Biochar-induced soil stability influences phosphorus retention in an agricultural field in Quebec

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December, 2013

A thesis submitted to McGill University in partial fulfillment of
the requirements of the degree of
MASTER OF SCIENCE

Abstract

M.Sc. Vanita Sachdeva Natural Resource Sciences

Surface runoff from agricultural fields is the largest non-point source of phosphorus (P) that pollutes surface water in humid temperate regions. Best management practices have attempted to reduce P loading and improve P retention in agricultural soils but significant losses continue to occur, emphasizing the need for novel solutions. The objective of this research project was to determine whether biochar amendments in an agricultural soil could reduce P loss in surface runoff by increasing water infiltration or by improving soil stability. Experimental plots were established in St-Francois-Xavier-de-Brompton, Quebec, Canada on an agricultural field amended with three types of biochar (Dynamotive, Pyrovac, and Basques) applied at two application rates (5 and 10 t ha^{-1}), and one unamended control plot. First, a 30-minute rainfall simulation was conducted using the Cornell Sprinkle Infiltrometer to assess runoff volume, time-until-ponding, infiltration rate, and water holding capacity (WHC), as well as P concentration and load in runoff. Second, soil samples from the experimental plots were fractionated using a wet-sieve method to determine the proportion of macro- and micro-aggregates. Each fraction was analyzed for total organic C and total P to locate biochar presence and determine whether additional P was retained in macro- or micro-aggregate fractions of biochar-amended soils. Water dynamics in the rainfall simulation showed no significant differences, however, runoff contained significantly less ortho-P in soil amended with Dynamotive biochar at 5 t ha^{-1} ($p=0.048$) and significantly less particulate P from soil amended with

Pyrovac biochar at 5 t ha^{-1} ($p=0.012$) and Dynamotive and Basques biochars at 10 t ha^{-1} ($p=0.024$ and $p=0.047$, respectively). Soils amended with biochar at 5 t ha^{-1} and 10 t ha^{-1} also had significantly greater microaggregate stability ($p=0.032$ and $p=0.046$, respectively), which corresponded to significantly more organic C content ($p=0.013$ and $p<0.01$, respectively). Macroaggregates from biochar-amended soils also contained significantly higher organic C and total P concentrations ($p<0.05$ for both biochar rates) than the control soil. This suggests that the reduction in particulate P concentration in runoff is the result of biochar integration within the microaggregate structure, which indirectly promotes P retention in macroaggregates.

Résumé

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Les eaux de ruissellement associées aux champs agricoles sont la plus grande source de charges diffuses de phosphore (P) affectant l'eau de surface en régions tempérées. Les pratiques de gestions optimales tentent de réduire la charge en polluants phosphorés ainsi que d'améliorer la rétention de P dans les sols agricoles, mais malheureusement, des pertes significatives continues d'être recensées, accentuant le besoin pour de nouvelles solutions. L'objectif de cette recherche était de déterminer si des amendements de biocharbon dans les sols agricoles pourrait réduire la charge en phosphore par ruissellement en augmentant l'infiltration de l'eau dans les sols et en améliorant la stabilité des sols. Des parcelles expérimentales ont été établies à St-Francois-Xavier-de-Brompton, Québec, Canada dans un champ agricole amendé avec trois types de biocharbon (Dynamotive, Pyrovac, and Basques) utilisant deux différentes doses d'application (5 et 10 t ha^{-1}), et un sans biocharbon. Premièrement une simulation de précipitation de pluie de 30 minutes a été effectuée à l'aide de Cornell Sprinkle Infiltromètre pour mesurer les niveaux de ruissellements, la durée avant le début du ruissellement, le taux d'infiltration, la capacité de rétention de l'eau, ainsi que la teneur et la charge total de P dans l'eau de ruissellement. Deuxièmement, des échantillons de sols des différentes parcelles on été fractionnées selon la technique de tamisage humide pour déterminer la proportion de macros et micros agrégats. Chacune des fractions a été analysé pour leur quantité de C organique ainsi que le niveau total de P afin de localiser la présence de biocharbon et afin de déterminer

si plus de P a été retenu dans les fractions contenant les macro ou les micros agrégats de sols amendés avec du biocharbon. La dynamique de l'eau dans la simulation de précipitation a démontré aucune différence significative, cependant le contenue du ruissellement avait significativement moins d'orthophosphate dans le sol amendé avec le biocharbon Dynamotive à 5 t ha^{-1} ($p=0.048$) et il y avait significativement moins de P particulaire avec le sol amendé avec le biocharbon Pyrovac à 5 t ha^{-1} ($p=0.012$), le biocharbon Dynamotive et le biocharbon Basques à 10 t ha^{-1} ($p=0.024$ et $p=0.047$, respectivement). Les sols amendés avec du biocharbon à 5 t ha^{-1} et à 10 t ha^{-1} avaient aussi, de façon significative, des micros agrégats plus stables ($p=0.032$ et $p=0.046$, respectivement), ce qui correspond à une plus grande quantité de C organique ($p=0.013$ et $p<0.01$, respectivement). Les macros agrégats des sols amendés en biocharbon avaient des concentrations de C organique et P totales plus élevées ($p<0.05$ pour les deux concentrations de biocharbon) que le sol témoin. Cela suggère que la réduction de la concentration de P particulaire dans l'eau de ruissellement est la conséquence de l'intégration du biocharbon dans la structure des micros agrégats, ce qui encourage la rétention dans les macros agrégats.

Contribution of Authors

This thesis is composed of a general introduction explaining the context of this research project, three chapters written in manuscript format according to the guidelines of the Graduate and Postdoctoral Studies Office, and a general conclusion to highlight key findings and future research needs.

The first chapter is a literature review summarizing current knowledge in the thesis research field. The project's hypotheses and objectives are presented at the end of the first chapter.

The second and third chapters present a field and lab experiment, respectively, that achieve the project objectives. A connecting paragraph joins the two chapters in order to maintain progression from one manuscript to the next.

Both manuscripts, as well as the literature review, were co-authored by the candidate and her supervisor, Dr. Joann K. Whalen. They are formatted for submission to Geoderma.

The experiments were all conducted on or with soil collected from a field experiment established by Barry Husk of BlueLeaf, Dr. Julie Major, and Dr. Don Smith. The experiments were designed by the candidate, Dr. J. K. Whalen, and Dr. J. Major. The candidate performed the experiments and processed the samples with the help of lab and field assistants. The candidate conducted data analysis and interpretation under the guidance of Dr. Pierre Dutilleul.

The manuscripts that compose the body of this thesis are in the following order:

Chapter 1. Sachdeva, V. and J.K. Whalen (2013). Literature Review.

Chapter 2. Sachdeva, V. and J.K. Whalen (2013). Application of biochar to Quebec agricultural soil reduces particulate phosphorus concentrations in simulated rainfall runoff (In preparation for Geoderma).

Chapter 3. Sachdeva, V. and J.K. Whalen (2013). Biochar amendments alter nutrient composition in aggregates from Quebec agricultural soil (In preparation for Geoderma).

Acknowledgements

I would like to thank my supervisor, Dr. Joann K. Whalen, for supporting, encouraging, and challenging me throughout this process. She has been a strong academic role model in my life and has provided numerous opportunities for me to succeed. I would also like to thank Dr. Don L. Smith for his invaluable wisdom about the scientific process, which was always complemented by colourful stories.

I am indebted to Barry Husk of BlueLeaf for initiating this project, providing funding, and always asking thought-provoking questions. I would additionally like to thank the Natural Sciences and Engineering Research Council of Canada (NSERC) and Centre SÈVE for scholarships and grants that enabled this research.

Thank you also to Dr. Pierre Dutilleul for statistical advisement, Dr. Julie Major for providing valuable direction, Dr. Dina Schwertfeger for coaching me through my experiments, François Gagné-Bourque and Guillaume Lamarche-Gagnon for translating the abstract, Hicham Benslim for processing samples, and Hélène Lalande for imparting her tremendous scientific expertise. Lastly, I would like to thank the entire Soil Ecology Group for sharing this experience with me, helping with so much work, and for being unconditionally supportive.

This thesis is dedicated to my amazing friends that carried me through the last two years.

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General Introduction

Agricultural production requires soil fertilization to maintain maximum crop yields because annual harvests deplete soils of natural nutrients stores. One crucial nutrient for plant growth is phosphorus (P), second only to nitrogen. In 2009, 23 Tg (1 teragram = 1 million metric tons) of P fertilizers were manufactured from mined geological phosphate reserves (Bennett et al. 2001; Bennett and Elser, 2011). Rock phosphate extraction is expected to peak in 2030 and reserves are forecasted to deplete within the century. In order to maintain sufficient food production, P efficiency must be improved by recycling and reducing loss through extraneous pathways.

P fertilizers have an inherently low plant uptake efficiency rate, leaving approximately half of every fertilizer application in the soil. The cumulative surplus in upland soils is not harmful for crop yields but it is vulnerable to environmental forces such as wind and rain (Daniel et al., 1994). Globally, precipitation running off the soil surface collects 2 Tg of solubilized P and 20 Tg of sediment-bound P each year (Bennett et al., 2001). The 22 Tg of eroded and leached P migrates into streams, lakes, and oceans and nourishes previously P-limited algal populations (Schindler, 1977). Algal blooms rapidly expand and vacuum available oxygen out of the aquatic ecosystem, a process called hypereutrophication. It results in widespread floral and faunal death that destroys potable water supply and renders the body of water unfit for recreational or industrial use.

P runoff from agricultural fields is responsible for 50%-60% of all nutrient deposits in freshwater, and croplands are ranked as the highest non-point source (Parry, 1998). Ongoing research is investigating methods to harvest and recycle P immobilized within algae and mechanisms to inhibit P mobility from fields to lakes. Many uncontrollable parameters moderate P movement including soil type, field slope, precipitation intensity, and crop production history. However current farm management practices are equally influential and can easily be modified to reduce P loss.

Farmers worldwide have adopted region-specific best management practices (BMPs) in an attempt to mitigate P and other nutrient losses from their fields without compromising crop yields (Frink et al., 1999). BMPs target loss as two phases: the source and in transport (Sharpley et al., 1994b). “Source” BMPs address fertilizer type, rate, timing, and application method. “Transport” BMPs try to capture migrating P in runoff before it reaches a watercourse by encouraging subsurface water flow (eg. tilling, tile drains) or creating physical barriers above the soil surface to slow movement (eg. crop residue, cover crop, buffer strips).

BMP success has been modeled and observed at the field scale. However, nutrient transport measurements at the watershed scale demonstrate their efficacy is limited. BMPs merely spatially, chemically, or temporally displace the underlying issue of nutrient loading and still result in comparable rates of nutrient loss (Sprague and Gronberg, 2012). Novel solutions must be explored in the scientific sphere to improve fertilizer efficiency and BMP success. Black carbon soil amendments have been proposed to retain soil nutrients for the purpose of environmental protection.

Incompletely combusted biomass in the form of charcoal, soot, or black carbon, was historically incorporated to Amazonian *Terra Preta* soil and proved to reduce nutrient loss, as well as promote overall soil fertility. The same material has been recently manufactured as a biofuel byproduct for carbon sequestration, referred to as biochar. Recent research has investigated whether biochar can serve as an innovative solution to address nutrient loss while maintaining or enhancing crop integrity (Lehmann et al. 2009). The limited research that exists was mostly conducted in tropical regions and it is uncertain whether biochar's success is viable in North American humid, temperate climates or soil types (Laird et al. 2010).

The American Midwest "Corn Belt", Southern Ontario, and Southeastern Quebec fall within humid, temperate climates. Cumulatively, they account for 46 million hectares of active cropland and receive on average 1000 mm of precipitation annually (van Bochove et al., 2006). In Quebec, Canada, 40% of the 3.5 million hectares of agricultural soil is characterized by high or very high risk for P loss, as defined by 3 or more mg P kg^{-1} soil yr^{-1} lost during a storm event, as of 2006 (van Bochove et al., 2006); thus making it a prime candidate for biochar research.

The objectives of this research project are: (1) to determine whether biochar application can reduce particulate P and dissolved P concentrations in runoff in a simulated rainfall on a field in Quebec, Canada and (2) to establish which biochar-mediated changes are influencing differences in P concentration in surface runoff.

Chapter 1: The potential of biochar application to mitigate phosphorus loss from agricultural fields: a review

Phosphorus fertilization on agricultural soil consumes 96% of all mined rock phosphate globally and is responsible for 60% of all P deposits in aquatic ecosystems. On-field nutrient management practices are a critical mediator of global P imbalance and subsequent aquatic pollution.

This literature review follows P movement from fertilizer application to waterborne loss: first, P fertilization requirements for agricultural crops are briefly described, followed by a summary of the various P pools existing in soil, how fertilization contributes to them, and their vulnerability to hydrological loss. Second, the scientific principles of common BMPs used in humid temperate climates are explained, along with an evaluation of their concurrent success and failures at reducing waterborne P loss. Biochar is proposed as an additional method to slow P loss from agricultural fields and relevant biochar properties are described. Physical, chemical, and biological mechanisms of biochar-induced P immobilization are hypothesized, using research from tropical regions as evidence. Lastly, Quebec is used as a model region to investigate biochar effects on P movement in humid temperate climates, due to its thriving agricultural industry and accelerated eutrophication problem compared to other North American regions.

Section 1: Phosphorus dynamics in agricultural soils

1.1.1 Phosphorus in agriculture

Agricultural soils are typically fertilized with inorganic and organic P to increase crop yields. Phosphorus is vital for plant growth because it serves as the structural backbone of nucleic acids (sugar-phosphates) and cell membranes (phospholipids), moderates energy transfer reactions during photosynthesis (phosphorylation), and facilitates nutrient metabolism. Phosphorus (mostly in the form of phytic acid) accumulates within plant seeds for long-term energy storage at concentrations up to 4000 mg P kg^{-1} (Marschner, 1995).

Plants with mild P deficiency possess smaller leaf surface area from stunted foliar growth, and those with more severe deficiencies result in lower crop quality and yield. Plants require P at early stages of crop growth; the impact of inadequate P may occur before any symptoms are exhibited on the plant and deficiencies cannot be retroactively remediated with P fertilizers (Barry and Miller, 1989). It is crucial to ensure adequate P reserves within agricultural soil to achieve optimal yields. Timing of P fertilizer application, generally at planting, and placement of P fertilizer in proximity to the root zone (eg. band application) are source strategies to avoid P deficiencies.

An average corn crop (*Zea mays* L.) in a humid temperate climate receives 12 kg P ha^{-1} yr^{-1} of mineral fertilizers and 14 kg P ha^{-1} yr^{-1} of organic fertilizer to meet its biological requirements of P. However, plants have low fertilizer-use efficiency (~ 50% in annually cropped systems, (Richardson et al., 2011)) caused by rapid chemical fixation of plant-available P, which results in a net accumulation of approximately 6 kg P ha^{-1} yr^{-1}. The cumulative surplus, referred to as legacy P, collects in the topsoil; soils may become P-saturated from repeated fertilizer applications over the course of many years.

Legacy P exists in inorganic and organic forms that are mildly soluble (labile) to highly insoluble (recalcitrant), all found within a continuous, dynamic cycle (Figure 1-1). Ecosystems that are generally undisturbed (eg. grassland and forests) have predictable quantities of legacy P pools from chemical equilibrium that stabilizes over geological timespans (Figure 1-2). Conversely, managed agroecosystems have greater annual P flux, which continually shifts the balance of P between plant-available and unavailable forms. As a result, the crop demand may exceed the existing soil P supply and the latter cannot be relied upon to adequately replenish the soil solution, thus requiring fertilization in spite of historical P build-up.

1.1.2 Soil P pools and their dynamics

Constant inputs (from fertilizer) and losses (from plant uptake) perpetually disturb the chemical equilibrium of the P cycle and alter plant-available concentrations that are found only in the soil solution (Richter et al., 2006). Shifts between inorganic P fractions are largely moderated by a series of pH-dependent abiotic reactions (DeLuca et al., 2009), while microbial enzymes control organic P immobilization and mineralization. Given that P has multiple chemical forms within soil, various pools are conceptually partitioned, quantified and characterized by their chemical affiliations and propensity to refurnish plant-available P. The major pools of this model are presented in order of bioavailability: (1) soil solution P (2) organic P, (3) secondary P, (4) occluded P, and (5) primary P.

(1) Soil solution P is an inorganic soluble form of orthophosphoric acid (often called ortho-P) that comprises approximately 1% of total soil P, yet is the only plant-available form of P (Bolan, 1991). It's speciation ranges from H_3PO_4 to PO_4^{3-}, with the relative concentrations of each ionic form changing according to soil pH. These ions are readily available for plant, algal, or microbial assimilation (Correll et al., 1998) and prone to leach, so this pool is regarded as the most environmentally potent. Inorganic fertilizers and soluble P compounds from manure and other organic residues contribute directly soil solution P.

