Assessment of Exceptional Quality Biosolids for Urban Agriculture

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ACADEMIC ABSTRACT

Biosolids have been used as soil conditioners and fertilizers in agriculture and mine land reclamation, but application of Exceptional Quality (EQ) biosolids to rehabilitate anthropogenic soils for urban agriculture is recent and requires greater study to ensure their appropriate use. The objectives were: 1) to quantify plant available nitrogen (PAN) of new EQ biosolids in a greenhouse bioassay; 2) to quantify PAN of EQ biosolids applied to an urban degraded subsoil via tall fescue N fertilizer equivalency, and compare field results to laboratory tests; 3) to investigate EQ biosolids and inorganic fertilizer effects on urban soil properties, vegetable yields, and potential N and phosphorus (P) loss. Biosolids evaluated were products of thermal hydrolysis plus anaerobic digestion (BLOOM), blending with woody mulch (BM) and sand/sawdust (BSS), composting (LBC), and heat-drying (OCB). Organic N mineralization of new blended biosolids products ranged between 20-25% in the greenhouse bioassay. Products BLOOM, BM, and OCB had the highest organic N mineralization as estimated by the 7-day anaerobic incubation, and this test and soil nitrate-N had the highest correlations with tall fescue N uptake (r=0.49 and r=0.505, respectively). We conducted a two-year field study with four growing seasons (fall 2016-2017 and summer 2017-2018) in an urban disturbed subsoil where EQ biosolids were applied seasonally at agronomic N rates, and yearly at reclamation rates (5x agronomic N). Cabbage yields were greater with reclamation rates (~3.0 kg m^{-2}) and bell pepper yields were greater with BLOOM reclamation rate (~1.0 kg m^{-2}) than with the inorganic fertilizer (1.0 kg m^{-2} and 0.2 kg m^{-2}, respectively) during

second year growing seasons. Soil carbon (C) accumulation (%C remaining in the soil) two years after biosolids additions ranged between 37 to 84%. Soil N availability and mineralization were limited most likely due to lack of residual soil C and N, and high clay content. Nitrogen leaching losses from reclamation rates were not greater than agronomic N rates. Leachate P was below detection during most of the experiment. Despite limiting soil conditions, biosolids amendment at reclamation rates showed greatest potential to increase vegetable yield and improve soil properties after two years of application, while not impairing water quality.

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GENERAL AUDIENCE ABSTRACT

Exceptional Quality (EQ) biosolids are by-products of wastewater treatment plants that have been processed to destroy pathogens, reduce attraction by disease-spreading organisms (e.g. flies, mosquitoes, rodents, etc.), and limit heavy metal concentrations. These characteristics make EQ biosolids safe for use by home gardeners for growing food crops. There is limited information on optimal recommended rates at which these products should be applied to urban gardens. The purpose of our research was to determine optimum application rates of EQ biosolids to urban gardens based on their essential plant nutrient (esp., nitrogen and phosphorus) availability. We learned that the EQ biosolids we studied are less concentrated in plant available nitrogen and phosphorus than biosolids applied to conventional agricultural fields. This is because we diluted our biosolids with sawdust, sand, and woody mulch to facilitate their storage, handling, and ease of application. We learned that high EQ biosolids application rates reduce soil compaction and increase essential plant nutrient availability and crop yields for agriculture practiced in urban soils. The high application rates of EQ biosolids accomplished such soil-improving and yield-increasing benefits without impairing local water quality.

DEDICATION

To my parents,
Diego Álvarez and Odiney Campos,
I love you with all my heart

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1. Introduction

1.1. Background

World population has grown rapidly in the last decade adding nearly one billion people since 2005 (UN, 2017). Approximately 55% of the world's population lives in urban areas, and an increase of up to 68% is projected to live in these areas by the year of 2050 (UN, 2018). North America is one of the most urbanized regions in the world with around 82% of people living in urban areas (UN, 2018). As world population and urbanization continue to grow, cities are faced with an increasing challenge to attain sustainable development. One of the most important ways to promote sustainability in the cities is by enhancing land-use planning and integrating environmental infrastructure for improved water and solid resource management.

Urban agriculture is a land management practice that can promote sustainability in cities by providing multiple ecosystem services and contributing to household food security (Thompson et al., 2007). Allotment or community gardens have become more common due to the greater availability of vacant lands left by past manufacturing industries and old residential neighborhoods, and a shift towards a more sustainable way of living (Palmer, 2018). The use of community gardens has proven to enhance people's quality of life by being a source of local, nutritious, fresh produce, and by helping to reduce family's food expenses (Thompson et al., 2007; USEPA, 2011). Community gardens also create opportunities for recreation and physical activity; stimulate social interaction among diverse intergeneration and cultural groups; and are a tool to educate people in topics of sustainable production and natural resource conservation (Thompson et al., 2007; USEPA, 2011).

While there are many societal benefits from community gardens, urban agriculture provides a vast amount of environmental benefits. For instance, urban agriculture generates

green space that reduces the amount of impervious urban land area, which not only lessens city heat zones, but also aids in storm water management (USEPA, 2011). Increased biodiversity, improved air quality, erosion control, and rehabilitation of urban degraded soils are other important ecosystem services linked to urban agriculture (USEPA, 2011).

The latter mentioned ecosystem service is also one of the greatest limitations of urban agriculture. While urban agriculture can help rehabilitate urban soils over time, many urban soils are highly degraded, which makes them more difficult to manage in order to grow crops. Urban soils are generally compacted as a result of vehicle traffic, physical disturbance, and have been greatly impaired due to the removal of topsoil, incorporation of foreign fill materials, added contaminants, and influence of intensive water management practices from lawn irrigation and drainage systems (Beniston and Lal, 2012; Craul, 1985). All of these activities can lead to low quality urban soils with low organic matter content, low fertility, high bulk density, decreased water holding capacity, and reduced microbial activity, which ultimately limit agricultural yields and urban agricultural production (Beniston and Lal, 2012; Craul, 1985). Thus, the success of urban farming largely depends on the implementation of management practices that can rehabilitate urban degraded soil and sustain plant growth.

The application of organic residuals as soil amendments is a management practice that can be used to restore properties of urban disturbed soils (Scharenbroch, 2009; Cogger et al., 2005; Basta et al., 2016; Kumar and Hundal, 2016). The benefits of organic amendment addition to soils has been demonstrated consistently. Organic materials improve soil physical, chemical, and biological properties by replenishing organic carbon, soil structure (i.e. porosity, aggregation, water holding capacity), microbial activity, and nutrient availability (Beniston and Lal, 2012). Urban areas can provide a tremendous amount of organic amendments through the

sustainable recycling of properly treated sewage sludge, which results in a by-product called biosolids.

Biosolids are organic, nutrient rich by-products generated by wastewater treatment processes, which can be used as organic amendments in agriculture (USEPA, 1994). Approximately 55% of biosolids are applied to agricultural fields, while the rest are disposed at landfills or incinerated (NEBRA, 2007). However, land application is the most sustainable disposal method because it returns carbon and nutrients to the soil, which can aid vegetation establishment and agricultural production, while minimizing the potential of greenhouse gas emissions compared with landfill disposal and incineration (USEPA, 2011a).

Land-applied biosolids are classified in two main categories: Class B and Class A biosolids. Class B biosolids are treated to achieve approximately 99% pathogen reduction through "Processes to Significantly Reduce Pathogens (PSRP)" (USEPA, 1994). Pathogens are detectable but at very low levels, which do not present a public health or environmental risk if exposure to biosolids is prevented right after their use. Class B biosolids are restricted to specific areas such as forests, roadsides, and reclamation sites, but have been most often applied in agricultural lands, where crop harvest and animal grazing are limited for certain periods of time after biosolids addition to the soil. (USEPA, 1994).

Class A biosolids are treated to levels where pathogens are below detectable levels by following "Processes to Further Reduce Pathogens (PFRP)" (USEPA, 1994). Their use is less restricted and are allowed in high public access sites, such as urban landscapes. Class A biosolids that not only meet, but also exceed reduction of pathogens, vector attractiveness, and alternative pollutant limit requirements are called Exceptional Quality or EQ biosolids (NEBRA, 2007;

USEPA, 1994). Exceptional Quality biosolids can be applied by following general requirements for land application and management practices like any other soil amendment or fertilizer.

The effects of EQ biosolids soil amendments obtained through established PFRP methods such as composting and heat-drying are well-known, and most research agrees that their use improves urban soil properties and vegetation such as in the establishment of tall fescue grass (Schnell et al. 2009; Sullivan et al. 2010; Basta et al., 2016; Kumar and Hundal, 2016). However, there are recently developed PFRP treatment methods being adopted by wastewater treatment industries, which then generate new EQ biosolids products that have still not been tested and compared to previously established EQ biosolids products.

The rising popularity of urban agriculture coupled with the need to improve properties of degraded soils provides a unique opportunity to locally reuse EQ biosolids products in urban areas. However, in order to appropriately use new EQ biosolids products in urban agriculture, it is vital to understand their nitrogen (N) availability and develop methods for estimating N mineralization of such biosolids in urban soils. There is little research regarding the effect of established and new EQ biosolids products on vegetable production in urban degraded soils. The extent of N and phosphorus (P) losses after EQ biosolids application to urban soils has also not been studied in the past. Given the increasing popularity of urban agriculture and the potential use of EQ biosolids in these settings, research is needed to better determine the potential benefits and risks associated with the application of EQ biosolids to grow vegetables in urban agriculture.

1.2. Objectives

The overall goal of this research was to investigate the suitability of EQ biosolids products for their use in urban agriculture and develop recommendations for their application to rehabilitate degraded urban soils. The specific objectives were:

1) To develop and evaluate the capability of new EQ biosolids blended products to support plant establishment and growth. [Chapter 3]

2) To estimate plant-available N and organic N mineralization rate of EQ biosolids products capable of use in urban agriculture practiced on infertile, degraded soil. [Chapter 4]

3) To compare the effects of EQ biosolids products and inorganic fertilizer on soil physiochemical properties and vegetable production and quality grown in an infertile, degraded urban soil. [Chapter 5]

4) To compare the effects EQ biosolids products and inorganic fertilizer applied at varying rates to an infertile, degraded urban soil on N and P loss potential. [Chapter 6]

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2. Literature Review

2.1. Urban Agriculture Vegetable Production

Urban agriculture refers to agriculture that takes place within or on the fringe of a city or town (Mougeot, 2005). It is generally practiced in spaces of less than 0.5 hectare, and its main focus is often not only agricultural production, but also the provision of services such as health, education, recreation, and green space for more sustainable cities (Mougeot, 2005; Vejre and Simon-Rojo, 2015). However, in terms of crop production, urban agricultural practices are generally intensive because they aim to produce higher yields in smaller available urban spaces (Vejre and Simon-Rojo, 2015).

Among the most common types of crops grown in US urban agriculture are fresh vegetables (e.g. leafy greens, root crops, etc.), nursery plants (e.g. herbs, flowers, mushrooms, etc.), and fresh fruits (e.g. berries) (Oberholtzer et al., 2016). There are a few urban agriculture projects that have evaluated crop yields produced at urban farms. Results from the Farming Concrete project in New York City showed that approximately 40,000 kg of vegetables were produced in 6684 m^2 (~0.6 ha) from 67 gardens in 2010, which is around 6 kg m^{-2} (Farming Concrete Report, 2010). A study conducted in 226 community gardens in Philadelphia also showed a production of ~7 kg of vegetables (largely tomato) per m^2 (Vitiello and Nairn 2009). The total production form these gardens was estimated to be over 900,000 kg of vegetables and were worth more than $40,000,000. The amount of fresh produce that can be produced in small spaces of land in the cities is promising, yet there is a lack of agricultural studies specifically conducted to assess the agricultural potential of urban soils, which generally have distinct properties than conventional agricultural soils (Beniston and Lal, 2012).

2.2. Urban Soil Restoration

While natural and agricultural soils form gradually due to the influence of soil forming factors (i.e. climate, biota, topography, parent material, and time), urban soils can form as rapidly as construction activities take place in urbanized areas. Some of the main alterations that occur to soils located in urban areas are 1) compaction due to heavy vehicle traffic, 2) topsoil removal, 3) addition and burial of foreign materials (e.g. asphalt, bricks, cement, etc.), 4) addition of organic and inorganic pollutants (Beniston and Lal, 2012; Craul, 1985). The result of these is a soil that lacks a traditional layered soil profile, is highly disturbed, and has reduced ability to provide ecosystem services.

Urban soil properties vary among regions due to inherent properties of their location (i.e. original parent material, climate, etc.). However, in general, urban soils are characterized by low porosity and aeration, high bulk density, low organic matter and nutrient availability, and a higher risk of nutrient runoff due to reduced infiltration and drainage as a result of soil compaction (Beniston and Lal, 2012, Craul, 1985). A common practice that can be used to rehabilitate the degraded properties of urban soils is the addition of organic residuals. Organic amendment can improve urban soil physical (i. e. porosity, aggregation, water holding capacity), chemical (i.e. carbon and nutrient content), and biological properties (i.e. microbial activity and vegetation growth) (Beniston and Lal, 2012; Basta et al., 2016; Kumar and Hundal, 2016). Their addition can also help remediate contaminated urban soils by providing a clean growing medium that dilutes contaminants, and increases organic matter, which in combination can help reduce bioavailability of trace elements (e.g. lead [Pb], arsenic [As], cadmium [Cd], copper [Cu], etc.) (Ge et al., 2000; Grimes et al., 1999; Brown et al., 2016). The use of organic residuals to amend existing urban soils for agricultural production also provides a means of capitalizing on excess

waste produced in urban areas to obtain environmental benefits, such as increased soil water storage and carbon sequestration (Nehls et al., 2015; Brown et al., 2012). Biosolids are among the organic residuals commonly produced in large amounts in urban areas, and these by-products can be used to restore disturbed urban soils.

2.3. Biosolids Types and Production Methods

Biosolids are the by-products obtained from the treatment of wastewater sludge. Biosolids amendments historically used in agriculture, forestry and reclamation of mine lands are called Class B biosolids. These biosolids are treated through Processes to Significantly Reduce Pathogens (PSRPs) such as lime stabilization, aerobic and anaerobic digestions, air drying, and composting at 40°C or higher and maintained for 5 days (USEPA, 1994). Class B biosolids have very low levels of pathogens and present a low risk of affecting public health. However, to ensure safety, these biosolids are more restricted in terms of where they can be applied (i.e. agricultural land, forests, roadsides, and mine land reclamation sites) and how long after application crops can be harvested and animal can graze (USEPA, 1994).

The second type of biosolids are called Class A. These biosolids are treated through Processes to Further Reduce Pathogens (PFRPs) and can be applied in urban landscapes because their pathogen levels are below detection, which makes them safe for high public access sites such as parks, golf courses, and gardens. Some of the most traditional PFRPs include thermal drying and composting at 55°C or higher for 3 days or longer; while more recently advanced processes include thermal hydrolysis followed by anaerobic digestion (Cambi^{TM}), and pasteurization of digested wastewater sludge (USEPA, 2006; Abu-Orf et al., 2012).

Under the Class A biosolids types, there is an additional classification that is referred to as Exceptional Quality or EQ biosolids. Class A, EQ biosolids are not only treated by PFRPs, but

also treated to reduce vector attraction and to meet pollutant concentration limits (USEPA, 1994). Such biosolids can be safely applied to urban landscapes by following requirements and appropriate management practices used for other organic amendments and fertilizer applications.

While EQ biosolids are sometimes applied as dewatered "cake" materials, it is becoming more common for these biosolids to be further air-dried, pelletized or bended with other organic residues such as sawdust, woody wastes, and sands in order to reduce their moisture content and facilitate their handling during application. The City of Tacoma in Washington (Tagro™; https://www.cityoftacoma.org/cms/one.aspx?pageId=16884) and the City of Vancouver in British Columbia (Nutrifor™; http://www.metrovancouver.org/services/liquid-waste/innovation-wasterwater-reuse/biosolids/Topsoil/Pages/default.aspx) are producing such biosolids blended products. The District of Columbia Water and Sewer Authority (DC Water) has also recently adopted and installed the first Cambi™ thermal hydrolysis operation in the United States. The process produces an EQ biosolids, which upon various degrees of air-drying, is being marketed as Bloom soil amendment (http://bloomsoil.com/about-bloom/).

2.4. Biosolids Use

Biosolids have been used to improve soil physiochemical and biological properties of agricultural, forestall, and mine lands for many years (Brown and Chaney, 2000; Sopper and Kerr, 1982; Binder et al., 2002). The beneficial effects of biosolids application on crop yield can often surpass those of an inorganic fertilizer alone, because the addition of organic matter helps improve not only nutrient availability and microbial activity, but also soil structure and water holding capacity (Brown et al., 2011; Cogger et al., 2013). Cogger et al. (2013) reported that medium and high (13.4 and 20.1 Mg ha^{-1} yr^{-1}) biosolids application rates significantly increased grain yield of wheat (*Triticum aestivum* L.) and protein compared with anhydrous NH_3 inorganic

fertilizer. These results were largely attributed to an increase in soil N availability with repeated applications of biosolids, as well as increases in soil C and lowered bulk density. Greater tree height, diameter, and woody biomass have been shown with single applications of biosolids to forest soils (Brown and Chaney, 2000; Sopper and Kerr, 1982). Jaber et al. (2005) also found similar or better tomato and squash yields with biosolids compost application compared with an inorganic fertilizer to agricultural fields.

Biosolids use in agriculture and forestry has normally been based on N crop requirement or agronomic N rate. By contrast, biosolids application to mine lands are recommended at higher than agronomic N rates due to greatly reduced physical, chemical and biological functions of such highly disturbed soils. High applications rates can result in short-term nitrate leaching, which is permitted under regulation because the benefits of high biosolids application rates needed to restore soil productivity outweigh the detrimental environmental effects (Sopper, 1993; Brown and Chaney, 2000). Daniels and Haering (1994) showed that applying a semi-composted mix of 1/3 anaerobically stabilized biosolids cake and 2/3 composted wood chips at 184, 368, and 552 Mg ha^{-1} to a Virginia mine land resulted in greater forage yields than an inorganic fertilizer after two growing seasons. Similarly, Hinesly et al (1982) reported rapid establishment and growth of tall fescue, perennial ryegrass, and western wheat grass in a disturbed mine land after the application of high biosolids rates (224, 448, and 896 Mg ha^{-1}).

Best management practices for the use of EQ biosolids products in urban landscapes could be either based on the traditional agronomic N rate or higher reclamation rates depending on the degraded status of the urban soil. Basta et al. (2016) reported improved soil quality, nutrient availability, soil enzymatic activity, and vegetative growth of a native seed mix (grasses, legumes, and forbs) after the application of biosolids at 202 Mg ha^{-1} to a disturbed urban soil.

Scharenbroch et al. (2013) also showed increased tree growth after a relatively low biosolids application (25 Mg ha^{-1}) compared to a non-fertilized control. However, tree growth was not greater than an inorganic N treatment. Nevertheless, the biosolids treatment in this study did increase soil organic C and N mineralization of a silt loam and clay compacted soil in comparison to both the control and inorganic N treatment.

2.5. Carbon Sequestration in Urban Soils

Sequestering organic C in soils can contribute to decreased atmospheric carbon dioxide (CO_2) and combat climate change (Lal, 2004). Short-term carbon storage doesn't imply C sequestration, since soil C sequestration refers to long-term storage of C in soils. Biosolids typically contain essential nutrients (N, P, K [potassium], Ca [calcium], Mg [magnesium], Zn [zinc], among others) and ~50 to 70% organic matter. Thus, biosolids long-term continuous application has the potential to contribute to C sequestration and has been shown to increase soil organic C (Lu et al., 2011; Cogger et al., 2013; Li and Evanylo, 2012).

Cogger et al. (2013) reported that, after 10 years of biosolids application, approximately 28% of the biosolids C added was stored in the soil. Moreover, the increase in soil C was maintained 9 years after biosolids application had ceased. Spargo et al. (2008) showed that sites with a history of biosolids application increased soil C storage up to 4.19 Mg C ha^{-1} in the top 15 cm of soil at the Virginia Coastal Plains. Li and Evanylo (2012) also found that soil C accumulation (%C remaining of C added) increased in surface soils with long-term application of various biosolids amendments to Virginia Coastal Plains and Piedmont soils. In this study, a biosolids compost applied at the agronomic N rate resulted in soil C accumulation of 11% at 0 to 7.5 cm soil depth, while increasing rates of aerobically and anaerobically digested biosolids increased C accumulation ranging from 4.8 to 11.5% at 0 to 7.5 cm soil depth. This study and

previous literature has also found that the ability of soils to store C and reach a soil C saturation limit might be influenced by soil properties such as soil texture (Li and Evanylo, 2012; Hassink, 1997). For instance, after long-term cessation (7-27 years) of biosolids applications, soil C accumulations were greater in two clay loam soils than a sandy loam soil. While the sandy loam soil reached C saturation at lower C input, the clay loam soils did not reach saturation even at higher C inputs, indicating that these soils could continue to store C (Li and Evanylo, 2012). These differences in soil C sequestration potential were likely due to the physical protection of organic matter in finer-textured soil particles and aggregates, which would help retain C in the soil (Hassink, 1997; Li and Evanylo, 2012).

These results are evidence that long term application of biosolids to agricultural soils can result in potential C sequestration; however, less is known about the C sequestration potential of urban disturbed soils. Research was conducted in Washington State, USA to assess C sequestration potential from the application of organic amendments to agricultural and urban soils. Their study found that 40 to 65 Mg ha^{-1} total C supplied with biosolids and compost treatments, resulted in greater soil C increases in a disturbed urban land along a highway roadside than an agricultural soil previously under row crop productions (Brown et al., 2012). Mean C stored as a percent of the amount of C added in the highway roadside was of 48% for yard debris compost and 81% for biosolids-wood mulch, while 19% to 27% C was stored in the soil with compost and Class A biosolids applied to sites that had historically been used to grow crops. However, these results were based on short term measurements of soil C accumulation, which does not imply long term C sequestration.

2.6. Biosolids Nitrogen Mineralization

As is the case for many other organic residuals, a large fraction of biosolids N (50-90%) is found in the form of organic compounds; therefore, biosolids plant N availability depends largely on the amount of organic N that can mineralize over time. (Sommers, 1977; Lu et al., 2012). Environmental conditions such as soil texture, moisture content, temperature, pH, aeration, and the presence of microorganisms influence biosolids N mineralization rates and plant available N (Sommers, 1977; Gilmour et al., 2003; Lu et al., 2012). Warmer temperatures and an adequate balance between soil moisture and air can accelerate N mineralization. Soils with higher clay contents can also physically protect C- and N-containing substrates, resulting in reduced organic N mineralization (Griffith, 2008). Practices such as the incorporation of biosolids into the soil can also minimize the loss of N via ammonia volatilization (Quemada et al., 1998).

Biosolids N mineralization is also strongly dependent on the wastewater treatment process used create the biosolids product (Rigby et al., 2016). Composted biosolids have lower organic N mineralization rates than thermally-dried or digested biosolids because the composting process stabilizes organic matter into more recalcitrant C and N forms of lower availability (Rigby et al., 2016). After studying the plant availability of organic N from a large variety of biosolids, Gilmour et al. (2003) estimated that organic N mineralization rates were10% for composted biosolids and 40% for thermally dried biosolids during their first year of application. A review article of N mineralization of biosolids-amended soils found similar organic N mineralization values for composted (10-24.5%) and heat-dried (~40%) biosolids (Rigby et al., 2016). However, as new EQ biosolids product are developed such as those produced by the

Cambi^{TM} process or blended with other organic and/or mineral materials, additional studies are required to understand their N availability and organic N mineralization.

2.6.1. Indicators of Nitrogen Mineralization

Estimating N mineralization from biosolids is vital to recommend accurate application rates that will maximize crop production while minimizing the risk of N loss. Methods for estimating N availability and organic N mineralization from organic residuals range from greenhouse and field bioassays to chemical and biological laboratory tests. The N equivalency method is the most common procedure used to quantify N availability and mineralization in both greenhouse and field settings (Muñoz et al., 2004; Rigby et al., 2016). During this method, the apparent N recovery or amount of N assimilated by the plant is compared between a known organic amendment rate application and increasing rates of an inorganic N fertilizer (Muñoz et al., 2004; Rigby et al., 2016). The N equivalency method is one of the most accurate methods to estimate N availability of organic residuals; however, it is time-consuming and labor intensive, requiring a whole growing season to obtain better estimates.

Identifying a rapid soil test that could provide reliable estimates of biosolids N mineralization without the need of an entire growing season would significantly contribute to improve assessments of biosolids N fertilizer value and recommendations for their application in urban soils. Rapid chemical evaluations of N mineralization could extract N fractions such as nitrate (NO_3-N), ammonium (NH_4-N), or soluble organic N by using alkali, neutral or acidic solutions (Ros et al., 2011). A meta-analysis conducted to identify the best chemical method for estimating potential N mineralization concluded that neutral and acidic extractions provided more accurate estimates of the amount of available N and mineralizable N from soils (Ros et al.,

2011). Although chemical tests are practical, they do not consider the critical role that microbes play in the process of N mineralization; thus, biological tests have also been evaluated.

Biological tests generally involve the measurement of the amount of inorganic N or carbon dioxide (CO_2) released in the soil after incubation due to microbial activity. Soil incubations have been shown to accurately estimate N mineralization; however, some of them can also be time consuming and labor intensive, which precludes their adoption by commercial labs (Bremner, 1965; Drinkwater et al., 1996). The 7-day anaerobic incubation (7-AI) is one of the fastest and most frequently used biological incubation tests (Waring and Bremner, 1964; Curtin et al., 2017). The test is conducted on soil under water-logged conditions, which eliminates the task of maintaining a specific soil water content throughout the incubation (Waring and Bremner, 1964; Drinkwater et al., 1996). In addition, nitrification is prevented due to soil saturation, thus all N mineralized during the incubation is in the form of NH_4-N. A drawback to this method is that anaerobic conditions are not representative of field soils, and slow oxygen diffusion reduces microbial mineralization. Nevertheless, the 7-AI has been used for decades and is recommended as an indicator of PMN (Doran and Parkin, 1994).

Aerobic incubations have also been used as more realistic methods to estimate PMN due to their maintenance of optimum temperature and moisture conditions. However, they are more labor intensive than anaerobic incubations and are commonly conducted for several weeks to allow for the mineralization of both labile and stable organic N pools (Stanford and Smith, 1972; Drinkwater et al., 1996). Because N mineralization is a decomposition process that generates carbon dioxide (CO_2), evolution of CO_2 during aerobic incubations was also proposed as a means to estimate N mineralization (Franzluebbers et al., 2000; Haney et al., 2001; Haney et al., 2008). Researchers have observed that a flush of CO_2 evolved during 1 to 3 days after rewetting

of an air-dried soil is highly correlated to N mineralization from 24 day aerobic incubations (Franzluebbers et al., 2000; Haney et al., 2001). More recently, a 1-day flush of CO_2 was found to be related to soil N mineralization and field corn yield response (Franzluebbers, 2018).

The commercial Solvita CO_2 Burst test (https://solvita.com/co2-burst/), which measures CO_2 evolution during a period of 1-day after rewetting an air-dried soil, was developed from laboratory studies that identified relationships between CO_2 evolution and N mineralization (Haney et al., 2001; Haney et al., 2008). The Solvita CO_2 Burst test is a simple and rapid test that has been adopted by a few commercial laboratories since the Natural Resources Conservation Service (NRCS) is offering incentives for the use of this soil testing method for estimating N mineralization in some states, increasing demand from farmers. Nevertheless, this test has not been studied in a wide range of soils and climatic regions, and its accuracy for estimating potentially mineralizable N in urban soils after biosolids application has never been tested.

Few studies have evaluated the use of short-term biological tests such as Solvita CO_2 burst and 7-AI to quantify mineralizable N after biosolids application. Bamber et al. (2018) tested the Solvita CO_2 burst method for quantifying potentially mineralizable N after application of anaerobically digested and lime stabilized biosolids for winter wheat production. While the test was unable to accurately quantify surface-applied, unincorporated biosolids N mineralization in no-till management systems, it did accurately predict mineralizable N in the unamended control soils.

The 7-AI has been used to estimate N mineralization in many soils, but very few studies have used this method to estimate organic N mineralization from biosolids-amended soils, neither have they used it on urban soils. Thangarajan et al. (2015) used the 7-AI to study N mineralization from biosolids-amended Australian soils and other wastes. This study found N

availabilities of 38-48% and strong correlations between the results obtained with the 7-AI and a 28 day aerobic incubation. Gomez-Munoz et al., (2017) used the 7-AI to determine N mineralization of various agricultural and urban wastes, including sewage sludge, from a field with a 11 years of organic amendment application at both agronomic N rates and three times the agronomic N rate (based on statutory 45% N fertilizer replacement values). This study determined very low N availabilities of 1.8% and 2.2% for agronomic and three times the agronomic N rate, respectively. More recently, White et al. (2018) employed the 7-AI to determine coefficients of N availability of various biosolids types applied at four increasing rates to four agricultural soils of different soil texture. Nitrogen availability coefficients obtained from this study were extremely variable, ranging from-13% to 86%.

2.7. Biosolids Phosphorus Solubility

Phosphorus loss from agricultural lands has been one of the major contributing factors to surface water eutrophication in the United States (Correll, 1998). Of particular concern are the risks of P losses from soils that have received fertilizer, manure, and biosolids applications over a long period of time (Maguire et al., 2001). As mentioned earlier, biosolids are typically applied at rates that supply crop N requirements with the purpose of achieving adequate plant growth, while avoiding nitrate leaching to groundwater sources (USEPA, 1994). This practice generally leads to a greater risk of P accumulation and runoff after organic amendment application, since most crops have greater N than P requirements, and an even greater risk of P loss would be expected from biosolids applied at high application rates (Jameson et al., 2016; O'Connor et al., 2004).

