co)	7.1	-	mg/kg
Copper (Cu):	430	1500	mg/kg
Iron (Fe):	86000	-	mg/kg
Lead (Pb):	49	300	mg/kg
Manganese (Mn):	360	-	mg/kg
Mercury (Hg):	< 1.0	17	mg/kg
Molybdenum (Mo):	11	75	mg/kg
Nickel (Ni):	26	420	mg/kg
Selenium (Se):	3.9	36	mg/kg
Zinc (Zn):	720	2800	mg/kg

Size Distribution	
MM	% by weight
> 50	0.0
25 to 50	0.0
16 to 25	0.0
9.5 to 16	0.0
6.3 to 9.5	0.0
4.0 to 6.3	1.8
2.0 to 4.0	1.6
< 2.0	96.6

Analyst: Assaf Sadeh

*Sample was received and handled in accordance with TMECC procedures.

Figure S4 *Lab analysis of Cured Bloom (provided by DC Water).*



Leafgro

LABORATORY ANALYSIS

Comparison to Maryland Department of Agriculture's and EPA's acceptable ranges

Analyte	MDA Limits	Leafgro®
pH	6.0 –8.0	7.65
Metals	mg/kg dry wt.	mg/kg dry wt.
Arsenic	41	5.66
Cadmium	39	1.17
Chromium	1200	16.4
Copper	1500	47.9
Lead	300	25.4
Mercury	17	ND
Molybdenum	18	2.34
Nickel	420	10.1
Selenium	36	ND
Zinc	2800	177
Total Nitrogen		1.29 %
Phosphorous		0.25 %
Potassium		0.95 %

Western Branch
Prince George's County

Figure S5*.Lab analysis of Leafgro (provided by Leafgro representative).*

Bibliography

Adegbidi, H. G., Briggs, R. D., Volk, T. A., White, E. H., & Abrahamson, L. P. (2003). Effect of organic amendments and slow-release nitrogen fertilizer on willow biomass production and soil chemical characteristics. *Biomass and Bioenergy*, *25*(4), 389-398.

American Biogas Council, Biogas Opportunities Roadmap (USDA, EPA, DOE, 2014), EPA AgSTAR 2016, EPA LMOP 2017, Water Environment Federation "Enabling the Future".

Aoi, Y., Masaki, Y., Tsuneda, S., & Hirata, A. (2004). Quantitative analysis of amoA mRNA expression as a new biomarker of ammonia oxidation activities in a complex microbial community. *Letters in applied microbiology, 39*(6), 477-482.

Barbarick, K. A., Doxtader, K. G., Redente, E. F., & Brobst, R. B. (2004). Biosolids effects on microbial activity in shrubland and grassland soils. *Soil Science, 169*(3), 176-187.

Belyaeva, O. N., & Haynes, R. J. (2009). Chemical, microbial and physical properties of manufactured soils produced by co-composting municipal green waste with coal fly ash. *Bioresource technology*, *100*(21), 5203-5209.

Bengtsson, G., Bengtson, P., & Månsson, K. F. (2003). Gross nitrogen mineralization-, immobilization-, and nitrification rates as a function of soil C/N ratio and microbial activity. *Soil Biology and Biochemistry*, *35*(1), 143-154.

Borken, W., Davidson, E. A., Savage, K., Gaudinski, J., & Trumbore, S. E. (2003). Drying and wetting effects on carbon dioxide release from organic horizons. *Soil Science Society of America Journal*, *67*(6), 1888-1896.

Brady, N. C., & Weil, R. R. (2008). *The nature and properties of soils* (Vol. 14). Upper Saddle River, NJ: Prentice Hall.

Brankatschk, R., Töwe, S., Kleineidam, K., Schloter, M., & Zeyer, J. (2011). Abundances and potential activities of nitrogen cycling microbial communities along a chronosequence of a glacier forefield. *The ISME journal*, *5*(6), 1025.