(2) Secondary P minerals are a transient pool that is an intermediate between soil solution P and occluded P. They form when P chemisorbs to Al^{3+} and Fe^{2+} ions in acidic soils or sorbs to Ca^{2+} in alkaline soil, which become immobile sesquioxides that are immobilized from water-borne loss (Smeck, 1985). If there is insufficient soil solution P to meet the biological P requirements of plants and microbes, secondary P minerals easily dissociate and reform ortho-P ions, making it a desirable pool. Absorbable Al^{3+} and Fe^{2+} cations exist in finite quantities within the soil and, depending on the soil type, the threshold for immobilization can be as low as 10-20% (as assessed by P/Al or P/Al+Fe, Mehlich-III extraction)

(Beauchemin and Simard, 1999). Once the degree of phosphorus saturation (DPS) reaches its maximum, additional ortho-P remains in the soil solution pool, where it is mobile and susceptible to transport processes (Sims et al., 2002).

(3) Organic P constitutes a wide range of compounds of varying complexities that can quickly or slowly replenish soil solution P with ortho-P ions. The most common organic P forms are inositol-bound P, nucleic acids, phospholipids, organic matter, and humus, presented in increasing order of complexity and stability determined by the number of phosphoester bonds. Soil microbial enzymes, like phosphatase, are responsible for moderating the rate of mineralization from organic P to inorganic P. Since secondary minerals dissociate to form ortho-P faster than organic compounds, inorganically fertilized soils often have slower organic P turnover, and accumulate large quantity of small organic particles in the soil.

(4) Occluded P compounds are recalcitrant inorganic complexes that exist along a continuum of chemical resistance. Secondary P minerals often serve as the foundational structure of occluded P compounds, but more complex bonds are forged with other elements or organic matter (particularly inositols) that renders the entire compound inert. Occluded P are generally thought to be a P-sink, though in extreme cases when all other P pools are exhausted, the reaction can be reversed to supply the soil solution.

(5) Primary P, also known as apatite, is a collection of phosphate

minerals that slowly weather and release soluble P over the course of decades, but is not replenished within the pedologic timeframe (Smeck 1985). It is often mined to manufacture inorganic fertilizers, but natural weathering itself is not considered a significant contributor to bio-available soil solution P in agricultural soils.

Inorganic fertilizer use efficiency is low due rapid transformation from soil of ortho-P released from dissolving fertilizer granules to secondary P minerals prior to plant uptake. Organic fertilizer use efficiency is low because microbes must breakdown organic P before secondary mineral P is transformed into a plant-usable form. As a result, applied fertilizer is often cycled through multiple pools over the course of multiple growing seasons before it ultimately reaches plants or becomes occluded in organic or inorganic forms (Smeck, 1985). With each additional fertilizer application, the quantity of soil solution P, as well as the organic P, secondary P mineral, and occluded P pools get larger. This results in a greater concentration of P vulnerable to loss from soil.

1.1.3 Losses of agricultural soil P via surface runoff and leaching

Soil hydrology dictates how P moves out of the soil and into waterways. Precipitation, including rainfall, snowfall, and snowmelt, makes contact with the soil surface and soil infiltrability determines the water pathway: incoming water can leave the soil via runoff (surface flow) or leaching (subsurface flow) (McDowell et al., 2001).

When expressing P movement from agricultural fields, certain pools are combined to reflect their environmental potency and the methods used to quantify them. Phosphorus compounds in solution are referred to as dissolved P (DP) but can be split into dissolved reactive P (DRP) and dissolved unreactive P (DUP). "Reactivity" refers to the interaction between ortho-P and pigmented reagents used during analysis. Therefore DRP denotes only ortho-P, while DUP includes all non-ortho-P, including dissolved inorganic or soluble organic P. Secondary P minerals, occluded P, and organic P associated with any colloidal sediment ($\geq$ 0.45 μm) are reduced to a single category called particulate P (PP). Total P (TP) is the sum of all three.

Subsurface flow, or leaching, refers to precipitation that has infiltrated the soil surface, moved past the active root zone, and will migrate down stream through artificial drainage systems (eg. tile drains) or groundwater (Sims et al., 1998). Subsurface water movement occurs either via (1) matrix flow or (2) preferential flow.

> (1) Matrix flow is water percolation through the soil pore space, with a high degree of soil-water contact (McDowell et al., 2001). Different pore sizes within the soil determine the pathway of the water; mesopores usually move water from high moisture to low moisture, while micropores tend to hold water in place via capillarity forces (Tseng and Tseng, 2006).
>
> Historically, leaching contributed negligible amounts of P to waterways. This was because the high degree of soil-water contact chemisorbed most DP on Al^{3+} and Fe^{2+} cations and trapped PP before it reached steams, thus

preventing P loss to waterways (Major et al., 2010a). However, many soils that received repeated, long-term application of P fertilizer have become P-saturated, and surpassed soil's P-sorption mechanisms. Research in shows that DP losses via matrix flow are now high enough to accelerate eutrophication (Ryden et al., 1977).

(2) Preferential flow occurs when water travels rapidly through soil cracks and small tunnels created by plant roots and earthworms burrows that connect to tile drains or directly to groundwater reserves. Water collects sediments and soil particles from the soil surface and topsoil that are especially rich in P (PP) from surface applied fertilizer. Particulate P in preferential flow pathways bypasses the soil matrix and circumvents soil contact. Therefore, there are fewer opportunities for P interception before it exits the soil profile and moves into drains making preferential flow responsible for 10 - 75% of subsurface P loss (Jarvis, 2007).

As precipitation continues, eventually it is unable to infiltrate the soil profile and runs off toward an alternate drainage pathway, like a ditch or stream. Runoff is generated through four possible pathways (Naef et al., 2002): (1) compact, impermeable soil immediately repels water, (2) soil slowly saturates and eventually exceeds its water holding capacity (WHC), causing subsequent water to move overland, (3) a dense or rocky subsurface layer inhibits drainage and forces lateral flow, or (4) groundwater recharges toward the surface. Geological characteristics (eg. soil and subsoil type, water table height, and topography) or management practices (eg. paving, tilling, tile drainage, cover crops) determine the dominant active pathway. Most agricultural soils have a well-developed macropore network that has a large water holding capacity and promotes preferential flow, resulting in delayed runoff (pathway #2).

Agricultural soils in humid temperate areas experience significant runoff events during spring snowmelt and a few major rainfalls over the season. Despite the infrequency of surface runoff, it is responsible for 60% of P removal from cultivated land (Jamieson, 2003). The impact of water droplets first detaches PP from the soil surface and then makes contact with the top 5 cm of soil, called the "effective depth of interaction" (McDowell et al., 2001), which collects DP. Both DP and PP move off the field at rates significantly higher (20%) than the total P lost via both subsurface flow pathways (Macrae et al., 2007).

For example, a Quebec watershed experienced an annual average of 1088 mm of precipitation and subsequently lost 1.1 kg ha^{-1} yr^{-1} of total P from runoff and leaching (Enright and Madramootoo, 2004). Tile drains accounted for 79% of the drainage but only 39% of the P export (0.060 mg/L to 0.365mg/L), while 21% of precipitation left as surface runoff but carried 61% of total P with it (0.196 mg/L to 2.146 mg/L) (Enright and Madramootoo, 2004). This represents a 9.6-fold increase in surface runoff P concentrations to leaching P concentrations. The proportion of suspended sediment (same as PP), TP, and ortho-P from the surface and subsurface flow was also fractionated (Table 1-1), with PP from surface runoff clearly presenting the largest environmental threat. Surface runoff from agricultural soils, especially soils that were recently fertilized, is the primary contributor of nutrient overenrichment in aquatic ecosystems (Schindler, 2006)

1.1.4 Fate of P in aquatic ecosystems

Once P migrates from the field, regardless of its pathway, it reaches streams, rivers, lakes, and ultimately oceans. Aquatic ecosystems respond quickly to minor biotic and abiotic disturbances like temperature, salinity, and nutrient concentrations. In freshwater, P is the limiting nutrient for algal growth and any P input rapidly expands algae population, referred to as hypereutrophication (McDowell et al., 2001). Algae immediately utilize DP, creating an instantaneous burst of growth, while PP sediments slowly release P to sustain high growth rates (Sharpley et al., 1994b). Large algal blooms consume dissolved oxygen and effectively suffocate other aquatic species, causing a trophic cascade effect. Some ecosystems lose resilience and eventually become dead zones, as seen along the Gulf Coast of North America.

1.1.5 Soil management to prevent P loss

Producers implement best management practices (BMP) strategically to reduce P migration from their fields, based on the soil's risk level. The risk for potential P loss is best gauged through soil test P (STP) analyses, because STP concentration and DRP concentration in runoff are proportionally correlated (r^2=0.73; Sims et al. (2002)). Soil test P is determined from a chemical extracting solution that is calibrated to regional soil type and the crop P requirements, and indicates the probability of responding to P fertilizer inputs (Simard et al., 1996; Heckman et al., 2006;). Soil test P determines the baseline P concentration within a soil sample and allows producers to build suitable nutrient management plans that calibrate their fertilizer rates to achieve maximum crop yields without posing an environmental risk. If the STP concentration is approaching saturation, it is critical to implement BMPs to reduce the load of legacy P within the soil and to hinder movement (called source-BMPs and transport-BMPs, respectively) (Table 1-2).

Source BMPs strive to reduce fertilizer application rates without compromising crop yields because greater fertilizer applications are positively correlated with both higher crop yields and also higher P loss from agroecosystems (Romkens and Nelson, 1974). Three critical aspects of fertilizer application are targeted: timing, type, and application method. In humid temperate regions, the majority of P loss usually occurs during spring snowmelt and 1-2 discrete rainfall events, particularly when soil is exposed (Sharpley et al., 1994b). Therefore fertilizer applications should ideally not coincide with periods of historically high precipitation (Sharpley et al., 1994c). Allowing a longer duration of time between fertilization and precipitation can significantly reduce P loss by allowing rapid transformation of P into chemically inaccessible pools to occur. For example, Westerman and Overcash (1980) observed a 90% reduction of P concentration in runoff from 1h to 3d after manure application. Further, applications should preferably be synchronized with plant growth stages that uptake large quantities of P, to maximize P use efficiency.

Fertilizer type and application methods, particularly for manure, can have an equal impact on P concentration in runoff. Diammonium phosphate contains 80% DP while dairy manure, poultry manure, and swine slurry only contain 33, 26, and 27% DP, respectively (Kleinman et al., 2002). Soils with broadcast fertilizer, regardless of type (diammonium phosphate, dairy manure, swine manure, or poultry manure), exhibit 55% more DRP and 37% more TP in runoff than soils with the same amount of fertilizer incorporated by banding/injecting/plowing/tilling in the field (Kleinman et al., 2002). Despite reducing DRP and TP, incorporating manure concurrently increases PP concentration by 200% because the incorporation process exposes loose soil particles, while broadcasted manure creates a protective layer over the soil surface to seal it from erosion (Kleinman et al., 2002).

Transport BMPs aim to prevent, slow, or trap migrating DP and PP before it enters surface water. They have variable influence on P and often must be paired with a suitable source BMP for noticable results. The practices most helpful for controlling P loss in runoff include leaving crop residues, seeding cover crops, plowing, conservation tillage, and adding soil amendments (Sharpley et al., 2006). The idea is to ultimately improve soil drainage by ideally developing a healthy soil structure that favors water infiltration. Infiltration reduces the volume of runoff, which contains exponentially higher P concentrations than leaching due to more opportunities for P capture.

Crop residues and cover crops shield and stabilize the soil surface from precipitation, particularly rainfall, by creating a physical barrier to P transport in surface runoff. Continuous impact of raindrops slowly disassembles the soil surface into its primary particles that disperse across the soil surface and eventually dry out to create a surface crust. Soil crusts eventually thicken and seal the soil, resulting in 100% of water removal via surface runoff, thus facilitating PP erosion (Moore and Singer, 1990). Residues and cover crops also function by promoting soil aggregate formation, which is associated with improved infiltration and resistance to erosion.

Soil aggregates are the foundational unit of good soil stability (Bronick and Lal, 2005). Soil aggregates are formed when negatively charged clay platelets flocculate together using cation bridges to become a central nucleus for organic matter attachment. A small (<250 μm) clay-cation-organic matter complex is formed, called a microaggregate. Multiple microaggregates become bound using various binding agents of different strengths, like microbially secreted polysaccharides, bacterial/fungal debris, fungal hyphae, or plant roots, in increasing order of strength (Tisdall and Oades, 1982). It results in discrete soil bundles that open soil pore networks and improve water infiltration by limiting detached sediments that clog pores and slow drainage. The aggregates are able to hold colloidal particulate matter, like PP, and protect it from rainwater or snowmelt erosion.

Aggregates are the ideal foundational structure for efficient soil drainage, though improved soil drainage can be artificially recreated by tilling and installing tile drains. Tilling promotes subsurface flow because it opens the soil surface, disrupts preferential flow pathways to revert to matrix flow, and creates depressions for water collection. Water that percolates through the soil matrix will filter out PP travelling through the soil surface, thus sparing it from erosion. However, tilling paradoxically dismantles soil aggregates thus exposing particulate matter to erosion once soils cease draining and begin to runoff. As a result, conservation tillage or no-till systems have been implemented as a practice to sustain soil aggregation and reduce PP, but can increase DP and ortho-P concentrations by allowing P to accumulate within the top layers of the soil surface rather than mixing them into the soil (Sharpley et al., 1994a; Gaynor and Findlay, 1995).

Soil amendments that temporarily immobilize P can also be integrated into topsoil (Stout et al., 1998). These products contain some form of P sorbing (Al or Fe) or precipitating agent (Ca or Mg) that shift P away from the soil solution, which supplies DP in runoff (McDowell et al., 2001), and induces clay flocculation for highly-tensile aggregates (Barral et al., 1998). These products can extend P holding capacity by providing more binding sites and delaying saturation, however these products must be consistently applied each season otherwise the soil solution re-equilibrates to the same concentration of soil solution P.

Each BMP varies in practicality depending on region, equipment, crop type, and associated costs, in conjunction with its ability to reduce P loss. Many regional networks and government policies financially incentivize BMP adoption, especially for practices that would otherwise have no associated cost savings for farmers, such as changes in fertilizer application time. A meta-analysis by Gitau et al. (2001) calculated the overall effectiveness of individual BMPs and saw total P loss decreases between 10% and 75% at the field-scale, but Sprague and Gronberg (2012) observed less success at the watershed level. They suggest that BMPs cannot overcome prior accumulation of P within the soil and attributes research results to mere shifts in the chemical P equilibrium resulting in different forms, appearing as a superficial reduction. The disparity amongst BMPs highlights the importance of improving exiting BMPs, as well as developing new feasible practices to address rising eutrophication rates. One such novel soil amendment for the purpose of P reduction in runoff and erosion may be biochar.

Section 2: Biochar to reduce nutrient loss

1.2.1 Overview of biochar

Biochar is a carbon-rich product made from the incomplete combustion of biomass. It is similar to black carbon and charcoal and differs solely because it is intentionally made as a co-product of biofuel production, with a value associated to it as a soil amendment. It is a relatively recalcitrant material in the soil, with recorded lifespans upward of 7000 years (Verheijen et al., 2010). Given its stability in soil, biochar application contributes to a pool of C that has a long residence time in soil and increases soil C sequestration.

The use of biochar (as black carbon or charcoal) in agricultural systems has been popular in tropical regions for decades, historically known as the *Terra Preta* soils (an Oxisol), as well as a byproduct from slash-and-burn practices. Biochar benefits soil properties physically, chemically, and biologically, to ultimately increase crop yields. A meta-analysis by Jeffery et al. (2011) compiled studies that examined the effect of biochar application on crop productivity (yield or above-ground biomass), considering important variables including biochar application rate, soil pH, soil texture, crop type, biochar production method, and experimental design. The grand mean, regardless of variable, saw a 10% increase in crop productivity, though there was a wide range (-28% to 39%), with the best results seen in acidic and coarse-textured soils. For example, Major et al. (2010b) observed a 140% increase in maize yields 4 years after amending with 20 t ha^{-1} in an Oxisol in Columbia, while Gaskin et al. (2010) observed a linear decrease in grain yields with biochar application (up to 22 t ha^{-1}) to a loamy sand soil in the

American Lower Coastal Plains. Biochar is also explored as a method for bioremediation of heavy metals and/or agrochemicals, a replacement medium for green roofs, and greenhouse gas reduction.

1.2.2 Biochar Production

Biochar is produced in an oxygen-limited or oxygen-free environment, by pyrolysis of biomass. When pyrolyzing biomass, most non-carbon species are removed, resulting in some labile matter (including salts), ash, and extremely stable aromatic carbon ring as the foundational structure with reactive functional groups on its surface. The final stage of biochar manufacture often entails an "activation" process, meant to amplify the size and number of the pores using high-pressure steam, CO_2, or O_2 or washed in a strong oxidizing agent such as KOH or H_3PO_4 (Azargohar and Dalai, 2008; Verheijen et al., 2010). This large and reactive surface area is responsible for many of biochar's positive effects in soil.