However, research has shown that biosolids P is less soluble compared to fertilizers and manures due to processes used to treat biosolids in order to meet pathogen reduction

requirements. Processes that decreased P solubility of biosolids include stabilization through alkalization or addition of lime, which precipitates biosolids P as calcium phosphate ($Ca\text{-}PO_4$); and the addition of iron (Fe) and aluminum (Al) based chemicals to precipitate P as Fe- and Al-PO_4 (Maguire et al., 2001; Penn and Simms, 2002; White et al., 2010; Jameson et al., 2016). Biosolids physical moisture reduction via dewatering techniques (e.g. gravity, drying beds, centrifugation, belt presses, etc.) can also contribute to reduce soluble P in biosolids (Jameson et al., 2016; Brandt et al., 2004).

In addition to biosolids P solubility, other factors that affect P losses are P retention characteristics of the soil receiving the biosolids amendment (Ebeling et al., 2003). For example, the presence of Fe and Al oxides, calcium carbonates, soluble organic ligands, and competing ions, can have an effect on P solubility (Ebeling et al., 2003; O'Connor et al., 2004). Thus, it is important to initially assess the soil being amended because different soil properties are expected to influence P solubility from biosolids in different ways.

2.7.1. Indicators of Phosphorus Loss

Despite the low P solubility of many biosolids, long-term applications and the degree of P saturation of the amended soil can increase the risk of P loss. Thus, the use of relevant indicators of soil P status after biosolids application is important in order to minimize environmental P losses. The soil P saturation ratio (PSR) is one of the most common indicators of risk of P loss (Nair, 2014). Soil PSR measures the amount of oxalate extractable P relative to oxalate extractable Fe and Al ([mmol kg^{-1} P] / [mmol kg^{-1} Fe + mmol kg^{-1} Al]) with the purpose of evaluating the capacity of a soil to retain P in Fe and Al phosphate forms. Oxalate Fe and Al extractions are used for this calculation because phosphate ions are attracted to Fe and Al, making P insoluble in water and minimizing its risk of loss (Nair, 2014).

A high soil PSR value indicates that high amounts of P are bound to the Fe and Al binding sites in the soil. Such high "saturation" of P-binding sites more easily permits a greater risk of P loss. Standards and criteria for P application vary across states. In Virginia, the most commonly employed P application rates criteria are regulated by the environmental threshold method that determines the soil Mehlich-1 P concentration at which no additional P can be applied (Virginia DCR, 2014). The environmental threshold criteria establishes that Virginia ridge and valley soils with Mehlich 1-P concentrations between 55 and 162 mg/kg can receive P application based on crop uptake, while soils with concentrations greater than 162 mg/kg should not receive any additional P fertilization. Use of PSR for environmental P assessment indicates that P can be applied based on crop uptake until 35% PSR is reached; however, no additional P fertilizer should be applied if PSR is greater than 65%.

Water extractable P (WEP) has been shown to be an effective predictor of dissolved reactive phosphorus concentrations in runoff from fields that had previously received manure fertilization (Pote et al., 1996). Strong correlations have also been found between WEP in manures and P concentrations in runoff and leaching from manure-amended soils (Kleinman et al., 2002). Thus, WEP has been proposed as a useful environmental indicator of P loss (Pote et al., 1996; Kleinman et al., 2002). Brandt et al. (2004) found that the amount of WEP from biosolids was related to wastewater treatment processes used in biosolids production. For instance, WEP was inversely correlated to high Fe and Al contents, and high concentrations of these metals reduced P solubility in biosolids-amended soils. Research has also shown that biosolids treated with amendments that reduce P solubility (e.g. $FeCl_3$, lime) did not increase soil P saturation and reduced runoff of dissolved reactive P and bioavailable P (White et al., 2010;

Maguire et al., 2000). Such biosolids applications may increase Fe, Al, and Ca in the soil and might mitigate P runoff or leaching by increasing P sorption capacity of the amended soils.

Overall, the use of biosolids as soil amendments contributes to improve soil physiochemical and biological properties by increasing N availability, soil organic C, water retention, among others. Such improvements have been observed historically in agriculture and mine land reclamation, and more recently on urban soils. However, little information is available regarding the use of new EQ biosolids products in urban agriculture, their effect on vegetable yields, and their potential influence on carbon sequestration in urban soils. The purpose of using EQ biosolids products in urban agriculture is to use a local source of organic residuals as soil amendments to help restore degraded urban soils, and also provide nutrients for crop growth. The first step towards using these recently developed EQ biosolids products as organic amendments in urban agriculture should focus on estimating their N availability and organic N mineralization. Evaluating N availability of new EQ biosolids products is important because new treatment processes can create biosolids with variable N availabilities, and N mineralization rates can be influenced by the unique properties of urban soils. A better understanding of N mineralization of these biosolids products will help ensure appropriate N recommendations that will benefit agronomic responses, while minimizing nutrient loss. Additionally, quantifying the effects of biosolids application on N and P loss in urban agriculture is needed in order to assess the risk of N and P loss over time after various biosolids application

2.8. References

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3. Development and Assessment of Exceptional Quality Biosolids Products for Urban Gardens

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3.1. Abstract

Exceptional Quality (EQ) biosolids may be developed into products that can rehabilitate disturbed urban soils for the production of garden vegetables. The objectives of this study were to compare newly developed EQ biosolids products specially tailored for urban soil use with those of established products for the purpose of identifying their capability to support germination and plant growth, as well as to quantify their plant available nitrogen (N) and phosphorus (P). Seven EQ biosolids products and an inorganic fertilizer control were compared in greenhouse bioassays employing soybean (*Glycine max* L.) and tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons) to assess product quality and nutrient availability. The EQ biosolids were derived from treatment processes such as thermal drying, composting, and blending with complementary organic and mineral materials. The EQ biosolids products applied at an estimated equivalent agronomic N rate enabled adequate germination and plant growth. The N uptake by tall fescue grown with the biosolids amendments compared with known rates of inorganic N confirmed organic N availability to be approximately 40%, 20%, and

15% for thermally-dried, blended, and composted EQ biosolids products, respectively. The application of these products at agronomic N rates to a soil testing adequate in P increased soil P saturation to 20-35%, a normal range for soil not excessively enriched with P. The availability of N and P in the EQ biosolids products will permit their agronomically beneficial and environmentally sound use in urban soils.

3.2. Introduction

Land application of biosolids has been encouraged since 1993 when the EPA created Title 40 of the Code of Federal Regulations (CFR), Part 503, which promulgated the Standards for Use or Disposal of Sewage Sludge (USEPA, 1994a). Class B biosolids (99% pathogen reduction level) have been used for many decades in agricultural lands, mine land reclamation sites, roadsides, and forests. Standards for Class B biosolids are achieved through Processes to Significantly Reduce Pathogens (PSRP), such as lime stabilization, aerobic and anaerobic digestion, and air drying (USEPA, 1994a).

Wastewater treatment/water reclamation facilities are increasingly adopting Processes to Further Reduce Pathogens (PFRP), which generate Class A biosolids. Class A biosolids have had pathogens reduced to non-detectable levels (USEPA, 1994a). Composting is a well-established PFRP for Class A biosolids use in urban soils (i.e. for turfgrass establishment and maintenance, ornamental and vegetable gardens, etc.) due to its ability to create "Exceptional Quality" or EQ biosolids. "Exceptional Quality" refers to Class A biosolids that additionally meet appropriate USEPA vector attraction reductions standards and Pollutant Concentration Limits (USEPA, 1994a). The reduction of pathogens, vector attraction, and pollutant concentrations to achieve EQ designation permit the safe application of EQ treated biosolids to public sites, such as gardens and lawns.

In addition to composting, biosolids are being treated by other PFRP methods such as thermal-drying, pasteurization, irradiation, thermal hydrolysis followed by anaerobic digestion, etc. that may permit these products to be used in urban settings without composting. Thermal-drying refers to the direct or indirect contact with hot gases to reduce biosolids moisture content to around 10% or lower (USEPA, 1994a). Pasteurization subjects biosolids to temperatures of 70°C or higher for 30 or more minutes, while irradiation exposes biosolids to beta or gamma rays. The process of thermal hydrolysis followed by anaerobic digestion consists on the treatment of dewatered solids with high pressure (5-8 bar) and temperature (150 °C to 170 °C) for 30 min, and then treating the hydrolyzed solids by diluting them in water and introducing them to a mesophilic anaerobic digester (Wilson et al., 2008). These emerging processes in biosolids treatments reduce the dry mass of biosolids, thus reducing the amount of space needed for biosolids storage before their use as soil amendments. The process of hydrolysis improves subsequent digestion of the biosolids by reducing hydraulic digestion time and increasing gas production for renewable power. Nevertheless, these emerging treatments can add much higher energy costs to wastewater treatment plant operations (Kelly, 2006). Biosolids use costs can be reduced by distributing and applying the products near their source of generation in, typically, urban areas.

Composted biosolids and heat-dried biosolids have been successfully used in urban soils for establishment and maintenance of lawns, trees, and ornamentals. Establishment and growth of Kentucky bluegrass (*Poa pratensis* L.) and perennial ryegrass (*Lolium perenne* L.) was enhanced in a disturbed urban soil in Columbus (OH) with the application of two local composted biosolids (Loschinkohl and Boehm, 2001). The improved establishment and growth were attributed to the plant available nitrogen (PAN) and phosphorus added with the application

of this soil amendment. Milwaukee's heat-dried pelletized biosolids product, Milorganite®, and Nitrohumus®, composted biosolids from Los Angeles County, CA, wastewater treatment facilities, have been used to establish and maintain grass in sports stadiums throughout the United States. Earthgro®, composted biosolids from Philadelphia, has also been used to grow ornamentals in pot containers (USEPA, 1994b). Scharenbroch et al. (2013) attributed N availability of EQ biosolids to increase growth of two tree plant species (*Acer saccharum* and *Gleditsia triacanthos*) in a simulated urban compacted soil.

While some PFRP methods such as composting and heat-drying provide an end product with low moisture content, dewatered EQ biosolids generated by other PFRP methods can have moisture contents that preclude easy handling and applying. To overcome high moisture content and facilitate storage and handling, further reduction of moisture via air-drying or blending with lower moisture byproducts, such as sawdust, ground/shredded woody wastes, and mineral-based substrates, may be employed. Exceptional Quality biosolids blended products have attributes (e.g. carbon stability, nitrogen and phosphorus availability) for value as beneficial soil amendments; however, the nutrient availability of these novel and diverse products is not well-understood.

Organic N comprises 50% to 90% of total N biosolids. This means that plant N availability will depend largely on the amount of organic mineralizable N present in biosolids (Lu et al., 2012). The plant availability of organic N from a variety of biosolids during the year of application ranges from 10% for composted to 40% for thermally-dried biosolids (Gilmour et al., 2003). Nitrogen mineralization is also dependent on environmental factors (i.e. moisture, temperature, soil texture) and treatment processes used to produce biosolids, which influences

their C: N ratio (Gilmour et al., 2003; Lu et al., 2012). Blending biosolids with woody byproducts (sawdust, mulch) will increase the C:N ratio, resulting in reduced PAN.

Like most organic amendments, biosolids are commonly applied at rates that supply crop N requirements for the purpose of achieving optimum plant growth, while avoiding excessive nitrate leaching to groundwater (USEPA, 1994a). Biosolids typically have an effective N: P ratio of 1:2 because some N may be lost or immobilized, while P is conserved during treatment processes (Jameson et al., 2016; O'Connor et al., 2004). Crops have greater N than P requirements; thus, the application of biosolids on a crop N requirement basis results in increases in soil P and the risk of P runoff. However, biosolids P is less soluble than P in inorganic fertilizers and manures due to the higher concentrations of P-binding iron (Fe), aluminum (Al), and calcium (Ca) (White et al. 2010). Iron- and Al-based treatment processes produce hydrated oxides of Fe and Al, which have great affinities for P.

The addition of mineral and organic substrates to biosolids will modify chemical and physical attributes that can affect soil properties and plant response (Frederick et al., 2004; Brown and Chaney, 2000). Key properties include pH, soluble salts/electrical conductivity (EC), cation exchange capacity (CEC), C stability, essential plant nutrient content and availability, porosity, water holding capacity (WHC), and plant available water (PAW). Organic C additions with biosolids byproducts to the soil can provide both potential beneficial (increased WHC, PAW, and CEC, pH buffering) and detrimental (reduced soil oxygen due to decomposition of unstable C, reduced relative PAN:P) effects (Brown and Cotton, 2011). Reduced N mineralization rate can be detrimental by slowing the generation of plant available N or beneficial by providing a slow-release pool of PAN less prone to leaching and denitrification. However, tailor-made biosolids byproducts with mineral (sand, quarry "fines") additions can

also improve application homogeneity by increasing granulation and can fortify potassium (K) concentrations, which are normally low in biosolids, making them more effective soil amendments (Brown and Chaney, 2000).

Exceptional Quality biosolids blended products have potential for rehabilitating disturbed soils, improving vegetation establishment, and increasing garden vegetable yield and quality in urban areas. TAGRO soil®, EQ biosolids cake mixed with sand and sawdust from Tacoma (WA), improved soil physical (e.g., water infiltration, soil bulk density) and chemical (e.g., total C, total N, extractable P, electrical conductivity) properties of an urban community garden soil relative to the non-amended control soil (McIvor et al., 2012). As the number of new EQ biosolids products increases, there is a greater need to understand N and P availability and potential plant growth retarding factors in these products. Information on nutrient availability and release can be used to ensure that plants receive the appropriate amount of nutrients for adequate growth, while reducing the risk of environmental impairment due to N and P loss in the cities. The goal of our study was to develop EQ biosolids blended products appropriate for urban landscape use and compare their properties (nutrient content and availability, pH, EC, etc.) and plant response with standard products in the marketplace (i.e. composted biosolids, and thermally-dried biosolids) in a controlled greenhouse study. The objective of our study was to identify the capability of new EQ biosolids blended products to support germination and plant growth, as well as to obtain an estimate of their PAN and P.

3.3. Materials and Methods

3.3.1. Materials

Exceptional Quality biosolids from DC Water's Blue Plains (BP) advanced wastewater treatment plant were used as the basis for two blended products designed to be used as

amendments for urban agriculture and turfgrass management. Based on previous recipe testing (Yu, 2014), BP EQ biosolids were blended with sand and sawdust at a ratio of 1.5 biosolids : 1.0 sawdust : 1.0 sand (dry weight basis) and designated as BSS. Sand was used to modify the structure of the amendment and make it easier to handle and spread, while sawdust was used to add carbon to the blend and balance the nitrogen from the biosolids. A second EQ biosolids blended product composed of a ratio of 0.75 biosolids : 1.0 shredded, woody mulch (dry weight basis) was designated as BM. The BSS and BM were blended to provide an initial calculated C:N ratio of 13:1 and approximately 50% moisture content. Each blended product was cured in a 130-L loosely covered container for four weeks, during which time the mixtures were turned two times per week. Curing time allowed for further decomposition and stabilization of active carbon, and immobilization of a portion of ammonium into organic N for future slow release. The BSS and BM products were compared with five other EQ biosolids products by laboratory testing and greenhouse bioassays. A list of the EQ biosolids products by product name, abbreviation used in our experiment, generation location, and manufacturing process is shown in Table 3.1.

All EQ biosolids products were analyzed by Waypoint Analytical Laboratories (Richmond, VA) for total and volatile (organic matter) solids (SM2540G) (APHA, 1992); total Kjeldahl N (TKN; SM-4500-NH3C-TKN) (APHA, 1992); ammonia + ammonium N (NH_4-N; SM-4500-NH3C) (APHA, 1992); nitrate N (SM-4500NO3F) (APHA, 1992); organic nitrogen (calculated as difference between TKN and NH_4-N); P, potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), aluminum (Al), copper (Cu), zinc (Zn) (SW-6010C) (USEPA, 2000a); and pH (SW-9045D) (USEPA, 2000b). Samples of BSS and BM products were analyzed four times over a period of two years to evaluate if there were any major changes

in stability/composition of the BP products during storage. This is important because biosolids blended products should ideally be stable over long periods of time, since they are often kept in storage, rather than used immediately after their production.

An Orangeburg loamy sand (fine-loamy, kaolinitic, thermic Typic Kandiudults) topsoil was used as the soil growth medium in all greenhouse studies. This soil had pH of 6.0 and Mehlich I-extractable P, K, Ca, and Mg of 29 mg/kg, 84 mg/kg, 266 mg/kg, and 63 mg/kg, respectively. Inorganic N fertilizer, as ammonium nitrate (NH_4NO_3), was used as the N control comparison for all greenhouse experiments. Ammonium nitrate fertilizer was chosen because it provides readily available nitrogen for plant uptake. Greenhouse bioassay amendment application rates were based on the assumption that organic N availability (i.e., mineralization rate) was 20% for blended products, 15% for composted biosolids, and 40% for heat-dried biosolids.

3.3.2. Soybean Bioassay

Seven EQ biosolids products (BM, BSS, TM, TSS, ABM, LBC, and OCB) were used as soil amendments in a soybean bioassay to assess the capability of different biosolids products to support germination and emergence, seedling vigor, plant growth, and potential phytotoxic effects. Soybeans were selected for product quality assessment because of their high sensitivity to the presence of phytotoxic organic compounds and soluble salts. Daniels and Evanylo (2011) have successfully employed such soybean bioassays in their program to evaluate if industrial byproducts meet requirements for registration as soil amendments in the Virginia Department of Agriculture and Consumer Services.

The inorganic fertilizer control and each EQ biosolids' product were applied to the soil at a rate calculated to supply 75 mg PAN/kg soil (i.e., 150 lbs N/acre). The purpose of the

amendment rate was not to provide N needs, since soybeans are N-fixing legumes; however, the N application rate was used to assess potential growth limitations at common field application rates to row and other crops typically fertilized with biosolids. Four additional treatments using EQ biosolids products as the entire growth medium (100% application rate) were employed for BP (BM100 and BSS100) and Tagro products (TM100 and TSS100). Thus, the 12 treatments evaluated were seven biosolids products applied at 75 mg PAN/kg soil (designated as BM1x, BSS1x, TM1x, TSS1x, ABM1x, LBC1x, and OCB1x), four biosolids blended products applied as the entire growth medium (designated as BM100, BSS100, TM100, and TSS100), and inorganic fertilizer (designated as F1x). Treatments were replicated four times and arranged in a completely randomized design.

Five soybean seeds were sown into the Orangeburg topsoil amended with each EQ biosolids product, and placed in 15-cm diameter plastic bag-lined pots. Deionized water was used for irrigation to maintain moisture at approximately 80% of the medium capacity of all treatments. Medium capacity (MC) was determined for the soil and soil-amendment mixes by placing one kilogram of amended soil media in 15-cm diameter drained pots and adding water to each of the pots until saturation. The weight of the pots was recorded after 24 hours of drainage. Soil MC, or the amount of water held in the soil after 24 hours of drainage, was calculated as the difference between the initial and final pot weights. This 100% MC value was multiplied by 0.8 to calculate the amount of water necessary at 80% MC. The plastic bag-liners were used to prevent leaching loss.

Soybeans were planted on the first week of February 2016, and were grown for a month. Plants were grown under natural sunlight and greenhouse temperatures were maintained between 25-30 °C. Seed germination percentage was determined 7 days after initial emergence and

soybean shoots were thinned to two plants per pot. The remaining plants were allowed to grow 28 to 35 days, during which time visual assessment of plant growth and quality was performed. Aboveground soybean biomass was harvested and dried at 60 °C for dry weight biomass determination.

3.3.3. Tall fescue Bioassay

The purpose of the tall fescue bioassay was to estimate plant available nitrogen (PAN) and bioavailable phosphorus (BAP) provided by the seven EQ biosolids products. This information is necessary to determine amendment application rates in the field. The method employed to calculate N mineralization and PAN compared plant N uptake from increasing inorganic N rates to plant N uptake from organic amendment of known organic and inorganic N content (Bowden et al., 2007). The inorganic N fertilizer was applied at 0, 37.5, 75, 150, and 300 mg N/kg soil (dry weight basis) to generate a calibration curve (plant N uptake vs N rate). Seven biosolids products (BM, BSS, TM, TSS, ABM, LBC, and OCB) were applied at an estimated 75 mg N/kg soil, and the biosolids products of BM, BSS, TM, and TSS products were also applied at an estimated 150 mg N/kg soil. Thus, this study contained 16 treatments with four replicates in a completely randomized design.

The biosolids' application rates were calculated based on estimated PAN from previous recipe development studies and available literature (Gilmour et al. 2003; Cogger et al. 2004). Ammonium (NH_4-N) was assumed to be 100% available for all amendments because the amendments had been stabilized in storage. Availability of organic N was considered variable due to the differences in treatment processes that were used to make each EQ biosolids product. Plant available N application rate calculations assumed organic N availability to be 20% for biosolids blended products (BM, BSS, TM, and TSS), 15% for composted biosolids products

(ABM and LBC), and 40% for heat-dried biosolids (OCB). Actual plant N availability was determined employing tall fescue N uptake response to the biosolids and fertilizer N treatments.

Orangeburg soil was weighed into 15-cm diameter plastic pots lined with plastic bags to avoid N and P leaching loss. The inorganic fertilizer and organic amendments were added at the rates previously described, and tall fescue was grown at greenhouse temperatures between 25-30 °C under natural sunlight. Deionized water was provided to each pot to maintain approximately 80% MC as described previously. Equal masses of tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons) seeds were evenly distributed on the soil surface in the pots and watered daily until germination and seedling establishment occurred. The tall fescue was clipped four times, or approximately once every two weeks, until two months after establishment. Tall fescue was clipped to a height of 5 cm during the initial three cuttings and to 1 cm at the final cutting.

Leaf clippings of each treatment were dried at 60 °C and weighed to determine above-ground biomass yield. Samples from all sampling times for each treatment replicate were combined for nutrient analyses because there was not enough plant tissue to perform separate N analysis at each clipping. Leaf samples were oven-dried at 65 °C for 24 hours and ground through a 0.85 mm screen with a Wiley mill press (Arthur H. Thomas Co., Philadelphia, PA). Tissue total N concentration was analyzed using a Vario Max CNS macro elemental analyzer (Elementar, Hofnau, Germany).

Tall fescue N uptake was calculated as the product of leaf nutrient concentration and above-ground biomass. Nitrogen fertilizer uptake values were plotted versus inorganic N rates to develop a linear regression function ($y = 0.3094x + 49.392$, $R^2 = 0.9911$), from which the fertilizer N equivalent of the EQ biosolids were calculated with the use of their N uptake values.

(Delin et al. 2012; Bowden et al., 2007). The fertilizer N equivalent was then used to determine % N mineralization with the following equation:

% N mineralization =

$$\frac{[(N\ eq_{trt}\ * 1\ kg\ soil) -\ Inorganic\ N\ added_{trt}] * 100}{Organic\ N\ added_{trt}}$$

Where N eq_{trt} is the N equivalency of the treatment, Inorganic N $added_{trt}$ is the amount of inorganic N added with the treatment, and Organic N $added_{trt}$ is the amount of organic N added with the treatment.

3.3.4. Soil Analyses

Soil samples were collected from soybean and tall fescue pot experiments after their final harvest. Soil samples were air-dried and homogenized through a 2-mm sieve. Soil pH and EC were analyzed on saturated paste extracts. This method consists of adding distilled water to a previously weighed soil until near saturation and filtering through a Buchner funnel under vacuum after having allowed the saturated paste to stand for 2 hours (Rhoades et al., 1982).

Soil samples from the tall fescue experiment were also analyzed for P by several methods at the end of the experiment. Total P was determined by USEPA 3051 method, which consists of a microwave assisted nitric acid digestion (USEPA, 2007). Analyses were also performed after extracting the amended soils with Mehlich 1 for P (Maguire and Heckendorn, 2011) and ammonium oxalate for P, Fe, and Al (Ross and Wang, 1993). Extracts were analyzed by the Virginia Tech Soil Testing Laboratory on a Thermo Elemental ICAP 61E. Oxalate extractions were used to determine the ratio of P to Fe + Al or P Saturation Ratio (PSR), which is a useful indicator of bioavailable P (Brandt et al., 2004).

3.3.5. Statistical Analysis

Data was analyzed using JMP® software (SAS Institute Inc., 2015). Normality and equal variance assumptions were tested, and nonparametric transformation methods were used to normalize the data when necessary. Analysis of variance (ANOVA) and Tukey-Kramer HSD method for mean separation was used to determine treatment effects of EQ biosolids products on soybean germination and yield of tall fescue. Soil P concentration differences between treatments were also determined using ANOVA and Tukey-Kramer HSD method. Treatment effects were considered significant at p-value < 0.05. Regression analysis was conducted on the results obtained for plant N uptake by tall fescue in order to calculate the plant available N supplied by these organic amendments.

3.4. Results and Discussion

3.4.1. Biosolids Chemical Composition

The pH of biosolids products ranged from slightly acidic to alkaline (pH 6.5 to 9; Table 3.2). Total solids (TS) ranged from 600 to 700 g/kg, except for OCB that had TS of 949 g/kg. The OCB has the highest TS because this product is heat-dried. The other biosolids products were mixed with substrates drier than the dewatered biosolids to improve amendment handling and applying.

Organic N was the largest N fraction all biosolids (Table 3.2). We purposefully designed the recipes for the BM, BSS, for lower C:N ratios than the similarly blended TAGRO products TM and TSS in order to provide higher N mineralization rates. The composted biosolids (LBC) had total N and C:N ratio similar to the woody-blended biosolids products BSS, BM, and ABM. The non-organic matter-amended OCB had the highest concentration of total N and the lowest C:N ratio and was, therefore, expected to provide the highest fraction of PAN.

The OCB generally had higher nutrient concentrations among all the biosolids products because it was not diluted by organic or mineral substrates. All amendments had relatively low K, with LBC and ABM having the highest concentrations (Table 3.2). Of the P-binding constituents, ABM and OCB had the highest Al concentrations, and BSS and BM had the highest Fe concentrations. The higher concentration of Fe in BM and BSS is due to the addition of ferric chloride to wastewater by BP to precipitate P and reduce its concentration in the effluent released to surface water (Maguire et al., 2001).

Chemical analyses of the BP biosolids products over time showed that both were relatively stable. The nutrients most susceptible to temporal change are N and C, due to their possible microbial transformations; however, all forms of N, C and C:N ratio were remarkably consistent over time (Table 3.3). Phosphorus, K, Ca, Mg, Fe, and Al – elements not susceptible to changes in total concentrations due to microbial transformations – did not vary much over time, except for Al.

3.4.2. Product Quality

Soybean germination ranged between 60 and 100% for all treatments, except for the inorganic fertilizer (F1x) and TSS100 (Table 3.4). The two BP blends at 100% of the growth media, BSS100 and BM100, supported moderate seed germination (60-70%) but poor biomass production. Only the TAGRO® TSS100 blend and the inorganic fertilizer (F1x) performed as poorly. Applying the biosolids at a typical field rate elicited excellent biomass response, but the biosolids products used as the entire root zone media produced poor biomass.

Growth medium pH was non-limiting (pH 6.5-7.5) for biosolids amendment treatments, while treatments comprising the entire root zone had pH ranging between 4.5 and 6.0 (data not shown) due to lack of mineral soil buffering. When biosolids products were used as soil

amendments, the concentration of soluble salts, expressed as electrical conductivity (EC) was low (<2.5 dS/m) and ideal for plant growth (Table 3.4). However, all 100% treatments had high concentrations of soluble salts that ranged between 3.0 and 7.5 dS/m, which likely inhibited germination, emergence, and growth of soybeans (Maas and Hoffman, 1977). Soluble salt accumulation occurred most likely due to existing salinity levels in biosolids, and accumulation of salts due to lack of drainage (USDA, 2011). Although the biosolids-sawdust-sand blended products are not intended for use as an entire rooting medium, the bioassay was designed to learn potential negative effects at high application rates. Overall, there was no phytotoxic effect with the use of EQ biosolids products as soil amendments, but care must be taken to ensure appropriate water drainage when these amendments are used as a high proportion of the potting medium.

3.4.3. Plant Available Nitrogen

Tall fescue biomass increased with inorganic N rate up to 75 mg PAN/kg soil (Fig. 3.1). However, the application of inorganic fertilizer beyond F1x rate resulted in the same (F2x) or lower (F4x) tall fescue biomass, as fitted with $y=-0.0011x^2 + 0.2752x + 51.87$, $R^2 = 0.89$ quadratic regression equation. The pH of all treatments ranged from 6 to 8, except for F4x whose pH was 4.5 (data not shown). Similarly, all treatments had soil EC values below 2.5 dS/m, except for F4x whose soil EC>4.5 dS/m (Table 3.5).

The reduction of tall fescue biomass with F4x was likely due to excessive soluble salts and growth-limiting acid soil pH, due to N mineralization and nitrification of the organic N (Swift and Koski, 2013). Tall fescue total biomass was not different between F1x and all amendments applied at estimated 1x agronomic N rate (Table 3.5). Biosolids blended with sand and sawdust (BSS and TSS) and with mulch (BM and TM) outperformed the fertilizer and all

other treatments when applied at estimated 2x agronomic N rate. The product of tall fescue leaf clipping N concentration and above ground biomass was used to calculate plant N uptake (Fig. 3.1). Tall fescue grown in soil amended with biosolids blended products applied at 150 mg/kg (BSS2x, BM2x, TSS2x, and TM2x) assimilated more N than that grown in soil fertilized with inorganic N at 150 mg/kg (F2x). However, all EQ biosolids products applied at 75 mg/kg resulted in the same plant N uptake as with inorganic N applied at the same rate (F1x).