Brown, S. L., Henry, C. L., Chaney, R., Compton, H., & DeVolder, P. S. (2003). Using municipal biosolids in combination with other residuals to restore metal-contaminated mining areas. *Plant and Soil*, *249*(1), 203-215.

Camacho, P., Ewert, W., Kopp, J., Panter, K., Perez-Elvira, S. I., & Piat, E. (2008). Combined experiences of thermal hydrolysis and anaerobic digestion–latest thinking on thermal hydrolysis of secondary sludge only for optimum dewatering and digestion. *Proceedings of the water environment federation, 2008(15)*, 1964-1978.

Caton, I. R., Caton, T. M., & Schneegurt, M. A. (2018). Nitrogen-fixation activity and the abundance and taxonomy of nifH genes in agricultural, pristine, and urban prairie stream sediments chronically exposed to different levels of nitrogen loading. *Archives of microbiology*, *200*(4), 623-633.

Charles, W. B., & Harris, R. E. (1972). Tomato fruit-set at high and low temperatures. *Canadian Journal of Plant Science*, *52*(4), 497-506.

Cogger, C. G., Forge, T. A., & Neilsen, G. H. (2006). Biosolids recycling: Nitrogen management and soil ecology. *Canadian Journal of Soil Science*, *86*(4), 613-620.

Coleman, D. C., Callaham, M. A., & Crossley Jr, D. A. (2017). *Fundamentals of soil ecology*. Academic press.

D.C. Water. (2019). Blue Plains Wastewater Treatment Plant. Retrieved from: www.dcwater.com/sites/default/files/documents/Blue_Plains_Plant_brochure.pdf

Di, H. J., Cameron, K. C., Shen, J. P., Winefield, C. S., O'callaghan, M., Bowatte, S., & He, J. Z. (2009). Nitrification driven by bacteria and not archaea in nitrogen-rich grassland soils. *Nature Geoscience*, *2*(9), 621.

Diacono, M., & Montemurro, F. (2011). Long-term effects of organic amendments on soil fertility. In *Sustainable Agriculture Volume 2* (pp. 761-786). Springer, Dordrecht.

Evanylo, G.K. 2006. Land application of biosolids. In: K.C. Haering and G.K. Evanylo, editors, e Mid-Atlantic nutrient management handbook. Mid-Atlantic Regional Water Program, Pennsylvania State Univ., State College, PA. p. 226–252.

Felipe de Mendiburu (2019). agricolae: Statistical Procedures for Agricultural Research. R package version 1.3-1. https://CRAN.R-project.org/package=agricolae

Fernández, J. M., Plaza, C., García-Gil, J. C., & Polo, A. (2009). Biochemical properties and barley yield in a semiarid Mediterranean soil amended with two kinds of sewage sludge. *Applied soil ecology*, *42*(1), 18-24.

Franzluebbers, A. J., Haney, R. L., Hons, F. M., & Zuberer, D. A. (1996). Determination of microbial biomass and nitrogen mineralization following rewetting of dried soil. *Soil Science Society of America Journal*, *60*(4), 1133-1139.

García-Gil, J. C., Plaza, C., Senesi, N., Brunetti, G., & Polo, A. (2004). Effects of sewage sludge amendment on humic acids and microbiological properties of a semiarid Mediterranean soil. *Biology and Fertility of Soils*, *39*(5), 320-328.

Gubry-Rangin, C., Nicol, G. W., & Prosser, J. I. (2010). Archaea rather than bacteria control nitrification in two agricultural acidic soils. *FEMS microbiology ecology*, *74*(3), 566-574.

Hargreaves, S. K., Roberto, A. A., & Hofmockel, K. S. (2013). Reaction-and sample-specific inhibition affect standardization of qPCR assays of soil bacterial communities. *Soil Biology and Biochemistry*, *59*, 89-97.

Hochmuth, R. C. (2015). Greenhouse cucumber production—Florida greenhouse vegetable production handbook, Vol. 3. *IFAS Extension, University of Florida. Florida, EEUU. HS790*.