The feedstock and temperature of pyrolysis creates biochars with vastly different structural and chemical compositions (Verheijen et al., 2010; Streubel et al., 2011). Feedstocks can be any lignocellulosic biomass that are ideally waste material. The pyrolysis process often maintains the structural skeleton of the parent material (Wu et al., 2009); therefore the biochar's physical characteristics, like porosity, are primary dictated by feedstock. Biochars made from wood, paper pulp, wood chips, and poultry litter produced significantly higher crop yields, while biosolid-derived biochar negatively impacted yields (Jeffery et al., 2011).

Pyrolysis temperature should be selected based on the intended purpose of the biochar. Higher temperatures (fast pyrolysis) volatilize non-carbon species like C=O (via decarboxylation) and C-H (via dehydration) functional groups and produces a higher proportion of inert aromatic structures (Novak et al., 2009b). The resulting biochar is extremely recalcitrant and a good candidate for long-term C sequestration. However, it possesses fewer exchange sites for possible adsorption of nutrients (Wang T. et al., 2012a), OM, pesticides (Wang et al., 2010), or heavy metals (Uchimiya et al., 2010). Lower temperatures (slow pyrolysis) maintain more of the ion exchange groups native to its feedstock that allow a variety of diverse binding opportunities, which is ideal for nutrient retention or bioremediation. Such accommodating binding sites make the biochar more vulnerable to mineralization, which can benefit microbial communities and nutrient turn over, but significantly reduces the mean-residence time.

Biochar properties can be very diverse and their effects are often specific to soil type (Novak et al., 2009b), and can occasionally have an adverse effect on soil health due to volatile organic compounds (Spokas et al., 2011) or polycyclic aromatic hydrocarbons (Chen et al., 2011b; Fabbri et al., 2012). Ongoing research is being done to optimize the biochar production method, develop a characterization index, and understand how diverse biochars react in different soil types. These aspects of biochar will not be discussed further in this review and it will be assumed that the proper type of biochar was applied for the given soil type.

1.2.3 Physical properties of soil altered by biochar application

Biochar application changes soil physical properties, due largely to high porosity within its particles. Micro- and mesopores within the biochar structure retain water, with reports of biochar holding up to ten times its own weight in moisture (Kinney et al., 2012). Most biochar particles are larger than the existing soil pore space, and therefore displace soil particles, which lowers soil bulk density and creates more pore space in a given volume of soil (Verheijen et al., 2010). Improved soil porosity allows greater aeration and drainage within the soil, which creates an aerobic environment that favors crop growth and reduces anaerobic microenvironments where N_2O and CH_4 are produced (Taghizadeh-Toosi et al., 2011; Wang J. et al., 2012).

1.2.4 Chemical properties of soil changed by biochar application

Biochar is not an inert compound; it interacts with the surrounding soil particles because of its large and reactive surface area. The chemical interaction between biochar and soil particles evolves over time, such that fresh and aged biochar can significantly alter the soil chemistry in different manners. Fresh biochar is thought to have an inherently hydrophobic surface from its many carboxyl (-COOH) groups, therefore during this stage binding sites attract nonpolar compounds, including many agrochemicals (Sun et al., 2011). Biochar weathers with time, undergoing deprotonation and forming a negatively charged reactive site ($-COO^-$), attracting positively charged ions (cations). The binding strength appears to be strong enough to prevent nutrient leaching (Laird et al.,

2010) but weak enough to allow nutrient exchange (ex. desorb PO_4-P and NH_4-N) for plant uptake (Hale et al., 2013).

Biochar also has a pH range of 6.2 to 9.6 and a substantial ash fraction, allowing it to act as a liming-agent (Verheijen et al., 2010). Higher pH in acidic soils improves nutrient solubility and availability and strengthens the anion and cation exchange capacity (AEC/CEC), which are measures of how strongly nutrients are bound to the soil matrix (Lee et al., 2010). Together, nutrients not associated with the biochar surface are also more plant-available but less likely to leach.

1.2.5 Soil biota populations altered by biochar

Soil amended with biochar positively impacts bacterial and fungal activity through direct interaction between biochar particles and microbes or indirectly, via changes in soil properties. Lehmann et al. (2011) extensively reviewed biochar's effects on soil biota, compiling studies that used a variety of methods to measure microbial populations, activity, and community composition. The mechanisms connecting biochar to biotic life in the soil are still largely unknown. Warnock et al. (2007) broadly summarized the possible hypotheses as follows: (1) biochar promotes growth in certain (or all) groups of bacteria and fungi by providing additional substrates and nutrients. (2) Changes in community composition (caused by mechanism #1) subsequently directly or indirectly shifts the overall community function. (3) Biochar interrupts or enhances plant-microbe or inter-microbial chemical signals and alters the function, growth rate, or movement of certain species. (4) Physically, biochar pores provides more habitat and creates refuge for microbes from predators, which allows uninhibited growth.

Section 3: Biochar: a soil amendment to protect aquatic systems from nonpoint source P pollution?

There is growing evidence that the changes in soil physical, chemical, and biological properties induced by biochar are beneficial for agricultural crop production. However, there is limited research regarding biochar for environmental protection, particularly pertaining to nutrient management in humid temperate regions. A variety of mechanisms have been theorized regarding biochar's ability to alter P movement and possibly support other BMPs to protect aquatic systems from nonpoint source P pollution. This section outlines possible ways biochar may influence P loss from soil and provides evidence for or against such mechanisms from the primary scientific literature.

1.3.1 Biochar to prevent P pollution: Physical, chemical and biological mechanisms

Biochar may prevent P loss from soils through its direct and indirect effects on soil physical, chemical, and biological, as outlined in section 2. Biochar amendments may enhance soil structure and stability to physically occlude particulate P, chemically sorb P, and biologically shift relative concentrations of P fractions by changes in microbial communities.

Biochar has exhibited ability to change physical properties relevant to P loss. By creating a better-connected pore network, biochar amendments promote water infiltration and water holding capacity that subsequently delay runoff, which is responsible for 60% of P loss (Macrae et al., 2007). In the *Terra Preta* soils, which are sandy soils that contain up to 35% black carbon built up from long term exposure (Glaser et al., 2000), Glaser et al. (2004b) observed 5% to 11% increase in macropores (>50 μm) in, which translated into a 18% higher field capacity. Ayodele et al. (2009) reported an 88% increase in infiltration and 50% less runoff on loamy sand soil in Ghana that was exposed to charcoal (unknown concentration) from slash-and-burn agriculture. Improved water infiltration in sandy soils has been established but water infiltration rates appear to be unchanged or even slowed as soil clay content increases (Asai et al., 2009; Busscher et al., 2010; Major et al., 2012; Bell and Worall, 2011). This relationship exists beyond tropical regions too; Podsolic soils in humid temperate regions in the American northeast had 18% higher water retention in sandy soil but a 20% reduction in clayey soil (Tryon, 1948).

Improved infiltration and water holding capacity would reduce the overall runoff volume by delaying saturation, resulting in lower P losses. Improved infiltration from biochar application is also theorized to be due in part to increased aggregate formation, caused by deposition and attachment of clay, silt, organo-mineral, and organic matter in biochar pores (Zimmerman et al., 2011; Major et al., 2012). Soil aggregates are largely made up of clay particles that have surface chemistry dominated by SiO_2, Al-, and Fe- sesquioxides, all of which can attract and bind soluble P ions (Edzwald et al., 1976). Therefore biochar application should theoretically contribute to greater P retention in aggregates and may reduce the probability of P loss in runoff (dissolved P and eroded sediment P) during rainfall events.

Fresh biochar is a P source in soil. Pyrolysis is capable of thermally decomposing most chemical bonds and volatilizes most other elements, but it is only capable of compromising C-P bonds (DeLuca et al., 2009). Once applied to the soil, this residual P can solubilize into the soil solution (Stevenson and Cole, 1999), or precipitate with Ca, Mg, Fe, or Al, also added from biochar ash (Wang T. et al., 2012b). Downie et al. (2007) showed that biochar made from chicken litter removed 52% of residual P by precipitating with Ca^{2+}, 70% of which was was accessible for plant-uptake, but immobilized from leaching (Neri et al., 2005).

Phosphorus adsorption to the biochar surface has also been observed. A number of researchers examined nutrient loss from leaching in biochar-amended soils, particularly in highly weathered tropical soils that are prone to leaching. Biochar application reduced ammonium (Lehmann et al., 2003), Ca, Mg, Mn, Zn, (Novak et al., 2009a) and P (Major et al., 2012; Laird et al., 2010) concentration in leachate because biochar's chemically reactive surface area provides additional exchange sites for these nutrients to bind. Hale et al. (2013) argues that biochar's P binding capacity is quickly saturated by P imported from the biochar's ash fraction, provides little opportunity for legacy P to adsorb, and it is still unclear as to how long biochar retains P on its surface.

Biochar-induced microbial activity affects P movement within the soil indirectly, though not necessarily positively. Anderson et al. (2011) saw a 6% increase in P solubilizing bacteria in a 12 week incubation experiment using a silt-loam soil and biochar application rates of 15 and 30 t ha^{-1}. Conversely, Warnock et al. (2010) saw a 58% decrease of arbuscular mycorrhizal fungi abundance with

2% biochar application rates. This corresponded to a 28% decline in soil solution P because arbuscular mycorrhizal fungi help plants absorb more P (Bolan, 1991). Biochar application at rates as low as 2 t ha^{-1} reduced acidic para-nitrophenyl (PNP) phosphatase activity from 25 to 21 mg PNP kg^{-1} h^{-1} and biochar application at 40 t ha^{-1} reduced phosphatase activity to 12 mg PNP kg^{-1} h^{-1} (Kumar et al., 2013). Reduced phosphatase slows the conversion of organic P (PP) to soluble solution P (DP), which can be beneficial in regions with high leaching potential.

Biochar application is generally associated with increased plant growth likely from improvements in soil fertility. More plant biomass requires more nutrients, including P, to grow and sustain the tissue. This means that plants take up more soluble P that is exported during crop harvest, which leaves less P in the soil, where it would be susceptible to loss from leaching or in runoff.

1.3.2 Quebec as a candidate for biochar application to resolve agroenvironmental P problems

Quebec soils are a prime candidate for biochar application. As of 2006, 3.5 million hectares of land were dedicated to agriculture in Quebec, with 72% of all P input as manure and 40% of the farmland classified as high to very high risk of P export due to an approaching P-saturation (van Bochove et al., 2006). The province contains 3% of the global freshwater reserves and eutrophication is particularly rampant in the region, with upward of 200 small and medium sized lakes exhibiting symptoms of excess cyanobacterial growth. For example, one severely impacted lake is Petit Lac St Francois (PLSF), found in the St Francis

River watershed. It is classified as hyper-eutrophic, surpassing Quebec guidelines (30 μg/L) for P concentration nearly twelve-fold with values as high as 350 μg/L. Excess phosphorus loading in this region likely comes from non-point sources like agricultural fields, which account for 52% of the land use within the watershed.

Quebec receives on average more than 1000 mm of precipitation annually, providing ample opportunity for waterborne P loss from agricultural land. Further, the dominant soils in Quebec are Podzolic and Gleysolic soils that tend to be dominated by non-expanding illite clay (de Kimpe 1970). Illite is prone to dispersion due to repulsion between clay particles with a mildly negative surface charge, which means that flocculation and aggregation are more difficult to attain and poor soil structure is inherent to the region. As a result, surface runoff is more likely to occur and contain a higher concentration of PP in Quebec agricultural soils than other regions with similar agricultural management practices.

Section 4: Conclusions and Future Direction

Despite producers' best efforts to reduce P losses from their agricultural fields, current recommendations for best management practices are inadequate to prevent P transport to aquatic systems. Novel technologies are required to address this issue. Historic evidence and recent research focuses mostly on tropical regions that demonstrate that biochar application (black carbon or charcoal) to agricultural soils reduces P leaching. It is not known whether biochar is also effective in retaining P in runoff, particularly in temperate soils. Mechanisms for P retention in a clay-loam soil in a temperate humid climate, such as Quebec, are hypothesized. To date, no studies have examined whether biochar reduces P loss in surface runoff.

My thesis research is based on a field study in the St Francis River watershed, Quebec, Canada. A field experiment was done to determine whether application to a clayey soil could reduce P concentrations in runoff using a rainfall simulation. A follow-up laboratory study was conducted to determine whether improved soil aggregation following biochar application was responsible for changing the P concentration in runoff. Together, these results provide support for biochar application, in conjunction with other practices, to reduce P runoff from intensively managed agricultural fields in humid temperate regions.

Table 1-1: Partitioning of annual precipitation between surface and subsurface drainage pathways, as well as the particulate P (PP), dissolved unreactive P (DUP), and dissolved reactive P (DRP) loss via surface and subsurface pathways in Quebec (adapted from Enright and Madramootoo (2003) and Jamieson (2003)).

	Precipitation		**PP**		**DUP**		**DRP**	
	mm yr^{-1}	%	Loss (kg/ha)	%	Loss (g/ha)	%	Loss (g/ha)	%
Surface	252	21	75	71	34	52	57	62
Subsurface	948	79	31	29	32	48	34	38
Total	**1200**		**106**		**66**		**91**	

Table 1-2: Best management practices recommended to farmers by the United States Department of Agriculture-Agricultural Research Service (USDA-ARS) and their impact on total P (TP), dissolved P (DP), and particulate P (PP) loss (adapted from Sharpley et al., 2006).

Management Practice	**Explanation**	**Impact on P loss**
Rate of application	Match crop needs	↓ TP
Timing of application	Avoid application to frozen ground and apply during season with low runoff probability	↓ TP
Method of application	Application can be through incorporation, banding, or injecting in soil	↑ PP ↓ DP
Source application	P sources can differ in their solubility	↓ DP
Crop residue	Harvest residues to reduce soil nutrients	↓ TP
Crop cover	Do not leave soil bare during winter	↓ TP
Invert stratified soil	Redistribute surface P through profile by plowing	↑ PP ↓ DP
Conservation tillage	Reduced and no-till cropping can increase infiltration and reduce soil erosion	↑ DP ↓ PP
Buffer strips	Remove transport of sediment-bound nutrients	↓ PP
Soil drainage	Tile drains and ditches enhance removal and reduce erosion	↓ PP
Soil amendments	Fly ash, Fe oxides and gypsum reduce P solubility	↓ DP

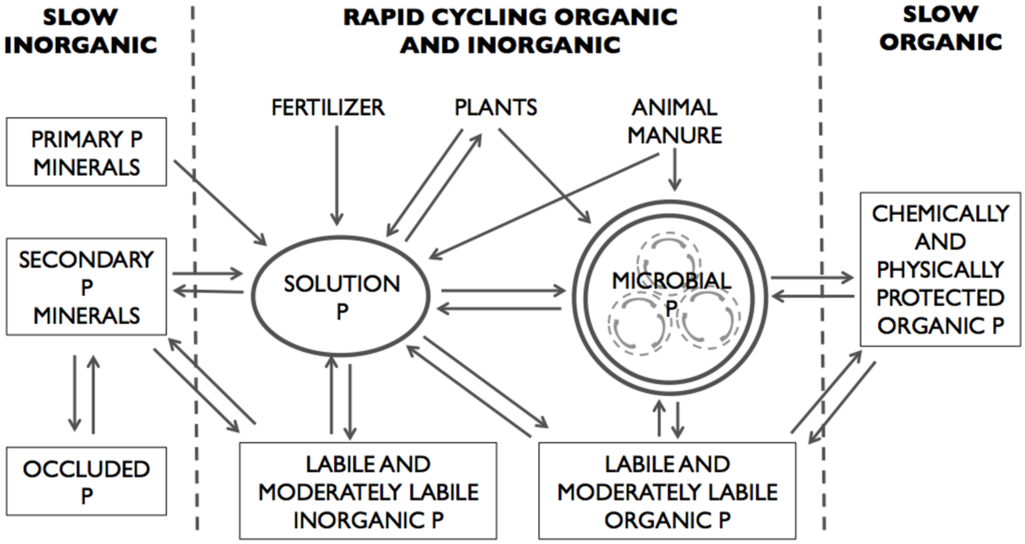

Figure 1-1: The phosphorus cycle and the dynamics between each fraction (from Sharpley and Rekolainen, 1997).