Due to the fact that F2x- and F4x-limited tall fescue biomass growth and N uptake, the linear regression equation describing the relationship between plant N uptake and inorganic N rate was developed using only the first three N rates (F0x, F0.5x, and F1x) ($y = 0.3094x + 49.392$, $R^2 = 0.9911$). The linear response obtained from the relationship between N uptake and 0x, 0.5x, and 1x inorganic fertilizer rates was used to calculate plant available N (PAN) for each EQ biosolids product by plugging the N uptake ("y") value for each biosolids product into the equation and solving for N rate ("x"). The PAN equivalent of all EQ biosolids products was greater or similar to the estimated N rate (75 mg/kg), except for ABM1x and TM1x. Products BSS1x, BM1x, TSS1x, and LBC1x supplied 90 mg/kg, 83 mg/kg, 108 mg/kg, and 87 mg/kg PAN, respectively; while ABM1x and TM1x supplied 50 mg/kg, and 26 mg/kg PAN, respectively (Table 3.6). The variability in calculated compared to estimated PAN demonstrates the difficulty in estimating plant available N from organic amendments.

3.4.4. Bioavailable Phosphorus

Both EQ biosolids from DC Water applied at 2x rate (BSS2x and BM2) resulted in the greatest increase in soil P of all treatments due to the high concentrations of P in the amendments (Table 3.7). Both TAGRO® products applied at 2x rate (TSS2x and TM2x) and the OCB resulted in the greatest increase in Mehlich 1-P of all treatments. Approximately 9% to 16% of

soil P was extractable by Mehlich 1 for most EQ biosolids products applications. Only TSS and TM biosolids applications resulted in as much as 20% of soil P extractable by M1-P. The small portion of soil P that was extractable by Mehlich 1 P suggests that the biosolids products studied may have low P availability and/or the biosolids P is strongly bound to Fe and Al. Such forms of P could lower the risk of P loss that could impair the quality of nearby water bodies after field application. Mehlich 1 soil test method does not accurately predict available P in environments with high Fe concentrations (Dao et al., 2005). In high Fe environments and for these types of biosolids products, the PSR may be a more appropriate indicator of P availability.

Blue Plains products applied at the 2x rate (BM2x and BSS2x) and the OCB gave the greatest oxalate extractable P concentrations (Table 3.7). Livingston compost (LBC) had the lowest oxalate extractable P, which was similar to the inorganic fertilized soil that received no P fertilizer. Products from Blue Plains also had the highest concentrations of oxalate extractable Fe. The higher amount of oxalate extractable Fe found with the addition of both EQ biosolids from DC Water is due to the large amount of Fe salts that are added during their wastewater treatment process (Maguire et al., 2001).

The P availability of EQ biosolids products was further assessed by calculating soil PSR from oxalate extractable P, Fe, and Al values. Treatment OCB1x had a higher PSR (43%) than all other EQ biosolids treatments (24%-37%). Although BSS and BM products added higher amounts of P to the soil than most EQ biosolids treatments, the use of Fe salts for P removal during DC's wastewater treatment generates an EQ biosolids product with high Fe which increased Fe in the soil, and helped reduce P solubility. Thus, EQ biosolids products can contain considerable amounts of P, but in contrast to many animal manures, the P solubility of biosolids

is generally reduced by Fe, Al, or lime that is commonly added during wastewater treatment processes.

3.5. Conclusions

Newly developed EQ biosolids products blended with organic and mineral substrates can be high quality soil amendments; however, use rates of these products must consider potentially plant growth-limiting factors such as pH and soluble salt concentrations/electrical conductivity. The use of these products will also be determined by the variable plant available N supplied upon organic N mineralization. Biosolids compost N availability has previously been established as 5-15% of organic N, which we validated as ~15% for the LBC. The PAN of newly developed products blended with organic and mineral substrates were in the range between composted biosolids (15%) and anaerobically digested biosolids (30-35%). Field research should be performed to further refine PAN estimates for such EQ biosolids products.

A concern of the use of EQ biosolids in urban landscapes (turfgrass, vegetables, ornamentals) is that unregulated application may increase soluble soil P to concentrations that will pose surface water runoff risks. The use of products that contained high concentrations of Fe and/or Al did not increase indicators of bioavailable soil P to excessive levels, as PSR was 24-37% in all amended soils, except OCB. Monitoring of P forms that pose water quality risks will continue to be important for the sustainable use of EQ biosolids in urban landscapes.

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Table 3.1. Description of Exceptional Quality (EQ) biosolids products evaluated.

Product code	Product name	Producer Location	Description
BSS	Biosolids-sand-sawdust	Blue Plains DC Water	EQ biosolids (CambiTHP™) blended with sand and sawdust.
BM	Biosolids-mulch	Blue Plains DC Water	EQ biosolids (CambiTHP™) blended with mulch.
TSS	TAGRO® soil amendment	Tacoma, WA	Anaerobically digested biosolids blended with sawdust and sand.
TM	TAGRO potting soil®	Tacoma, WA	Anaerobically-digested biosolids blended with woody mulch.
LBC	Livingston Compost®	Spotsylvania County, VA	Composted anaerobically-digested biosolids.
ABM	George's Old Town Blend®	Alexandria Renew Enterprises in Alexandria, VA	Anaerobically digested biosolids blended with woody mulch.
OCB	OceanGro®	Ocean County, NJ	Thermally-dried and pelletized biosolids.

Table 3.2. Selected composition variables of seven EQ biosolids products tested in this greenhouse study.

	Unit	BM[a]	BSS	TM	TSS	ABM	LBC	OCB
pH[bc]		6.52	6.07	7.77	9.00	7.44	7.98	7.30
Total solids[c]	g kg^{-1}	666	699	666	625	591	606	949
Moisture[c]	g kg^{-1}	334	301	334	375	409	394	51
Bulk density	g cm^{-3}	0.29	0.36	0.30	0.29	0.26	0.26	0.71
TKN	g kg^{-1}	26.9	22.3	10.0	9.8	17.2	23.1	49.1
NH_4-N	g kg^{-1}	3.6	2.8	2.6	3.1	6.2	7.8	5.7
Organic N	g kg^{-1}	23.3	19.5	7.4	6.7	11.0	15.3	43.4
NO_3-N	mg kg^{-1}	5.96	3.39	12.6	< 2	< 2	< 2	7.21
C	g kg^{-1}	388	247	265	180	317	364	383
C:N		14.4	11.1	26.5	18.4	18.5	15.8	7.8
P	g kg^{-1}	17.0	14.5	5.1	6.9	11.4	9.9	26.6
K	g kg^{-1}	2.0	0.9	1.5	1.3	3.0	4.0	1.4
S	g kg^{-1}	14.1	10.5	2.5	2.5	4.1	2.9	16.3
Ca	g kg^{-1}	29.3	14.1	8.5	9.5	14.5	10.9	28.4
Mg	g kg^{-1}	3.5	1.8	2.7	4.6	2.8	2.3	4.3
Na	g kg^{-1}	0.4	0.4	0.6	0.6	0.6	0.6	1.2
Fe	mg kg^{-1}	58300	52500	15500	16200	30700	17500	32800
Al	mg kg^{-1}	5400	3600	8000	13300	14500	8900	14700
Mn	mg kg^{-1}	431	248	286	267	514	699	2030
Cu	mg kg^{-1}	241	208	93	114	139	119	535
Zn	mg kg^{-1}	535	468	277	217	347	221	1110
As	mg kg^{-1}	7.0	<3.0	5.0	3.0	5.0	8.8	7.0
Pb	mg kg^{-1}	41	36	43	14	29	14	22
Cd	mg kg^{-1}	<2.0	<2.0	<2.0	<2.0	2.0	<2.0	2.0
Cr	mg kg^{-1}	88	169	344	520	152	24	35

[a] BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust, TM=Tagro[tm] potting soil, TSS=Tagro[tm] soil amendment, ABM= George's Old Town Blend from Alexandria Renew Enterprises biosolids compost, OCB=OceanGro thermally-dried, and LBC=Livingston Compost.

[b] saturated paste method

[c] all values are on a dry weight basis except as noted

Table 3.3. Selected chemical and physical properties of Blue Plains EQ biosolids products over time.

		BM[a]				BSS			
	Unit	12/7/2015	5/12/2016	7/27/2016	6/6/2017	12/7/2015	5/12/2016	7/27/2016	6/6/2017
pH[b]		6.52	6.52	6.85	5.96	6.07	6.5	6.79	6.03
Total Solids[c]	g kg^{-1}	666.3	733.9	770	713.8	699.3	794.2	755.7	930
Moisture[c]	g kg^{-1}	333.7	266.1	230	286.1	300.7	205.8	244.3	70
TKN	g kg^{-1}	26.9	27.8	28.7	27.6	22.3	25.8	27	25.8
NH_4-N	g kg^{-1}	3.6	4.9	4.9	4.4	2.8	4.3	6	4.3
Organic N	g kg^{-1}	23.3	22.9	23.8	23.2	19.5	21.5	21	21.5
NO_3-N	mg kg^{-1}	5.96	162	13.8	1290	3.39	12.6	143	63.1
C	g kg^{-1}	388	319	391	369	247	229	278	230
C:N		14.4	11.5	13.6	13.4	11.1	8.9	10.3	8.9
P	g kg^{-1}	17	15.7	15.7	14.3	14.5	14.2	15.9	20.2
K	g kg^{-1}	2	2.1	1.9	1.5	0.9	0.8	0.8	0.7
Ca	g kg^{-1}	29.3	29.1	25.5	25.4	14.1	14.1	14.6	18.9
Mg	g kg^{-1}	3.5	4	3.5	2.8	1.8	1.7	1.8	2.1
Fe	mg kg^{-1}	58300	54300	47900	43400	52500	49800	52400	61400
Al	mg kg^{-1}	5400	6300	4800	2010	3600	3200	3100	2170

[a] BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust.
[b] saturated paste method
[c] all values are on a dry weight basis except as noted

Table 3.4. Effect of organic amendment treatments and an inorganic fertilizer control on soybean germination, above ground biomass, soil pH, and soil electrical conductivity (EC) of the soybean bioassay. Values with same letters in columns are not significantly different (P<0.05).

Treatment[a]	Germination (%)	Dry Weight Biomass (g)	Soil pH	Soil EC (dS m^{-1})
F1x	42 c	0.55 cd	6.2 cde	2.2 cd
BSS1x	63 bc	1.50 ab	6.0 cde	1.5 de
BM1x	94 a	1.43 ab	6.4 bc	1.1 def
TSS1x	81 ab	1.74 a	7.4 a	0.8 f
TM1x	69 abc	1.74 a	7.3 a	0.6 f
ABM1x	63 bc	1.60 ab	7.3 a	0.6 f
LBC1x	81 ab	1.41 ab	7.1 a	1.0 ef
OCB1x	75 ab	1.51 ab	7.0 ab	1.2 def
BSS100	63 bc	0.27 d	5.8 de	7.2 a
BM100	69 abc	0.56 cd	5.6 e	6.3 ab
TSS100	13 d	0.21 cd	6.3 cd	3.4 bc
TM100	88 ab	1.31 abc	4.6 f	4.2 abc

[a] F= Ammonium nitrate inorganic fertilizer control, BSS=Blue Pains EQ biosolids + sand + sawdust, BM=Blue Pains EQ biosolids + mulch, TSS=Tagro[tm] soil amendment, TM=Tagro[tm] potting soil, ABM= George's Old Town Blend from Alexandria Renew Enterprises biosolids compost, LBC=Livingston Compost, and OCB=OceanGro thermally-dried biosolids.

Table 3.5. Effect of organic amendment treatments and various inorganic fertilizer control application rates on tall fescue above ground biomass and soil electrical conductivity (EC) of the tall fescue bioassay. Treatments with same letters within a column are not significantly different ($p<0.05$).

Treatment[a]	Dry Weight Biomass g pot^{-1}		Soil EC dS m^{-1}	
F0x	1.33	f	0.23	e
F0.5x	1.59	ef	0.18	e
F1x	2.01	cd	0.18	e
F2x	1.97	cde	0.45	de
F4x	1.38	f	4.54	a
BSS1x	2.07	cd	1.26	c
BSS2x	2.86	a	1.93	b
BM1x	2.01	cd	1.14	c
BM2x	2.64	a	1.81	b
TSS1x	2.18	bc	0.44	de
TSS2x	2.94	a	0.66	d
TM1x	1.85	cde	0.25	e
TM2x	2.57	ab	0.36	de
ABM1x	1.72	def	0.31	de
LBC1x	2.02	cd	0.67	d
OCB1x	2.06	cd	0.36	de

[a] F= Ammonium nitrate inorganic fertilizer control, BSS=Blue Pains EQ biosolids + sand + sawdust, BM=Blue Pains EQ biosolids + mulch, TSS=Tagro[tm] soil amendment, TM=Tagro[tm] potting soil, ABM= George's Old Town Blend from Alexandria Renew Enterprises biosolids compost, LBC=Livingston Compost, and OCB=OceanGro thermally-dried biosolids.

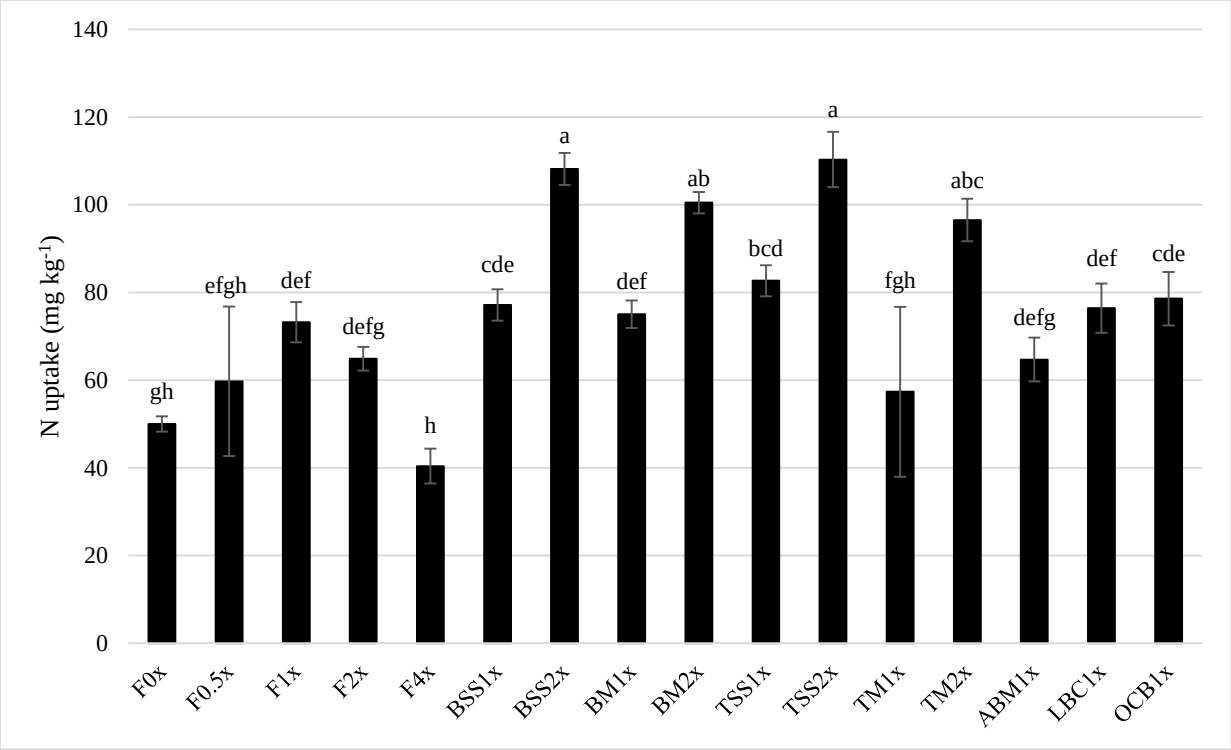

Figure 3.1. Effect of organic amendment treatments and various inorganic fertilizer control application rates on tall fescue N plant uptake. Values of treatments with same letters are not significantly different ($p<0.05$). F= Ammonium nitrate inorganic fertilizer control, BSS=Blue Pains EQ biosolids + sand + sawdust, BM=Blue Pains EQ biosolids + mulch, TSS=Tagro^{tm} soil amendment, TM=Tagro^{tm} potting soil, ABM= George's Old Town Blend from Alexandria Renew Enterprises biosolids compost, LBC=Livingston Compost, and OCB=OceanGro thermally-dried biosolids.

Table 3.6. Calculated plant available N applied with organic amendment treatments.

Treatment	Available N Applied (mg kg^{-1})
BSS1x	90
BM1x	83
TSS1x	108
TM1x	26
ABM1x	50
LBC1x	87
OCB1x	94

BSS=Blue Pains EQ biosolids + sand + sawdust, BM=Blue Pains EQ biosolids + mulch, TSS=Tagro^{tm} soil amendment, TM=Tagro^{tm} potting soil, , ABM= George's Old Town Blend from Alexandria Renew Enterprises biosolids compost, LBC=Livingston Compost, and OCB=OceanGro thermally-dried biosolids.

Table 3.7. Effect of organic amendment treatments and various inorganic fertilizer control application rates on soil total phosphorus (P), Mehlich 1-P, oxalate extractable P, Al, and Fe, and P saturation ratio (PSR). Values having same letters within columns are not significantly different ($p<0.05$).

Treatment[a]	P (mg kg^{-1})		Mehlich 1-P (mg kg^{-1})		Ox Ext P (mg kg^{-1})		Ox Ext Al (mg kg^{-1})		Ox Ext Fe (mg kg^{-1})		PSR (%)	
F0x	178	ef	25	e	81.3	ef	234	bc	197	hi	22	d
F0.5x	155	f	24	e	81.4	ef	231	bc	192	hi	22	d
F1x	154	f	25	e	77.7	ef	226	bc	185	hi	20	d
F2x	159	f	26	e	61.7	f	190	c	159	i	21	d
F4x	162	f	23	e	60.0	f	193	c	153	i	19	d
BSS1x	322	c	36	d	166	abc	237	bc	457	cd	31	bc
BSS2x	546	a	49	bc	246	a	247	ab	698	a	37	b
BM1x	363	bc	36	d	169	abc	248	ab	469	bc	31	bc
BM2x	453	ab	42	cd	233	ab	239	bc	651	ab	37	b
TSS1x	238	d	49	bc	122	cde	252	ab	209	hi	30	c
TSS2x	330	c	71	a	151	bc	271	ab	214	ghi	35	bc
TM1x	241	d	48	bc	131	cd	263	ab	247	fgh	30	c
TM2x	366	bc	76	a	187	abc	317	a	321	def	35	bc
ABM1x	288	cd	38	cd	149	bc	257	ab	304	efg	32	bc
LBC1x	220	de	27	e	84.3	def	218	bc	178	hi	24	d
OCB1x	365	bc	60	ab	232	ab	275	ab	393	cde	43	a

[a] F= Ammonium nitrate inorganic fertilizer control, BSS=Blue Pains EQ biosolids + sand + sawdust, BM=Blue Pains EQ biosolids + mulch, TSS=Tagro[tm] soil amendment, TM=Tagro[tm] potting soil, ABM= George's Old Town Blend from Alexandria Renew Enterprises biosolids compost, LBC=Livingston Compost, and OCB=OceanGro thermally-dried biosolids.

4. Plant available nitrogen estimation tools for a biosolids-amended, clayey urban soil

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4.1. Abstract

Improving mineralizable nitrogen (N) estimates from new exceptional quality (EQ) biosolids products is important for making more reliable supplemental N recommendations to rehabilitate disturbed urban soils for vegetative production. The objectives of this study were to compare the N fertilizer equivalency method and several chemical (NH_4-N, NO_3-N, total N, and organic N) and biological (7 day anaerobic incubation [7-AI] and Solvita CO_2 Burst) tests for quantifying plant available N (PAN) and organic N mineralization of EQ biosolids products used to grow tall fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons) in a clayey urban degraded soil. The EQ biosolids were products of thermal hydrolysis plus anaerobic digestion (BLOOM), blending of BLOOM with woody mulch (BM), blending of BLOOM with sand and sawdust (BSS), composting (LBC), and heat-drying (OCB). The EQ biosolids were applied at agronomic N rates, and the inorganic fertilizer was applied at four N rates in a field study. The N fertilizer equivalency method predicted considerably lower organic N mineralization than calculated from previous study for BM (7.1%), BSS (-12%), and LBC (4.6%). BLOOM, BM, and OCB had the highest 7-AI organic N mineralization. Correlations between tall fescue N uptake and soil N tests showed that soil NO_3-N and 7-AI were the best indicators of biosolids N availability in our urban

soil. However, the relatively low correlations between soil N indicators and tall fescue N uptake was likely due to low residual soil N, high soil clay content, and possible low microbial activity of the low organic matter-containing anthropogenic soil.

4.2. Introduction

Class A biosolids are by-products of treated domestic wastewater sludge obtained via processes to further reduce pathogens (PFRPs). Class A biosolids with low pollutant concentrations and decreased attraction to vectors that can transmit pathogens to humans (e.g. flies, mosquitoes, rodents, etc.) are called Exceptional Quality, or EQ, biosolids (USEPA, 1994). Well-established PFRPs include thermal drying and composting, while more recently developed PFRPs include pasteurization and the Cambi^{TM} thermal hydrolysis (https://www.cambi.com/) (USEPA, 1994; USEPA, 2006; Abu-Orf et al., 2009). A recent advance in EQ biosolids product development includes their blending with organic amendments and/or mineral byproducts to reduce moisture and improve friability for easier spreading. Such EQ biosolids can safely be used in urban landscapes for turfgrass, vegetable, and ornamental crop production (USEPA, 1994).

The use of EQ biosolids as sources of organic matter and nutrients to rehabilitate disturbed urban soils for vegetative production is increasing (Kumar and Hundal, 2016; Basta et al., 2016). Urban soils are typically characterized by low organic matter and carbon cycle-associated nutrients (e.g., nitrogen [N], phosphorus [P], sulfur [S]), compaction, and unusual pH due to routine topsoil removal during construction operations (Craul, 1985). Biosolids, among other organic-based byproducts, are particularly valuable amendments for urban landscapes because they are a local source of organic matter that can be applied to improve degraded soil physical properties (i.e., compaction, aggregation destruction, macropore reduction, plant

available water-holding capacity reduction) in addition to supplying essential plant nutrients (McIvor et al., 2012; Lu et al., 2012). The greatest limitations to using these recently developed EQ biosolids on urban landscapes are (1) their unknown N availability, which is the normal basis for application rate calculation, and (2) understanding N transformations in disturbed, low organic matter anthropogenic soils.

Methods for estimating N availability of organic byproducts include bioassays, and chemical and biological tests. One of the most common bioassay used to quantify N availability of organic materials is the N fertilizer equivalency method. This method compares the amount of N assimilated by a crop at various rates of inorganic N fertilizer with intermediate rates of organic amendments (Muñoz et al., 2004; Rigby et al., 2016). Although the N equivalency method can accurately estimate N mineralization, it is time-consuming and labor intensive because it generally requires a growing season to obtain representative results.

Additional research has focused on developing rapid soil N tests for estimating potentially mineralizable N (PMN). Chemical tests include extracts of N fractions such as nitrate (NO_3-N), ammonium (NH_4-N), or soluble organic N by using alkali, neutral or acidic solutions (Ros et al., 2011). Biological tests involve soil incubations that measure the amount of inorganic N or carbon dioxide (CO_2) released in the soil after incubation due to microbial activity. The 7-day anaerobic incubation (7-AI) is one of the fastest, most frequently used and recommended indicators of PMN (Waring and Bremner, 1964; Curtin et al., 2017). Although the 7-AI has been used to estimate N mineralization in many soils, very few studies have used this test to estimate N mineralization from biosolids-amended soils. Moreover, most studies evaluating chemical and biological tests to estimate mineralizable biosolids N have not studied EQ biosolids applied to anthropogenic soils. Gomez-Muñoz et al. (2017) used the 7-AI to determine potential

mineralizable N of various agricultural and urban wastes from a field that had received 11 years of agronomic N rates and three times the agronomic N rate, based on statutory 45% N fertilizer replacement values. Nitrogen availability of sewage sludge expressed as % NH_4-N released from soil total N of the long-term treated soils was 1.8% for the agronomic N rate and 2.2% for three times the agronomic N rate. White et al. (2018) more recently used the 7-AI to determine N availability coefficients (plant available N as a percent of total N applied) of various biosolids types applied to four agricultural soils of different soil textures. Nitrogen availability coefficients in this study varied from -13% to 86%.

The evolution of CO_2 during shorter aerobic incubations has also been proposed as a means to estimate N mineralization (Franzluebbers et al., 2000; Haney et al., 2001; Haney et al., 2008). Researchers have observed that a flush of CO_2 evolved during 1 to 3 days after rewetting of an air-dried soil is highly correlated to N mineralization from 24 day aerobic incubations and to field corn yield responses (Franzluebbers et al., 2000; Haney et al., 2001; Franzluebbers, 2018). A commercial version, the Solvita CO_2 Burst test (https://solvita.com/co2-burst/), was developed from laboratory studies that identified relationships between 1 day CO_2 evolution and N mineralization (Haney et al., 2001; Haney et al., 2008). This test has not been studied in a wide range of soils and climatic regions, and its accuracy for estimating PMN in urban soils after biosolids application has never been tested.

Given the importance of estimating mineralizable N of new EQ biosolids and the lack of knowledge regarding the effectiveness of quick chemical and biological tests to estimate mineralizable N from EQ biosolids amended urban soils, the objectives of this study were: (1) to use the N fertilizer equivalency method to quantify plant (tall fescue, *Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons) available N and organic N mineralization of various EQ biosolids

products applied to an urban degraded soil, and (2) to compare N fertilizer equivalency method with several chemical and biological tests for estimating mineralizable N.

4.3. Materials and Methods

4.3.1. Biosolids

Five EQ biosolids products were evaluated. Three biosolids were developed either directly or indirectly from the Blue Plains Advanced Wastewater Treatment Plant (DC Water, Washington, DC; https://www.dcwater.com/blue-plains), and one product each came from the Livingston Composting Facility (Spotsylvania County, VA; http://www.spotsylvania.va.us/Compost), and the Ocean County Utilities Authority (Bayville, NJ; http://www.ocua.com/SitePages/Home.aspx). The products were: 1) BLOOM, an air-dried biosolids from DC Water produced by the Cambi^{TM} process, which is a proprietary procedure employing thermal hydrolysis followed by anaerobic digestion; 2) a blended product composed of 0.75 BLOOM: 1.0 shredded, woody mulch (BM); 3) a blended product composed of 1.0 BLOOM: 1.0 sand: 1.0 sawdust (BSS); 4) Livingston's composted anaerobically digested biosolids (LBC); and 5) a thermally-dried, pelletized biosolids marketed as OceanGro® (OCB) obtained from Ocean County Utilities Authority in Bayville, New Jersey. The formulation for the blended BLOOM products were based on dry weight ratios of BLOOM, woody mulch, sand, and sawdust as described in a previous greenhouse study (Alvarez-Campos et al., 2018).

Each of the biosolids products was analyzed by Waypoint Analytical Laboratories (Richmond, VA; http://www.waypointanalytical.com/) for total solids; whole volatile solids which relate to organic matter (SM2540G; APHA, 1992); total Kjeldahl N (TKN; SM-4500-NH3C-TKN; APHA, 1992); ammonia + ammonium N (NH_4-N; SM-4500-NH3C; APHA, 1992); nitrate N (SM-4500NO3F; APHA, 1992); organic nitrogen (calculated as difference between

TKN and NH_4-N); phosphorus (P), potassium (K), sulfur (S), calcium (Ca), magnesium (Mg), sodium (Na), iron (Fe), aluminum (Al), copper (Cu), zinc (Zn) (SW-6010C; USEPA, 2000a); and pH (SW-9045D; USEPA, 2000b). Chemical properties of the biosolids are shown in Table 4.1.

Table 4.1. Selected composition variables of EQ biosolids products applied to the tall fescue field study bioassay.

	Unit	BLOOM†	BM	BSS	LBC	OCB
pH‡§		5.17	6.22	6.34	6.86	7.3
Total Solids§	g kg^{-1}	570	850	730	840	950
Moisture§	g kg^{-1}	430	150	270	160	50
C	g kg^{-1}	248	354	256	334	383
C:N		8.1	12.7	13.2	12.0	7.8
TKN	g kg^{-1}	30.8	27.9	19.4	27.7	49.1
NH_4-N	g kg^{-1}	1.9	4.5	3.4	3.1	5.7
Organic N	g kg^{-1}	28.9	23.4	16.0	24.6	43.4
NO_3-N	mg kg^{-1}	1240	785	495	1170	7.21
TP	g kg^{-1}	30.9	18.9	20.8	13.3	26.6
K	g kg^{-1}	1.5	1.6	1.3	4.9	1.4
Ca	g kg^{-1}	24.7	20	21.7	26.6	28.4
Mg	g kg^{-1}	4.1	2.8	2.9	3.2	4.3
Fe	mg kg^{-1}	82600	54400	56400	26700	32800
Al	mg kg^{-1}	11900	5600	5700	15300	14700

† BLOOM= air-dried Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust, LBC=Livingston Compost, and OCB=OceanGro thermally-dried.

‡ saturated paste method

§ Values are on a dry weight basis except as noted.

¶ All elemental values are total concentrations of one sample.

4.3.2. Tall Fescue (*Schedonorus arundinaceus* (Schreb.) Dumort., nom. cons) Field Study

Study Site and Experimental Design

The tall fescue field research study was conducted at the Virginia Tech Turfgrass Research Center (TRC) in Blacksburg, VA. Blacksburg is located in Montgomery County approximately 634 m above sea level. The site is located within the USDA cold hardiness zone 6b and the main climate is warm temperate according to the Koppen-Geiger climate classification (https://planthardiness.ars.usda.gov; Kottek et al., 2016). Precipitation from March to October 2017 was 840 mm and was gathered from Virginia Tech weather station located approximately 1 km from the site.