Hsu, S. F., & Buckley, D. H. (2009). Evidence for the functional significance of diazotroph community structure in soil. *The ISME journal*, *3*(1), 124.

Janssen, B. H. (1996). Nitrogen mineralization in relation to C: N ratio and decomposability of organic materials. In *Progress in Nitrogen Cycling Studies* (pp. 69-75). Springer, Dordrecht.

Ju, F., Guo, F., Ye, L., Xia, Y., & Zhang, T. (2014). Metagenomic analysis on seasonal microbial variations of activated sludge from a full-scale wastewater treatment plant over 4 years. *Environmental microbiology reports*, *6*(1), 80-89.

Koutroubas, S. D., Antoniadis, V., Fotiadis, S., & Damalas, C. A. (2014). Growth, grain yield and nitrogen use efficiency of Mediterranean wheat in soils amended with municipal sewage sludge. *Nutrient cycling in agroecosystems*, *100*(2), 227-243.

Latare, A. M., Kumar, O., Singh, S. K., & Gupta, A. (2014). Direct and residual effect of sewage sludge on yield, heavy metals content and soil fertility under rice–wheat system. *Ecological Engineering*, *69*, 17-24.

Liang, C., Das, K., & McClendon, R. (2003). The influence of temperature and moisture contents regimes on the aerobic microbial activity of a biosolids composting blend. *Bioresource Technology, 86*(2), 131-137. doi:10.1016/S0960-8524(02)00153-0

Lu, Q., He, Z. L., & Stoffella, P. J. (2012). Land application of biosolids in the USA: A review. *Applied and Environmental Soil Science, 2012.*

Mabuhay, J. A., Nakagoshi, N., & Isagi, Y. (2006). Microbial responses to organic and inorganic amendments in eroded soil. *Land Degradation & Development, 17*(3), 321-332.

Maietta, C. E. (2017). Soil microbial processes and community structure in natural and restored tidal freshwater wetlands of the Chesapeake Bay, Maryland, USA (Doctoral dissertation).

Mazzarino, M. J., Oliva, L., Abril, A., & Acosta, M. (1991). Factors affecting nitrogen dynamics in a semiarid woodland (Dry Chaco, Argentina). *Plant and Soil, 138*(1), 85-98.

Mercado-Luna, A., Rico-García, E., Lara-Herrera, A., Soto-Zarazúa, G., Ocampo-Velázquez, R., Guevara-González, R., ... & Torres-Pacheco, I. (2010). Nitrogen determination on tomato (Lycopersicon esculentum Mill.) seedlings by color image analysis (RGB). *African Journal of Biotechnology, 9*(33).

Miller, A. E., Schimel, J. P., Meixner, T., Sickman, J. O., & Melack, J. M. (2005). Episodic rewetting enhances carbon and nitrogen release from chaparral soils. *Soil Biology and Biochemistry, 37*(12), 2195-2204.

Mincer, T. J., Church, M. J., Taylor, L. T., Preston, C., Karl, D. M., & DeLong, E. F. (2007). Quantitative distribution of presumptive archaeal and bacterial nitrifiers in Monterey

Bay and the North Pacific Subtropical Gyre. *Environmental microbiology*, *9*(5), 1162-1175.

Naeini, S. M., & Cook, H. F. (2000). Influence of municipal compost on temperature, water, nutrient status and the yield of maize in a temperate soil. *Soil use and Management*, *16*(3), 215-221.

NEBRA (2007). A National Biosolids Regulation, Quality, End Use & Disposal Survey. North East Biosolids and Residuals Association.

Pascual, J. A., García, C., & Hernandez, T. (1999). Lasting microbiological and biochemical effects of the addition of municipal solid waste to an arid soil. *Biology and fertility of soils*, *30*(1-2), 1-6.

Pastor, J., Stillwell, M. A., & Tilman, D. (1987). Nitrogen mineralization and nitrification in four Minnesota old fields. *Oecologia, 71*(4), 481-485.