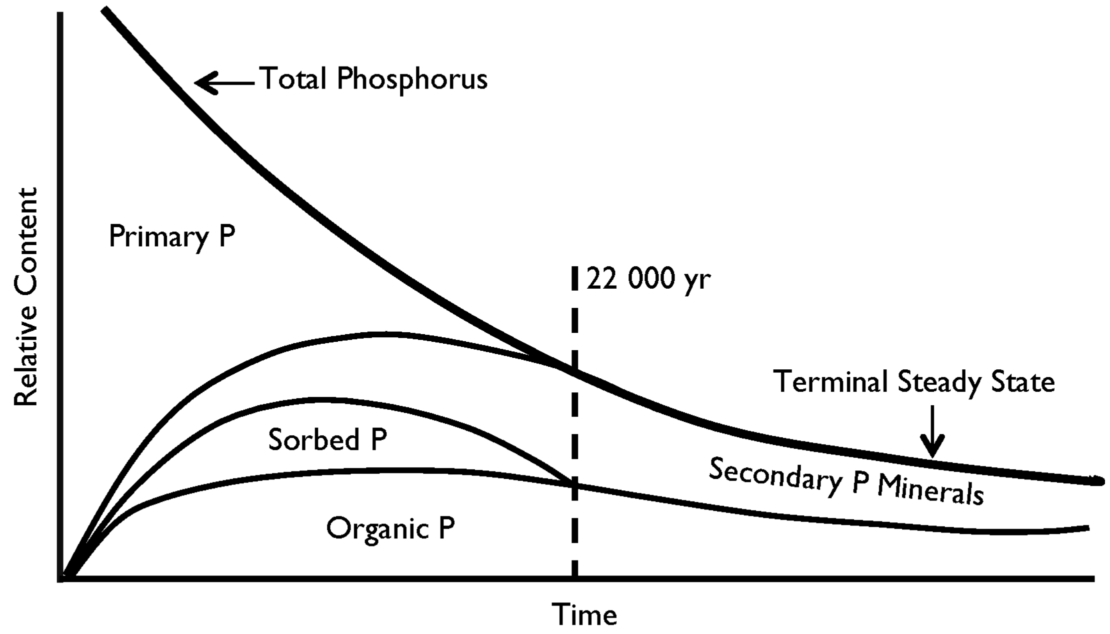

Figure 1-2: Evolution of P fraction concentrations in a geological timeframe from a low-disturbance system (adapted from Walker and Syers, 1976; Smeck 1985)

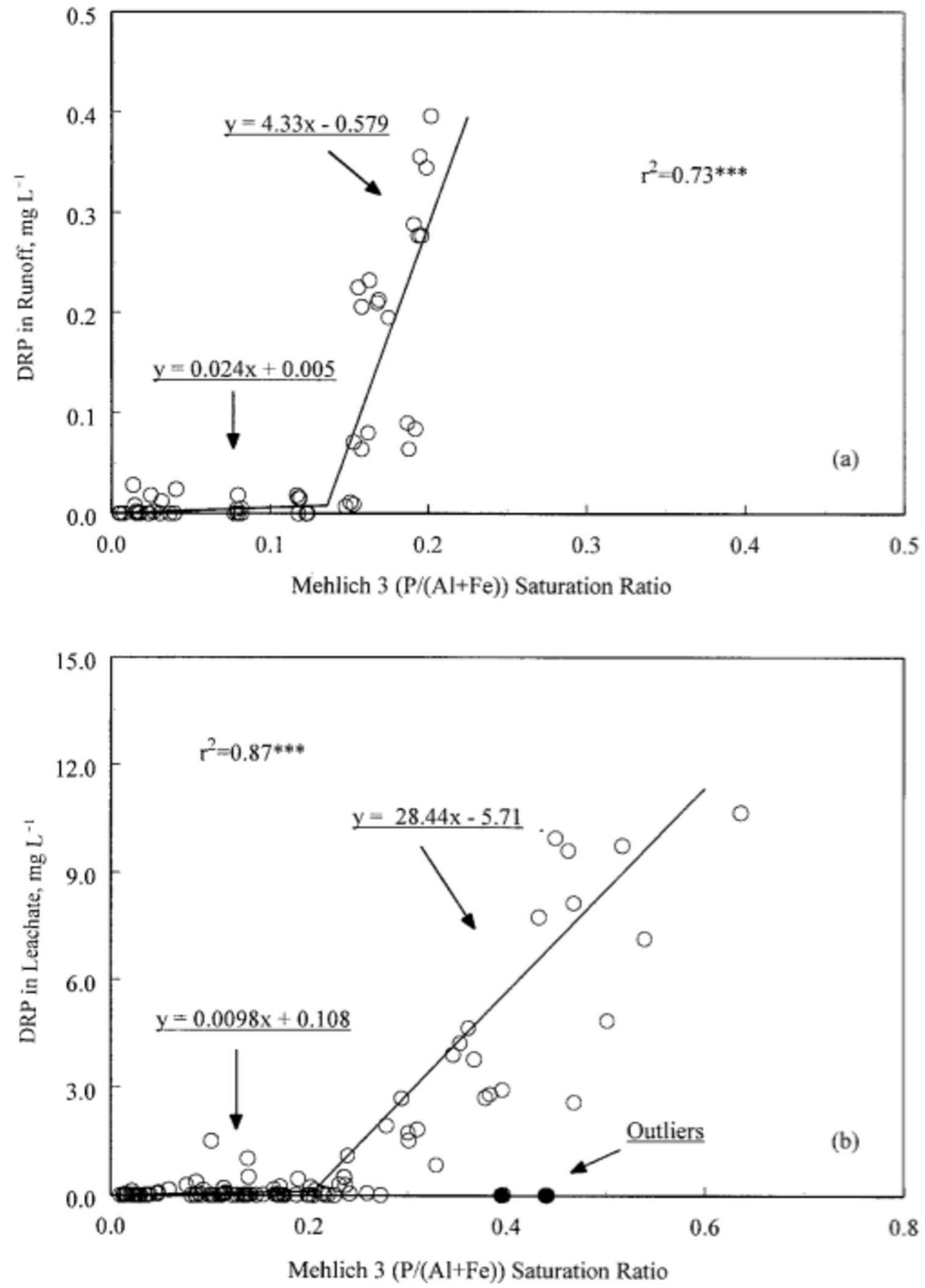

Figure 1-3: The relationship between soil test P concentrations and dissolved reactive P (DRP) in leachate or runoff (adapted from Sims et al., 2002).

Chapter 2: Application of biochar to Quebec agricultural soil reduces particulate phosphorus concentrations in simulated rainfall runoff

Abstract

Surface runoff from agricultural fields collects dissolved and particulate phosphorus (P) and deposits it in surface water, causing eutrophication and sedimentation. Management practices that improve water infiltration and storage in the soil profile are expected to reduce P loading to waterways. The objective of this study was to determine whether biochar amendments reduce P loss from a clay-loam soil primarily by improving water infiltration and water holding capacity (WHC) to reduce runoff volume or, secondarily, by reducing P concentrations in runoff. A 30-minute simulated rainfall was conducted using a Cornell Sprinkle Infiltrometer in April 2012 on a biochar-amended field (3 types of biochar, applied at 5 and 10 t ha^{-1}) located in St-Francois-Xavier-de-Brompton, Quebec. Runoff volume, time-until-ponding, infiltration rate, and WHC content were similar in biochar-amended soils and the unamended (control) plots. Runoff water samples were analyzed for ortho-P, dissolved P (DP), particulate P (PP), and total P (TP) loads. There was a significant decrease in ortho-P in soil amended with Dynamotive biochar at 5 t ha^{-1} (P=0.048) and a significant decrease in particulate P for Pyrovac biochar 5 t ha^{-1} and Dynamotive and Basques biochar at 10 t ha^{-1} (P=0.012, 0.024, and 0.047, respectively). The reduction in P concentration in runoff indicates that biochar amendments have potential to retain soluble and particulate P compounds but the exact mechanism remains unknown.

2.1 Introduction

Runoff water moving over exposed soil dislodges nutrient-rich organo-mineral particles and solubilizes nutrients that erode into surface water. Phosphorus (P) exported in this manner is of great concern due to its ability to accelerate eutrophication in freshwater streams and lakes. Agricultural soils without much residue cover in spring, before crop emergence, are vulnerable to P loss from runoff, particularly when they have a history of fertilization with manure, which tends to cause soil P enrichment. Consequently, more than half (58%) of P deposited in aquatic ecosystems originates from agricultural soil globally (Sharpley et al., 1994a), and up to 90% of the annual P load comes from runoff (Jamieson et al., 2003). The P load in agricultural runoff is partitioned into PP and DP, which contribute 70-95% and 5-30%, respectively (Enright and Madromootoo, 2003). Particulate P contains fine sediments enriched with P caused by strong binding associations between P and clay and organic matter. Dissolved P, like inorganic ortho- and polyphosphates and organic inositol hexaphosphate (Broberg and Persson, 1988), is strongly correlated to the soil test P concentration (determined by Mehlich-III, Olsen, Bray, or other test methods) (Sims et al., 2002).

To reduce the P lost in runoff, producers are encouraged to improve drainage because water that moves through the soil matrix has higher water-soil surface contact, which can intercept PP and adsorb DP because it leaves the field. Infiltrating water draining from subsurface outlets had 80% lower P concentration than surface runoff (Jamieson et al., 2003). Soils with tile drainage systems possess larger water-holding capacities (WHC), which sustain high hydraulic conductivity in the soil profile and draws water through the soil quickly to prevent saturation (Renck and Lehmann, 2004). Therefore, farm managers have adapted practices to improve soil infiltrability, increase WHC, and expedite drainage to reduce runoff volume and curb P loss through runoff (Sprague and Gronberg, 2012). Despite these interventions, the agricultural sector continues to be the largest non-point source contributor of P to aquatic ecosystems and further measures must be explored to reduce P loss from agricultural fields.

Biochar is a novel soil amendment made from pyrolyzed carbon that can alter physical soil properties affecting the soil-water relationship. Research shows biochar can hold water within its micro- and mesopores and increase infiltration by contributing to the formation of macropores and macroaggregates, which expands the soil pore network (Renck and Lehmann, 2004; Asai et al., 2009; Major et al., 2009). Karhu et al. (2011) reported an 11% increase in WHC following 0.4% (*w/w*) biochar application on agricultural soil in southern Finland; Glaser et al. (2004a) observed an 18% rise in WHC in *Terra Preta* soils, where 10% of organic carbon is black carbon. Both studies noted a 2-7% increase in the pore network (>50 µm), determined from soil bulk density measurements (Glaser et al., 2001; Karhu et al., 2011). However, Glaser et al. (2004b) reports that changes in drainage associated with improved aggregation only occur decades after biochar application, which Liang et al. (2006) attributed to the amount of time needed for biochar to weather and become integrated into the soil aggregates. The biochar-mediated improvement to WHC and porosity may facilitate water infiltration, thereby reducing the volume of runoff water leaving agricultural fields (Bronick and Lal, 2005).

Further, biochar also has direct and indirect effects on soil test P concentrations as well. The residual ash in fresh biochar is P source (DeLuca et al., 2009), with Ca^{2+} and Mg^{2+} that can precipitate approximately 33-100% DP in the soil solution (Parvage et al., 2013). However, this is expected to have a short-term effect and soil test P concentrations may equilibrate within one year (DeLuca et al., 2009). The reactive surface of biochar can directly sorb phosphate ions to anion exchange sites on the biochar surface (Chen et al., 2011a; Hale et al., 2013) or via associated organo-minerals, clay, and cation bridges (Laird et al., 2010). This mechanism was purported to be responsible for reducing ortho-P concentration in leachate by 90% (Borchand et al., 2012). Phosphorus immobilization in microbial biomass and P assimilation by plants may be stimulated in biochar-amended soils (Kumar et al., 2013), which would tend to reduce soil test P (in the short-term).

It is hypothesized that water infiltration will occur for a longer period of time in biochar-amended soils than unamended soils, thus increasing the time before runoff begins and lowering the overall volume of water running off the soil in a set time interval. It is also hypothesized that biochar-amended soil will retain more dissolved P and reduce P concentration in runoff, compared to unamended soil. Together, these mechanisms will reduce the P load in runoff from biochar-amended soil. This field experiment investigated P loss in simulated rainwater runoff from biochar-amended and unamended plots in an agricultural in Quebec, three years after biochar application.

2.2. Methods and Materials

2.2.1 Site description

The experimental site was located on a working dairy farm in the St-Francis-River watershed, Quebec (45°30’ N, 72°01 W, elevation 243 m). The monthly mean temperatures ranged from -10^{o}C in January to 21^{o}C in July, with a mean annual precipitation of 1230 mm, based on a 30 yr average data (1980-2010) from Environment Canada (2012). The soil is part of the Brompton stony loam series, classified as a poorly drained Podzol (Cann et al., 1947). Soil surface (0-12 cm) is a black loam above a compact grey white sandy loam with many small stones (5-17 cm). Surface soil contains 190 g kg^{-1} of clay, 380 g kg^{-1} of sand, and 430 g kg^{-1} of silt, with 33 g total C kg^{-1}, 2.3 g total N kg^{-1}, 5.2 g total P kg^{-1} and pH of 5.9.

2.2.2 Experimental Design

The experiment began in spring 2008. The field was cultivated with a moldboard plough and harrowed, then a single application of biochar was made using a tractor and programmable spreader. The experiment was a randomized complete block design, with three blocks. Each block had 7 treatments: 3 types of biochar at two application rates (5 t ha^{-1} and 10 t h^{-1}), and one unamended (control) plot that did not receive biochar, for a total of 21 experimental units (Appendix A). Plots were 5 m by 6 m with a 1 m buffer between each plot within a block, as well as a 2 m buffer between each block.

Biochars were obtained from commercial producers that use different materials and production techniques (Table 2-1), however detailed information about their manufacture is proprietary. Selected physical and chemical characteristics were determined by an independent laboratory and provided in Appendix B. However, biochar is not sufficiently characterized to correlate results to their different properties.

The year before this study, in 2011, the field was sown with cereals (oat/barley; *Avena sativa/Hordeum vulgare*) that were cut and fed as forage to cattle. The crop rotation in the plots followed the producer's management in the field, which was a corn-soy-cereal rotation. Plots received inorganic fertilizer to meet the NPK requirements according to provincial fertilizer recommendations, namely 50 kg N (urea) ha^{-1}, 30 kg P_2O_5 ha^{-1}(diammonium phosphate), and 20 kg K_2O ha^{-1} (muriate of potash). Manure was applied at a rate of 50 t ha^{-1}. No herbicides or fungicides were applied to the cereals grown 2011.

2.2.3 Rainfall Simulation and Infiltration Rate

Drop-forming rainfall simulators are often used to assess soil infiltrability, runoff rate, and particle detachment for the prediction of potential erosion rates. They sprinkle water at a given rate to determine time-until-ponding, which is a function of sorptivity, matric flux potential, water volume, and rainfall rate (White and Perroux, 1987). A portable rainfall simulator (Cornell Sprinkle Infiltrometer, Cornell University, Ithaca, United States) was used in this experiment to determine soil infiltrability and collect runoff from the soil surface (Figure 2-1).

The apparatus consists of an airtight 20L vessel filled with water that sits on a metal ring (area = 457.3 cm^2) inserted at a depth of 7 cm (Ogden et al., 1997). Capillary tubes that perforate the bottom of the reservoir drip water from a height of 15 cm above the soil surface. The rainfall rate was adjusted by sliding a bubbling tube that changes the internal pressure head (Ogden et al., 1997).