In preparation for the establishment of the simulated urban (anthropogenic) soil, existing soil at the TRC research location was excavated to a depth of approximately 45 cm. The excavated soil was replaced with a clayey subsoil fill obtained from a local construction site. The original soil map unit description of the soil at the construction site was Groseclose-Urban land complex, identified with a clayey subsoil of slow permeability, high shrink/swell potential, and small stones. The geology of the region is characterized by the presence of sedimentary rocks such limestone and high lime shales. The soil was tilled with a tractor-mounted rear-tine tiller to break apart large clods and level the soil.

The experimental design was a completely randomized design for our 9 treatments x 3 replications. Individual experimental units in the 77.4 m^2 constructed anthropogenic soil site were 1 m x 1 m. Establishment of alleyways of 60-cm width between experimental units prevented movement of nutrient- and biosolids-amended soil across treatments. Four synthetic fertilizer control treatments, as urea (46-0-0) at 0, 112, 224 and 336 kg N ha^{-1}, were applied and incorporated into the soil to establish a calibration curve for plant N uptake response to N rate.

The five other treatments consisted of each of the biosolids products (BLOOM, BM, BSS, LBC, and OCB) applied and incorporated into the soil at a rate estimated to provide 224 kg PAN ha^{-1}. All biosolids and inorganic fertilizer treatments were hand-applied and incorporated into the soil to a depth of 10 cm with a roto-tiller during the last week of March 2017.

Calculation of PAN to determine biosolids application was based on organic N mineralization rate estimates in previous literature (Gilmour et al., 2003) and the results of a preliminary greenhouse study on various EQ biosolids (Alvarez-Campos et al., 2018). Organic N mineralization rates (% available N of total organic N) for the growing season were approximated as 20% for blended, 15% for composted, and 40% for heat-dried biosolids. The amounts of biosolids added on a dry weight with the purpose of supplying 224 kg PAN ha^{-1}, and the amount of total N, organic N, and inorganic N with these applications are shown in Table 4.2.

Table 4.2. Biosolids addition, and amount of total N, organic N, and inorganic N added with EQ biosolids treatment to supply 224 kg PAN ha^{-1}.

	BLOOM†	**BM**	**BSS**	**LBC**	**OCB**
Biosolids addition (Mg ha^{-1}) dw	21.2	24.5	33.9	36.2	9.7
Biosolids total N added‡ (kg N ha^{-1})	653	685	657	1003	478
Biosolids organic N added§ (kg N ha^{-1})	612	576	541	889	422
Biosolids inorganic N added¶ (kg N ha^{-1})	41	109	116	114	55

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust, LBC=Livingston Compost, and OCB=OceanGro thermally-dried.

‡ Total N of biosolids multiplied by biosolids addition (dw).

§ Total organic N of biosolids multiplied by biosolids addition (dw).

¶ Total inorganic N of biosolids multiplied by biosolids addition (dw).

Soil Sampling and Analysis

The processes we employed (i.e., dumping and consistent spreading of subsoil clay; intensive and extensive tillage and grading of the site; removal of unearthed rocks and anthropogenic materials, such as plastic, asphalt, and trash; settling of constructed soil for 6 months) for developing a simulated anthropogenic soil from the construction site fill was conducted to ensure uniform site distribution of soil. Benchmark soil samples were collected before EQ biosolids application to quantify chemical properties and nutrient status via soil testing (Maguire and Heckendorn, 2011) for supplementation with additional inorganic fertilizer for adequate tall fescue growth (Table 4.3). A soil corer (2 cm diameter) was used to obtain a composite sample of eight cores from four random sampling spots across the field to a 10 cm depth. Soil samples were air-dried, sieved through a 2-mm mesh, and analyzed for routine soil testing using Mehlich 1 extraction by the Virginia Tech Soil Testing Laboratory on a Thermo Elemental ICAP 61E. Spatial sampling for routine soil test variables (i.e., pH, electrical conductivity, Mehlich 1-extractable P, K, Ca, Mg, Mn, and Zn) demonstrated site homogeneity.

Table 4.3. Summary of textural content and nutrient concentration of benchmark soil samples obtained from the urban soil used in this study. Four replicate samples used to obtain each average.

Soil testing indices	
Chemical properties	
pH†	7.7
Olsen P‡, mg kg^{-1}	11
P§, mg kg^{-1}	3
K, mg kg^{-1}	56
Ca, mg kg^{-1}	2594
Mg, mg kg^{-1}	656
Zn, mg kg^{-1}	2.3
Mn, mg kg^{-1}	21
Cu, mg kg^{-1}	1.3
Fe, mg kg^{-1}	8.4
B, mg kg^{-1}	0.4
Total organic C¶, g kg^{-1}	6.7
Total N, g kg^{-1}	0.4
Particle size distribution#	
Clay, g kg^{-1}	630
Silt, g kg^{-1}	150
Sand, g kg^{-1}	220

† 1:1 (v/v) soil to water ratio.
‡ 1:20 (w/v) soil to 0.5 M sodium bicarbonate solution ratio extraction.
§ Macro and micronutrient availability based on 1:1 (v/v) soil to Mehlich 1 extraction.
¶ Hydrochloric acid fumigation to remove carbonates prior to total C analysis.
# Hydrometer method.

Soil pH and Mehlich 1 analyses revealed that soil P levels were very low (3 mg kg^{-1}), and that pH and Ca/Mg concentrations were high (Table 4.3). The two latter soil properties are indicative of calcareous soils that most likely formed from limestone parent material that has not yet weathered to the typically acid pH of most fine-textured Virginia subsoils (Creggar et al., 1985). Based on this information, we proceeded to use an Olsen extraction (0.5 M sodium bicarbonate), which is recommended for determining soil P levels of calcareous soils. Olsen extraction was conducted by adding 40 ml of Olsen solution to 2 g of soil, shaking in a reciprocating shaker for 30 minutes, and filtering through Whatman 40 filter paper (Olsen, 1957). The extraction was sent to the Virginia Tech Soil Testing Laboratory to be analyzed for P. Based on Olsen P and routine soil K testing results, supplemental inorganic P (64 kg P ha^{-1}) and K (84 kg K ha^{-1}) were applied in the forms of triple superphosphate (0-46-0) and potash (0-0-60), respectively, based on soil test recommendations for tall fescue (Maguire and Heckendorn, 2017).

The hydrometer method was used to perform particle size analysis (Gee and Bauder, 1986). Soil total C and N of benchmark soil samples were analyzed by combustion with a Vario MAX CN macro elemental analyzer (Elementar, Hanau, Germany). A hydrochloric acid (HCl) fumigation procedure was conducted prior to analysis to remove inorganic C and obtain organic C concentrations (Harris et al., 2001).

Field soil sampling was conducted five days after biosolids and inorganic fertilizer treatment applications were incorporated, and these samples were used for chemical and biological laboratory analyses. Sampling was performed by using a 2 cm soil corer with a 6-core composite obtained from each experimental unit. The soil samples collected from each treatment

plot were air-dried and passed through a 2 mm sieve to separate any rock fragments or debris in preparation for chemical analysis of soil N forms and biological incubations.

Tall Fescue Maintenance and N Uptake Calculations

Tall fescue seeds were planted in March at 3.63 kg seeds 93 m^2 (8 lbs seeds 1000 ft^2), which is approximately 20,000 seeds m^2, by spreading the seeds evenly throughout the plots to ensure tall fescue full coverage during establishment. Plots were manually irrigated to provide ample moisture for germination, emergence, and growth. Tall fescue stands were mowed to a height of ~7.6 cm every 3 weeks from May to October 2017, and clippings from each experimental unit was collected. Leaf clipping samples were oven-dried at 65°C for 24 hours, weighed to determine aboveground biomass dry matter yield, and ground through a 0.85 mm Wiley mill press in preparation for N analysis. Leaf samples were analyzed with a Vario Max CNS macro elemental analyzer (Elementar, Hofnau, Germany) for determination of N concentration. Tall fescue aboveground N uptake was calculated as the product of leaf N concentration and biomass dry matter.

The amounts of PAN or fertilizer equivalency provided by biosolids treatments were estimated using the quadratic equation ($ax^2 + bx + c = 0$) obtained from the N uptake of 0, 112, 224, and 336 kg ha^{-1} N rates ($y = -0.000271x^2 – 0.2169x – 2.3603$, $R^2 = 0.9028$), which best described the data. The N uptake for each biosolids product ("y") was used to solve for the actual applied N rate or PAN ("x"). Because N biomass and uptake from the 0 N rate treatment were negligible, the difference between biosolids treatments N uptake and N uptake of the 0 N rate was not used to calculate PAN. Thus, percent PAN was calculated by dividing the resulting PAN observed from each biosolids treatment or fertilizer equivalency by the total N added (Muñoz et al., 2004)**:**

$$PAN\ (\%) = \frac{PAN\ observed}{total\ N\ added}\ x\ 100$$

Organic N mineralization was calculated by dividing PAN observed by the total amount of organic N added:

$$Organic\ N\ mineralization\ (\%) = \frac{PAN\ observed}{organic\ N\ added}\ x\ 100$$

4.3.3. Soil Laboratory Analyses

Chemical Analysis of Soil N Forms

Soil NH_4-N and NO_3-N were measured after extracting with 2 M KCl at a 1:10 soil to KCl solution ratio. The solution was mixed in a constant speed reciprocal shaker (Eberbach Corporation, Ann Arbor, Michigan) at 180 rpm for 30 minutes. The supernatant was filtered through a 0.45 µm filter paper, and analyzed by flow injection on a Lachat 8500 to determine NO_3-N (QuikChem Method 12-107-04-1-B; Knepel, 2001), and NH_4-N (QuikChem Methods 12-107-06-3-B; Hofer, 2001).

Sieved soil samples were further ground in an electric mortar grinder RM200 (Retsch, Inc., Newtown, PA) and passed through a 180 µm sieve in preparation for total N analysis. Approximately one gram of soil was weighed and analyzed in a Vario Max CNS macro elemental analyzer (Elementar, Hofnau, Germany). Organic N was calculated by subtracting inorganic nitrogen (NH_4-N and NO_3-N) from soil total N.

Anaerobic Incubation

A 7 day anaerobic incubation (7-AI) was conducted to determine potentially mineralizable N in the biosolids-amended soils (Curtin et al., 2017; Curtin and Campbell, 2007). Soil samples were air-dried and sieved to 2 mm. Five grams of soil with two replicate samples from each treatment were weighed in 50 ml centrifuge tubes and waterlogged by adding 10 ml of

deionized water. The headspace of the tube (~30 m^3) was purged with N_2 gas for 60 seconds, and immediately capped with a rubber stopper (#6) to cover the tube, which was then sealed with electrical tape to ensure anaerobic conditions. Soil samples were placed in a Precision Model 815 Low Temperature Incubator (Thermo Fisher Scientific, Marietta, Ohio) for 7 days at constant temperature of 40°C (Curtin and Campbell, 2007). After 7 days, 30 ml of 2.67 M KCl were added to the centrifuge tube with incubated soil and extracted by shaking the solution in a constant speed reciprocal shaker (Eberbach Corporation, Ann Arbor, Michigan) at 180 rpm for 30 minutes. The supernatant was then filtered through a 0.45 µm filter paper, and extracts were frozen until NH_4-N analysis.

On the start date of the anaerobic incubation (day 0), another set of soil samples was also prepared for extraction by weighing 5 grams of soil with two replicate samples from each treatment into 50 ml centrifuge tubes and adding 40 ml of 2 M KCl. The solution was then mixed in a reciprocal shaker and filtered as explained previously, and frozen until NH_4-N analysis. Extracts were analyzed on a Lachat 8500 to determine NH_4-N (QuikChem Methods 12-107-06-3-B; Hofer, 2001). The amount of N mineralized was calculated as the difference in NH_4-N between extractions done at day 7 and day 0 (Drinkwater et al., 1996). The N that mineralized during the anaerobic incubation was divided by the organic N of the soil to obtain an organic N mineralization rate estimate.

Solvita CO_2 burst Soil Test

The Solvita CO_2 burst Soil Test (https://solvita.com/co2-burst/) was used to measure CO_2 evolution after 24 hours of aerobic soil incubation. Forty grams of air-dried, 2 mm sieved soil were weighed into a beaker (50 cm^3) with three holes in the bottom. The beaker was placed inside a glass jar (250 cm^3) and the soil in the beaker was wetted to obtain approximately 50%

water filled pore space which was determined based on the settled volume of 40 grams of soil in the beaker, as suggested in the CO_2 burst protocol. A pH-sensitive gel embedded in a rectangular plastic holder with a paddle was also placed inside the jar. The Solvita gel technology uses an alkali trap to absorb an amount of CO_2 that is proportional to the total concentration of CO_2 in the incubation jar. The samples were kept in in a Precision Model 815 Low Temperature Incubator (Thermo Fisher Scientific, Marietta, Ohio) incubator at 20°C for 24 hours. After 24 hours, the reactive gel indicator paddles were read in a digital color reader (DCR) which detects color intensity of red, green, and blue. The Solvita test color provides a measurement of the level of CO_2 detected within the Solvita gel technology during the incubation. Thus, the Solvita DCR provides a Solvita color number and the level of CO_2 as mg kg^{-1}. The concentration of CO_2 emitted is assumed to be proportional to PMN (Haney et al., 2008). The Solvita online PMN calculator was used to determine PMN in kg ha^{-1} as estimated by the Solvita CO_2 Burst test.

4.3.4. Statistical Analyses

All statistical analyses were conducted using JMP® software (SAS Institute Inc., 2015). Data was approximately normal as determined with goodness-of-fit method. Analysis of variance (ANOVA) and Fisher's LSD method for mean separation was used to determine differences in treatment N availability obtained by biological (7-day anaerobic incubation and Solvita) and chemical (soil NO_3, soil NH_4, soil organic N, a total N) methods for estimating potentially mineralizable N. Non-parametric spearman correlations were determined between field N fertilizer equivalency results, and both biological and chemical based potential mineralizable N indicators with the Multivariate method. Linear relationships between field tall fescue N uptake and all N indicators were evaluated using bivariate fit line. A p-value of $\alpha = 0.05$ was used to assess for significance.

4.4. Results and Discussion

4.4.1. Tall Fescue N uptake

Cumulative tall fescue dry biomass (data not shown) and N uptake (Figure 4.1) increased quadratically with inorganic N fertilizer rates (0, 112, 224, and 336 kg N ha^{-1}). The quadratic response was likely due to near attainment of maximum yield by the highest fertilizer N rate boundary. Neither biomass nor N uptake was measured for the tall fescue at the 0 N rate due to negligible biomass production in the anthropogenic, low residual N soil.

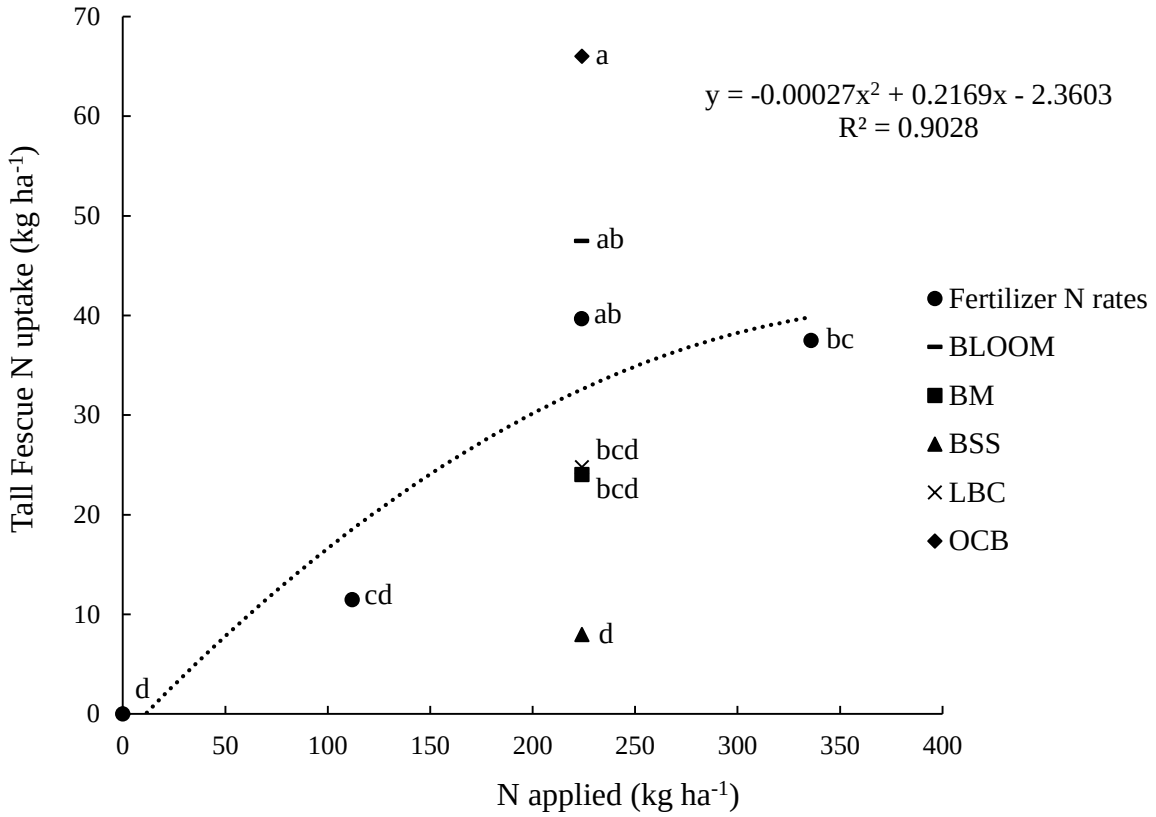

Figure 4.1. Effects of inorganic N fertilizer applied at 0, 112, 224, and 336 kg N $ha^{-1}$and EQ biosolids products applied at the agronomic N rate (224 kg ha^{-1}) on cumulative (March to October 2017) tall fescue N uptake. Different lower case letters represent differences between tall fescue N uptake of biosolids and the actual fertilizer rate.

4.4.2. Biosolids Plant Available Nitrogen

The amounts of PAN provided by the biosolids were calculated by solving the fertilizer N uptake quadratic equation ($y = -0.000271x^2 – 0.2169x – 2.3603$, $R^2 = 0.9028$; Figure 4.1) for "x" upon insertion of measured N uptake ("y") for each biosolids source. Measured N uptake and calculated PAN for each biosolids product are presented in Table 4.4. Tall fescue N uptake from BLOOM and OCB were greater than any fertilizer N rate, which prevented precise calculation of PAN for these two treatments. We estimated that the PAN would have been >400 kg ha^{-1}, which was the maximum point of the quadratic function. Based on the relative N uptake of biosolids compared to the same rate of inorganic fertilizer, we underestimated organic N mineralization rates for BLOOM (20%) and OCB (40%) and overestimated them for BM (20%), BSS (20%), and LBC (15%).

Table 4.4. Cumulative tall fescue N uptake of biosolids treatments, and plant available N (PAN), fertilizer N equivalent, and organic N mineralization calculated from cumulative tall fescue N uptake values.

	BLOOM†	BM	BSS	LBC	OCB
Tall fescue N uptake (kg ha^{-1})	47.5	24.0	8.0	24.8	66.0
PAN, calculated‡ (kg N ha^{-1})	≥400	150	51	155	≥400
Fertilizer N equivalent§ (%)	61	22	7.8	16	84
Organic N, calculated¶ (kg N ha^{-1})	359	41	-65	41	345
Organic N mineralization# (%)	59	7.1	-12	4.6	82

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust, LBC=Livingston Compost, and OCB=OceanGro thermally-dried.

‡ Calculated with the quadratic equation obtained from increasing rates of inorganic fertilizer.

§ Percent PAN of total N

¶ Difference between calculated PAN and inorganic N measured in biosolids.

Percent organic N that became plant available.

The non-woody amended biosolids products of BLOOM and OCB had the highest fertilizer N equivalent, most of which formed via high organic N mineralization rates (~59% and 82%, respectively; Table 4.4). Organic N mineralization rates of BLOOM and OCB were greater than previously published mean N mineralization for anaerobically digested biosolids (~30%) and heat-dried biosolids (~40%; Rigby et al., 2016). Treatments BM and LBC had fertilizer N equivalents of 22% and 16%, respectively, while their organic N mineralization rates were of 7.1% and 4.6%, respectively. The organic N mineralization rate of our compost treatment (LBC) was very similar to the mean value (~6.7%) and within range (10-24.5%) of previously published N mineralization rates for composted biosolids (Rigby et al., 2016).

The BSS product had a fertilizer N equivalent of 7.8% and a net negative (-12%) organic N mineralization rate. There are few previously published N mineralization rates for woody- and mineral- blended biosolids products, such as BM and BSS. A previous greenhouse experiment had suggested an organic N mineralization rate of ~20% for these products (Alvarez-Campos et al., 2018). However, different experimental conditions of the previous greenhouse study (e.g. controlled temperature and moisture, loamy sand soil) likely resulted in different N mineralization rates than those obtained in our field study.

Factors that likely contributed to lower N mineralization rate calculations for the field bioassay were common soil N transformations and losses (i.e., immobilization, denitrification, NO_3-N leaching) that we did not measure in the field, and the very low background N and possible low microbial activity of our low organic matter-containing anthropogenic field soil. Undisturbed natural soils often have an intact topsoil and well-established C-N cycle that mineralizes 1-3% of the native organic N annually (Pierzynski et al., 2005). The residual soil N of undisturbed soils can contribute to the overall N provisioning. As was observed from the 0 N

rate of the tall fescue N uptake curve (Figure 4.1), our field soil had little to no residual N or C (Table 4.3). These unique properties of anthropogenic soils likely contribute to reduced plant available N, even upon addition of an N source.

The high clay content of our soil may also have physically protected C- and N-containing substrates from microbial decomposition, thus reducing organic N mineralization (Griffin, 2008). Some studies have reported lower biosolids N mineralization rates in finer- than coarser-textured soils, while other studies have reported greater N mineralization rates in soils with higher clay (Hernandez et al., 2002; Rigby et al., 2009). However, the increased N mineralization in high clay soils was usually associated with high organic matter concentrations (Rigby et al., 2009).

4.4.3. Chemical and Biological Nitrogen Indicators

Analysis of inorganic N of the fertilized and biosolids-amended soils shortly after treatment application shows that NO_3-N comprised nearly the entire pool of inorganic N (Table 4.5). This is not surprising as nitrification of NH_4-N is very rapid in aerobic environments (Weil and Brady, 2017). Only in the unamended control did NH_4-N contribute anywhere close to 20% of the inorganic N, while in all other treatments NH_4-N comprised ~2-4% of the inorganic N. The concentration of soil inorganic N increased linearly with N fertilizer rate. All biosolids applied to supply 224 kg PAN ha^{-1} (except OCB) generated the same concentration of soil NO_3-N as the fertilizer rate designed to supply 112 kg N ha^{-1}. The OCB provided the same soil inorganic N concentration as that of the 224 kg ha^{-1} fertilizer rate.

Table 4.5. Concentrations of N forms in N-fertilized and biosolids-amended field soils used to compare 7-day Anaerobic Incubation (7-AI) and Solvita CO_2 Burst tests as indices of potentially mineralizable N (PMN). Values with same letters in columns are not significantly different ($p<0.05$). Table averages obtained from laboratory duplicates.

Treatment	NH_4-N	NO_3-N	Inorganic N	Organic N	Total N	7-AI	Solvita CO_2 Burst	Solvita PMN‡
	----------	----------	----------	mg kg^{-1}	----------	----------	----------	kg ha^{-1}
F0	0.93 d	4.1 d	5.0 d	360 c	365 c	0.32 c	4.33	1.01
F112	1.4 cd	60 c	61 c	580 bc	641 bc	6.9 bc	5.80	3.08
F224	1.7 bcd	110 b	112 ab	740 abc	852 ab	1.7 bc	7.37	4.06
F336	3.1 a	141 a	144 a	591 bc	735 abc	5.2 bc	5.13	2.11
BLOOM†§	2.2 abc	49 c	51 c	1072 a	1123 a	25 a	14.0	9.23
BM	1.8 bc	52 c	54 c	947 ab	1001 ab	19 a	15.9	9.69
BSS	1.6 cd	44 c	46 c	646 bc	691 bc	5.0 bc	8.97	3.96
LBC	2.0 bc	56 c	58 c	918 ab	976 ab	13 ab	12.1	7.60
OCB	2.5 ab	95 b	98 b	737 abc	834 ab	21 a	15.7	8.08

† F=fertilizer, BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust, LBC=Livingston Compost, and OCB=OceanGro thermally-dried.

‡ Calculated with the online Solvita potential mineralizable nitrogen (PMN) calculator.

§ All biosolids treatments applied at the agronomic N rate (224 kg ha^{-1})

Total N concentration in the biosolids-amended soils were equal to that in the soil fertilized with 224 kg fertilizer N ha^{-1} (Table 4.5). The largest fraction of total N in our soil was organic N. Total N of the unamended soil (360 mg kg^{-1}) was within the range reported for urban soils (200-7100 mg kg^{-1}; Beyer et al., 1995), albeit on the low side for even disturbed urban soils, whose topsoil N concentrations are typically lower than those of average agricultural soils (1500 mg kg^{-1}; Weil and Brady, 2017; Schomberg et al., 2009). Treatments BLOOM, BM, and OCB gave the greatest differences between final and initial 7-AI NH_4-N, demonstrating higher organic N mineralization than in the other treatments (Table 4.5). However, organic N mineralization rates estimated with the 7-AI as the amount of N mineralized divided by the organic N content, were 2.3% for BLOOM, 2% for BM, 0.8% for BSS, 1.4% for LBC, and 2.8% for OCB. Most of these values are much lower than the estimates obtained from the tall fescue N uptake field experiment (Table 4.4). The PMN measured with the 7-AI ranged from 0.32 to 25 mg kg^{-1}, which are at the lower end of values reported in other studies. Schomberg et al. (2009) and Soon et al. (2007) reported 7-AI values of 4 to 115 mg kg^{-1} and 2.9 to 80.8 mg kg^{-1}, respectively, for soils of agricultural lands.

There were no differences in Solvita CO_2 burst and Solvita PMN among all N fertilized and biosolids-amended soils (Table 4.5). Values of Solvita PMN (kg ha^{-1}; Table 4.5) were lower than organic N mineralization estimates obtained in the tall fescue N uptake field experiment for BLOOM (359 kg N ha^{-1}), BM (41 kg N ha^{-1}), LBC (41 kg N ha^{-1}), and OCB (82 kg N ha^{-1}; Table 3). The BSS treatment had the lowest Solvita PMN; however, this value was greater than results from the tall fescue field experiment (-65 kg N ha^{-1}; Table 4.4). This is because the Solvita CO_2 Burst test permitted CO_2 evolution and N mineralization to occur without the potential N losses (i.e. immobilization, denitrification, NO_3-N leaching) that most likely occurred in the tall fescue

N uptake field experiment. Solvita CO_2 Burst concentration results obtained in our 24 hour incubation (Table 4.5) were also lower than those reported in the literature for a loamy sand (~32 mg kg^{-1}; Tu, 2016), a clay loam (~97 mg kg^{-1}; Tu, 2016) soil, and various soil types from the Midwestern US (16-150 mg kg^{-1}; Yost et al., 2018).

4.4.4. Relationships between soil N indicators and tall fescue N uptake

Soil NH_4-N ($r = 0.593$, p-value = 0.0014) and NO_3-N ($r = 0.505$, p-value = 0.0072) showed the strongest positive significant correlation with tall fescue N uptake (Table 4.6). However, soil NH_4-N is not particularly useful as a PAN indicator because concentrations are very low under oxic conditions and have very little contribution to available N. Additionally, soil NO_3-N could better serve as a PAN indicator as most soil inorganic N occurred as NO_3-N (Table 4.5). Other studies have also found strong correlations between soil NO_3-N and plant N uptake (Magdoff et al., 1984). Tall fescue N uptake also had a moderate positive significant correlation with 7-AI ($r = 0.490$, p-value = 0.011; Table 4.6), suggesting that there is a relationship between these parameters and that the 7-AI has the potential to be a good indicator of N availability in disturbed, urban soils. In contrast, soil organic N, total N and Solvita CO_2 Burst showed no significant relationship with tall fescue N uptake.

Table 4.6. Spearman correlation coefficients for the associations among field tall fescue (TF) N uptake and chemical and biological N indicators of N availability in an urban soil. P-values shown in parenthesis. Treatment laboratory duplicates used for analysis.

N indices	TF N uptake	7-AI‡	NH_4-N	NO_3-N	Inorganic N	Organic N	Total N
7-AI	0.490 (0.011)						
NH_4-N	0.593 (0.0014)	0.640 (0.0006)					
NO_3-N	0.505 (0.0072)	0.251 (0.2169)	0.607 (0.001)				
Inorganic N	0.509 (0.008)	0.273 (0.1879)	0.607 (0.0011)	1 (<0.0001)			
Organic N	0.309 (0.1246)	0.901 (<0.0001)	0.577 (0.0021)	0.186 (0.3621)	0.186 (0.3621)		
Total N	0.326 (0.0968)	0.896 (<0.0001)	0.668 (0.0002)	0.257 (0.1956)	0.324 (0.1059)	0.9822 (<0.0001)	
Solvita CO_2 Burst	0.228 (0.252)	0.775 (<0.0001)	0.520 (0.0065)	0.154 (0.4441)	0.182 (0.3725)	0.670 (0.0002)	0.662 (0.0002)

‡ 7 day anaerobic incubation (7-AI)

Soil NH_4-N, but neither NO_3-N nor inorganic N, correlated well with 7-AI (Table 4.6), because the 7-AI test method measures only NH_4-N. The 7-AI is, therefore, strongly influenced by the amount of potentially mineralizable soil organic N. Moreover, 7-AI also correlated well with total N because most N is in organic form as can be evidenced by the strong significant correlation between soil organic N and total N ($r = 0.9822$, p-value = <0.0001; Table 4.6) and data in Table 4.5. The 7-AI correlated well with Solvita CO_2 Burst results ($r = 0.775$, p-value = <0.0001), and Solvita CO_2 Burst was strongly correlated with soil NH_4-N, organic N, and total N. The CO_2 measured by the Solvita CO_2 Burst method corresponds to CO_2 released as microbial decomposition generates NH_4-N from organic N. Thus, it is expected that CO_2 released would be related to the amount of organic N that can be mineralized to NH_4-N. The strong correlations observed between 7-AI, Solvita CO_2 Burst, and organic N suggest that these N indicators show promise to identify an available N fraction after biosolids application. However, calibration of these tests would be needed for estimation of plant growth field responses on urban degraded clayey soils and a greater variety of urban soil types.