Pereira e Silva, M. C. P., Schloter-Hai, B., Schloter, M., van Elsas, J. D., & Salles, J. F. (2013). Temporal dynamics of abundance and composition of nitrogen-fixing communities across agricultural soils. *PLoS One*, *8*(9), e74500.

R Core Team (2019). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ros, M., Hernandez, M. T., & García, C. (2003). Soil microbial activity after restoration of a semiarid soil by organic amendments. *Soil Biology and Biochemistry*, *35*(3), 463-469.

Rösch, C., Mergel, A., & Bothe, H. (2002). Biodiversity of denitrifying and dinitrogen-fixing bacteria in an acid forest soil. *Appl. Environ. Microbiol.*, *68*(8), 3818-3829.

Sharma, B., Sarkar, A., Singh, P., & Singh, R. P. (2017). Agricultural utilization of biosolids: A review on potential effects on soil and plant grown. *Waste Management*, *64*, 117-132.

Smith, S. R., & Durham, E. (2002). Nitrogen release and fertiliser value of thermally-dried biosolids. *Water and Environment Journal*, *16*(2), 121-126.

Sollins, P., Spycher, G., & Glassman, C. A. (1984). Net nitrogen mineralization from light- and heavy-fraction forest soil organic matter. *Soil Biology and Biochemistry*, *16*(1), 31-37.

Storer, D. A. (1984). A simple high sample volume ashing procedure for determination of soil organic matter. *Communications in Soil Science and plant analysis*, *15*(7), 759-772.

Sullivan, D. M., Cogger, C. G., & Bary, A. I. (2015). Fertilizing with Biosolids (Publication). A Pacific Northwest Extension Publication. Washington State University, Oregon State University, and the University of Idaho.

Sullivan, T. S., Stromberger, M. E., Paschke, M. W., & Ippolito, J. A. (2006). Long-term impacts of infrequent biosolids applications on chemical and microbial properties of a semi-arid rangeland soil. *Biology and Fertility of Soils*, *42*(3), 258-266.

U.S. EPA. (1994). "A plain English guide to the EPA part 503 biosolids rule," Tech. Rep. EPA/832-R-93-003, Office of Wastewater Management, Washington, DC, USA.

U.S. EPA. (2011). "Water: sewage sludge (biosolids)" Retrieved from http:// water.epa.gov/polwaste/wastewater/treatment/biosolids/ index.cfm.

Van Ploeg, D., & Heuvelink, E. (2005). Influence of sub-optimal temperature on tomato growth and yield: a review. *The Journal of Horticultural Science and Biotechnology*, *80*(6), 652-659.

Vance, E. D., Brookes, P. C., & Jenkinson, D. S. (1987). An extraction method for measuring soil microbial biomass C. *Soil biology and Biochemistry*, *19*(6), 703-707.

Wakelin, S. A., Gupta, V. V. S. R., & Forrester, S. T. (2010). Regional and local factors affecting diversity, abundance and activity of free-living, N2-fixing bacteria in Australian agricultural soils. *Pedobiologia*, *53*(6), 391-399.

Wang, H., Kimberley, M. O., & Schlegelmilch, M. (2003). Biosolids-derived nitrogen mineralization and transformation in forest soils. *Journal of environmental quality*, *32*(5), 1851-1856.

Ward, G. M., & Miller, M. J. (1969). Magnesium deficiency in greenhouse tomatoes. *Canadian Journal of Plant Science*, *49*(1), 53-59.

Yao, H., Gao, Y., Nicol, G. W., Campbell, C. D., Prosser, J. I., Zhang, L., ... & Singh, B. K. (2011). Links between ammonia oxidizer community structure, abundance, and nitrification potential in acidic soils. *Appl. Environ. Microbiol.*, *77*(13), 4618-4625.

Zak, D. R., Holmes, W. E., MacDonald, N. W., & Pregitzer, K. S. (1999). Soil temperature, matric potential, and the kinetics of microbial respiration and nitrogen mineralization. *Soil Science Society of America Journal*, *63*(3), 575-584.