The rainfall simulation was done in April 2012 (before seeding) while the soil was bare and most vulnerable to surface erosion. The measurements reflect the highest potential erosion rates during the growing season, that a rainfall simulator can provide. The rainfall rate (r_t) was adjusted to 25-30 cm hr^{-1} to ensure ponding for each measurement. Each simulation lasted 0.5h (T_f), with runoff samples collect every 6 minutes in 125 ml polypropylene Nalgene bottles. They were transported in a cooler with icepacks to the laboratory and stored in a 4°C fridge prior to analysis. The total volume of simulated rain used on each plot (V_t, in L), the time to ponding, and the volume of runoff (V_{ro}, in mL) were measured to calculate runoff rate ($ro_{t,}$ L min^{-1}) and soil infiltration ($i_{t,}$ ml cm^{-2} hr^{-1}) using equations 1 through 3:

$$r_t = \frac{V_t}{T_f} \qquad (1)$$

$$ro_t = \frac{V_{ro}}{\text{area} \times T_f} \qquad (2)$$

$$i_{t=} r_t - ro_t \qquad (3)$$

Phosphorus concentration in runoff stabilizes within minutes (McDowell and Sharpley, 2001) so a composite water sample was made from equal volumes of water collected at each 6 minute interval during the simulation. A standard four-stage method was used to determine the total P concentration and the DRP, DUP, or PP concentrations (APHA, 1992). First, the water sample was split into two subsamples, one of which was filtered through a 0.45 μm Millipore filter using a vacuum pump and glass wool funnel to remove PP. Both the unfiltered sample and the filtered sample were oxidized to ortho-P using 400 μL of persulfate digestion reagent. The digestion reagent (450 μL of 13.2 g $K_2S_2O_8$ and 3 g NaOH in 1000 mL water) and sample (300 μL) were added to 1.1 ml glass vials (SiliCycle, Quebec City, Canada), sealed with a rubber stopper mat, clamped with aluminum plates, and autoclaved for 2 hours at 110°C (Shand et al., 2008). The digested (filtered and unfiltered) and undigested samples were analyzed by combining 200 μL of sample with 40μL of 14.2 mM ammonium molybdate tetrahydrate in 3.1 M sulfuric acid shaking for 10 min, and then adding 40 μL of malachite green, shaking more vigorously for another 20 min (D'Angelo et al., 2001). The samples were analyzed at 650 nm using a microplate reader (Biotech, Winooski, USA, Model EL312). The concentration of each P fraction was determined as follows: The unfiltered digested sample quantifies TP, while the filtered sample gives DP (DRP+DUP). The difference between TP and DP is PP. To distinguish DRP and DUP, the filtered, undigested sample gets analyzed to determine DRP, which can be subtracted from DP to isolate DUP (equation 4 and 5).

$$\mathrm{DP} = \mathrm{TP} - \mathrm{PP} \qquad (4)$$

$$\mathrm{DUP} = \mathrm{TP} - \mathrm{PP} - \mathrm{DRP} \qquad (5)$$

Phosphorus load is the concentration of TP in the runoff sample multiplied by the volume of runoff that left the field (equation 6). It measures total P (mg) lost in a discrete rainfall event regardless of the duration of the rainfall.

$$P_{load} = P_{concentration} \times V_{ro} \qquad (6)$$

2.24. Soil Moisture Content

Soil samples (from 0-12cm depth, composed of 6 cores, collected with a 3 cm (i.d.) hand-held soil probe), were collected after rainfall simulation experiment to determine whether biochar-amended soil contained more water than an unamended soil following saturation. The wet weight and dry weight, after drying for 48 h at 60°C, of soil were used to determine moisture content according to equation 7:

$$\text{Soil moisture content} = \frac{\text{soil wet weight (g)} - \text{soil dry weight (g)}}{\text{soil wet weight (g)}} \times 100 \qquad (7)$$

2.2.5 Statistical Analysis

Analysis of variance (ANOVA) with the PROC GLM procedure of the SAS software (version 9.2) was used to determine the effect of biochar type and application rate on soil water dynamics, P concentration, and the P load in runoff. Normal distribution of the residuals was verified using the UNIVARIATE procedure and no transformations were required. Pre-planned orthogonal contrasts were done to compare the effect of biochar type, biochar rate, and all biochar treatments versus the control on the P concentration and P load in runoff. Effects were considered significant $\alpha = 0.05$.

2.3 Results

The water dynamics in biochar-amended soil were not significantly different than the control, based on moisture content, time-until-ponding, runoff volume, and infiltration rate (Table 2-2). Soil moisture content was the same in all plots, regardless of the treatment. As there was no effect of biochar on soil water dynamics, the P load was not significantly different between biochar-amended and unamended plots.

Total P concentration in runoff was lower in four of the biochar-amended treatments than the control, namely those receiving Dynamotive biochar (5 and 10 t ha^{-1}) and plots amended with Pyrovac and Basque biochar at 10 t ha^{-1} (Table 2-2). When the DRP, DUP, and PP components were examined individually (Figure 2-2), only Dynamotive at 5 t ha^{-1} had significantly less DUP than the control (p=0.048), and there was no other difference in DRP and DUP concentrations between biochar-amended and unamended plots. Particulate P was significantly lower in Pyrovac at 5 t ha^{-1} and Dynamotive and Basques 10t ha^{-1} (P=0.012, 0.024, and 0.047, respectively). These changes were not substantial enough to lower the P load in runoff.

2.4 Discussion

2.4.1 Soil water dynamics

It was hypothesized that biochar-amended soil would exhibit greater infiltration due to enhanced WHC, but the results showed no effect of biochar on soil water dynamics. One reason may be that biochar application rates of 5 and 10 t ha^{-1} (also express as 0.23 and 0.45% (*w/w*), respectively) were not sufficiently high to induce changes in soil water dynamics. Previous studies that reported effects had soils amended with 2 to 45% (*w/w*) biochar (equivalent to 45 to 1080 t ha^{-1}; Novak et al., 2009a; Kameyama et al., 2012). Fine-textured soils amended with biochar do not show much change in water dynamics (Asai et al., 2009; Laird et al., 2010; Major et al., 2012), probably due to the high inherent WHC in such soils, as compared to sandy soils (Sohi et al., 2010).

The mechanisms involved in biochar-mediated moisture retention are still unknown but Major et al. (2009; 2012) hypothesized that water molecules directly interact with biochar through two pathways; (1) capillary action that draws water into biochar micropores (Kinney et al., 2012) or (2) negatively charged binding sites on the biochar surface adsorb and hold water molecules (Novak et al., 2012). Soil water dynamics can also be influenced indirectly by biochar application from increased organic matter, aggregation, or plant uptake.

Considering the direct effect of biochar only, the accumulation of water in biochar micropores via capillarity relies on unobstructed access to the micropore network. Images of biochar-amended soil taken with a scanning electron microscope and ^{13}C CP-NMR indicate that silt, clay, and organic matter particles accumulate in biochar macropores within one season of application (Joseph et al., 2010). Given that this experiment occurred three years after biochar application, it is possible that biochar micropores are no longer accessible to sequester water through capillarity.

Fresh biochar has accessible micropores but its surface is initially hydrophobic. As biochar weathers, its surface oxidizes to deprotonate alkyl groups, which disperses biochar's hydrophobic properties (Kinney et al., 2012) and creates negatively charged carboxyl groups on its surface ($-COO^-$) (Cheng et al., 2008). Water molecules possess a positive pole that could weakly associate on aged biochar. However, in the presence of cations, that are ubiquitous in soil (Na^+, Ca^{2+}, Al^{3+}, Mg^{2+}), water would be quickly displaced for a more thermodynamically favorable ionic bond. This hypothesis is consistent with results seen of Kinney et al. (2012), where biochar alone held 10 times its weight in water but the WHC was not maintained in the biochar + soil mixture. They suggested that surface adsorption is disrupted when biochar particles are dispersed with cations present in the soil solution.

2.4.2 Phosphorus dynamics

It was hypothesized that biochar amendments would reduce the P load in part by retaining DP and reducing the P concentration in runoff. There was significantly lower TP concentration in 4 of 6 biochar treatments but this did not produce a significant reduction in P load given the fact that the runoff volume was similar in the biochar-amended plots, relative to the control plots. Particulate P reduction is likely due in part to sediment accumulation, as described above by Joseph et al. (2010).

The major finding of this study is that biochar had relatively no effect on DP in runoff water, which is in contrast to many reports of TP and DP in leachate from biochar-amended soil (Lehmann et al., 2003; Laird et al., 2010; Borchand et al., 2012; Major et al., 2012; Yao et al., 2012). Every paper attributed this reduction directly to adsorption of phosphate ions on the biochar surface (evidenced by a decrease in DP), or indirectly to plant uptake, microbial immobilization, precipitation, or change in pH that altered P solubility. My findings show clearly that the PP fraction was diminished in runoff from biochar-amended field soils, four years after biochar was applied. I attribute this finding to deposition or sedimentation of PP within the biochar micropores. If my hypothesis is correct, then it suggests that physical stabilization of P within aggregates consisting of biochar, cations, colloidal soil P, and organic debris can retain PP and prevent its loss in runoff.

2.5 Conclusion

Biochar does not alter soil physical properties enough to change WHC, runoff volume, or infiltration rate. As a result, it does not reduce P loss through reduction in runoff. Rather, it may reduce PP concentration in runoff by sequestering particulate matter enriched with P within its pores and creating an aggregate. Provided biochar becomes economically accessible to farmers, it may serve as a tool to help reduce P losses via runoff, while being potentially beneficial to crop yields. It remains undetermined whether aggregation can cause reductions in PP, given that most leachate studies cite adsorption to biochar as the main retention mechanism.

Table 2-1: Physical and chemical properties of biochar applied to experimental field plots in the St-Francois-River watershed.

	Dynamotive	**Pyrovac**	**Basques**
Parent material[2]	Hardwood	Softwood bark	Hardwood
Temperature of pyrolysis[2]	>500°C	>500°C	<500°C
Length of pyrolysis[2]	Short	Short	Long
Texture	Fine powder	Granular	Powder
pH	8.65	8.29	7.77
Organic Carbon, g kg^{-1}	740	460	670
Total Nitrogen, g kg^{-1}	4.2	5.9	5.1
Total Phosphorus, mg kg^{-1}	300	978	1763
Ash content, g kg^{-1}	980	442	109

[1] Data obtained from Soil Control Laboratory (Watsonville, CA).

[2] Specific details not provided due to propriety rights

Table 2-2: Water infiltration, runoff dynamics and P loss in runoff from simulated rainfall applied with a Cornell Sprinkle Infiltrometer to plots in the St-Francis River watershed. Values for each treatment are the mean ± standard deviation (n=3).

		Water Dynamics[1]				**P loss**	
Treatment	Rate (t ha^{-1})	Moisture content (g kg^{-1})	Time to ponding (min)	Runoff volume (mL)	Infiltration rate (ml cm^{-2} hr^{-1})	P concentration (mg L^{-1})	P load (mg)
Control	0	345 ± 69.0	8:34 ± 1:23	1780 ± 487	13.8 ± 4.21	0.255 ± 0.137	0.993 ± 0.624
Dynamotive	5	335 ± 55.1	5:10 ± 1:44	2860 ± 296	15.0 ± 6.62	0.143 ± 0.055*	0.894 ± 0.351
	10	352 ± 85.4	2:30 ± 1:11	3000 ± 479	12.5 ± 2.23	0.143 ± 0.134*	0.938 ± 0.613
Pyrovac	5	363 ± 53.7	4:25 ± 3:53	3220 ± 432	14.1 ± 3.67	0.222 ± 0.116	1.563 ± 0.548
	10	385 ± 84.1	5:00 ± 0:42	2272 ± 245	13.9 ± 5.52	0.156 ± 0.128 *	0.775 ± 0.373
Basques	5	364 ± 82.9	5:00 ± 2:26	2883 ± 512	17.8 ± 6.89	0.215 ± 0.130	1.355 ± 0.642
	10	373 ± 105	6:14 ± 2:22	3170 ± 866	12.6 ± 2.73	0.158 ± 0.119 *	1.095 ± 0.985

[1] All data insignificant (p>0.05) using a contrast analysis to compare treatments against the control

*Values are the mean and standard errors (n=3). Columns with different capital letters had significantly (P<0.05, contrast analysis) lower PP concentration than the control. Columns with different lowercase letters had significantly (P<0.05, contrast analysis) lower DRP concentration than the control.

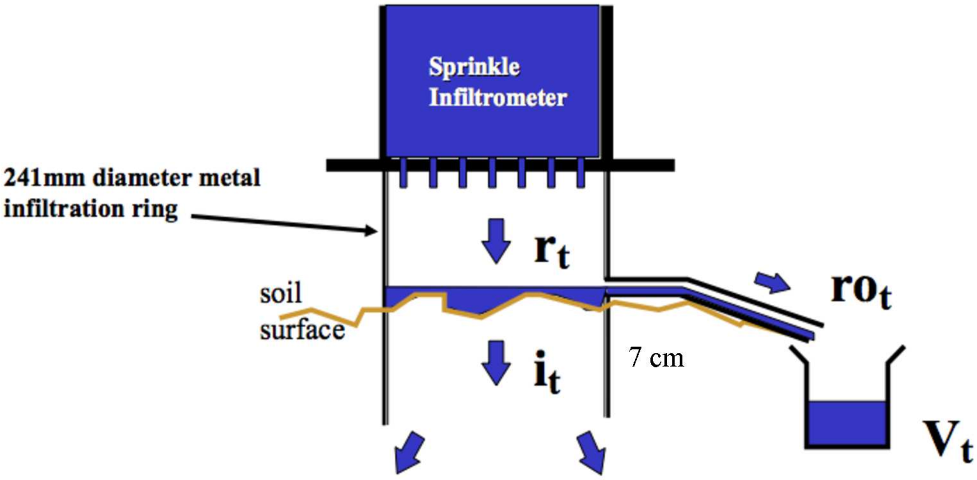

Figure 2-1: A diagram of the Cornell Sprinkle Infiltrometer, where r_t represents the rainfall rate and V_{ro} is the volume of runoff collected. The two values were used to calculate runoff rate (ro_t) and soil infiltration (i_t) (Cornell Soil Health, 2013).

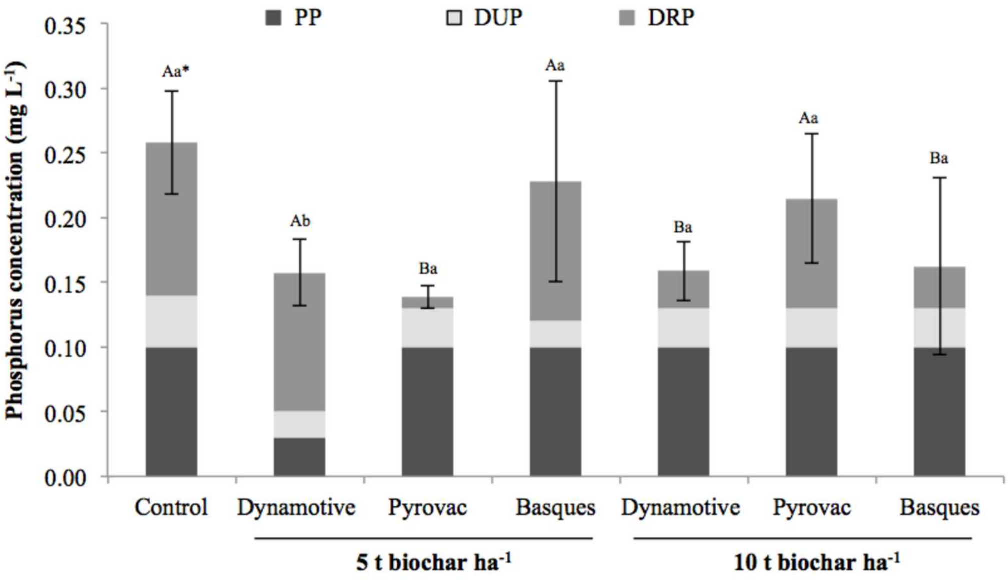

Figure 2-2: Particulate P (PP), dissolved reactive P (DRP), and dissolved unreactive P (DUP) concentrations (in mg L^{-1}) in surface runoff after a 0.5h rainfall simulation on field plots amended with biochar or without biochar (control).

*Values are the mean and standard errors (n=3). Columns with different capital letters had significantly ($P<0.05$, contrast analysis) lower PP concentration than the control. Columns with different lowercase letters had significantly ($P<0.05$, contrast analysis) lower DRP concentration than the control.

Connecting paragraph

The previous work found a reduction in particulate P concentration in surface runoff, but it was not related to changes in the measured soil physical properties (eg. soil infiltration and soil bulk density) or soil P chemistry (eg. Mehlich-III P). Small-scale processes occurring within the soil structure (eg. within aggregates) could be responsible for occluding PP and preventing it from being carried off the field via surface runoff. The largest non-point source of P pollution is particulate P in runoff from agricultural fields. The next step is to determine whether biochar induces aggregate formation, improves aggregate stability, or alters aggregate composition.

Chapter 3: Biochar amendments alter nutrient composition in aggregates from Quebec agricultural soil

Abstract

Soil aggregates bind loose particles together and resist dispersive forces, thus retaining organic carbon (C) and other nutrients that would otherwise be susceptible to loss in eroded sediments. The objective of this study was to determine whether biochar amendments improve soil aggregate formation, strength, or composition for the purpose of reducing C and P loss. Biochar-amended soil was collected from an experimental field site in St-Francois-Xavier-de-Brompton, Quebec. The soil had been amended with 3 types of biochar (Dynamotive, Pyrovac, and Basques) at 2 application rates (5 and 10 t ha^{-1}) two years prior to the experiment. Soil samples were fractionated into unaggregated (<150 μm), microaggregated (150-250 μm), and macroaggregated (>250 μm) classes using a wet-sieve method. Each fraction was analyzed for total organic C and total P concentration. There was no difference in the proportion of un-, micro-, or macroaggregates or the mean-weight diameter in biochar amended soil. Biochar treatments increased microaggregate stability ($p=0.032$ and $p=0.046$ for 5 t ha^{-1} and 10 t ha^{-1}, respectively), which corresponded to more organic C ($p=0.013$ and $p<0.01$, for 5 t ha^{-1} and 10 t ha^{-1}, respectively). Macroaggregates contained higher organic C concentrations ($p=0.061$ and $p=0.028$ for 5 t ha^{-1} and 10 t ha^{-1}, respectively). Macroaggregates also contained higher P concentrations ($p=0.035$ and $p=0.043$). This suggests that biochar becomes integrated within the

microaggregate structure and indirectly promotes P retention within macroaggregates.