The Solvita CO_2 Burst test has been shown to have significant and strong correlations with bermudagrass and tall fescue N uptake, and corn grain yield (Moore, 2018; Tu, 2016; Yost et al., 2018). These results were obtained in agricultural soils with established C-N cycles, not in a highly disturbed anthropogenic soil with little residual N and C. Yost et al. (2018) determined that omitting sites with low soil organic matter improved the strength of the relationship found between Solvita CO2 burst test and the economically optimum N rate of corn from 17 soil sites in eight Midwestern states. Tu (2016) also observed that Solvita CO_2 Burst test gave better correlations with other soil tests (64 day aerobic incubation, permanganate oxidizable carbon, and 2M KCl extractable NH_4-N and NO_3-N) in coarse- than fine-textured soils. Thus, the urban

soil's clay texture and lack of established C-N cycle due to soil disturbance, most likely increased the difficulty to estimate biosolids N mineralization with the N indicators evaluated in this study.

4.5. Conclusions

Estimating plant available N and organic N mineralization rates for newly developed EQ biosolids products is important in order to ensure that crops and other plants receive adequate, but not excessive, amounts of available N. The N fertilizer equivalency method of quantifying biosolids plant available N allowed us to confirm that biosolids produced by heat-drying (OCB) and the Cambi^{TM} process (BLOOM) have higher plant available N and organic N mineralization rates than biosolids blended with woody byproducts. Such woody-amended biosolids have lower available N due to dilution of N concentration and reduction of mineralization rate by the high C:N ratio substrate. The positive correlations between tall fescue N uptake and soil inorganic N (especially NO_3-N), and 7-AI show that these tests were the best indicators of biosolids N availability in our urban soil. The positive correlations between the 7-AI, Solvita CO_2 burst, and organic N suggest that these N indicators may be able to identify available N fractions after biosolids application, but further calibrations of these tests is needed for their use to estimate field N uptake responses on urban degraded clayey soils and more urban soil types. The overall reliability of laboratory N tests may have been reduced due to the low C- and N-, and high clay-containing urban soil. Further assessments of rapid tests with the purpose of identifying a routine test for estimating organic N mineralization of new EQ biosolids could allow more precise recommendations for the use of these products on urban soils for vegetation establishment and agriculture, while reducing potential negative environmental impacts due to N losses.

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5. Exceptional Quality biosolids amendments for vegetable production in urban agriculture

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5.1. Abstract

Exceptional Quality (EQ) biosolids may be developed into products that can rehabilitate disturbed urban soils for the production of vegetables in urban gardens. The objectives of this study were to investigate the effects of various EQ biosolids products on urban soil chemical properties and vegetable plant yields. Field research was conducted at Blacksburg, VA, in an urban subsoil fill from a nearby construction site to simulate common degraded urban soil properties. The experimental design was a randomized complete block design with eight fertility treatments and four replicates. Three EQ biosolids were applied seasonally at agronomic nitrogen (N) rates (fall 2016-2017, and summer 2017-2018), and yearly at land reclamation rates (5x agronomic N rate; fall 2016-2017); while a heat-dried EQ biosolids and inorganic fertilizer control were applied at the agronomic N rate only. Soil bulk density decreased two years after the yearly fall application of EQ biosolids at the reclamation rate. Soil C accumulation two years after biosolids applications ranged between 37 to 84%, highlighting the potential use of these products to sequester carbon in urban degraded soils. Soil N availability was limited, even after cumulative biosolids rates. Reduced effect of cumulative biosolids rates was likely due to lack of residual soil C and N, and high clay content; which limited organic N mineralization. Despite

limiting physical, chemical, and biological conditions of our soil, the addition of biosolids at higher than agronomic rates (i.e. reclamation rate) showed great potential to increase vegetable yield after two years of application.

5.2. Introduction

Urban agriculture has been part of city life for many years, but the growth of urban agriculture in more recent years is due to a greater availability of vacant lands that have the potential to be used to grow crops, and an overall shift towards the need of a more sustainable way of living in urban areas (Lawson et al., 2003; Palmer, 2018). Agriculture in the cities can have a strong influence on creating more sustainable cities through the offering of multiple ecosystem services. Urban agriculture can help provide food to the most vulnerable populations in a city, contributing to increased food security (Altieri et al., 1999). It may also promote social interaction, community development, and more active lifestyles (Thompson et al., 2007; van den Berg et al., 2010). Beyond these provisioning and cultural services, urban agriculture may also provide regulating services such as storm water management, increased biodiversity, recycling of organic residues, carbon sequestration, and rehabilitation of urban degraded lands (USEPA, 2011; Kulak et al., 2013; Matteson et al., 2008).

Among the greatest challenges of urban agriculture are the commonly degraded nature and potential presence of contaminants in urban soils. In contrast to natural soils, human activities strongly influence urban soils through the removal of topsoil, physical disturbances, heavy vehicle traffic, and addition of foreign materials (i.e. asphalt, bricks, glass, plastic, among others), which ultimately results in a highly impaired, heterogeneous soil. (Craul, 1985; Gregory et al., 2006; Beniston and Lal, 2012). These disturbed soils are characterized by soil compaction, increased bulk density, reduced water infiltration rates and aeration, low water holding capacity,

low organic matter content, and low fertility, which ultimately limit crop growth and yields, and create major constraints for productive urban agriculture (Gregory et al., 2006; Beniston and Lal, 2012).

Organic amendment application is one of the practices that can restore urban degraded soils and improve growing conditions. The use of organic amendments can replenish soil organic carbon (C), which will favor microbial activity and the mineralization of essential nutrients such as nitrogen (N) and phosphorus (P) (Bot and Benites, 2002; Beniston and Lal, 2012). Organic matter additions have helped restore soil structure by contributing to lower bulk density, and increase soil porosity, aggregation, water holding capacity, and plant available water (Brown et al., 2011; Garcia-Orenes et al., 2005; Khaleel et al., 1981). In addition to these benefits, organic matter application to urban soils provides an opportunity to recycle locally-generated urban wastes, such as exceptional quality (EQ) biosolids products.

Class A Exceptional Quality biosolids are by-products from wastewater sludge treatment. Class A biosolids are obtained by Processes to Further Reduce Pathogens (PFRPs). Some PFRP treatments methods include well-known processes such as composting and thermal drying, and newer treatment processes such as pasteurization and thermal hydrolysis followed by anaerobic digestion (USEPA, 2006; Abu-Orf et al., 2012). The designation of Exceptional Quality (EQ) derives from the characteristic low pollutant concentrations and reduced vector attraction of these biosolids (USEPA, 1994). Such attributes permit the safe use of EQ biosolids in urban landscapes. More recently developed EQ biosolids have been mixed with sources of dry organic and/or a mineral matter with the purpose of reducing moisture content, enhancing structure, and improving ease of handling and spreading.

Biosolids have been used as soil conditioners and fertilizers in forestry, agriculture, and reclamation of mine land (Brown and Chaney, 2000; Kerr and Sopper, 1982; Binder et al., 2002). Positive responses on tree height and diameter, and woody biomass have been reported with single applications of biosolids to forest soils (Brown and Chaney, 2000; Kerr and Sopper, 1982). Cogger et al. (2013) found that medium to high biosolids rates resulted in greater grain yield and protein increase of wheat than anhydrous NH_3 additions. Comparable or better tomato and squash yields were also determined with biosolids compost application compared to an inorganic fertilizer (Jaber et al., 2005), while greater tomato yields were obtained with heat-dried biosolids application than composted biosolids (Ozores-Hampton et al., 2004). Thus, biosolids generated by different processes may also result in distinct crop yield responses due to their diverse influences on soil properties and nutrient availability. Heat-treated biosolids generally provide greater available N and P due to lower C: N ratio and generally higher nutrient concentration, which promotes microbial activity and nutrient mineralization. In contrast, composted biosolids have a higher C: N ratio and slower N mineralization; however, they also add more organic matter which can have a greater effect on improvement of soil physical properties (i.e. bulk density, aggregation, etc.).

Application of biosolids in agriculture and forestry are based on N crop requirement or agronomic N rate, while biosolids applications to mine lands are performed at higher than agronomic rate for the purpose of accelerating the rehabilitation of these disturbed lands. For instance, greater forage yields were observed with high applications of a mix of 1/3 anaerobically stabilized biosolids cake and 2/3 composted wood chips (184, 368, and 552 Mg ha^{-1}) than with an inorganic fertilizer and unfertilized control in southwest Virginia mine lands after two growing seasons (Daniels and Haering, 1994). Rapid establishment and growth of tall

fescue, perennial ryegrass, and western wheatgrass was also shown after high biosolids rate applications to a disturbed mine land (224, 448, and 896 Mg ha^{-1}; Hinesly et al., 1982). Such high biosolids application rates to mine lands may result in short-term nitrate N (NO_3-N) leaching loss; however, this is typically permitted by regulation because it is needed in order to restore the productivity of the mine land soil (Sopper, 1993; Brown and Chaney, 2000).

The application of EQ biosolids in urban agriculture could be based on either crop agronomic N rate or soil reclamation rate, depending on the extent of soil degradation. Agronomic N rates might be appropriate in less degraded urban soils, but previous studies have shown that large application rates of organic amendments (e.g. compost) to urban degraded soils have consistently improved soil physical properties under tree and ornamental production. This suggests that such magnitudes of organic matter additions can benefit urban agriculture (Scharenbroch, 2009; Weindorf et al., 2006; Cogger et al., 2005). Improved soil quality, nutrient availability, soil enzymatic activity, and vegetative performance of a native seed mix (grasses, legumes, and forbs) were also reported after a high biosolids application rate (202 Mg ha^{-1}) to an urban degraded soil (Basta et al., 2016). Most studies have focused on evaluating the effect of biosolids on soil properties, and growth of trees and grasses in urban areas; however, agricultural studies conducted to assess vegetable agricultural potential of urban degraded soils are lacking.

There is no peer-reviewed, published research that has demonstrated the effect of biosolids application on mineral and vitamin content of vegetables. Variable results of vegetable nutrient and vitamin contents have been found for organic amendment and inorganic fertilizers (Warman and Havard, 1996; Woese et al., 1997). A comparison between inorganic and organic amendments used to grow various crops showed no differences in vitamin content of potato and sweet corn after three years of treatment application; however, potatoes had greater phosphorus

(P), magnesium (Mg) and boron (B) content when grown with organic amendment applications compared to inorganic fertilization (Warman and Havard, 1996). Greater cabbage vitamin C was achieved with organic amendment addition than inorganic fertilizer on the first year of a field study, but the opposite was true for the second year of this study (Warman and Havard, 1996). Cabbage P and B contents were higher with organic amendments than inorganic fertilizer during the first two years of this study, yet the inorganic fertilizer showed higher contents of these nutrients in the third year. A literature review conducted to compare the effects of organic amendment and inorganic fertilizer use on vitamin and mineral content of crops demonstrated a lack of effect of such treatments on vitamin B1, B2, and A. Half of the studies showed that crops have higher vitamin C when grown with organic amendment additions than with inorganic fertilizers, while the other half showed no major differences between inorganic and organic fertilized amendments (Woese et al., 1997).

The effects of biosolids have largely been studied on agronomic crops, forestry, and re-vegetated mine lands, but less is known about the effects of such recently developed EQ biosolids products on vegetable yield and nutrient/vitamin content in urban agriculture. Due to the increasing importance of urban agriculture and the potential to use local EQ biosolids to improve degraded urban soils, the objectives of this study were to compare the effects of various EQ biosolids products and inorganic fertilizer on vegetable yield and nutrient content, and soil chemical and physical properties of an urban soil.

5.3. Materials and Methods

5.3.1. Site description

The urban garden field research was conducted at the Virginia Tech Turfgrass Research Center (TRC) in Blacksburg, VA, which is approximately 634 m above sea level. The site is

within the USDA cold hardiness zone 6b, and is referred to as mountain temperate or humid continental (Daly et al., 2012). The pre-existing site soil was excavated to a depth of 45 cm and replaced with a subsoil fill from a nearby construction site to simulate an urban degraded soil. The soil map unit description of the subsoil fill used was a Groseclose (Fine, mixed, semiactive, mesic Typic Hapludults) urban land complex characterized with a clayey texture and slow permeability.

The area of the field research site was 253 m^2 (22 m long x 11.5 m wide). The fill soil was tilled several times in preparation for plot layout and vegetable planting. A tractor- mounted rear-tine tiller was used to break apart large clods of soil. Large rocks and pieces of plastic, asphalt, and trash were removed from the site, as these materials came to surface after tilling. Soil preparation was finished during the summer of 2016.

5.3.2. Biosolids sources

Four EQ biosolids were evaluated in this field study. Two products were obtained from Blue Plains Advanced Wastewater Treatment Plant (DC Water) in Washington, DC (https://www.dcwater.com/blue-plains). One of their products is an air-dried EQ biosolids (BLOOM^{TM}), which is produced via thermal hydrolysis followed by an anaerobic digestion (Cambi^{TM} process). A second product from DC Water is a blend of 0.75 BLOOM:1.0 shredded, woody mulch (dry weight basis) (BM). The biosolids compost was generated by Livingston Composting in Spotsylvania County, VA (http://www.spotsylvania.va.us/Compost; LBC) from an anaerobically digested biosolids. The heat-dried, pelletized biosolids was obtained from Ocean County Utilities Authority in Bayville, NJ (http://www.ocua.com/SitePages/Home.aspx), marketed as OceanGro®, and identified as OCB in our study.

Each biosolids product was sampled prior to field application at the beginning of each growing season and sent to Waypoint Analytical Laboratories to be analyzed for total solids (SM-2540G) (APHA, 1992); Total Kjeldahl Nitrogen (SM-4500-NH3C-TKN) (APHA, 1992); ammonia-nitrogen (SM-4500-NH3C) (APHA, 1992); nitrate (SM-4500NO3F) (APHA, 1992); organic nitrogen (calculation); phosphorous, potassium, sulfur, calcium, magnesium, sodium, iron, aluminum, copper, zinc (SW 6010C); heavy metal concentrations (SW-6010C)(USEPA, 2000); and pH (SW-9045D) (USEPA, 2000). Total carbon (C) and nitrogen (N) were determined by dry combustion with a Vario Max CNS macro elemental analyzer (Elementar, Hofnau, Germany) at Virginia Tech SPES research laboratory facilities.

5.3.3. Experimental design

The field study was established in a randomized complete block design with eight fertility treatments in each block, and four replicate blocks for a total of 32 plots. Treatment plots were 4.5 m^2 (1.8 m long x 2.5 wide), and blocks were separated by a 0.6 m alley. Treatments consisted of BLOOM, BM, and LBC applied at the agronomic N rate (1x) and at five times the agronomic N rate (5x) or reclamation rate. The inorganic fertilizer (urea, 46% N) and OCB were applied at the agronomic rate only. Agronomic N rates were meant to supply adequate N for vegetable production according to Virginia Tech Soil Testing Laboratory recommendations for agricultural soils (Maguire and Heckendorn, 2017), while the high application rate was based on mine land reclamation rates that consist of a single large application of biosolids to facilitate rapid restoration of degraded soil (Sopper, 1993; Daniels and Haering, 1994). Treatments applied at the agronomic rate were applied at the beginning of each cropping season, while treatments applied at 5x agronomic N rate were applied once a year before the beginning of the fall cropping season. Our calculations for EQ biosolids applications were based on organic N

mineralization rates of 30% for BLOOM, 20% for BM, 15% for LBC, and 40% for OCB, which were based on the results of previous studies (Alvarez-Campos et al., 2018; Gilmour et al., 2003).

5.3.4. Supplemental fertilization

Supplemental P and K were applied in the forms of triple super phosphate (0-46-0) and muriate of potash (0-0-60) based on soil test nutrient recommendations for adequate vegetable growth. The application of EQ biosolids at the estimated agronomic N rate and reclamation rate supplied adequate P (98 kg P ha^{-1}) for vegetable growth in the initially P deficient soil. However, inorganic K fertilizer was needed to provide sufficient K (93 kg K ha^{-1}). The agronomic N rate application of EQ biosolids supplied adequate P (86 kg P ha^{-1}) during the summer of 2017, but supplemental P was added to reclamation rate treatments since soil P concentrations were still below critical levels and these treatments were not applied during the summer seasons. Inorganic K additions (140 kg K ha^{-1}) were required for both agronomic and reclamation rates in the summer of 2017. Supplemental P was not needed in the fall of 2017 and summer of 2018, but supplemental K was required for agronomic rates in the fall of 2017 and for BM and BLOOM reclamation rates in the summer of 2018.

5.3.5. Vegetable planting and agronomic management

A gasoline-powered, 4-stroke, single cylinder rotary tiller (23 cm tilling width and 20 cm maximum tilling depth) and a digging fork were used to incorporate the soil amendments within one week of planting seeds/transplanting seedlings, and to prepare an adequate bed for planting of the vegetable crops. A rake was used to level the surface of the soil and a hoe was used to prepare the soil for planting at the appropriate depth.

Fall Garden

Black seeded simpson lettuce (*Lactuca sativa*), siberian kale (*Brassica napus* L. var. *pabularia*), red meat/watermelon radish (*Raphanus sativus*), red ace beets (*Beta vulgaris*), and storage No 4 cabbage (*Brassica oleracea L. var. capitata L*) were crops selected for fall garden planting. Lettuce, kale, radish, beets, and cabbage were grown in 2016, and cabbage, kale and beets were grown in 2017. The agronomic N requirements for these vegetables range from 84 kg to 140 kg N/ha (75 to 125 lbs N $acre^{-1}$). The inorganic fertilizer and biosolids products were applied to supply the mean N rate of 112 kg PAN ha^{-1} and 560 kg PAN ha^{-1} for reclamation rate treatments. Treatment applications were made during the second and fourth weeks of August 2016 and 2017, respectively. Vegetable crops (as seeds or transplants) were planted 3-5 days after biosolids application each year.

In 2016, each plot had lettuce planted in 60-cm row with 10-cm spacing between plants for a total of 6 plants per plot. Kale was planted in a 90-cm row with 30.5 cm spacing between plants for a total of 3 plants per plot. Radishes were also planted in a 60-cm row, but with a spacing of approximately 6-cm between plants for a total of 10 radishes per plot. Similarly, beets were planted with 6-cm distance between plants and in 120-cm rows for a total of 20 plants per plot. Cabbage was germinated in the greenhouse and transplanted to the field a month after germination. Three cabbages were planted with approximately 50-cm distance between plants in 150-cm row at each of the plots. The spacing between rows was of 45.7-cm for a total of 3 rows per plot for every growing season. In 2017, crops were planted in 213-cm rows, at the same spacing between plants.

Fall vegetable crops were harvested in early November 2016 and 2017. Each cabbage head, and kale and lettuce leaves were cut at the base with a sharp knife. Cabbage head and kale

and lettuce biomass wet weight were recorded for each treatment plot. Radishes and beets were carefully pulled out of the ground and washed. Their weight was recorded as below-ground biomass.

Summer Garden

Vegetables were grown during spring and summer 2017 and 2018. Summer vegetable crops included raven (F1) zucchini squash (*Cucurbita pepo*), gourmet (F1) bell pepper (*Capsicum annuum*), and bush-type cosmos green beans (*Phaseolus vulgaris)*. We applied the EQ biosolids products and inorganic fertilizer at 112 kg PAN/ha (100 lbs PAN/acre). Treatments were applied on the third week of May and vegetable crops were planted two days after their application. All three crops were planted in a 213-cm row. Zucchini was planted with 60-cm spacing between plants for a total of 3 plants per plot. Bush beans were planted with approximately 7.5-cm spacing between plants for a total of 28 plants per plot. Poor germination of the summer 2017 crops required thinning to 10 plants per plot, i.e., the stand count of plants in the lowest germinated plots. Green bean germination was greater in the summer of 2018; thus, all plots were thinned to 20 plants in order to match the amount of plants of the plot with lowest germination. Bell peppers were germinated in the greenhouse and transplanted to the field a month after germination. The spacing between bell pepper plants was approximately 50-cm apart for a total of four plants per plot. Zucchini and bush beans were harvested throughout the growing season, approximately 2-3 times a week. Bell peppers were harvested in the first week of August. During harvest, the produce was carefully detached from the plant and the wet weight by plot was recorded.

An electric fence powered with solar panels was installed at the beginning of the experiment to prevent rodents and deer from gaining access to the garden. Additionally, a

chicken wire was installed surrounding the field site in the summer of 2017. To maintain adequate soil moisture for germination and vegetable growth during dry periods, sprinkler irrigation was used during fall 2016, and drip irrigation was used during 2017 and 2018. Throughout the experiment, pest control included the use of *Bacillus thuringiensis* (BT) to control for the cabbage looper (*Trichoplusia ni*), and hand picking/killing of Mexican bean beetle and larvae (*Epilachna varivestis* Mulsant). Weed control was performed manually throughout most of the experiment, except for the summer of 2018 when Segment herbicide was applied to control for grass weeds.

5.3.6. Soil and plant sampling

A soil corer (2-cm diameter) was used to collect eight 15-cm depth composite random benchmark soil samples across each replicate block before treatment application for the purpose of characterizing soil benchmark properties and identifying potential nutrient deficiencies requiring remediation. Soil samples were air-dried and sieved through a 2-mm mesh in preparation for routine soil testing analysis.

Soil samples were collected approximately eight weeks after EQ biosolids products application during each growing season to determine amendment effects and nutrient requirements for the next growing season. A 2-cm diameter soil corer was used to collect eight soil samples from each treatment plot to depth of 10 cm. Soil samples from each treatment plot were mixed to obtain a single representative soil sample. Subsequently, soil samples were air-dried and ground to pass through a 2-mm sieve in preparation for soil chemical analyses.

Zucchini and green beans harvested from BLOOM-1, BLOOM-5 and inorganic fertilizer treatment on several dates during summer 2018 were subsampled and processed for vitamin and

mineral analyses. Sliced zucchini and whole green beans were frozen immediately upon transporting to the laboratory.

5.3.7. Soil and plant analyses

Soil chemical properties

Soil chemical analyses included pH; total organic carbon (TC); total nitrogen (TN); ammonium N (NH_4-N); nitrate N (NO_3-N); Mehlich I extractable P, K, Ca, Mg, Zn, Mn, Cu, and Fe (Maguire and Heckendorn, 201); and Olsen P (Olsen, 1957). Soil pH was determined on a 1:1 (w/v) soil to water mixture (Maguire and Heckendorn, 2011). Soil NH_4-N and NO_3-N were measured in a 2.0 M KCl extract at a 1:10 soil to KCl solution ratio. The solution was extracted by shaking in a reciprocating shaker (Eberbach Corporation, Ann Arbor, Michigan) at 180 rpm for 30 minutes. The supernatant was filtered through a 0.45 µm filter paper, and analyzed through flow injection on a Lachat 8500 to determine NO_3-N (QuikChem Method 12-107-04-1-B; Knepel, 2001) and NH_4-N (QuikChem Methods 12-107-06-3-B; Hofer, 2001). Mehlich 1 P, K, Ca, Mg, Zn, Mn, Cu, and Fe were extracted (4 cm^3 of soil to 20 ml of Mehlich 1 extracting solution) by the Virginia Tech Soil Testing Lab (Maguire and Heckendorn, 2011). Olsen P was determined by extracting 2 g of soil with 40 ml of 0.5 M sodium bicarbonate, shaking in a reciprocating shaker for 30 minutes, and filtering through a Whatman 40 filter paper (Olsen, 1957). The resulting Olsen extraction was sent to the Virginia Tech Soil Testing Laboratory to be analyzed for P in a Thermo Elemental ICAP 6IE.

Soil TC and TN were analyzed by combustion with a Vario MAX CN macro elemental analyzer (Elementar, Hanau, Germany). Because the soil was calcareous, a hydrochloric acid (HCl) fumigation procedure was conducted to remove inorganic C, and obtain organic C with C analysis. The soil was ground in an electric mortar grinder RM200 (Retsch, Inc., Newtown, PA)

and passed through a 180 µm sieve. Fumigation was performed by adding 3 ml of deionized water to 3 g of soil placed in a 50 ml glass beaker to moisten the soil to approximately field capacity. The beakers with soil were placed in a desiccator, and a 150-ml glass beaker containing 100 ml of 12M HCl was placed in the center of the desiccator (Harris et al., 2001). Air was extracted from the desiccator, and the samples were exposed to HCl vapor for 6 hours. After fumigation, soil samples were rinsed with deionized water until the pH of the rinse was raised from 2 to >6 in preparation for combusting in the CN analyzer. Soil samples were then dried at 60 °C for 24 hours and manually ground with a mortar and pestle. Soil carbon stored was calculated as follows:

$Cs=\rho b \times C_c \times d \times 10^{-3}$

where Cs is the soil C stored (kg ha^{-1}), ρb is the soil bulk density (kg m^{-3}), C_c is the total soil organic C concentration (mg kg^{-1}), and d is the soil sampling depth (m). Then, the % soil C accumulation was determined by:

$$CA = \frac{Csa - Csc}{Cr} x100$$

where, Csa is the C stored in the amended soils, Csc is the C stored in the control soil, and Cr is the C application rate (kg ha^{-1})

Soil physical properties

Soil texture was determined by hydrometer method (Gee and Bauder, 1986). Analyses of physical properties were conducted at the end of the experiment because physical properties generally require a longer period of time to show changes after organic amendment addition. Soil bulk density was determined by using the excavation method (Page-Dumroese et al., 1999). Four soil samples of approximately 15-cm diameter and 10-cm depth were excavated from each treatment plot. The volume of the hole was determined by lining the hole with plastic and

recording the amount of water needed to fill the hole. The weight of each soil sample was recorded. Approximately 50 g of soil was oven-dried at 105 ℃ to determine the moisture content of the soil sample. This value was used to estimate the oven-dry weight of the whole sample, and calculate the bulk density as the oven-dry weight divided by the volume of the soil. Particle density was assumed to be 2.86 Mg m^3 due to the significant amounts of clay present in the urban soil (Schjønning et al., 2017). Soil bulk density and particle density were used to calculate total porosity as one minus the bulk density divided by particle density (Hao et al. 2008).

Field capacity (FC) and permanent wilting point (PWP) were determined by using pressure extraction (Klute, 1986). Soil samples obtained via excavation method were air-dried and sieved to < 2 mm to remove rock fragments, and pieces of asphalt and brick that were present in the disturbed urban soil. Retainer rings were placed in a ceramic plate, and approximately 10-15 g of sieved soil was place in each ring. Water was added to cover the plate (not the rings) in order to saturate the samples overnight, and saran plastic wrap was used to cover the plates with the samples to prevent evaporation. The plate was placed in the pressure-plate extractor the next day and the desired pressure was applied until the samples equilibrate (i.e. water flow ceases to emit from outflow tube). Field capacity was obtained by applying a pressure of -33 kPa, while permanent wilting point was obtained by applying a pressure of -1500 kPa. Available water holding capacity (AWHC) was estimated as the difference between FC and PWP.

Vegetable mineral and vitamin content

Vegetable samples were sent to Exova laboratories (https://www.exova.com/) in Portland, Oregon to be analyzed for protein content, Ca, P, Fe, Mg, Zn, and vitamins A, B2, and B6. Each sample was blended to ensure a homogeneous sample and then digested to use for

extraction of the target nutrients and vitamins. Protein content was determined using a Kjeldahl N acid digestion followed by titration (Method 981.10; AOAC, 2016). Calcium, iron, magnesium and zinc were obtained by acid digestion followed by Flame Atomic Absorption Spectrometry (Method 7000B; USEPA, 2007). Phosphorus was determined by acid digestion followed by spectrophotometric reading at 400 nm (Method 965.17; AOAC, 2016). Vitamin B2 was obtained by liquid extraction followed by fluorometric detection (Method 970.64; AOAC, 2016), and vitamin B6 (Mann et al., 2005) and C (Iwase, 2000) were determined by liquid extraction followed by high-performance liquid chromatography (HPLC) analysis. Ethanolic KOH saponification, liquid extraction and HPLC analysis was used to determine Vitamin A and Vitamin E (Method 2001.13; AOAC, 2016).

5.3.8. Statistical Analyses

Analyses of variance and multiple comparisons (Tukey HSD) were performed with the GLIMMIX procedure in SAS 9.4 (SAS Institute, 2011) to determine statistically significant differences in vegetable yield and soil chemical parameters after EQ biosolids and inorganic fertilizer treatment applications over time. Vegetable mineral/vitamin results and soil physical parameters were analyzed for a single sampling date. Treatment differences were considered significant at the $p < 0.05$ level. Treatment, harvest, and soil sampling dates were analyzed as fixed effects. All data was normally distributed as determined by the distribution of studentized residuals, except for kale yield which had to be log transformed.

5.4. Results and Discussion

5.4.1. Biosolids

The EQ biosolids products had a pH that ranged from acidic to slightly basic (Table 5.1). The moisture content of these products is low, which facilitates their handling and application for

urban agriculture by urban farmers, community gardeners, and homeowners. Most N was found in organic form that would require mineralization to become available for plant uptake. The products BLOOM and OCB had lower C:N ratios than BM and LBC due to the presence of woody material in BM and LBC (Table 1); thus, BLOOM and OCB were expected to have greater short term mineralizable N.