3.1 Introduction

Quebec, like other humid temperate regions, experiences high annual precipitation >1000 mm, with rapid disappearance of snow in the early spring, and episodic, heavy rainfall events during the frost-free period. Water moving on the soil surface transports primary particles (clay and small sediments >0.45 μm) and organic matter (OM) from agricultural fields. Annually cropped fields that are left bare during the pre-crop emergence and post-harvest periods are especially prone to erosion. For example, agricultural Podzols and Brunisols in Quebec with minimal slope lost 1.6 - 10.5 t soil ha^{-1} yr^{-1} (Kirby and Mehuys, 1987), and Dube (1975) attributed up to 69% of annual soil loss from similar soils that were bare during springtime.

Soil erosion has environmental implications both on and off the field. Primary particle loss dismantles the soil structure, reducing soil water holding capacity, aeration, plant-available nutrient concentrations, and ultimately crop output. Primary particles are often enriched with phosphorus (P) compared to surrounding soil due to strong clay-P associations (Römkens et al., 1973) and historic fertilizer applications that slowly saturate soil with P (Sharpley et al., 1994). Phosphorus-enriched primary particles >0.45 μm in size, referred to as particulate P or PP, migrate into rivers and estuaries and cause sedimentation and eutrophication of surface waters (Schindler et al., 1987). Jamieson et al. (2003)

measured 75 kg PP ha^{-1} lost from a bare agricultural field during a single snowmelt event in Quebec's Pike River watershed, a region notoriously affected by eutrophication.

Nutrient-rich soil particles, including PP, can resist erosive forces by hierarchically binding small particulate matter (<53 μm) together to form larger microaggregates (150-250 μm) and eventually stable macroaggregates (>250 μm) (Le Bissonnais, 1996). Multiple microaggregates bind together with transient, temporary, or persistent C-derived binding agents to create larger macroaggregates. The primary binding agents are all carbon based, thus soil organic carbon (SOC) is correlated to macroaggregate formation (Six et al., 2002). The most common cementing compounds are microbially-secreted polysaccharides, followed by bacterial debris, fungal debris, hyphae, and roots, listed in order from smallest to greatest binding strength determined by their ability to resist decomposition (Tisdall and Oades, 1982). Strength is crucial to maintain aggregate integrity after a disturbance like rainfall or cultivation, though macroaggregates inherently disassemble because of OM decomposition (Bronick and Lal, 2005). Macroaggregates also contain partially decomposed plant and animal residues as particulate organic matter, which are not bound in clay-OM complexes but occluded within the macroaggregate structure. Consequently macroaggregates contain higher concentrations of organic C, N, and P than microaggregates (Elliot, 1986). Compared to organic C and N, which are lost readily in gaseous and soluble forms as a result of microbial activity, P is retained within macroaggregates (Linquist et al., 1997); therefore macroaggregate formation is expected to curb PP loss due to wind and water erosion.

Many soil management practices are implemented to promote macroaggregate formation and/or strength and prevent the release of nutrients (see review by Bronick and Lal, 2005). Incorporation of plant residues, animal manure, and compost, as well as perennial crops and reduction in tillage intensity are all effective in promoting macroaggregate formation (Le Bissonnais, 1996). Biochar is an organic soil amendment rich in organic C that is produced from pyrolyzed biomass, and biochar has been hypothesized to promote soil aggregation (Major et al., 2012). It is made up of highly stable aromatic C rings (Novak et al., 2009b) that allow less than 1% of C to mineralize, resulting in a mean residence time of 1000+ years in soil (Lehmann et al., 2009). Biochar's large and reactive surface area provides ample binding sites for clay plates or OM to flocculate together and integrate biochar into the clay-cation-OM complex of microaggregates (Brodowski et al., 2011). Biochar has large pores that shelter niche microbial and fungal communities, resulting in greater microbial activity in biochar-amended soils than non-amended soils (Lehmann et al., 2011). The growth and activity of decomposer microorganisms generate byproducts that contribute to macroaggregate formation (Zimmerman et al., 2011). Biochar may change the nutrient proportion within aggregates by preferentially attracting certain organic or mineral sediments enriched with P (Hale et al., 2013).

The objective of this experiment was to determine whether biochar applied to an agricultural Podzol would promote macroaggregate (1) formation and (2) stability and (3) change the nutrient composition of macroaggregates, particularly the retention of P. It is hypothesized that biochar will create more aggregates that are stronger and contain a higher concentration of total P.

3.2 Methods and Materials

3.2.1 Site description and experimental design

The experiment was conducted on soil collected from a field described in chapter 2 of this thesis. Briefly, plots were amended three years prior to this experiment with three types of biochar (Dynamotive, Pyrovac, and Basques) at 0, 5, and 10 t ha^{-1}. Treatments were arranged in a randomized complete block design formation with 3 replicates. The experimental plots were cultivated with a moldboard plough and sown with barley in the 2011 growing season, and received manure and inorganic fertilizers and no herbicide.

3.2.2 Soil sampling

One undisturbed soil sample was taken from the plough layer (15 cm depth) of each plot after crop harvest, before fall plowing (October, 2011) by excavating a soil block (15 cm x 15 cm) with a shovel. Soil was carefully transferred into a plastic storage container (15 L Rubbermaid container) to prevent compaction or desiccation. Storage containers were kept at 4^{o}C until analysis, which was completed within one week of collection.

3.2.3 Aggregate separation and analysis

A sample of field-moist soil (40 g) was gently crumbled by hand to break up clods, and sieved through a 6 mm mesh sieve. It was distributed evenly atop of a nest of 5 sieves, with mesh sizes 2 mm, 850 µm, 500 µm, 250 µm, and 150 µm arranged from top to bottom. The sieves and soil were slowly submerged in water for 10 min to allow the sample to hydrate through capillary action, then were repeatedly immersed 3.7 cm deep, 29 times per min for 10 min using an apparatus similar to that described by Kemper and Rosenau (1986). The soil remaining on each sieve represented the aggregates of the given size range. Each macro- and microaggregate fraction was collected and transferred to filter paper to dry at 105°C for 24 h, and was then weighed. A separate sample of field-moist soil (5 g) was also weighed and dried (105°C for 24 h) to determine soil moisture content.

A subsample (0.065g) of each soil aggregate fraction underwent analysis for C and P. Organic C was determined using a Flash EA 1112 NC soils analyzer (Carlo-Erba, Milan, Italy), assuming that total C was equivalent to organic C. Total P concentrations in soil were determined with H_2SO_4/H_2O_2 digestion (Parkinson and Allen, 1975) and analyzed colorimetrically using a Lachat Quick-Chem AE flow injection auto analyzer (Lachat Instruments, Milwaukee, WI, United States).

The remaining soil macro- and microaggregate samples underwent dispersion with 50 ml of 0.5% (w/v) sodium hexametaphosphate solution by shaking at 60 rpm for 45 min. The sample was passed through the same sieve to extract the water-stable aggregates. The samples were transferred onto filter paper, dried at 105°C for 24 h and weighed.

3.2.4 Calculations

The water-stable aggregates ($\text{WSA}_{i,}$ expressed as a % for each aggregate fraction) and mean-weight diameter (MWD, in mm) were calculated according to Van Bavel (1949):

$$(1)\ \text{WSA}_i = \frac{w_{2i} - w_{3i}}{\frac{w_1}{1+wc} - \sum_{i=1}^{n} w_{3i}}$$

$$(2)\ \text{MWD} = \sum_{i=1}^{n} x_i\, \text{WSA}_i$$

Where $i = 1, 2, 3 \ldots, n$ and corresponds to each size fraction

A modification was made to the w_{3i} variable to compensate for the subsample taken for the C:N:P analyses. The percentage of soil lost during the dispersion was calculated and was used to estimate the original weight of the W_{3i} fraction. The final data are expressed in terms of macroaggregates, microaggregates, and unaggregated fractions, which are the combination of multiple sieve sizes. Macroaggregation is represented by sieves of mesh size 2000 µm, 850 µm, and 500 µm, and 250 µm, while 150 µm is microaggregates, and the soil that passed through all the sieves is a combination of microaggregated and unaggregated soil.

Despite biochar's high C content, it still contains small amounts of P (11.9 – 300 mg total P kg^{-1}), reported in the Soil Control Lab Biochar Analysis (Appendix I). Total P digestions cannot discern P originating in the biochar itself or from OM and clay sediments surrounding the biochar. To correct biochar-added P, the C differential between amended and non-amended soil was attributed to biochar and the expected P content from the present biochar was extrapolated using the P values from the biochar analysis and the sample calculation found in Appendix II. The difference between total P and biochar-P was assumed to be increased P-laden clay sediments, organic matter, or microbial matter.

3.2.5 Statistical Analysis

The effect of biochar on MWD, wet-sieved aggregate distribution, aggregate strength, and C and P content were analyzed using a two-way MANOVA with PROC MIXED using SAS statistical software (version 9.2) to find within-treatment and between-treatment effects. Preplanned orthogonal contrast analyses ($\alpha=0.05$) were conducted to compare individual treatments, pooled treatments by rate, pooled treatments by type, or all biochar against the control.

3.3 Results

3.3.1 Aggregate distribution

Between 93%-97% of the soil was recovered during the aggregation separation. The proportion of water stable aggregates (Figure 3-1) and MWDs (Table 3-1) did not significantly differ for any of the biochar treatments individually, or when pooled by type or rate, compared to the control.

3.3.2 Aggregate stability

The proportion of undispersed water-stable microaggregates was significantly higher compared to the control ($p = 0.039$). The control maintained 3.8% of its microaggregates, while the biochar amended plots sustained more microaggregates in all 3 types of biochar, with more variation derived from rate rather than type of biochar; the 5 and 10 t ha^{-1} treatments held 5.4% ($p=0.046$) and 6.1% ($p=0.032$) more microaggregates, respectively (Table 3-1). There were significant block effects for aggregate strength ($p = 0.02$) for block 1 compared to block 2 and 3.

3.3.3 Aggregate composition

Biochar rate, but not biochar type, affected nutrient composition; therefore the data across biochar type was pooled to isolate rate effects. Microaggregates contained 40.0% ($p=0.013$) and 67.6% ($p<0.01$) more organic C in biochar amended plots at 5 and 10 t ha^{-1} than the unamended control (Fig. 2). Macroaggregates contained 26.3% ($p=0.061$) and 31.9% ($p=0.028$) more organic C in soils amended with biochar at 5 t ha^{-1} and 10 t ha^{-1}, respectively (Fig. 2). The P concentration in macroaggregates was significantly higher ($p=0.035$ and $p=0.043$ for 5 t ha^{-1} and 10 t ha^{-1}, respectively) compared to the control but was unchanged in the microaggregate fraction (Fig. 2). Unaggregated soil had similar C or P concentrations in biochar amended and unamended soil.

3.4 Discussion

3.4.1 Aggregate Formation

Biochar amendments did not increase aggregate MWD or the distribution of soil mass in macroaggregate and microaggregate fractions. I attribute these findings to the fact that chemical recalcitrance of the aromatic ring structure causes slow mineralization and is unable to serve as an adequate microbial substrate. This refractory nature may explain why aggregation does not respond in the same manner to biochar as other organic C inputs, despite being classified as one (Lin et al., 2012). My results are consistent with Watts et al. (2005) and Busscher et al. (2010), who found that fresh charcoal did not alter aggregate distribution after 28-day and 70-day incubations, respectively. Soil from this experiment was amended 3 years earlier, but Major et al. (2012) speculated biochar would require several decades to form larger aggregates (as seen in Glaser et al., 2004b).

3.4.2 Aggregate Strength

Aggregate strength was significantly higher in microaggregate fractions of biochar-amended soils compared to the control. This could suggest that biochar particles were integrated into the microaggregate structure and served as a structural skeleton, which replaces the traditional electrostatic stabilizing forces in the clay-cation-OM complex. It could occur because biochar surfaces oxidize to create cation exchange sites (Glaser et al., 2000; Smernik et al., 2000; Skjemstad et al., 2002) that have a tremendously high affinity for organic matter (Sobek et al., 2009) and for localized hydrophobic patches within the clay lattice structure (Sposito et al., 1999). This hypothesis is consistent with findings from Brodowski et al. (2006), who determined that microaggregates preferentially sequester black carbon, over other forms of SOM, creating a biochar core.

The data are not able to conclusively confirm this hypothesis, because strength is determined by using a solution that promotes clay dispersion by altering ionic concentration, but is unable to disperse the biochar structure or its chemical affiliations with OM. It is difficult to determine whether larger, loose biochar fragments that are unaffiliated with any soil but unable to pass through the sieve are mistakenly counted as water-stable aggregate, thus confounding the data. Between 61.9% and 70.7% of three biochar types' particles were <250μm, yet there was no increased presence in either microaggregate or unaggregated particles. Further analysis, like those done in Brodowski et al. (2006), is needed to determine the exact location of biochar to determine conclusively whether it beneficially contributes to aggregate strength.

A reduction in macroaggregate strength may have been caused by OM becoming encapsulated within biochar pores where it stabilizes through cation bridges. This renders the attached OM unmineralizable, in a process called sorptive protection (Kaiser and Guggenberger, 2000; Jastrow et al., 2007; Kasozi et al., 2010) that causes negative priming (Zimmerman et al., 2011). As a result, both the biochar C and native SOC are not decomposed by microbes, and less microbially-secreted cementing agents to hold together macroaggregates are produced.

3.4.3 Aggregate Nutrient Composition

Both biochar-amended micro- and macroaggregates contain significantly higher C due to biochar presence, with microaggregates containing more organic C than macroaggregates. I explain this data using a soil C input model that shows C preferentially associates with different fractions as it shifts along a conceptual C-saturation continuum (Six et al., 2002). Carbon-inputs into soil initially form chemical bonds with organo-mineral complexes such as silt and clay but once this fraction becomes saturated, additional C becomes compartmentalized within microaggregates. Brodowski et al. (2006) corroborates the idea that biochar is being located within the microaggregate center, though I also suggest that some of the C-enriched microaggregates are participating in the hierarchical formation of macroaggregates.

Phosphorus concentrations did not correlate to biochar location. There was significantly higher P found within the macroaggregates though, which suggests biochar may be facilitating additional P retention within the macroaggregate (Zimmerman et al., 2011). It is possible OM receiving sorptive protection against decomposition may also serve as a dual mechanism to accumulate organic P or that biochar indirectly alters microbial decomposition rates or community composition to change P turnover, which explains increased P in macroaggregates, but not microaggregates. Parvage et al. (2012) suggested biochar amendments induce precipitation of DP through added Ca, Mg, and K ions from residual biochar ash and the colloidal precipitate flocculates to form PP.

3.5 Conclusion

Biochar does not increase microaggregate presence but may be the core of their structure, which improves strength and potentially improves its resistance to erosion. Biochar does not improve macroaggregate formation nor their strength, but is able to increase the P concentrations in macroaggregates compared with unamended soils. Higher P retention in macroaggregates may prevent P loss via erosion, and is a likely explanation for the lower PP concentrations found in simulated runoff from the previous chapter.

It must be noted though that biochar application may not be a primary solution to promoting soil stability. The process of incorporating biochar into soil can unnecessarily disrupt aggregates and concurrently facilitate OM and biochar decomposition by exposing previously sequestered substrate to microbes (Kuzyakov et al., 2009). Black carbon can also preferentially erode shortly after incorporation because of its light weight and weak associations to surrounding soil particles (Rumpel et al., 2009). The extent of the biochar-P relationship must be further investigated to know what form P is bound and if the relationship changes over time before it can be implemented as a tool for environmental protection.

Table 3-1: The proportion of Water Stable Aggregates (WSA) as a percentage (%) that remained intact after the dispersion in each fraction and the overall mean weight diameter (MWD, in mm) of soil aggregates affected by three types of biochar and two application rates.