Phosphorus concentration was also greater in BLOOM and OCB because these products did not include woody materials to dilute P concentration. Potassium concentrations were low in all biosolids. Due to their low K concentrations, inorganic K fertilization was required to supply adequate plant-available K for vegetable production. Biosolids from DC Water (BLOOM and BM) had higher Fe concentrations than LBC and OCB due to the addition of ferric salts for P removal during wastewater treatment processes (Jameson et al., 2016). Zinc and Cu concentrations were higher in non-woody amended biosolids (BLOOM and OCB), while Al was lowest in the BM product (Table 5.1).

Table 5.1. Chemical properties of EQ biosolids products applied to the urban garden field study.

	Unit	BLOOM†	BM	LBC	OCB
pH‡		5.17	6.18	6.86	7.3
Total Solids‡	g/kg	567	870	892	950
Moisture‡	g/kg	435	130	108	50
C	g/kg	248	355	334	383
C:N		8.1	12.5	12.0	7.8
TKN	g/kg	30.8	28.4	27.7	49.1
NH_4-N	g/kg	1.9	4.7	3.1	5.7
Organic N	g/kg	28.9	23.7	24.6	43.4
NO_3-N	mg/kg	1240	719	1170	7.21
TP	g/kg	30.9	19.3	13.3	26.6
K	g/kg	1.5	2.1	4.9	1.4
Ca	g/kg	24.7	20.7	26.6	28.4
Mg	g/kg	4.1	2.9	3.2	4.3
Zn	mg/kg	824	511	168	1110
Cu	mg/kg	388	262	302	535
Fe	mg/kg	82600	56100	26700	32800
Al	mg/kg	11900	8400	15300	14700

† BLOOM= air-dried Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried.

‡ Values are on a dry weight basis except as noted.

§ All elemental values are total concentrations.

The amounts of biosolids applied to the urban garden after four growing seasons (fall 2016, summer 2017, fall 2017, and summer 2018) ranged from 16 Mg ha^{-1} to 68 Mg ha^{-1} for biosolids applied at the agronomic rate; and 116 Mg ha^{-1} to 176 Mg ha^{-1} for biosolids applied at the reclamation rate (Table 5.2). The seasonal application rate of biosolids was designed to supply N at an agronomic N rate, while the single annual rates were designed to supply 5x the agronomic N rate. Agronomic rates were deigned to supply 112 kg N ha^{-1}cropping $season^{-1}$. Biosolids rates were based on target N rate and organic N mineralization rate assumptions of 30% for BLOOM, 20% for BM, 15% for LBC, and 40% for OCB. We did not estimate residual (i.e., after first year) mineralizable N contributions for calculating second year biosolids application rates. The amounts of P applied with biosolids treatments were higher than that applied with the inorganic fertilizer treatment. Application of biosolids also added considerable C, especially at the reclamation rates, after the four vegetable growing seasons (Table 5.2).

Table 5.2. Cumulative amount of biosolids, total organic carbon (C), nitrogen (N), and phosphorus (P) applied during the period August 2016 to May 2018 as measured in 2018.

Treatment†	Biosolids addition (Mg ha^{-1})	TN applied (kg ha^{-1})	P applied (kg ha^{-1})
Fertilizer		448	185
BLOOM-1‡	52	1358	1407
BM-1	65	1427	711
SBC-1	68	1636	425
BLOOM-5	116	3394	3155
BM-5	144	3552	1578
SBC-5	176	4469	1115
OCHDB	19	955	547

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, 5=reclamation rate (dry weight basis)

5.4.2. Soil Physical Properties

Particle size analysis of the benchmark soil sample revealed that the soil's textural class was clay (630 g kg^{-1} clay, 150 g kg^{-1} silt, and 220 g kg^{-1} sand). Such high clay-content soils, often present at new construction areas, will typically limit vegetation establishment and growth success. Soil bulk density was lower and porosity was higher with the reclamation rate treatments than with the inorganic fertilizer by the end of the expeirment (Table 5.3). Reclamation rate treatments, BM-1, and BLOOM-1 increased water-holding capacity on a weight (g g^{-1}), but not volume (cm^3 cm^{-3}), basis at FC compared to the inorganic fertilizer and OCB treatments. This was likely due to differences between field and laboratory bulk densities, since laboratory measurements were conducted with disturbed, sieved samples rather than intact cores. Water-holding capacity at PWP did not change with any treatment application. Soil AWHC (g g^{-1}) increased on a weight, but not volume, basis with BLOOM-5, LBC-5, and LBC-1 compared to the inorganic fertilizer (Table 5.3).

Soil physical parameters improved with reclamation rates, likely due to the high organic matter inputs. Improvements in soil physical parameters such as bulk density, aggregate stability, available water, water holding capacity at -33 kPa (FC) and -1500 kPa (PWP), and infiltration rate have been reported after the addition of biosolids to disturbed urban soils (Bary et al., 2016; Garcia-Orenes et al., 2005; Hinesly et al., 1982). For instance, aggregate stability and available water holding capacity increased after high applications of biosolids to a clay loam mine land spoil bank in Fulton County, Illinois (Hinesly et al., 1982). Incorporating biosolids to a compacted highway roadside in Tacoma, Washington, also decreased bulk density by greater than 50% compared to the control with no amendments (Bary et al., 2016).

Table 5.3. Soil bulk density (BD), porosity, field capacity (FC), permanent wilting point (PWP), and available water holding capacity (AWHC) two years after the application of EQ biosolids products to an urban degraded soil. Values with same letters in columns are not significantly different ($p<0.05$).

Treatments†	BD	Porosity	FC	FC§	PWP	PWP	AWHC	AWHC
	g cm-^{3}	%	g g^{-1}	cm^3 cm^{-3}	g g^{-1}	cm^3 cm^{-3}	g g^{-1}	cm^3 cm^{-3}
Fertilizer	1.34 a	0.53 c	0.311 b	0.321	0.218	0.25	0.09 b	0.072
BLOOM-1†	1.28 ab	0.55 bc	0.338 a	0.315	0.229	0.25	0.108 ab	0.070
BM-1	1.27 abc	0.56 abc	0.336 a	0.316	0.226	0.24	0.110 ab	0.080
LBC-1	1.26 abc	0.56 abc	0.331 ab	0.308	0.208	0.23	0.122 a	0.078
BLOOM-5	1.15 c	0.60 a	0.350 a	0.319	0.226	0.23	0.124 a	0.087
BM-5	1.21 bc	0.58 ab	0.350 a	0.322	0.236	0.24	0.114 ab	0.082
LBC-5	1.16 bc	0.59 ab	0.348 a	0.315	0.219	0.24	0.129 a	0.077
OCB	1.27 abc	0.56 abc	0.313 b	0.301	0.205	0.24	0.112 ab	0.069

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, and 5=reclamation rate.
§ Field capacity (FC) expressed on a weight (g/g) and volume (cm^3/cm^3) basis to show the influence of the altered bulk density of sieved soil samples on FC results on a volume basis.

5.4.3. Soil Chemical Properties

Analysis of the benchmark soil samples gave a pH of 7.7, 6.7 g total organic C kg^{-1}, 0.4 g total N kg^{-1}, and Mehlich 1 results of 3 mg P kg^{-1}, 56 mg K kg^{-1}, 2594 mg Ca kg^{-1}, 656 mg Mg kg^{-1}, 2.3 mg Zn kg^{-1}, 21 mg Mn kg^{-1}, and 1.3 mg Cu kg^{-1}. Fertilizer recommendations for Virginia soils were obtained from Virginia Tech Soil Testing Laboratory calibrations for Mehlich 1-extractable elements (Maguire and Heckendorn, 2017). The Mehlich I extraction has been developed for application to southeastern U.S. acid soils. The initial high pH, and Ca and Mg concentrations are indicative of a calcareous soil, which only occur in humid Virginia as subsoils formed from limestone parent material (Creggar et al., 1985).

The very low Mehlich 1-P, even upon amending with high applications of biosolids further demonstrated the calcareous nature of the subsoil (Table 5.2 and 5.4), whose free carbonates likely partly neutralized the acid-extracting properties of the Mehlich I extract. Therefore, we also performed Olsen P extractions, which are more appropriate for calcareous soils. The benchmark soil Olsen P concentration was 11mg/kg. Such an Olsen P soil test value is interpreted as low and in need of additional P for optimum plant growth (Franzen, 2007; Horneck et al., 2011; Manitoba Phosphorus Expert Committee, 2006).

Soil Olsen P and Mehlich 1 P increased with EQ biosolids application over time (Table 5.4 and 5.5). Reclamation rates resulted in significantly greater Olsen P and Mehlich P throughout the experiment. These results were expected since much larger amounts of P were applied with EQ biosolids applications (Table 5.2), while the inorganic P fertilizer was not applied once P concentrations were adequate based on soil test recommendations. There were no significant treatment effects on soil Mehlich 1 K that could not be accounted for by supplemented inorganic K fertilizer addition. Extractable Ca and Mg concentrations were very

high in the calcareous soil at the beginning of the study and remained so for the duration of the research (Table 5.4 and 5.5).

Table 5.4. Olsen phosphorus (P), and Mehlich 1 P (M1-P), potassium (M1-K), and calcium (M1-Ca) eight weeks after treatment application on fall 2016, 2017, and summer 2018 growing seasons.

Treatment	Olsen P			M1-P		
	----------mg kg^{-1}----------					
	Oct-16	**Oct-17**	**Jul-18**	**Oct-16**	**Oct-17**	**Oct-18**
Fertilizer	17 bc C	43 de A	32 f B	9.0 C	30 b B	52 b A
BLOOM-1†‡	18 bc C	52 d B	75 c A	7.3 A	15 b A	23 de A
BM-1	14 c C	35 e B	46 e A	5.8 A	10 c A	16 e A
LBC-1	15 c C	42 de B	63 d A	7.0 B	22 bc B	56 b A
BLOOM-5	36 a B	122 a A	117 a A	9.0 B	23 bc AB	33 cd A
BM-5	23 bc B	72 c A	70 cd A	11.3 B	27 bc AB	30 cde A
LBC-5	27 ab B	97 b A	98 b A	13.5 C	77 a B	100 a A
OCB	15 c B	37 e A	44 e A	5.5 B	18 bc B	42 bc A

Treatment	M1-K			M1-Ca		
	----------mg kg^{-1}----------					
	Oct-16	**Oct-17**	**Oct-18**	**Oct-16**	**Oct-17**	**Oct-18**
Fertilizer	77 B	110 ab A	78 ab B	2897 A	2917 A	2779 A
BLOOM-1	76 B	112 ab A	65 bc B	2759 A	2934 A	2653 A
BM-1	75 B	110 ab A	70 bc B	2879 A	3083 A	2947 A
LBC-1	69 B	94 b A	82 ab AB	2797 A	2930 A	2854 A
BLOOM-5	72 A	59 c B	85 ab A	3376 A	3432 A	2900 B
BM-5	68 A	49 c B	77 ab A	2662 A	2913 A	2828 A
LBC-5	84 B	126 a A	93 a B	2932 A	3011 A	2692 A
OCB	75 B	122 a A	61 c B	3102 A	3021 A	2592 B

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, and 5=reclamation rate.

Table 5.5. Analysis of variance F ratios and levels of significance of soil nutrient parameters for treatment, date, and interaction effects.

Parameters	Treatment		Date		T * D	
	F	**Pr>F**	**F**	**Pr>F**	**F**	**Pr>F**
Olsen P	65.27	<0.0001	493.07	<0.0001	18.77	<0.0001
M1-P	21.23	<0.0001	66.27	<0.0001	4.83	<0.0001
M1-K	6.33	0.0003	27.08	<0.0001	6.12	<0.0001
M1-Ca	0.83	0.5701	6.26	0.0039	0.86	0.6079
M1-Mg	0.77	0.6164	9.12	0.0004	1.38	0.2009
M1-Zn	36.94	<0.0001	123.03	<0.0001	9.47	<0.0001
M1-Cu	56.28	<0.0001	14.09	<0.0001	6.48	<0.0001
TC	12.16	<0.0001	111.17	<0.0001	7.58	<0.0001
TN	23.69	<0.0001	155.15	<0.0001	10.37	<0.0001
NO_3-N	4.54	0.0024	2.68	0.079	2.36	0.014
NH_4-N	2.22	0.0688	1.99	0.1476	2.2	0.0218

All EQ biosolids treatments increased Zn concentrations over time (Table 5.6), but Zn was never increased to deleterious concentrations for plant growth. Treatments BLOOM-5 and BM-5 elicited the greatest increases, having been provided 10x the agronomic N rate over the duration of the study. Soil Cu concentrations increased only with agronomic and reclamation rates of BLOOM and OCB. Although EQ biosolids treatments overall increased soil Zn, and BLOOM and OCB increased soil Cu, the concentrations of both metals in the soil are still well below annual pollutant loading rates limits as established by USEPA, Part 503 (USEPA, 1995). In addition, the relatively high pH of our urban soil would most likely maintain Zn and Cu in insoluble forms and would reduce their uptake by plants (Evanylo et al., 2006).

Table 5.6. Soil micronutrient concentration at fall 2016, 2017, and 2018 growing seasons with the application of various EQ biosolids treatments.

Treatment	Zn		
	----------mg kg^{-1}----------		
	Oct-16	**Oct-17**	**Oct-18**
Fertilizer	5.3 ab A	3.65 e A	5.5 e A
BLOOM-1†‡	3.4 ab C	9.4 c B	13.6 bc A
BM-1	3.1 b B	7.6 cd A	10.0 d A
LBC-1	2.7 b B	5.2 de AB	8.0 de A
BLOOM-5	6.6 a C	23.5 a B	28.3 a A
BM-5	5.1 ab B	15.6 b A	16.0 b A
LBC-5	4.2 ab B	10.2 c A	10.4 cd A
OCB	2.9 b C	7.1 cd B	10.5 cd A
Treatment	**Cu**		
	----------mg kg^{-1}----------		
	Oct-16	**Oct-17**	**Oct-18**
Fertilizer	1.1 b A	1.0 cd A	0.9 de A
BLOOM-1	1.1 b C	2.0 b B	2.8 b A
BM-1	1.3 b A	1.3 bc A	1.5 cd A
LBC-1	1.2 b A	0.9 cd A	0.5 e A
BLOOM-5	2.5 a C	4.0 a B	5.3 a A
BM-5	1.4 b A	1.6 bc A	1.8 c A
LBC-5	1.0 b A	0.5 d A	0.3 e A
OCB	1.1 b B	1.6 bc B	2.9 b A

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, and 5=reclamation rate.

The increase in soil P over time has become an environmental concern and commonly occurs where organic wastes, such as biosolids and manures, are routinely applied at agronomic N rates (Jameson et al., 2016; O'Connor et al., 2004). Biosolids P availability and loss can be reduced by addition of ferric salts during wastewater treatment (Jameson et al., 2016). We showed that high Fe-amended DC Water biosolids (BLOOM and BM) resulted in consistently lower soil extractable P than other biosolids products. The P retention characteristics of the soil also play an important role on the soil's ability to retain P. In our urban soil, P solubility and bioavailability were likely reduced by low soil P and high soil Ca concentrations, and the Fe added with some biosolids (BLOOM and BM).

Soil TOC and TN concentrations increased over time for all biosolids treatments, but not in the inorganic fertilizer treatment soil (Table 5.7). Reclamation rate treatments gave significantly greater soil TOC and TN than all other treatments in fall 2017 and summer 2018 (Table 5.7). Inorganic soil N as NH_4-N and NO_3-N was measured to determine the amount of N available for uptake by the vegetable crops. Soil NH_4-N and NO_3-N determinations also provided an indication of organic N mineralization after the application of biosolids treatments. Biosolids reclamation rates generally resulted in greater soil NO_3-N and NH_4-N than the inorganic fertilizer and biosolids applied at agronomic rates, especially during the second fall growing season (fall 2017). This greater N availability was not maintained into summer 2018, even after large biosolids fall applications.

Table 5.7. Soil total organic C (TOC), total N (TN), nitrate N (NO_3-N), and ammonium (NH_4-N) after initial treatment application (October 2016), after second year treatment application (October 2017), and by the end of the experiment (July 2018).

Treatment†	TOC			TN		
	g kg^{-1}					
	Oct-16	**Oct-17**	**Jul-18**	**Oct-16**	**Oct-17**	**Jul-18**
Fertilizer	9.7 A	7.2 c A	8.5 d A	0.8 bc A	0.6 d A	0.7 e A
BLOOM-1‡	13 B	12 b B	18 bc A	1.3 ab B	1.3 c B	2.0 c A
BM-1	9.6 B	11 bc B	17 bc A	0.8 bc B	1.0 cd B	1.5 d A
LBC-1	8.6 B	11 bc B	18 b A	0.8 bc B	1.1 cd B	1.6 d A
BLOOM-5	10 C	19 a B	24 a A	1.1 ab C	2.5 a B	3.0 a A
BM-5	13 C	22 a B	28 a A	1.3 a C	2.1 b B	2.6 b A
LBC-5	11 C	20 a B	25 a A	1.1 ab C	2.0 b B	2.3 bc A
OCB	8.7 B	7.8 bc B	13 c A	0.6 c B	0.8 de B	1.2 d A
Treatment	NO_3-N			NH_4-N		
	mg kg^{-1}					
	Oct-16	**Oct-17**	**Jul-18**	**Oct-16**	**Oct-17**	**Jul-18**
Fertilizer	5.5 bc A	2.8 c A	5.0 A	3.0 bc A	2.2 b A	3.9 A
BLOOM-1	5.0 bc A	6.3 bc A	5.2 A	4.5 ab A	3.2 b A	3.3 A
BM-1	4.0 c A	4.3 bc A	3.1 A	4.3 ab A	2.8 b A	4.1 A
LBC-1	4.1 bc A	3.3 c A	6.4 A	2.2 bc A	3.3 b A	3.8 A
BLOOM-5	9.8 b AB	13 a A	6.9 B	2.6 bc B	6.4 a A	3.6 B
BM-5	9.8 b A	7.9 bc A	5.0 A	5.4 a A	5.7 a A	4.0 A
LBC-5	17 a A	7.6 bc B	5.5 B	3.5 ab B	6.3 a A	3.6 B
OCB	4.6 bc A	8.9 ab A	6.1 A	1.9 c A	3.2 b A	2.6 A

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, and 5=reclamation rate

Our results show that there was a large amount of C accumulated in the soil after the two-year term of this experiment (Table 5.8). Reclamation rate application of biosolids resulted in greater C accumulation after two years (18,328 to 23,078 kg ha^{-1}; Table 5.8). All biosolids treatments had greater C accumulation than the inorganic fertilizer, which did not receive any C inputs. The amount of C increase attributed to biosolids application ranged from 37% to 84%, with little differences between these treatments. The high percent of soil C accumulation achieved with biosolids applications, and overall low residual soil N availability, even after high biosolids applications, suggests that microbial decomposition is being limited. The urban subsoil used in this experiment had little residual C and N and a high clay content. Such soil could have a tendency to adsorb organic matter compounds in the surface of clay minerals and Fe and Al oxides (Bingham and Cotrufo, 2016; Deb and Shukla, 2011; Kögel-Knabner et al., 2008). High clay content could also lead to occlusion of C and N compounds within aggregates or within small pores, which would have hindered access to microbes and prevented microbial decomposition and mineralization (Bingham and Cotrufo, 2016, Griffin, 2008).

Table 5.8. Total organic carbon (TOC) applied with biosolids, and TOC stored and accumulated in the degraded urban soil after two years of treatment applications.

Treatment†	TOC applied	TOC stored§	TOC accumulation¶
	kg ha^{-1}	kg ha^{-1}	%
BLOOM-1‡	15041	11536 bc	77 a
BM-1	12339	10331 bc	84 a
LBC-1	18927	12197 b	65 ab
BLOOM-5	37604	18328 a	49 bc
BM-5	30711	23078 a	75 a
LBC-5	58751	18969 a	37 b
OCB	7449	6201 d	83 a

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, and 5=reclamation rate.

§ Soil total organic C concentration at the end of two years (mg kg-1) multiplied by soil bulk density and sampling depth.

¶ Soil total organic C of the inorganic fertilizer control subtracted to soil total organic C stored in the amended soil at the end of two years, divided by treatment C application rate, and multiplied by 100.

Research on C storage of amended agricultural and urban soils has shown that urban soils might have a greater potential of sequestering C (Brown et al., 2012). A disturbed urban soil along a highway roadside showed a greater increase in soil C than an agricultural soil after biosolids were applied at 40 to 65 Mg ha^{-1}. The mean C accumulation as a percent of the amount of C added was 48% for yard debris compost and 81% for biosolids-wood mulch (Brown et al., 2012). These values are similar to the range of %C accumulation obtained in our study (37-84%).

5.4.4. Vegetable Yield

Of the cabbage, beets, radish, kale, and lettuce that were planted in the fall garden in 2016, we were only able to collect yield data for cabbage, beets, and radish due to poor lettuce germination and kale destruction by pests. There was an effect of reclamation rate treatments on cabbage yield during fall 2016, but this effect was more evident during fall 2017 (Table 5.9 and 5.10). Kale yield similarly showed greater yields after reclamation rate applications during fall 2017. Zucchini yield in 2017 was higher with the inorganic fertilizer than most biosolids amendments, except OCB (Table 5.10). The overall increase in green beans yield in summer 2018 compared to summer 2017 was due to improved seedling germination. Bell pepper and green beans yields increased in summer 2018 with the application of BLOOM-5 and LBC-1 (Table 5.10).

Table 5.9. Analysis of variance F ratios and levels of significance of vegetable yields for treatment, date, and interaction effects.

	Treatment		Year		T * Y	
	F	**Pr>F**	**F**	**Pr>F**	**F**	**Pr>F**
Cabbage	17.26	<0.0001	1.85	0.1867	2.83	0.0267
Beets	1.67	0.1649	5.93	0.0226	0.64	0.7212
Zucchini	3.15	0.0165	N/A	N/A	N/A	N/A
Bell Pepers	2.70	0.0325	24.02	<0.0001	1.77	0.1404
Snap Beans	1.52	0.2082	618.92	<0.0001	2.79	0.0285
Kale	11.95	<0.0001	N/A	N/A	N/A	N/A

Table 5.10. Fresh crop weight yield in each treatment for vegetable crops grown in fall (2016 and 2017) and summer (2017 and 2018) growing seasons. Different lowercase letters within columns indicate significant differences between treatments by harvest date. Different uppercase letters within rows indicate significant differences between harvest dates for each treatment.

	Fall				
	Cabbage		**Beets**		**Kale**
Treatment	----------	----------	**kg m^{-2}**	----------	----------
	2016	**2017**	**2016**	**2017**	**2017**
Fertilizer	1.56 cd A	1.01 b A	0.17 A	0.11 B	0.41 bc
BLOOM-1	1.09 d A	0.91 b A	0.10 A	0.05 B	0.20 c
BM-1	1.17 d A	0.58 b A	0.14 A	0.05 B	0.15 c
LBC-1	1.53 cd A	0.60 b B	0.13 A	0.03 B	0.17 c
BLOOM-5	2.19 bc B	3.12 a A	0.11 A	0.15 B	1.14 ab
BM-5	2.86 ab A	3.13 a A	0.19 A	0.17 B	1.10 ab
LBC-5	3.22 a A	3.51 a A	0.27 A	0.16 B	1.57 a
OCB	2.08 bc A	1.38 b A	0.25 A	0.08 B	0.34 bc
	Summer				
	Bell Pepper		**Green Beans**		**Zucchini**
Treatment	----------	----------	**kg m^{-2}**	----------	----------
	2017	**2018**	**2017**	**2018**	**2017**
Fertilizer	0.29 A	0.22 c A	0.09 B	1.84 b A	6.43 a
BLOOM-1†‡	0.24 A	0.48 bc A	0.23 B	1.60 b A	3.71 bc
BM-1	0.24 A	0.30 bc A	0.19 B	1.67 b A	3.54 c
LBC-1	0.18 B	0.58 b A	0.13 B	2.36 a A	4.28 bc
BLOOM-5	0.19 B	0.99 a A	0.19 B	2.36 a A	2.84 c
BM-5	0.21 B	0.48 bc A	0.19 B	1.87 b A	2.54 c
LBC-5	0.20 B	0.47 bc A	0.21 B	2.03 ab A	3.52 c
OCB	0.43 A	0.59 b A	0.24 B	1.63 b A	5.64 ab

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, and 5=reclamation rate

Reclamation rates provided greater organic C and available N and P after their application in the fall seasons, which likely contributed to increased yields. Improvements in soil physical properties (i.e. bulk density, porosity) after two reclamation rate applications of biosolids to the disturbed urban soil likely contributed to increased cabbage and kale growth. Nevertheless, improved soil physical properties were not sufficient to promote adequate growth of belowground crops in our experiment as was observed by the lack of treatment effects on beets yield during both fall seasons and decrease in yield during fall 2017 (Table 5.9 and 5.10).

Most biosolids agronomic N rate applications resulted in lower zucchini yield than the inorganic fertilizer in summer 2017. Agronomic N rates might have not been able to provide sufficient nutrients for adequate yield development later in the growing season, which is when vegetables have the largest N requirements (Sullivan et al., 2017; Pettygrove et al., 2003). Additionally, we could also have overestimated the amount of N mineralized from biosolids in this particular soil. Our estimates of organic N mineralization were based on our greenhouse studies (Chapter 3) and published literature; however, the clayey nature of our urban soil could have reduced the rate of mineralization from these products, as we observed in our N mineralization study (Chapter 4).

We also expected greater improvements in summer 2018 vegetable yields due to the high cumulative rates of biosolids applied. Residual soil N of agricultural and forested soils can contribute to an overall N mineralization of biosolids-amended soils (Singh et al., 2011); however, our soil was a disturbed urban soil with little to no residual C and N to contribute to N mineralization and N availability for plant uptake. The high clay content of the soil could have also physically protected C and N compounds from microbial degradation and reduced N mineralization and availability (Bingham and Cotrufo, 2016; Griffin, 2008). The poorly

weathered Groseclose subsoil used in this experiment could have 2:1 clays, which could lead to NH_4 fixation of mineralized organic N, reducing N availability (Weil and Brady, 2017).

5.4.5. Vegetable Mineral and Vitamin Content

Our results show little to no effect of agronomic and reclamation rate applications of biosolids on vegetable minerals and vitamins (Table 5.11). The agronomic rates of biosolids products and the inorganic fertilizer were applied to supply the same amount of plant-available N while providing adequate levels of P and K. Reclamation rate applications added a much higher amount of essential nutrients; thus, we anticipated a greater benefit from these treatments on mineral and vitamin content of harvested vegetables.

Published results on the influence of organic amendments on mineral and vitamin content of agricultural crops have been variable. Several studies showed that there was no benefit of organic amendments or inorganic fertilizers in increasing the vegetable content of minerals and vitamins (Svec et al., 1976; Warman and Harvard, 1996; Woese et al., 1997). Warman and Harvard (1996) did obtain higher concentrations of minerals and vitamins in vegetables with organic amendment than with inorganic fertilizer in some years but not others. Thus, the effects of organic amendment use on vegetable mineral and vitamin contents are not clear. We found little nutritional benefit of biosolids on vegetables compared to inorganic fertilizer despite the greater benefits of biosolids on soil physical properties, fertility, and vegetable yield. The degraded properties of our urban soil (i.e. limited N and P availability) could have reduced plant vigor and vegetable quality, even after high biosolids application rates.

Table 5.11. Mineral and vitamin testing results after the application of the inorganic fertilizer, BLOOM-1, and BLOOM-5 in the summer of 2018, and their comparison to the USDA National Nutrient Database.

Zucchini	Fertilizer	BLOOM-1	BLOOM-5	USDA National Nutrient Database
Protein (%)	0.625	0.650	0.650	1.160
Calcium (mg/100g)	17.5	18.2	18.4	18.0
Phosphorus (mg/100g)	20.9	20.3	19.7	28.0
Iron (mg/100g)	0.300	0.285	0.323	0.510
Magnesium (mg/100g)	12.4	12.2	12.0	13.0
Zinc (mg/100g	0.148	0.133	0.148	0.210
Vit. A (IU/100g)	183	196	225	198
Vit. B6 (mg/100g)	0.060	0.058	0.060	0.050
Snap beans	**Fertilizer**	**BLOOM-1**	**BLOOM-5**	**USDA National Nutrient Database**
Protein (%)	1.98	2.08	2.00	1.79
Calcium (mg/100g)	54.4 a	58.1 a	46.5 b	42
Phosphorus (mg/100g)	30.8	34.7	29.6	32.0
Iron (mg/100g)	1.45	1.21	0.82	0.85
Magnesium (mg/100g)	26.3	24.8	24.1	22.0
Zinc (mg/100g	0.315	0.330	0.290	0.260
Vit. B2 (mg/100g)	0.115	0.125	0.087	0.091
Vit. A (IU/100g)	46.3 b	72.5 a	61.8 ab	547
Vit. B6 (mg/100g)	0.096	0.090	0.092	0.044

5.5. Conclusions

While the conditions of urban soils can vary geographically, urban soils are often highly disturbed, infertile, and excessively acidic or basic, all of which can negatively affect their capacity for vegetable production. Our greatest challenges of producing vegetables on the soil we studied were high clay content and lack of established residual C and N cycles, which limited organic N mineralization and N availability. Despite the limiting physical, chemical, and biological conditions of our soil, the addition of biosolids at higher than agronomic rates (i.e. reclamation rate) showed great potential to increase vegetable yield after two years of application. However, greater benefits of biosolids occurred in soil properties (reduced bulk density, greater total N and P) than in plant productivity and vegetable quality.