Treatment	**Rate ($t\ ha^{-1}$)**	**WSA-size fraction (mm)**					
		>2	**2-0.85**	**0.85-0.5**	**0.5-0.25**	**0.25-0.15**	**MWD (mm)**
Control	0	49.6	51.5	50.4	2.83	3.81	0.73
Dynamotive	5	55.7	55.2	44.6	3.68	5.36	0.87
	10	65.0	65.4	55.6	3.69	6.52	0.83
Pyrovac	5	59.8	54.2	50.4	3.60	4.98	0.78
	10	49.6	54.6	51.8	3.71	5.04	0.97
Basques	5	59.2	54.5	47.3	3.60	5.87	0.70
	10	57.9	61.6	57.6	3.91	6.67	1.12
ANOVA results		NS	NS	NS	NS	P=0.038	NS
Contrast Analysis							
Control vs. biochar (5 $t\ ha^{-1}$)		NS	NS	NS	NS	P=0.032	NS
Control vs. biochar (10 $t\ ha^{-1}$)		NS	NS	NS	NS	P=0.043	NS

NS = not statistically significant (P>0.05)

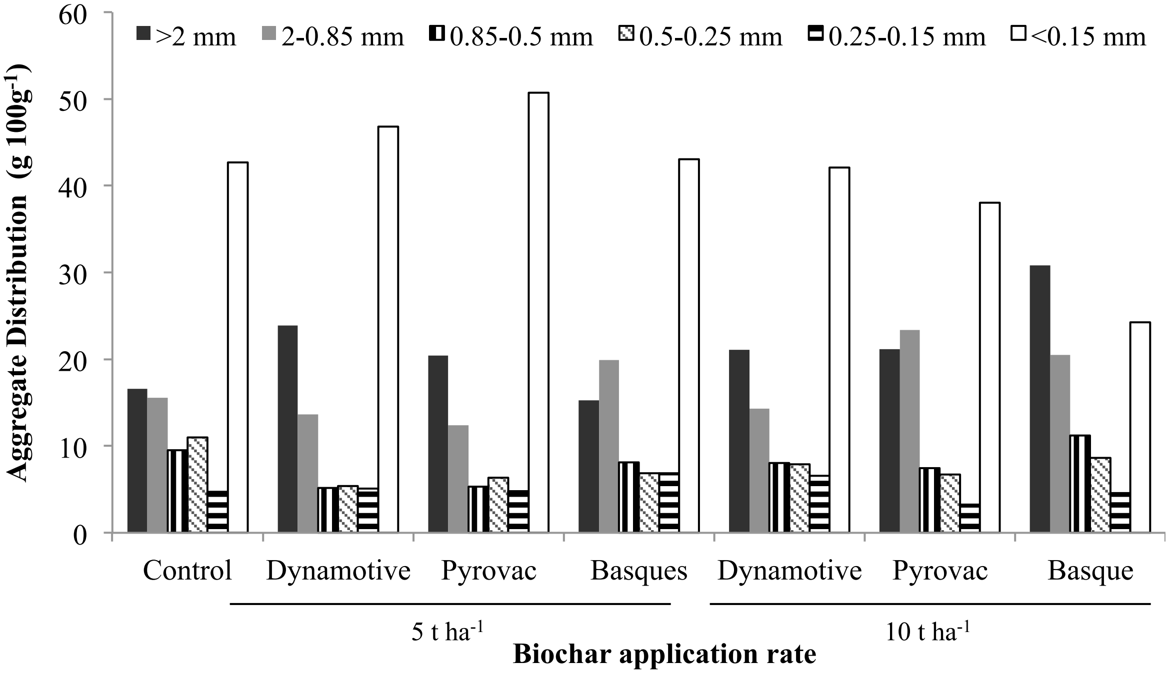

Figure 3-1: The distribution of water-stable aggregates in g $100g^{-1}$ prior to dispersion, according to biochar type and application rate. No significant difference was seen in distribution between treatments.

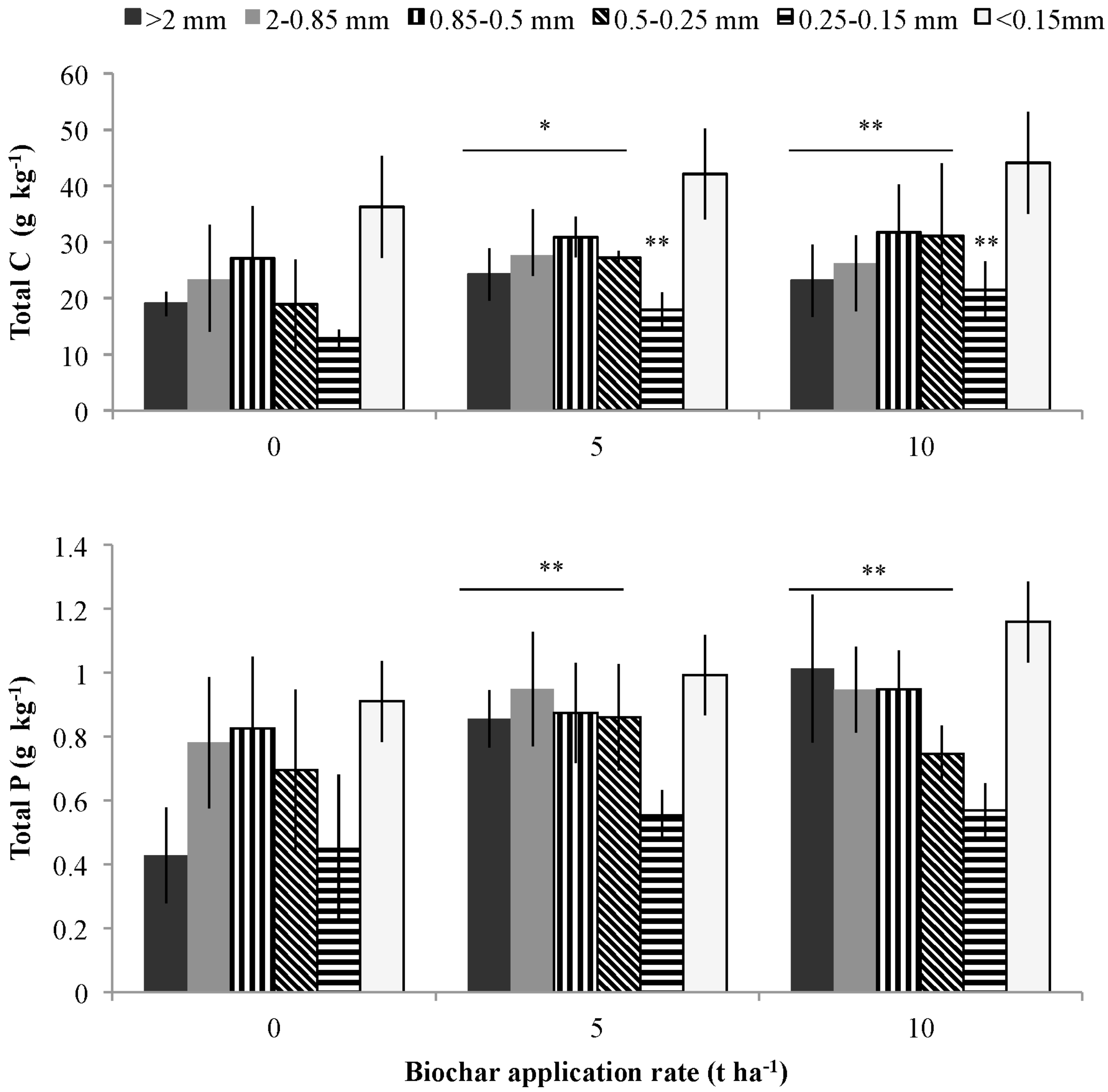

Figure 3-2 Total C and P content of aggregate size fractions collected from biochar-amended plots.

** indicates significant difference ($p<0.05$, contrast analysis) from the unamended plot (0 t ha^{-1}).

* indicates significant difference ($p<0.1$, contrast analysis) from the unamended soil (0 t ha^{-1})

General Conclusions

The objectives of my thesis research were to (1) investigate whether biochar reduced the P load in surface runoff from an agricultural soil found in a humid temperate climate region and (2) determine whether improved soil stability from soil aggregation could be a mechanism behind the observed reduction in P load. The first objective was achieved by performing a rainfall simulation on biochar-amended plots on an active Quebec agricultural field. The second objective was achieved by physically separating soil samples by size into macroaggregate, microaggregate, and unaggregated fractions and quantifying C and P content within each fraction.

Biochar application reduced PP concentration in runoff from a simulated rainfall on the experimental plots. Water-holding capacity and soil infiltration did not appear to contribute to this reduction. The results were attributed to biochar integration within the microaggregate structure, which translated to P retention at the macroaggregate scale. Clay and organic matter deposits in biochar pores, precipitation of DP to PP from biochar ash, and sorptive protection of OM from adsorption were all speculated as possible mechanisms that improve P concentration.

In order to determine which mechanisms are active, tests like Fourier Transform Infrared (FT-IR) spectroscopy, ^{31}P-Nuclear magnetic resonance (^{31}P-NMR), and Phosphorus K-edge X-ray adsorption near-edge structure (Xanes) spectroscopy may be performed. These tests characterize and quantify the organic and inorganic P forms associated with biochar and locate whether they exist primarily within biochar pores or on the biochar surface. This information will help identify how the biochar-phosphorus relationship is mediated by soil texture and whether phosphorus retention in biochar-induced aggregates persists through time. Once the biochar-phosphorus relationship is understood, methods to exploit the relationship and maximize P retention can be explored, for the ultimate purpose of environmental protection.

Before biochar is integrated into the repertoire of best management practices for P retention, further research must provide indisputable evidence that it reduces P losses from agricultural soils in humid temperate regions in both the short and long term, in dissolved and particulate forms, and from surface and subsurface flow. Once these variables are determined, biochar application may become one of many tools that improve P efficiency for the purpose of environmental protection and, ultimately, restoring balance to global P distribution.

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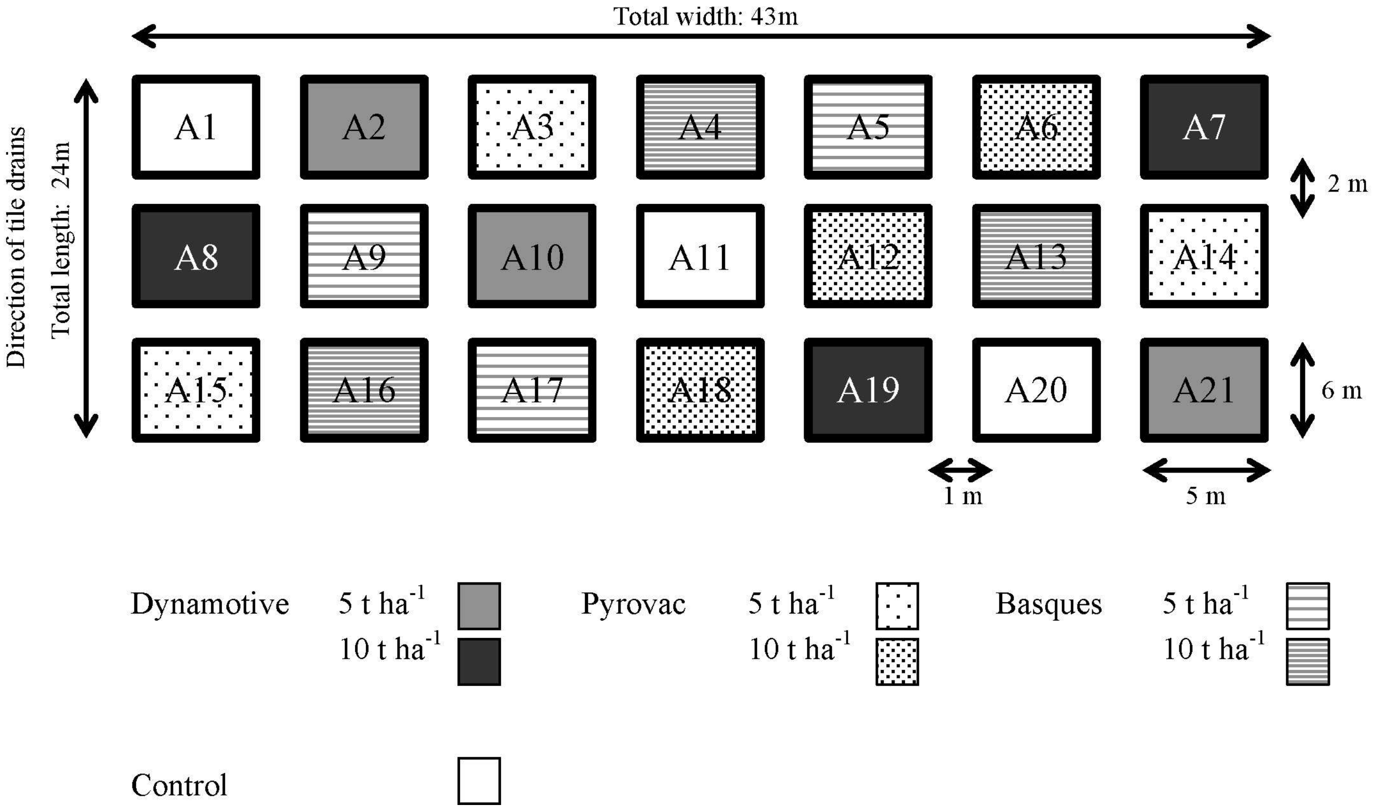
Appendix A: The field set up for the randomized complete block design experiment, with 3 blocks, in St-Francois-Xavier-de-Brompton, Quebec, Canada. The plots were amended with one type of biochar (Dynamotive, Pyrovac, or Basques) and applied at either 5 or 10 t ha^{-1}, with one plot at 0 t ha^{-1} as the control.



Appendix B: A report of chemical and physical property of biochar used in this experiment analyzed by Soil Control Lab (Watsonville, California). Found on next 9 pages.

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Account No.:
2010666 4 0
Batch
Nov. - Dec. 2011 17
CODE:
BioChar IBI

Barry Husk
BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received:	31 Jan. 12
Sample Id.:	Dynamotive biochar
Sample id. Number	4 2010666

International BioChar Initiative (IBI) Level I

	Wet	Dry			
Moisture	39.8	0.0	percent	Declaration	ASTM D1762-84 (105c)
Total Ash	5.9	9.8	percent	50% Max	ASTM D 1762-84 (750c)
Organic Carbon	44.0	74.0	percent	Declaration	CHN by dry combustion
Inorganic Carbon	0.07	0.12	percent	Declaration	HCl treated
Hydrogen/Carbon (H:C)	0.55	0.55	molar ratio	0.7 Max	
Hydrogen	2.0	3.4	percent	Declaration	CHN by dry combustion
Total Nitrogen (N)	0.23	0.42	percent	Declaration	CHN by dry combustion
pH value	8.65	NA	units	Declaration	Ahmedna et al (2002) (1997)
Liming (neut. value)	7.8	12.9	% CaCO3	Declaration	Rayment & Higginson
Liming (carbonate value)	0.59	0.98	% CaCO3	Declaration	ASTM D 4373
Activity Iodine or Butane)	2.6	4.4	g/100 g	Declaration	ASTM D 5742 (butane)

Particle Size Distribution (ASTM D5158 powdered) or (ASTM D2862 granular)

MM	Inch or Mesh #	% Retained	Fraction %
> 19	0.75	0.0	0.0
16 to 19	0.625	0.0	0.0
9.5 to 16	0.375	0.0	0.0
6.3 to 9.5	0.250	0.0	0.0
2.0 to 6.3	#10	0.0	0.0
0.850 to 2.0	#20	2.4	2.4
0.425 to 0.850	#40	13.8	11.4
0.250 to 0.425	#60	38.1	24.3
0.180 to 0.250	#80	59.6	21.5
0.150 to 0.180	#100	81.2	21.6
< 150	< #100	100.0	18.8
		SUM	100.0

Analyst: Frank Shields

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Date Received:	31 Jan. 12
Sample Id.:	Dynamotive biochar
Sample id. Number	4 2010666

International BioChar Initiative (IBI) Level II

			Results Wet Basis	Dry Basis	Maximum Allowed	Units	Method
Arsenic	(As)		0.43	0.26	13	mg/kg	B
Cadmium	(Cd)		< 0.01	< 0.01	1.4	mg/kg	A
Chromium	(Cr)		43	26	93	mg/kg	A
Cobalt	(Co)		0.082	0.049	34	mg/kg	B
Copper	(Cu)		15	9.3	143	mg/kg	A
Lead	(Pb)		5.6	3.4	121	mg/kg	A
Molybdenur	(Mo)		0.64	0.38	5.0	mg/kg	B
Mercury	(Hg)		0.020	0.033	1.0	mg/kg	A
Nickel	(Ni)		3.6	2.1	47	mg/kg	A
Selenium	(Se)		< 0.01	< 0.01	2.0	mg/kg	B
Zinc	(Zn)		22	13	416	mg/kg	A
Boron	(B)		32	19	Declaration	mg/kg	TMECC
Chlorine	(Cl)		22	37	Declaration	mg/kg	TMECC
Sodium	(Na)		122	73	Declaration	mg/kg	TMECC
Basic Soil Enhancement Properties							
Potassium	(K)	Total and available	6453	3884	Declaration	percent	E
Phosphorus	(P)	Total	300	180	Declaration	percent	E
Ammonia	(NH4-N)	Mineral	23	38	Declaration	mg/kg	R
Nitrate	(NO3-N)	Mineral	2.5	4.2	Declaration	mg/kg	R
Phosphorus	(P)	Available	10.6	17.5	Declaration	mg/kg	F
Electrical Conductivity		EC 1:5 w/w	4.420	NA	Declaration	S/m	R
Moisture			39.8	0.0	Declaration	percent	TMECC
pH value			8.65	NA	Declaration	units	TMECC

Methods

Method A	Amlinger, Faroino and Pollack (2004)
Method B	Bureau de normalisation du Quebec (2005)
TMECC	Test Methods for the Examination of Compost and Composting (2001)
Method E	Enders and Lehmann (2011)
Method R	Rayment and Higginson (1992)
Method F	Formic Acid procedure