Our research showed that a degraded urban soil has the potential to store large amounts of C in a short amount of time. Thus, the addition of EQ biosolids amendments to degraded soils in urban areas provides the opportunity to increase soil C storage and reduce C emissions. The impacts of such practice can have great potential to sequester C in urban soils. Restoration of degraded urban soils will likely benefit from multiple applications of biosolids amendments at high rates, depending on the degree of soil degradation.

5.6. References

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6. Environmental impact of exceptional quality biosolids use in urban agriculture

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6.1. Abstract

Exceptional Quality (EQ) biosolids may be used to rehabilitate anthropogenic soils for agricultural production, but research on the environmental ramifications of such products when applied at rates to improve such degraded soils are lacking. The objective of our study was to compare the impacts of EQ biosolids and inorganic fertilizer applied to supply organic matter and nutrients for rehabilitating anthropogenic soils on potential N and P water quality impairment risk. Three EQ biosolids (composted, air-dried, and blended with shredded woody mulch) were applied at 1x agronomic N rates twice per year (at the beginning of each cropping cycle) and once per year (fall 2016 and 2017) at a disturbed land, reclamation rate (5x agronomic N rate) for vegetable crop production to an anthropogenic, low fertility subsoil. Heat-dried EQ biosolids and inorganic fertilizer control treatments were applied at agronomic N rates only at the beginning of each cropping cycle (fall and spring, 2016 and 2017, respectively). While nitrate-N posed the greatest nutrient leaching risk, lysimeter mass loading of NO_3-N from reclamation rate treatments were not greater than inorganic fertilizer or biosolids applied at agronomic N rates. Leachate P was below detection during most of the experiment and soil water-extractable P (WEP) concentrations were low. These results suggest that reclamation rate applications of EQ

biosolids to anthropogenic, low fertility subsoils have low potential for N and P water quality impairment.

6.2. Introduction

Urban agriculture can contribute to restore urban soil ecosystem function reduce energy/fuel consumption needed for the transportation of vegetable produce to cities, and increase food security (Cooley and Emery, 2016). One of the main challenges of urban agriculture is that many soils in urban areas have been degraded and have difficulty supporting vegetation and ecosystem function (Kumar and Hundal, 2016). Topsoil removal and heavy vehicle traffic commonly occur during construction and industrial activities, which result in soil compaction, increased bulk density, reduced aeration and porosity, and reduced organic matter and soil fertility (Craul, 1985; Gregory et al., 2006; Beniston and Lal, 2012). Anthropogenically-altered soils may have their properties and functions restored with the addition of organic amendments.

The application of organic amendments to degraded soil, especially when applied at high rates, can help improve soil physical properties such as reducing compaction, and increasing porosity and water holding capacity (Punshon et al., 2002; McIvor et al., 2012). Organic amendment addition can also improve soil chemical and biological properties such as increasing organic matter, nutrient availability, microbial communities, and plant growth (Ozores-Hampton et al., 2011; Sydnor and Redente, 2002). Organic amendments available in urban areas provide the opportunity of locally recycling urban wastes and improving nutrient recovery.

Biosolids are by-products from municipal wastewater treatment and resultant sludge management practices. They have been commonly used in agriculture, forestry, and mine land reclamation. A recent increase in the development and use of Exceptional Quality (EQ) biosolids

have led to the use of biosolids in urban landscapes as well. Exceptional Quality biosolids are produced by processes to further reduce pathogens (PFRPs), which create Class A biosolids, and have reduced vector attraction and low pollutant concentrations (USEPA, 1994). Class A biosolids are being generated by processes such as thermal drying, and composting, pasteurization, and thermal hydrolysis followed by anaerobic digestion (Cambi^{TM}) (USEPA, 2006; Abu-Orf et al., 2012). Some EQ biosolids appropriate for application to urban landscapes are being produced by blending biosolids with drier organic and/or mineral byproducts to create a product with reduced moisture that is easier to handle and spread. The biosolids blended products generated by the City of Tacoma, Washington (https://www.cityoftacoma.org/cms/one.aspx?pageId=16884), and the City of Vancouver, British Columbia (http://www.metrovancouver.org/services/liquid-waste/innovation-wasterwater-reuse/biosolids/Topsoil/Pages/default.aspx), are examples of such products.

The use of EQ biosolids products as soil amendments can help improve urban soil properties and add essential nutrients needed for urban agriculture, but research on the environmental ramifications of such products when applied at rates to improve such degraded soils are lacking. Biosolids products must be applied appropriately to reduce the risk of nutrient loss that can negatively impact groundwater and contribute to eutrophication of surface water. Biosolids applied at agronomic nitrogen (N) rates have been generally used to grow agricultural and forestry crops, but considerably higher application rates have been used for reclamation of disturbed lands such as mine lands (Brown and Chaney, 2000; Sopper, 1993).

There is lower risk of N loss from soils by leaching as ammonium (NH_4-N) than nitrate (NO_3-N) due to fixation of NH_4-N in clay minerals or adsorption by negatively charged clays and organic matter (Pierzynski et al., 2005). Nitrate is also at higher concentrations in the soil

solution; thus, it is more likely for high biosolids application rates to cause a short-term increase in nitrate (NO_3-N) loss through leaching. However, this short-term NO_3-N loss is permitted by regulation for mine land reclamation because it promotes faster soil restoration and vegetation productivity that will ultimately prove to be more beneficial for the long-term management of the site (Brown and Chaney, 2000; Sopper, 1993).

Phosphorus (P) soil transport and loss is a common environmental concern where biosolids are repeatedly applied at agronomic N rates due to excessive soil P accumulation (Jameson et al., 2016; O'Connor et al., 2004). Factors that affect P losses from soil include both biosolids and soil properties. Phosphorus retention characteristics (i.e., presence of Fe and Al oxides, calcium carbonates, soluble organic ligands and competing ions) of the soil receiving an organic amendment affects P solubility (Ebeling et al., 2003; O'Connor et al., 2004). Biosolids P is less soluble than inorganic fertilizers and manures due to processes used to treat biosolids in wastewater treatment plants (Maguire et al., 2001; Penn and Simms, 2002; White et al., 2010). Such processes include alkaline stabilization and precipitation of P as Fe- and Al-PO_4 (Maguire et al., 2001; Penn and Simms, 2002; White et al., 2010). Thus, biosolids produced by various treatment processes should be assessed for their effect on amended soil.

Two laboratory methods for assessing the solubility and potential for P loss include quantifying the P saturation ratio (PSR) and water extractable P (WEP). The PSR is one of the most common methods for quantifying P loss risk (Nair, 2014). The PSR measures the amount of oxalate extractable P relative to oxalate extractable Fe and Al with the purpose of evaluating the capacity of a soil to retain P, mainly in Fe and Al phosphate forms. Oxalate Fe and Al are used for this calculation because phosphate ions are highly attracted to Fe and Al; thus, making P insoluble in water and minimizing the risk for P loss (Nair, 2014). A high PSR indicates that the

sites for soil P binding with Fe and Al are largely filled, which indicates that P loss can occur if additional P is added to the soil. The Virginia Nutrient Management Standards and Criteria (Virginia DCR, 2014) establishes P application rates to minimize P losses. If soil PSR is greater than 65%, then no P can be applied to the soil. If PSR is 30% or greater, P applications should not be greater than P crop removal. Finally, if PSR is below 30%, then P can be applied and managed in such way to minimize water quality impairment due to P losses.

Water extractable P is frequently used as an estimator of P in runoff (Pote et al., 1996; McDowell et al., 2001). Water is the main solvent and medium of transport after field rain events; thus, it can represent a good estimate of P loss via runoff. Past research has found strong relationships with P loss via runoff (McDowell et al., 2001). Water extractable P was also highly correlated with dissolved reactive P concentrations in runoff when compared to Mehlich 3, Olsen P, and Bray-Kurtz P (Hooda et al., 2000).

Land applied-biosolids treated with amendments that reduce P solubility (e.g., $FeCl_3$) have been shown not to increase soil P saturation and runoff of dissolved reactive P and bioavailable P (White et al., 2010). Maguire et al. (2001) found that biosolids treated with Fe, Al and/or lime had lower water soluble P, iron strip-bound P, and Mehlich-1 P concentrations than biosolids that had not received either of these treatments. The use of biosolids tends to result in a lower P loss risk than from manures and inorganic fertilizer applied at equivalent total P rates (White et al., 2010; Maguire et al., 2001).

Studies of N and P losses from urban/residential lawn management have been conducted in the past (Groffman et al., 2004; Toor et al., 2017); however, to our knowledge, this is the first report of N and P loss from an urban garden and with the use of EQ biosolids as soil amendments. With new EQ biosolids products targeted at urban agriculture, it is important to

establish their risks of N and P loss. The objective of our study was to compare the impacts of EQ biosolids and inorganic fertilizer applied to supply organic matter and nutrients for rehabilitating anthropogenic soils on potential N and P water quality impairment risk.

6.3. Materials and Methods

6.3.1. Site Description

The study was conducted at the Virginia Tech Turfgrass Research Center (TRC) in Blacksburg, VA, which is located in Montgomery County at approximately 634 m above sea level. The urban garden location was simulated by excavating the soil on site to a depth of 45 cm and replacing it with a subsoil fill from a nearby construction site with the purpose of recreating a common urban degraded soil of Virginia. The subsoil fill used to recreate the urban soil was from the Groseclose-Urban land complex soil map unit, characterized with a clayey texture and slow permeability. The urban field research site was approximately 253 m^2 (22 m long x 11.5 m wide). The urban soil was tilled multiple times with a tractor-mounted rear-tine tiller to break large soil clods. Large rocks and pieces of asphalt and plastic were removed from the site after each tilling, until only very small foreign materials were left behind.

6.3.2. Biosolids Sources

The EQ biosolids products used in this experiment included: 1) an air-dried EQ biosolids called BLOOM that is produced via thermal hydrolysis followed by anaerobic digestion (CambiTM) at Blue Plains Advanced Wastewater Treatment Plant (DC Water) in Washington, DC (https://www.dcwater.com/blue-plains); 2) a blended EQ biosolids product from DC Water, designated as BM, and made of 0.75 BLOOM: 1.0 shredded, woody mulch; 3) an anaerobically digested, composted EQ biosolids product from Livingston Composting Facility in Spotsylvania County, VA (http://www.spotsylvania.va.us/Compost), identified as LBC; and 4) a heat-dried,

pelletized EQ biosolids product from Ocean County Utilities Authority in Bayville, NJ (http://www.ocua.com/SitePages/Home.aspx), marketed as OceanGro and named OCB in this study.

Prior to their field application, a sample of each EQ biosolids product was sent to be analyzed for total solids, SM-2540G) (APHA, 1992); Total Kjeldahl Nitrogen (SM-4500-NH3C-TKN) (APHA, 1992); ammonia-nitrogen (SM-4500-NH3C) (APHA, 1992); nitrate (SM-4500NO3F) (APHA, 1992); organic nitrogen (calculation); phosphorous, potassium, sulfur, calcium, magnesium, sodium, iron, aluminum, copper, zinc (SW 6010C); heavy metal concentrations (SW-6010C)(USEPA, 2000); and pH (SW-9045D) (USEPA, 2000) at Waypoint Analytical Laboratories in Richmond. Chemical composition of EQ biosolids products applied are shown in Table 6.1.

Table 6.1. Selected composition variables of EQ biosolids products applied to the urban garden field study.

	Unit	BLOOM†	BM	LBC	OCB
pH§		5.17	6.18	6.86	7.3
Total Solids§	g kg^{-1}	567	870	892	950
Moisture§	g kg^{-1}	435	130	108	50
C	g kg^{-1}	248	355	334	383
C:N		8.1	12.5	12.0	7.8
TKN	g kg^{-1}	30.8	28.4	27.7	49.1
NH_4-N	g kg^{-1}	1.9	4.7	3.1	5.7
Organic N	g kg^{-1}	28.9	23.7	24.6	43.4
NO_3-N	mg kg^{-1}	1240	719	1170	7.21
TP	g kg^{-1}	30.9	19.3	13.3	26.6
K	g kg^{-1}	1.5	2.1	4.9	1.4
Ca	g kg^{-1}	24.7	20.7	26.6	28.4
Mg	g kg^{-1}	4.1	2.9	3.2	4.3
Fe	mg kg^{-1}	82600	56100	26700	32800
Al	mg kg^{-1}	11900	8400	15300	14700

† BLOOM= air-dried EQ biosolids from DC Water, BM=DC Water EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried.

§ Values are on a dry weight basis except as noted.

‡ All elemental values are total concentrations.

6.3.3. Experimental Design

The experimental design was a randomized complete block design with eight fertility treatments and four replications for a total of 32 plots. Treatment plots were 1.8 m long and 2.5 m wide (area = 4.5 m^2). Treatments comprised EQ biosolids BLOOM, BM and LBC applied at the agronomic N rate (1x), and at five times the agronomic N rate (5x), in addition to OCB and an inorganic fertilizer (urea, 46% N) applied at the agronomic N rate only.

Treatments applied at the agronomic N rates were intended to provide adequate N for vegetables growing in the experimental plots based on existing VT soil testing recommendations for vegetable crops (Maguire and Heckendorn, 2011), while the high application rate was selected based on mine land reclamation rates that are meant to be applied as a large application of biosolids to help restore degraded soils properties for vegetation establishment and growth (Daniels and Haering, 1994). Agronomic N rates (112 kg ha^{-1})were applied at the beginning of each growing season in August 2016, May 2017, August 2017, and May 2018; while high (5x) agronomic N rate treatments were applied once a year before the beginning of the fall crop season in August 2016 and 2017.

Biosolids application rates were based on organic N mineralization rate assumptions of 30% for BLOOM, 20% for BM, 15% for LBC, and 40% for OCB (Gilmour et al., 2003; Alvarez-Campos et al., 2018). Residual mineralizable N contributions were not taken into account for modifying second year biosolids application rate calculations. The cumulative amount of biosolids, total organic C (TOC), total N (TN), and P applied with biosolids rates and inorganic fertilizer applications throughout the experiment are shown in table 6.2.

Table 6.2. Cumulative amount of biosolids, total organic carbon (C), nitrogen (N), and phosphorus (P) applied during the period August 2016 to May 2018 as measured in 2018.

Treatment†‡	Biosolids addition (Mg ha^{-1})	TOC applied (kg ha^{-1})	TN applied (kg ha^{-1})	P applied (kg ha^{-1})
Fertilizer		0	448	185
BLOOM-1	52	15041	1358	1407
BM-1	65	12339	1427	711
LBC-1	68	18927	1636	425
BLOOM-5	116	37604	3394	3155
BM-5	144	30711	3552	1578
LBC-5	176	58751	4469	1115
OCB	19	7449	955	547

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, 5=reclamation rate (dry weight basis)

6.3.4. Vegetable Cropping System

Vegetables were grown in the urban garden field experiment during two fall (2016 and 2017) and two summer (2017 and 2018) growing seasons. Lettuce (*Lactuca sativa*), kale (*Brassica napus* L. var. *pabularia*), radish (*Raphanus sativus*), beets (*Beta vulgaris*), and cabbage (*Brassica oleracea L. var. capitata L*) were produced in the fall of 2016, while only cabbage, kale, and beets were grown in the fall of 2017. All vegetable seeds were sowed directly in the soil, except for cabbage that was germinated in the greenhouse and transplanted to the field a month after germination. Zucchini squash (*Cucurbita pepo*), bell peppers (*Capsicum annuum*), and green beans (*Phaseolus vulgaris)* were produced during the summers of 2017 and 2018. Zucchini squash and green beans were sown directly to the soil, while bell peppers were transplanted a month after germination. Vegetable crops were planted in rows with a spacing of 45.7 cm between rows and a total of three rows per plot during every growing season. Additional details about vegetable planting, agronomic management, and effects of EQ biosolids application on vegetable production and soil properties can be found in Chapter 5.

6.3.5. Soil Sampling and Analyses

A 2-cm soil corer was used to collect eight composite random benchmark soil samples to a 15-cm depth from each replicate block on June, 2016 before the beginning of the experiment. Samples were air-dried, sieved (2 mm mesh) and analyzed by the Virginia Tech Soil Testing Laboratory for routine soil test analysis (Maguire and Heckendorn, 2017). The hydrometer method was used to determine particle size analysis (Gee and Bauder, 1986). The soil had a clayey texture (630 g kg^{-1} clay, 150 g kg^{-1} silt, and 220 g kg^{-1} sand), a pH of 7.7, and Mehlich 1 P of 3 mg kg^{-1}, K of 56 mg kg^{-1}, Ca of 2594 mg kg^{-1}, and Mg of 656 mg kg^{-1}. The high pH, and Mehlich 1 Ca and Mg suggested that the soil is calcareous, which is typical of soils derived from

limestone parent material that has not been exposed to substantial weathering in western Virginia. Thus, we also used an Olsen extraction (0.5 M sodium bicarbonate) to determine soil P, since such extraction is recommended for determining soil P levels of calcareous soils. The Olsen extraction was performed with 2 g of soil to which 40 ml of Olsen solution was added. The solution was set in a reciprocating shaker for 30 minutes, and filtered through Whatman 40 filter paper (Olsen, 1957). The extraction was sent to the Virginia Tech Soil Testing Laboratory to be analyzed for P, and analysis showed 11 mg kg^{-1} of Olsen extractable P. Olsen P and routine soil K testing were used as basis for supplemental inorganic P (triple superphosphate, 0-46-0) and K (potash; 0-0-60, respectively, based on soil test recommendations for vegetable growth. (Maguire and Heckendorn, 2017). Phosphorus and K were added to all inorganic fertilizer treatment plots. Supplemental K was added to all EQ biosolids treatments, while supplemental P was not required during most of the experiment, except for reclamation rate treatments in the summer of 2017.

Soil samples were collected and analyzed to determine cumulative effects of treatments approximately eight weeks after treatment application in both fall growing seasons (October 2016 and 2017) and the final summer growing season (July 2018). Eight soil samples were collected from each treatment plot to a depth of 10-cm with a 2-cm diameter soil corer. Soil samples were air-dried and passed through a 2-mm sieve to separate from rock fragments and debris in preparation for soil analyses.

Ammonium oxalate extractable P, iron (Fe), and aluminum (Al) were determined by adding 40 ml of 0.2 M ammonium oxalate, adjusted to pH 3.0, to 1 g soil. The solution was extracted by shaking in the dark for 2 hours at 180 rpm in a reciprocating shaker (Eberbach Corporation, Ann Arbor, Michigan), centrifuging for 10 min at 2000 rpm, and filtering

(Whatman no. 40 filter paper; Pote et al., 1996). The P saturation ratio was calculated as the oxalate extractable P divided by the sum of the extractable Fe and Al content, and multiplied by 100. Water soluble P was determined by adding 25 ml of DI water to 1 g soil. The solution was extracted by shaking for 1 hour at 180 rpm on a reciprocating shaker, centrifuging for 10 min at 2000 rpm, and filtering through a 0.45 µm membrane filter (Pote et al., 1996). Concentrations of both oxalate and water extractable elements were determined by Inductively Coupled Plasma Atomic Emission Spectroscopy (Maguire and Heckendorn, 2011).

Soil TN and TOC were analyzed via combustion on a Vario MAX CN macro elemental analyzer (Elementar, Hanau, Germany). A hydrochloric acid (HCl) fumigation procedure was conducted before analysis to remove inorganic C from the calcareous soil (Harris et al., 2001). Soil was ground through an electric mortar grinder (RM 200, Retsch, Inc., Newton, PA) through a 180 µm sieve. The HCl fumigation was conducted by placing 3 g of soil moisten with 3 ml of deionized water in beakers in a desiccator with 100 ml 12M HCl in order to expose the samples to HCl vapor for 6 hours. Samples were rinsed after fumigation to increase their pH to 6-7, and were then dried at 60 °C for 24 hours, and manually ground with a mortar and pestle.

6.3.6. Water Monitoring Instrumentation

Zero-tension drainage lysimeters were installed at each treatment plot to collect water samples and determine N and P leachate. The lysimeters were constructed from a plastic snap end drainage pipe cap (product #0867AA N-12, Emco Waterworks,) measuring 20 cm tall and 20 cm in diameter, Kynar tubing (Kynar, Philadelphia, PA) was used to transport leachate from the lysimeter reservoir to the surface with aid of an electric pump (Lasley et al., 2010). One end of the Kyanar tubing was covered with a fine screen and placed at the bottom of the lysimeter. The lysimeter was filled up to 12.7 cm with coarse sand (well gravel pack; Drillers Service,

Inc.); thus, the screened tube was meant to prevent clogging from the coarse sand. The other half of lysimeter was filled with the subsoil fill. The top surface (rim) of the zero-tension lysimeters were installed approximately 15 cm below the soil surface and adjusted with a leveling device.

6.3.7. Water Sampling and Analyses

Two benchmark leachate samples were collected before application of treatment application in August 2016 to test lysimeters for leachate volume collection precision among treatments and to ensure that initial leachate N and P concentrations were low and uniform. The total water volume holding capacity of each lysimeter was approximately 9.4 L based on estimated pore volume of sand and clay soil fill. Fertility treatments first initially applied in August 2016, and leachate water samples were collected after significant rain events (>2.54 cm) until October 2018. Precipitation data was gathered from the Virginia Tech weather station located approximately 1 km from the site (Table 6.3).

Table 6.3. Actual and historical monthly precipitation collected from Virginia Tech weather station during the 26 month sampling period.

Month	Monthly Precipitation†	Historical Monthly Mean Precipitation‡
	cm	cm
Aug-16	11.4	9.1
Sep-16	17.0	7.9
Oct-16	5.5	7.1
Nov-16	3.6	7.3
Dec-16	7.9	7.5
Jan-17	10.4	7.8
Feb-17	3.3	7.1
Mar-17	7.8	9.2
Apr-17	14.8	8.8
May-17	16.0	11.0
Jun-17	7.4	10.2
Jul-17	8.4	10.8
Aug-17	6.4	9.1
Sep-17	3.7	7.9
Oct-17	19.6	7.1
Nov-17	3.1	7.3
Dec-17	1.6	7.5
Jan-18	4.1	7.8
Feb-18	10.8	7.1
Mar-18	11.7	9.2
Apr-18	11.5	8.8
May-18	15.2	11.0
Jun-18	3.8	10.2
Jul-18	5.9	10.8
Aug-18	13.8	9.1
Sep-18	19.9	7.9

† https://montgomery.weatherstem.com/vt

‡ Blacksburg monthly climate normal (1981-2010) from the National Weather Service website of the National Oceanic and Atmospheric Administration (NOAA).

The volume of water extracted from each lysimeter was recorded at every sampling event. Water samples were immediately placed in an ice-filled cooler in the field, and were transported to the laboratory for filtering (0.45 µm) and analysis or appropriate storage at 4°C. If water samples could not be analyzed within 48 hours, they were stored in a freezer (-12 °C) until analysis. Leachate water samples were analyzed for concentrations of nitrate (NO_3-N), ammonium (NH_4-N), and orthophosphate (Ortho-P). Concentrations of NH_4-N and NO_3-N in leachate water samples were determined by colorimetric flow injection on a Lachat 8500 (QuikChem Method 10-107-06-2-A and QuikChem Method 10-107-04-1-A; Wendt, 2000). Ortho-P was determined by using the ascorbic acid method and similarly analyzed on Lachat 8500 (QuikChem Method 10-115-01-1-A; Murphy and Riley, 1962). Method detection limits were 0.01 mg N/L for NO_3-N, 0.001 mg N/L for NH_4-N, and 0.01 mg P/L for ortho-P. The use of zero-tension lysimeters provided both the volume and concentration of nutrients leached, which were used to calculate NO_3-N, NH_4-N and Ortho-P loads draining below the root zone.

6.3.8. Statistical Analyses

Analyses of variance and multiple comparisons (Tukey HSD) were performed with the GLIMMIX procedure in SAS 9.4 (SAS Institute, 2011) to determine statistically significant differences of water NO_3-N, NH_4-N, and Ortho P concentrations, as well as soil P saturation ratios and water extractable P among EQ biosolids treatments. Treatment differences were considered significant at the $p < 0.05$ level. Treatment and water sampling date were analyzed as fixed effects. Regression analysis was conducted to determine the relationship between WSP and PSR. Data was normally distributed as determined by the distribution of studentized residuals. GraphPad Pad Prism 7 was used to plot nutrient concentrations in water (mg L^{-1}) by treatment over time (GraphPad Software, San Diego, California USA).

6.4. Results and Discussion

6.4.1. Environmental Phosphorus Indices

Soil oxalate P, Fe, and Al showed a significant interaction between treatment and date (Table 6.4). Oxalate P significantly increased with the application of all biosolids treatments, while it remained the same with the application of the inorganic fertilizer (Table 6.5). This is because inorganic P was not applied once soil test P levels were raised to adequate concentrations for plant growth, while the planned and performed biosolids applications continued to add P to the soil.

Table 6.4. Analysis of variance F ratios and levels of significance of soil nutrient parameters for treatment, date, and interaction effects.

Parameters	Treatment		Date		T * D	
	F	Pr>F	F	Pr>F	F	Pr>F
Oxalate P	87.08	<0.0001	273.62	<0.0001	23.64	<0.0001
Oxalate Fe	123.82	<0.0001	132.38	<0.0001	29.08	<0.0001
Oxalate Al	10.68	<0.0001	102.35	<0.0001	11.95	<0.0001
PSR	51.82	<0.0001	372.69	<0.0001	8.44	<0.0001
WSP	10.24	<0.0001	1.70	0.2044	3.42	0.0111

Table 6.5. Oxalate soil phosphorus (P), iron (Fe), and aluminum (Al) eight weeks after treatment application on fall 2016, 2017, and summer 2018. Different lowercase letters within columns indicate significant differences between treatments by harvest date. Different uppercase letters within rows indicate significant differences between harvest dates for each treatment.

Treatment	Oxalate P			Oxalate Fe		
	----------mg kg-1----------			----------mg kg-1----------		
	Oct-2016	**Oct-2017**	**Jul-2018**	**Oct-2016**	**Oct-2017**	**Jul-2018**
Fertilizer	101 c A	211 e A	149 f A	1181 c A	1111 e A	898 f A
BLOOM-1†‡	199 bc C	950 c B	1203 c A	1603 bc C	3331 c B	3970 c A
BM-1	138 c B	586 d A	712 d A	1353 c B	2546 d A	2835 d A
LBC-1	118 c B	385 de A	548 de A	1138 c A	1517 e A	1593 e A
BLOOM-5	709 a C	2821 a A	2379 a B	2599 a C	8000 a A	7109 a B
BM-5	410 b C	1802 b A	1492 b B	2089 ab C	5753 b A	4975 b B
LBC-5	270 bc B	1200 c A	1093 c A	1325 c B	2441 d A	2229 d A
OCB	105 c B	363 de A	338 ef A	1099 c A	1345 e A	1197 ef A
	Oxalate Al			**PSR**		
	----------mg kg-1----------			----------%----------		
	Oct-16	**Oct-17**	**Jul-18**	**Oct-16**	**Oct-17**	**Jul-18**
Fertilizer	1085 A	1025 c B	881c C	5.35 d B	11.7 e A	9.86 e A
BLOOM-1	1065 A	1052 bc A	909 c B	9.48 cd C	30.9 c B	36.9 b A
BM-1	1050 A	1019 c A	883 c B	7.05 d B	22.5 d A	22.1 cd A
LBC-1	1038 B	1108 bc A	1058 b AB	6.49 d C	18.2 d B	26.0 c A
BLOOM-5	1114 A	1128 b A	963 c B	26.0 a B	49.1 a A	47.1 a A
BM-5	1076 A	1080 bc A	904 c B	17.0 b B	39.8 b A	39.2 b A
LBC-5	1120 C	1407 a A	1270 a B	13.4 bc B	40.2 b A	40.5 b A
OCB	1054 A	1057 bc A	917 c B	5.81 d B	18.5 d A	19.7 d A

† BLOOM= air-dried Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, 5=reclamation rate (dry weight basis)

Oxalate Fe concentrations significantly increased with the agronomic and reclamation rate applications of BLOOM and BM (Table 6.5). The addition of ferric salts in wastewater treatment increases the biosolids' concentrations of Fe and P, which increased soil oxalate-extractable P and Fe with the annual reclamation application rates of BLOOM and BM. Soil oxalate Al did not increase with the first treatment application in the fall 2016, but Al concentrations increased from fall 2016 to 2017 with all treatments, except the inorganic fertilizer (Table 6.5). Since the soil used in this experiment had a pH ~7.5-7.9, there should be no exchangeable Al. Oxalate extractable Al measures non-crystalline Al, including short-range order Al hydroxides/oxyhydroxides and Al bound to organic matter (Cornell and Schwertmann, 2003). Based on results from the last soil sampling date, LBC had a greater potential to increase extractable Al.

Various studies have estimated soil PSR values that indicate higher potential of P release via runoff and leaching in Virginia soils (Beck et al., 2004; Maguire et al., 2001; Maguire et al., 2000). One study determined that a soil PSR greater than 40% had higher potential of P release via runoff and leaching (Maguire et al., 2000). Another study determined that a PSR lower than 20% was considered to represent an environmentally sound concentration of soil P that would be of little risk of P loss (Beck et al., 2004). Virginia standards and criteria for P application in Virginia soils indicate that no additional P fertilizer can be applied if soil PSR is greater than 65%. (Virginia DCR, 2014). Soil PSR values in our experiment increased with the application of EQ biosolids at the reclamation rate during the first year (October 2016), but all PSR values were lower than 26% (Table 6.5). Agronomic rate applications of BLOOM showed a PSR of 36.9% by the last sampling date (July 2018), while all other agronomic rate treatments had soil PSR $\leq$ 26%. The second year of biosolids reclamation rate application (October 2017) increased soil

PSR to ranges between 39.8% and 49.1%, and these levels were maintained in July 2018 (Table 6.5). These results indicate that BLOOM-1 and biosolids reclamation rates might pose a greater risk of P loss; however, soil PSR after application of these treatments was not near the 65% PSR limit at which no more P can be applied (Virginia DCR, 2014).

Continued application of biosolids is expected to increase soil PSR and the risk of P loss over time, but some wastewater treatment methods can reduce P solubility in biosolids and biosolids-amended soil. Maguire et al. (2000) found relatively low PSRs of biosolids-amended soils in Virginia, Maryland, and Delaware, even when soil oxalate extractable P was high. These seeming anomalies were due to consistently higher concentrations of biosolids-borne oxalate Fe and Al in the amended soils. Despite the high amount of Fe added with BLOOM (Table 6.6), the product's PSR (26%) was the highest among biosolids due to the greater amount of P added with both BLOOM reclamation (3155 kg ha^{-1}; Table 6.2) and agronomic rates (1407 kg ha^{-1}; Table 6.2) compared to other biosolids treatments (Table 6.2). The PSRs of LBC and BM were similar (Table 6.6). The lower amount of P added with LBC agronomic and reclamation rates than with BLOOM and BM treatments (Table 6.2), likely contributed to LBC's lower PSR (Table 6.6). Nevertheless, LBC and BM treatments resulted in similar soil PSR levels (Table 6.5), suggesting that the greater amount of Fe added with BM could have reduced the risk of P loss upon P-binding (Jameson et al., 2016; and Maguire et al., 2001). Treatment OCB had the highest PSR due to its low addition of Fe and Al in comparison to all other biosolids products (Table 6.6).

Table 6.6. Cumulative amount of iron (Fe) and aluminum (Al) applied, and biosolids PSR potential based on the relative amounts of Fe, Al, and P added from August 2016 to May 2018.

Treatment†‡	Total Fe applied (Mg ha^{-1})	Total Al applied (Mg ha^{-1})	Biosolids PSR (%)
BLOOM-1	4.3	0.6	29
BM-1	3.7	0.5	17
LBC-1	1.8	1.0	15
BLOOM-5	9.6	1.4	29
BM-5	8.1	1.2	17
LBC-5	4.7	2.7	15
OCB	0.6	0.3	59

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, 5=reclamation rate (dry weight basis)

Research in Mid-Atlantic soils of the US showed that a soil PSR > 30% was related to increased WEP and P losses in runoff (Pautler and Sims, 2000; Pote et al., 1996). In our experiment, WEP concentrations were below detection limit after treatment application in October 2016, at the same time that PSR values were all below 26% (Table 6.5). After the second year of treatment application (October 2017), WEP was detectable but at low concentrations (Table 6.7). The LBC applied at the reclamation rate gave greater WEP than all other treatments. These results suggest that two-year applications of LBC-5 may have a greater potential for increasing soluble soil P in runoff; however, most WEP concentrations were low and would likely pose a low risk of P surface transport with any of our treatments. The average WEP from various biosolids-amended soils across Virginia, Maryland, and Delaware was of 7.4 mg kg^{-1} (Maguire et al., 2000), while the average from our EQ biosolids amended plots was of 3.3 mg kg^{-1} in October 2017 and 3.0 mg kg^{-1} in July 2018. Our low WEP values suggest that soil PSR values might have overestimated P runoff risk, while still providing a meaningful indication of the relative risk of P loss from different treatments.

Table 6.7. Water soluble phosphorus (WEP) after the second year of application of various EQ biosolids treatments and an inorganic fertilizer. Different lowercase letters within columns indicate significant differences between treatments by harvest date. Different uppercase letters within rows indicate significant differences between harvest dates for each treatment.

Treatment	WEP	
	----------mg kg^{-1}--------	
	Oct 2016	**Jul 2018**
Fertilizer	2.98 b A	1.51 c B
BLOOM-1†‡	2.00 b A	1.85 bc A
BM-1	2.34 b A	1.37 c A
LBC-1	3.08 b A	2.77 bc A
BLOOM-5	1.78 b B	3.61 b A
BM-5	2.33 b A	3.05 bc A
LBC-5	8.93 a A	7.19 a B
OCB	2.44 b A	1.49 c A

† BLOOM= air-dried Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. ‡1= agronomic N rate, 5=reclamation rate (dry weight basis)

Soil PSR is routinely used to assess the risk of P loss in acidic soils. In calcareous soil, oxalic acid can precipitate Ca during the extraction and increase the pH of the acid buffer as it reacts with carbonates (Loeppert and Inskeep, 1996). Despite the possible shortcoming of evaluating the PSR value of calcareous soils, this method has been used successfully in neutral to calcareous soils in Canada and Belgium (Renneson et al., 2016; Tran and Giroux, 1987; Beauchemin, 1996). Beauchemin (1996) determined that PSR and WEP concentrations explained 82% and 78% of the risk of P loss as drainage water total P in calcareous soils. In our study, we found a linear relationship (r^2=03453; Figure 6.1) between WEP and PSR data from our three soil sampling dates combined, indicating that the relationship between these variables in our calcareous soil was low.

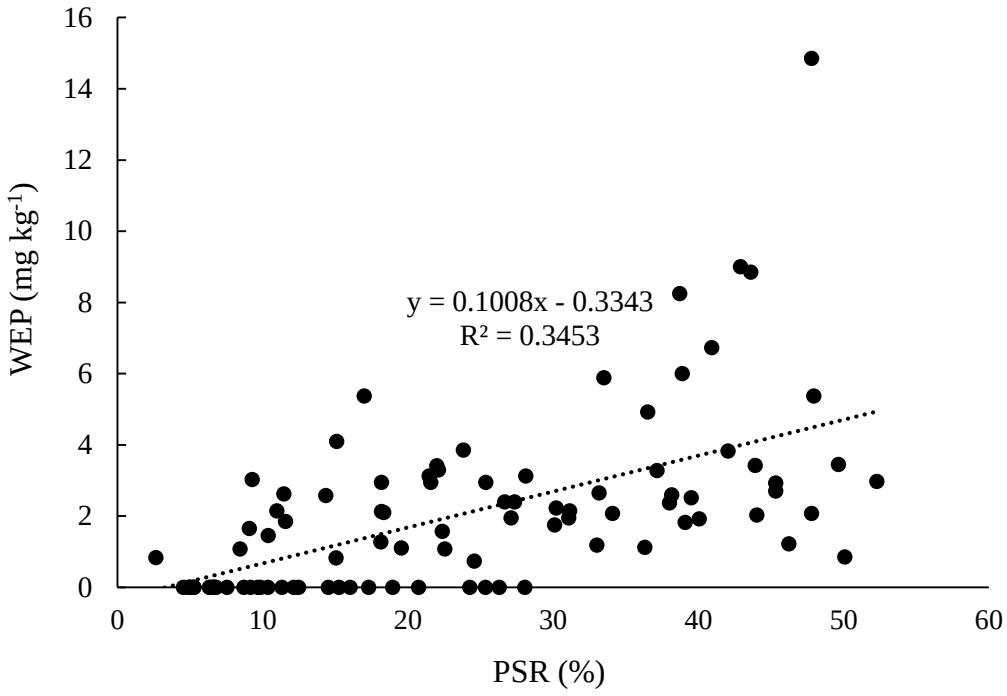

Figure 6.1. Relationship between water extractable phosphorus (WEP) and phosphorus saturation ratio (PSR) from treatment application based on all soil sampling dates.

6.4.2. Phosphorus and Nitrogen Loss in Leaching Water

The concentration of ortho-P in water samples was below detection limit (0.01 mg P/L) throughout most of the experiment. Only six water samples from LBC-5 and two water samples from OCB had P concentrations slightly above detection limit (range 0.0106-0.0195 mg L^{-1}) in 2018 (data not shown). These results show that a biosolids product applied at the reclamation rate (LBC-5) had similar water ortho-P compared to another biosolids applied at the agronomic rate (OCB). The low overall concentrations of ortho-P in leaching water confirms a low risk of P leaching even after high P applications (i.e., reclamation rates). While the risk of soil P leaching is generally low, this risk can increase as the soil becomes saturated with P from continuous P additions. Our data shows that PSR may be overestimating the risk of P loss in our urban soil, since PSR values increased, but both soil WSP and water ortho-P were very low throughout the two year experiment. The large amount of Fe added to the soil with the application of BLOOM and BM and the native soil Fe possibly contributed to bind some excess P applied with these products. The high soil pH and exchangeable Ca also may reduce P solubility and transport from high P application rates. Research conducted in coarse, medium, and fine-textured soils in Quebec found that the fine-textured soils had greater P sorption capacities than coarser-textured; thus, our clayey soils could have supported greater oxalate extractable P without increasing P leaching (Beauchemin, 1996).

As expected, NO_3-N concentrations in water samples increased during the beginning of each growing season with the applications of biosolids and inorganic fertilizer treatments (Figure 6.2). Reclamation rates and the inorganic fertilizer resulted in greater NO_3-N in water samples after treatment application for the fall growing seasons (September 2016 and 2017). Conversely, the inorganic fertilizer and BLOOM-1 gave greater NO_3-N concentrations in water samples after

their spring 2017 application, and only the inorganic fertilizer increased NO_3-N during spring-summer 2018. Nitrate-N concentrations decreased, and there were no differences between treatments with water samples taken during the winter and early spring (Figure 6.2).

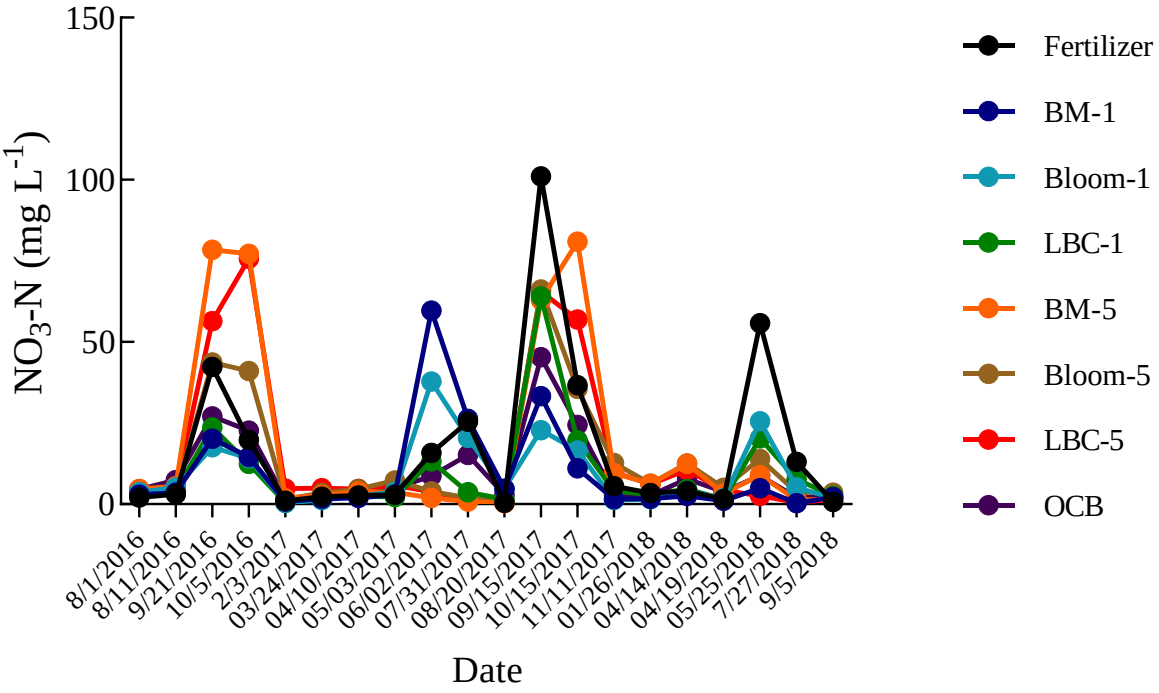

Figure 6.2. Nitrate (NO_3-N) concentrations over time from August 2016 to September 2019. Arrows in the x-axis indicate a significant increase in nitrate concentrations with treatments applied for fall 2016, summer 2017, fall 2017, and summer 2018 growing seasons. BLOOM= air-dried DC Water EQ biosolids, BM=DC Water EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. 1= agronomic N rate, 5=reclamation rate (dry weight basis)

The cumulative NH_4-N load was insignificant compared to the load of NO_3-N, and most of the NH_4-N detected in water samples came from the inorganic fertilizer treatment (Table 8). The cumulative concentration of NO_3-N by the end of the experiment was not significantly different among the inorganic N fertilizer and biosolids applied at the reclamation rates (Table 6.8). However, the inorganic fertilizer had greater cumulative NO_3-N load than BLOOM-1, LBC-1, and OCB. These results were unexpected, since the amount of PAN applied with reclamation rates of biosolids was estimated to be five times the agronomic N rates of biosolids and the inorganic fertilizer (and even greater differences in total N). Because N in biosolids is largely in organic N form, slow mineralization of organic N to soluble NH_4-N, and consequently NO_3-N, could have reduced the amount of NO_3-N lost via leaching. Organic N mineralization of EQ biosolids could have also been overestimated, minimizing the amount of NO_3-N loss; since most biosolids applied at the agronomic N rate had significantly lower cumulative NO_3-N load than the inorganic fertilizer treatment.

Table 6.8. Cumulative nitrate (NO_3-N) and ammonium (NH_4-N) load from leaching water obtained from August 2016 to September 2018.

Treatment†	NO_3-N‡	NH_4-N§	Total leachate N
	kg ha^{-1}	kg ha^{-1}	kg ha^{-1}
Fertilizer	135 a	6.01 a	139 a
BM-1	96 abc	0.18 b	97 abc
BLOOM-1	87 bc	0.25 b	88 bc
LBC-1	79 bc	0.63 b	80 bc
BM-5	117 ab	0.21 b	118 ab
BLOOM-5	92 abc	0.20 b	92 bc
LBC-5	99 abc	0.52 b	99 abc
OCB	65 c	2.56 ab	66 c

† BLOOM= air-dried Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. 1= agronomic N rate, 5=reclamation rate (dry weight basis).

‡ NO3-N and total N difference at 0.08 and 0.06 level, respectively. Fisher LSD mean separation.

§ NH4-N difference at 0.02 level. Tukey HSD mean separation.

Rationale for explaining the relatively low nitrate N losses from the high biosolids application rates could include denitrification of a portion of the N that was collected from the lysimeters. Fine-textured soils tend to have greater denitrification rates due to their poor drainage and high fraction of water-filled pores upon moisture inundation. Denitrification estimates in clay soils via field and laboratory methods have been measured as high as 87% (van der Salm et al., 2007). The C (Table 6.9) provided with biosolids application could have served as an electron donor for nitrate reduction in the lysimeters.

Conversely, large masses and fractions of total N remained in the soil after two years of amending with various biosolids treatments (Table 6.9). The fractions of applied N that were accounted for ranged from 42 to 82% (with the exception of the 133% outlier). High measured N accumulations offer proof evidence that significant portions of N (and associated C) can be accumulated in such anthropogenic fine-textured, clay soils. Such soils can adsorb organic matter compounds in the surface of clay minerals, and Fe and Al oxides, and physically protect C and N compounds within aggregates and small pores, which could have prevented accessibility and mineralization by microbes (Bingham and Cotrufo, 2016, Griffin, 2008). The Groseclose-derived, poorly weathered subsoil used in this experiment most likely had 2:1 clay minerals that are known to fix NH_4 in the soil and prevent its loss and transformations (Nommik and Vahtras, 1982).

Table 6.9. Total nitrogen (TN) applied, N accumulated, and soil total organic carbon (TOC) in July 2018.

Treatment†	N stored‡	N accumulation§	C stored¶	C accumulation#
	kg ha^{-1}	%	kg ha^{-1}	%
Fertilizer	184 e	42 c	0 d	0 c
BLOOM-1	1808 b	133 a	11536 bc	77 a
BM-1	1108 cd	78 b	10331 bc	84 a
LBC-1	1210 c	74 b	12197 b	65 ab
BLOOM-5	2749 a	81 b	18328 a	49 ab
BM-5	2402 a	68 bc	23078 a	75 a
LBC-5	2021 b	45 c	18969 a	37 b
OCB	787 d	82 b	6201 d	83 a

† BLOOM=Blue Plains EQ biosolids, BM=Blue Pains EQ biosolids + mulch, BSS=Blue Pains EQ biosolids + sand + sawdust, LBC=Livingston Compost, and OCB=OceanGro thermally-dried. 1= agronomic N rate, 5=reclamation rate (dry weight basis)

‡ N concentration (mg kg^{-1}) x soil bulk density (kg m^{-3}) x soil sampling depth (m) x 10^{-3}.
§ Difference between N stored in the amended and benchmark soil, divided by N application rate (kg ha^{-1}; Table 6.1).
¶ C concentration (mg kg^{-1}) x soil bulk density (kg m^{-3}) x soil sampling depth (m x and 10^{-3}.
# Difference between C stored in the amended and control soil, divided by C application rate (kg ha^{-1}; Table 6.1).

6.5. Conclusions

Biosolids have been used in agriculture, forestry and mine land reclamation for many years, but the land application of EQ biosolids in urban landscapes is more recent and requires greater study in order to ensure their appropriate use and minimize the risk of nutrient losses. Our study found that NO_3-N was the nutrient that posed the greatest risk of leaching. However, NO_3-N concentrations for high application rates (reclamation-type treatments) were not greater than agronomic inorganic fertilizer and biosolids application rates. This requires further study in order to identify other possible pathways in which NO_3-N may have been lost (e.g., denitrification). Soil PSR and WEP were low during the first year of the experiment, and it was not until the second year that P showed a greater risk of loss from soluble forms. Soil PSR results in our experiment seemed to overestimate the risk of P loss, since soil PSR levels of reclamation rates and BLOOM agronomic rate were greater than the recommended range (30%), but soil WEP and leaching water ortho-P concentrations from these and all other treatments were very low, indicating low P loss via runoff and leaching. Our data suggests that even after such high P applications and increases in soil oxalate extractable P, the increase in oxalate extractable Fe obtained from biosolids with high Fe concentrations could have reduced the risk of P loss. Urban soil properties (low initial P, and high pH, exchangeable Ca, and clay content) may have also contributed to lower the risk of P leaching. The use of low fertility subsoils exposed during construction activities can minimize the potential for soil P saturation and risk of soluble P loss, even after the addition of biosolids at high application rates for urban agriculture production. Further studies are required to understand N transformations and P losses with EQ biosolids applications to a greater selection of urban soils.

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7. Conclusions

Biosolids have been applied to improve soil physical, chemical, and biological properties in agriculture, forestry, and mine land reclamation for many years. The beneficial effects of biosolids application are largely due to the addition of organic matter, which improves nutrient availability, microbial activity, soil structure, and water holding capacity of soils. Exceptional Quality or EQ biosolids are products treated through processes to further reduce pathogens, vector attraction, and pollutant concentrations, which makes them safe for use in urban agriculture. The rising popularity of urban agriculture and greater production of EQ biosolids in urban areas provides a unique opportunity to recycle EQ biosolids locally into urban soils; however, there is limited research regarding the nitrogen (N) availability of newly developed EQ biosolids products. There is also little research about the effects of established and new EQ biosolids on vegetable production in urban degraded soils, and the extent of N and phosphorus (P) loss after their application. Chapter 3 and 4 of this dissertation focused on determining the quality and organic N mineralization of EQ biosolids products using greenhouse bioassays, a field tall fescue N uptake experiment, and rapid chemical and biological laboratory tests. Chapter 5 and 6 focused on evaluating the effects of four different EQ biosolids applied at agronomic N and reclamation rates (5x agronomic N rate) on properties of a degraded urban soil, vegetable production, and risk of N and P loss.

We used a soybean greenhouse bioassay to evaluate the quality of various well-established and new EQ biosolids products, and a tall fescue N uptake greenhouse bioassay to assess their N availability. We found that biosolids application based on crop N requirements or agronomic N rate served as a high quality soil amendment. Growth-limiting-factors (e.g. low pH and high soluble salt concentrations) were only observed when biosolids were used as the entire

growing media and this was likely enhanced by the concentration of soluble salts due to the lack of water drainage in the plastic-lined greenhouse pot. Nitrogen uptake by tall fescue grown with biosolids amendments compared with known rates of inorganic N confirmed organic N mineralization to be approximately 40%, 20%, and 15% for thermally dried, blended, and composted EQ biosolids products.

The greenhouse bioassay provided the basis for the tall fescue field experiment in which our aim was to estimate organic N mineralization from various EQ biosolids products applied to an urban degraded, clayey soil by using the N fertilizer equivalency method (tall fescue N uptake). Soil cores were obtained five days after biosolids application to conduct several chemical (i.e. soil NO_3-N, soil NH_4-N, total inorganic N, and organic N) and biological (i.e. 7 day anaerobic incubation and Solvita CO_2 burst) tests commonly used to evaluate N availability and mineralization. Field study and laboratory tests confirmed that biosolids produced by heat-drying and the thermal hydrolysis followed by anaerobic digestion (Cambi^{TM} process) have higher plant available N and organic N mineralization rates than biosolids blended with woody products and biosolids composts. Woody-amended biosolids and biosolids composts have lower available N due to dilution of N concentrations and higher C:N ratios. Heat-dried biosolids and biosolids produced via Cambi^{TM} process had greater organic N mineralization, while composted biosolids and blended products had lower organic N mineralization than was initially estimated.

Factors that could have reduced the accuracy of estimating plant available N and organic N mineralization rates based on field fertilizer equivalent method include losses of N from biosolids-amended soil. The lack of an established C-N cycle in anthropogenic soils limits the annual amounts of mineralized C and N generated, thus reducing native soil N contributions to plant available N. Most in situ N mineralization studies, including our own greenhouse bioassay,

are conducted in natural and agricultural soils that have larger reservoirs of C and N that annually mineralize. The fine-textured soil used in our study may have also physically protected C- and N-containing substrates from microbial degradation, thus reducing organic N mineralization. Comparisons between field tall fescue N uptake and laboratory test indicators revealed that total soil inorganic N, soil NO_3-N, and the 7 day anaerobic incubation (7-AI) were the best indicators of N availability in the urban soil used in this experiment. Positive correlations between the 7-AI, Solvita CO_2 burst, and organic N show that these N indicators are related to each other, but there is not enough evidence to suggest that Solvita CO_2 burst and organic N could effectively serve as N availability indicators. The reliability of these N tests may have also been reduced due to the low C- and N-, and high clay-containing urban soil.

We conducted a two year urban garden field study to evaluate the effects of EQ biosolids agronomic N rate and reclamation rate application on the properties of an urban degraded soil, vegetable production, and risk of N and P loss. The beneficial effects of biosolids application were evident in terms of improvements in soil properties such as reduced bulk density, and greater total N, P, and C. In fact, our research showed that degraded urban soils have the potential to sequester large amounts of C in a short amount of time (i.e. two years), implying that additions of EQ biosolids not only improve urban soils properties for urban agriculture production, but also have potential to sequester C in degraded soils of urban areas. Vegetable yields obtained with agronomic N rates of EQ biosolids were generally lower than yields obtained with reclamation rates. High soil compaction of the urban clayey soil and low initial concentrations of essential nutrients (N and P) most likely negatively impacted vegetable growth. Despite the limiting physical, chemical, and biological conditions of our soil, the addition of biosolids at higher than agronomic rates (i.e. reclamation rate) showed great potential to increase

vegetable yield after two years of application. Low orthophosphate concentrations in leaching water samples and water soluble P showed that there was very little risk of P loss even after high P applications with EQ biosolids treatments. These results were likely due to increases in soil oxalate extractable Fe with certain biosolids products, and the properties of our urban soil (low initial P, and high pH, exchangeable Ca, and clay content). Thus, we recommend the use of EQ biosolids products with high Fe to reduce the risk of P loss in urban agriculture production. Nitrate was the nutrient that posed the greatest risk of leaching; however, NO_3-N concentrations obtained from reclamation rate treatments were not greater than those found with biosolids and an inorganic fertilizer applied at the agronomic N rate. While some of the N applied was taken up by the vegetables grown in this field, N could have also been lost via denitrification or may have accumulated in the soil by occlusion within small pores and aggregates in the high clay soil.

Our urban garden field research showed overall high accumulations of C and N in the soil after two years of EQ biosolids applications. Future research should determine the mineralogy of the urban soil used in this experiment and aim to understand possible binding mechanisms and strength of organic-mineral interactions. Such information would be vital to determine if chemical stabilization of organic matter in the soil was preventing organic matter degradation by microbes. Dynamic force spectroscopy has been proposed as a technique that can quantitatively measure molecular binding at mineral surfaces. This technique can utilize both physical measurements and chemical analyses to further understand organic-mineral interaction under different environmental conditions, soil structure, and organic matter chemistry, which are all parameters that greatly affect C stabilization in the soil.

Further studies are also needed to evaluate the reliability of chemical and biological tests to estimate N mineralization in urban soils and understand the influence of low residual C and N,

and high clay contents on biosolids organic N mineralization rates. We also suggest the use of incubation and field experiments to obtain estimates of denitrification rates after EQ biosolids application to the clayey urban soil used in both the tall fescue and urban garden field studies.

While we used a soil that was representative of the often degraded properties of urban soils, future research should evaluate the impact of EQ biosolids applications on soils across a variety of urban landscapes because conditions of urban soils can differ geographically. Further assessment of EQ biosolids N mineralization in a greater variety of urban soils could lead to more accurate recommendations for EQ biosolids application in urban settings, and the possible development of correction factors to adjust laboratory N indicator test results to results obtained in field conditions.

Appendix

Appendix A. Soybean growth in greenhouse bioassay a) with incorporation of BM1x, TM1x, and Fertilizer1x; and b) BSS1x, TSS1x, and Fertilizer 1x.

Appendix B. Soybean growth in greenhouse bioassay a) with incorporation of BM100, TM100, and Fertilizer1x; and b) BSS100, TSS100, and Fertilizer 1x.

Appendix C. Field research site at the Turfgrass Research Center (TRC) in Blacksburg VA. Illustrates treatment application per plot before incorporation to the soil.

Appendix D. Vegetables grown on the field research garden at the Turfgrass Research Center (TRC) in Blacksburg VA during the fall of 2016.

Appendix E. Vegetables grown on the field research garden at the Turfgrass Research Center (TRC) in Blacksburg VA during the summer of 2017.

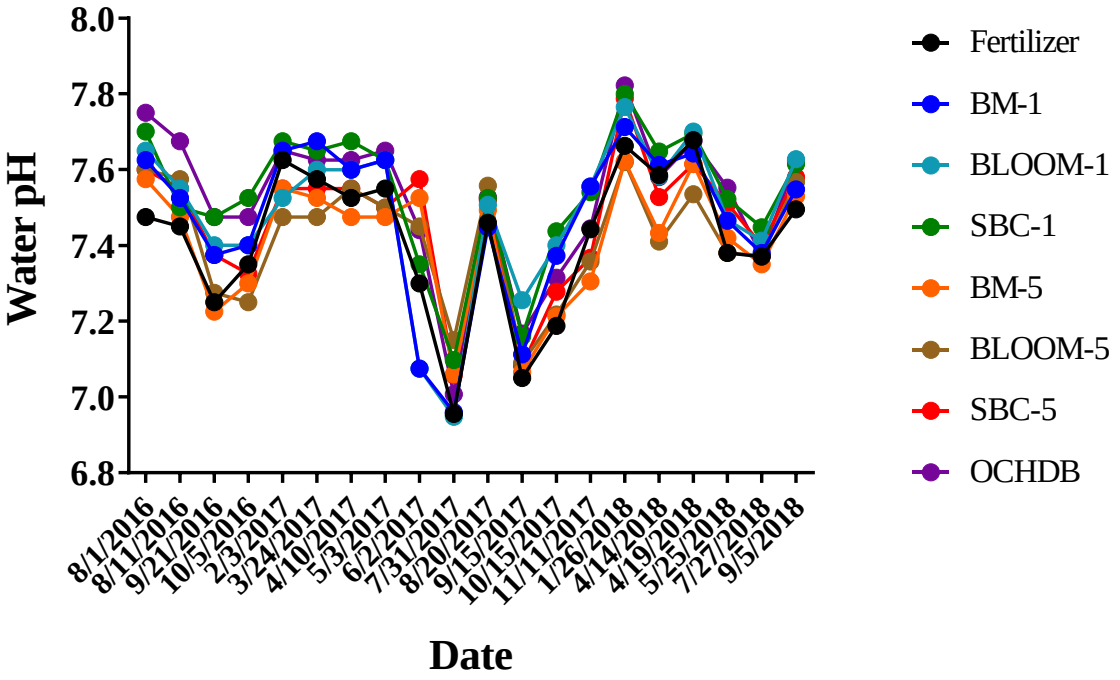
Appendix F. Vegetables grown on the field research garden at the Turfgrass Research Center (TRC) in Blacksburg VA during the fall of 2017.

Appendix G. Vegetables grown on the field research garden at the Turfgrass Research Center (TRC) in Blacksburg VA during the summer of 2017.

Appendix H. Soil pH eight weeks after treatment application on fall 2016-2017, and summer 2017-2018. Different lowercase letters within columns indicate significant differences between treatments by harvest date

	Soil pH			
Treatments	*Fall 2016*	*Summer 2017*	*Fall 2017*	*Summer 2018*
Fertilizer	7.70	7.77	7.93 a	7.92 a
BLOOM-1	7.56	7.58	7.84 a	7.76 abc
BM-1	7.61	7.68	7.88 a	7.83 ab
LBC-1	7.68	7.72	7.89 a	7.74 bcd
BLOOM-5	7.71	7.70	7.39 c	7.52 e
BM-5	7.59	7.65	7.56 bc	7.66 cde
LBC-5	7.63	7.69	7.69 ab	7.59 de
OCB	7.86	7.63	7.85 a	7.79 abc

Appendix I. Effect of treatments on pH of water samples collected from August, 2016 to September, 2018.



Appendix J. Effect of treatments on electrical conductivity (EC) of water samples collected from August, 2016 to September, 2018.