Analyst: Frank Shields

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Barry Husk
BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received: 31 Jan. 12
Sample Id.: Dynamotive biochar
Sample id. Number 4 2010666

Proximate Analysis

Sample Received DRY weight

Constituent	percent
Carbon (C)	74.0
Hydrogen (H)	3.2
Nitrogen (N)	0.42
Sulfur (S)	0.02
Oxygen (O)	12.6
Ash (total)	9.8
Water (200c)	0.0
Sum	100.0

as-Received WET weight

Constituent	percent
Carbon (C)	44.5
Hydrogen (H)	1.9
Nitrogen (N)	0.25
Sulfur (S)	0.01
Oxygen (O)	7.56
Ash (total)	5.92
Water (200c)	39.8
Sum	100.0

Moisture (200c) 39.8 percent

Analyst: Frank Shields

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Barry Husk
BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received:	31 Jan. 12
Sample Id.:	Pryovac biochar
Sample id. Number	4 2010666

International BioChar Initiative (IBI) Level I

	Wet	Dry			
Moisture	5.7	0.0	percent	Declaration	ASTM D1762-84 (105c)
Total Ash	41.7	44.2	percent	50% Max	ASTM D 1762-84 (750c)
Organic Carbon	44.1	46.7	percent	Declaration	CHN by dry combustion
Inorganic Carbon	0.37	0.40	percent	Declaration	HCl treated
Hydrogen/Carbon (H:C)	0.56	0.57	molar ratio	0.7 Max	
Hydrogen	2.1	2.2	percent	Declaration	CHN by dry combustion
Total Nitrogen (N)	0.54	0.59	percent	Declaration	CHN by dry combustion
pH value	8.29	NA	units	Declaration	Ahmedna et al (2002) (1997)
Liming (neut. value)	13.8	14.6	% CaCO3	Declaration	Rayment & Higginson
Liming (carbonate value)	3.1	3.3	% CaCO3	Declaration	ASTM D 4373
Activity Iodine or Butane)	4.2	4.5	g/100 g	Declaration	ASTM D 5742 (butane)

Particle Size Distribution (ASTM D5158 powdered) or (ASTM D2862 granular)

MM	Inch or Mesh #	% Retained	Fraction %
> 19	0.75	0.0	0.0
16 to 19	0.625	0.0	0.0
9.5 to 16	0.375	0.0	0.0
6.3 to 9.5	0.250	0.0	0.0
2.0 to 6.3	#10	0.0	0.0
0.850 to 2.0	#20	0.2	0.2
0.425 to 0.850	#40	16.8	16.6
0.250 to 0.425	#60	36.1	19.3
0.180 to 0.250	#80	50.3	14.3
0.150 to 0.180	#100	66.2	15.9
< 150	< #100	100.0	33.8
		SUM	100.0

Analyst: Frank Shields

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Date Received:	31 Jan. 12
Sample Id.:	Pryovac biochar
Sample id. Number	4 2010666

International BioChar Initiative (IBI) Level II

			Results Wet Basis	Dry Basis	Maximum Allowed	Units	Method
Arsenic	(As)		2.3	2.1	13	mg/kg	B
Cadmium	(Cd)		< 0.1	< 0.1	1.4	mg/kg	A
Chromium	(Cr)		4.6	4.4	93	mg/kg	A
Cobalt	(Co)		2.5	2.4	34	mg/kg	B
Copper	(Cu)		15	14	143	mg/kg	A
Lead	(Pb)		9.7	9.1	121	mg/kg	A
Molybdenur	(Mo)		< 0.1	< 0.1	5.0	mg/kg	B
Mercury	(Hg)		0.010	0.011	1.0	mg/kg	A
Nickel	(Ni)		10.0	9.4	47	mg/kg	A
Selenium	(Se)		< 0.1	< 0.1	2.0	mg/kg	B
Zinc	(Zn)		148	140	416	mg/kg	A
Boron	(B)		37	35	Declaration	mg/kg	TMECC
Chlorine	(Cl)		22	23	Declaration	mg/kg	TMECC
Sodium	(Na)		350	330	Declaration	mg/kg	TMECC
Basic Soil Enhancement Properties							
Potassium	(K)	Total and available	8411	7932	Declaration	percent	E
Phosphorus	(P)	Total	978	922	Declaration	percent	E
Ammonia	(NH4-N)	Mineral	23	24	Declaration	mg/kg	R
Nitrate	(NO3-N)	Mineral	2.3	2.4	Declaration	mg/kg	R
Phosphorus	(P)	Available	42.9	45.5	Declaration	mg/kg	F
Electrical Conductivity		EC 1:5 w/w	7.420	NA	Declaration	S/m	R
Moisture			5.7	0.0	Declaration	percent	TMECC
pH value			8.29	NA	Declaration	units	TMECC

Methods

Method A	Amlinger, Faroino and Pollack (2004)
Method B	Bureau de normalisation du Quebec (2005)
TMECC	Test Methods for the Examination of Compost and Composting (2001)
Method E	Enders and Lehmann (2011)
Method R	Rayment and Higginson (1992)
Method F	Formic Acid procedure

Analyst: Frank Shields

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BioChar IBI

Barry Husk
BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received:	31 Jan. 12
Sample Id.:	Pryovac biochar
Sample id. Number	4 2010666

Proximate Analysis

Sample Received DRY weight

Constituent	percent
Carbon (C)	46.7
Hydrogen (H)	2.2
Nitrogen (N)	0.59
Sulfur (S)	0.06
Oxygen (O)	6.3
Ash (total)	44.2
Water (200c)	0.0
Sum	100.0

as-Received WET weight

Constituent	percent
Carbon (C)	44.0
Hydrogen (H)	2.1
Nitrogen (N)	0.55
Sulfur (S)	0.06
Oxygen (O)	5.90
Ash (total)	41.68
Water (200c)	5.7
Sum	100.0

Moisture Content 5.7 percent

Analyst: Frank Shields

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Batch
Nov. - Dec. 2011 19
CODE:
BioChar IBI

Barry Husk
BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received: 31 Jan. 12
Sample Id.: Basques biochar
Sample id. Number 4 2010666

International BioChar Initiative (IBI) Level I

	Wet	Dry			
Moisture	66.0	0.0	percent	Declaration	ASTM D1762-84 (105c)
Total Ash	3.7	10.9	percent	50% Max	ASTM D 1762-84 (750c)
Organic Carbon	22.6	67.1	percent	Declaration	CHN by dry combustion
Inorganic Carbon	0.08	0.24	percent	Declaration	HCl treated
Hydrogen/Carbon (H:C)	0.67	0.67	molar ratio	0.7 Max	
Hydrogen	1.3	3.7	percent	Declaration	CHN by dry combustion
Total Nitrogen (N)	0.07	0.51	percent	Declaration	CHN by dry combustion
pH value	7.77	NA	units	Declaration	Ahmedna et al (2002) (1997)
Liming (neut. value)	17.1	50.3	% CaCO3	Declaration	Rayment & Higginson
Liming (carbonate value)	0.7	2.0	% CaCO3	Declaration	ASTM D 4373
Activity Iodine or Butane)	2.6	7.7	g/100 g	Declaration	ASTM D 5742 (butane)

Particle Size Distribution (ASTM D5158 powdered) or (ASTM D2862 granular)

MM	Inch or Mesh #	% Retained	Fraction %
> 19	0.75	0.0	0.0
16 to 19	0.625	0.0	0.0
9.5 to 16	0.375	0.0	0.0
6.3 to 9.5	0.250	0.2	0.2
2.0 to 6.3	#10	2.1	2.0
0.850 to 2.0	#20	7.0	4.9
0.425 to 0.850	#40	15.9	8.8
0.250 to 0.425	#60	29.3	13.4
0.180 to 0.250	#80	47.6	18.3
0.150 to 0.180	#100	71.1	23.5
< 150	< #100	100.0	28.9
		SUM	100.0

Analyst: Frank Shields

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Date Received:	31 Jan. 12
Sample Id.:	Basques biochar
Sample id. Number	4 2010666

International BioChar Initiative (IBI) Level II

			Results Wet Basis	Dry Basis	Maximum Allowed	Units	Method
Arsenic	(As)		3.4	1.2	13	mg/kg	B
Cadmium	(Cd)		4.6	1.6	1.4	mg/kg	A
Chromium	(Cr)		9.2	3.1	93	mg/kg	A
Cobalt	(Co)		1.6	0.56	34	mg/kg	B
Copper	(Cu)		26	8.9	143	mg/kg	A
Lead	(Pb)		14	4.6	121	mg/kg	A
Molybdenur	(Mo)		< 0.1	< 0.1	5.0	mg/kg	B
Mercury	(Hg)		< 0.1	< 0.1	1.0	mg/kg	A
Nickel	(Ni)		12	4.2	47	mg/kg	A
Selenium	(Se)		< 0.1	< 0.1	2.0	mg/kg	B
Zinc	(Zn)		461	157	416	mg/kg	A
Boron	(B)		87	30	Declaration	mg/kg	TMECC
Chlorine	(Cl)		21	62	Declaration	mg/kg	TMECC
Sodium	(Na)		429	146	Declaration	mg/kg	TMECC
Basic Soil Enhancement Properties							
Potassium	(K)	Total and available	9640	3279	Declaration	percent	E
Phosphorus	(P)	Total	1763	600	Declaration	percent	E
Ammonia	(NH4-N)	Mineral	56	165	Declaration	mg/kg	R
Nitrate	(NO3-N)	Mineral	2.5	7.4	Declaration	mg/kg	R
Phosphorus	(P)	Available	11.9	34.9	Declaration	mg/kg	F
Electrical Conductivity		EC 1:5 w/w	1.800	NA	Declaration	S/m	R
Moisture			66.0	0.0	Declaration	percent	TMECC
pH value			7.77	NA	Declaration	units	TMECC

Methods

Method A	Amlinger, Faroino and Pollack (2004)	Method E	Enders and Lehmann (2011)
Method B	Bureau de normalisation du Quebec (2005)	Method R	Rayment and Higginson (1992)
TMECC	Test Methods for the Examination of Compost and Composting (2001)	Method F	Formic Acid procedure

Analyst: Frank Shields

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BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received:	31 Jan. 12
Sample Id.:	Basques biochar
Sample id. Number	4 2010666

Proximate Analysis

Sample Received DRY weight		as-Received WET weight	
Constituent	percent	Constituent	percent
Carbon (C)	67.1	Carbon (C)	22.8
Hydrogen (H)	3.7	Hydrogen (H)	1.3
Nitrogen (N)	0.51	Nitrogen (N)	0.17
Sulfur (S)	0.09	Sulfur (S)	0.03
Oxygen (O)	17.7	Oxygen (O)	6.02
Ash (total)	10.9	Ash (total)	3.69
Water (200c)	0.0	Water (200c)	66.0
Sum	100.0	Sum	100.0

Moisture Content 66.0 percent

Analyst: Frank Shields

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Watsonville CA 95076
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Account No.:
2010666 4 0
Batch
Nov. - Dec. 2011 20
CODE:
BioChar IBI

Barry Husk
BlueLeaf Inc.
310 Chapleau Street
Drummondville Quebec CANADA J2B 5E9

Date Received:	31 Jan. 12
Sample Id.:	BlueLeaf biochar
Sample id. Number	4 2010666

International BioChar Initiative (IBI) Level I

	Wet	Dry			
Moisture	8.9	0.0	percent	Declaration	ASTM D1762-84 (105c)
Total Ash	15.7	17.3	percent	50% Max	ASTM D 1762-84 (750c)
Organic Carbon	68.7	77.0	percent	Declaration	CHN by dry combustion
Inorganic Carbon	0.46	0.50	percent	Declaration	HCl treated
Hydrogen/Carbon (H:C)	0.35	0.34	molar ratio	0.7 Max	
Hydrogen	2.0	2.2	percent	Declaration	CHN by dry combustion
Total Nitrogen (N)	0.51	0.56	percent	Declaration	CHN by dry combustion
pH value	9.35	NA	units	Declaration	Ahmedna et al (2002) (1997)
Liming (neut. value)	11.5	12.6	% CaCO3	Declaration	Rayment & Higginson
Liming (carbonate value)	3.8	4.2	% CaCO3	Declaration	ASTM D 4373
Activity Iodine or Butane)	8.7	9.6	g/100 g	Declaration	ASTM D 5742 (butane)

Particle Size Distribution (ASTM D5158 powdered) or (ASTM D2862 granular)

MM	Inch or Mesh #	% Retained	Fraction %
> 19	0.75	0.0	0.0
16 to 19	0.625	13.2	13.2
9.5 to 16	0.375	20.1	6.9
6.3 to 9.5	0.250	38.5	18.4
2.0 to 6.3	#10	52.4	13.9
0.850 to 2.0	#20	74.4	22.0
0.425 to 0.850	#40	83.8	9.4
0.250 to 0.425	#60	87.7	3.9
0.180 to 0.250	#80	92.6	4.9
0.150 to 0.180	#100	97.1	4.4
< 150	< #100	100.0	2.9
		SUM	100.0

Analyst: Frank Shields

A Division of Control Laboratories Inc.

ANALYTICAL CHEMISTS
and
BACTERIOLOGISTS
Approved by State of California

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International BioChar Initiative (IBI) Level II

			Results Wet Basis	Dry Basis	Maximum Allowed	Units	Method
Arsenic	(As)		0.77	0.70	13	mg/kg	B
Cadmium	(Cd)		1.6	1.5	1.4	mg/kg	A
Chromium	(Cr)		8.1	7.4	93	mg/kg	A
Cobalt	(Co)		0.48	0.43	34	mg/kg	B
Copper	(Cu)		12	11	143	mg/kg	A
Lead	(Pb)		3.7	3.4	121	mg/kg	A
Molybdenur	(Mo)		< 0.1	< 0.1	5.0	mg/kg	B
Mercury	(Hg)		< 0.1	< 0.1	1.0	mg/kg	A
Nickel	(Ni)		3.0	2.7	47	mg/kg	A
Selenium	(Se)		< 0.1	< 0.1	2.0	mg/kg	B
Zinc	(Zn)		105	95	416	mg/kg	A
Boron	(B)		28	25	Declaration	mg/kg	TMECC
Chlorine	(Cl)		16	18	Declaration	mg/kg	TMECC
Sodium	(Na)		126	115	Declaration	mg/kg	TMECC
Basic Soil Enhancement Properties							
Potassium	(K)	Total and available	4937	4496	Declaration	percent	E
Phosphorus	(P)	Total	901	820	Declaration	percent	E
Ammonia	(NH4-N)	Mineral	12	13	Declaration	mg/kg	R
Nitrate	(NO3-N)	Mineral	4.8	5.2	Declaration	mg/kg	R
Phosphorus	(P)	Available	11.9	13.0	Declaration	mg/kg	F
Electrical Conductivity		EC 1:5 w/w	3.560	NA	Declaration	S/m	R
Moisture			8.9	0.0	Declaration	percent	TMECC
pH value			9.35	NA	Declaration	units	TMECC

Methods

Method A	Amlinger, Faroino and Pollack (2004)
Method B	Bureau de normalisation du Quebec (2005)
TMECC	Test Methods for the Examination of Compost and Composting (2001)
Method E	Enders and Lehmann (2011)
Method R	Rayment and Higginson (1992)
Method F	Formic Acid procedure

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Proximate Analysis

Sample Received DRY weight		as-Received WET weight	
Constituent	percent	Constituent	percent
Carbon (C)	77.0	Carbon (C)	70.1
Hydrogen (H)	2.2	Hydrogen (H)	2.0
Nitrogen (N)	0.56	Nitrogen (N)	0.51
Sulfur (S)	0.02	Sulfur (S)	0.02
Oxygen (O)	3.0	Oxygen (O)	2.74
Ash (total)	17.3	Ash (total)	15.71
Water (200c)	0.0	Water (200c)	8.9
Sum	100.0	Sum	100.0

Moisture Content 8.9 percent

Analyst: Frank Shields

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Appendix C: Sample calculations to correct for P added from biochar particles in each aggregate fraction.

Sample calculation:

$$C_{biochar}\ (g\ kg^{-1}) = C\ in\ treatment - C\ in\ control$$

$$C_{dynamotive} = (78.5\ \pm 6.48) - (69.4\ \pm 20.4)$$

$$C_{dynamotive} = 9.1\ \pm 26.9\ g\ C\ kg^{-1}$$

$$= 0.0091\ \pm 0.0269\ kg\ C\ kg^{-1}$$

Dynamotive has 300 mg P kg^{-1}

$$P_{biochar}\ (mg) = C_{biochar}\ (kg\ C\ kg^{-1}) \times P\ (mg\ P\ kg^{-1})$$

$$P_{biochar} = (0.0091\ \pm 0.0269) \times 300$$

$$P_{biochar} = 0.273\ \pm 0.807\ mg$$

$$Reported\ values = P\ from\ digestion - P_{biochar}$$