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Examining the Effects of the Runoff Originating From Biosolids Amended Soil Plots on the Biogeochemical Nitrogen Cycle and Eutrophication

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EXAMINING THE EFFECTS OF THE RUNOFF ORIGINATING FROM BIOSOLIDS AMENDED SOIL PLOTS ON THE BIOGEOCHEMICAL NITROGEN CYCLE AND EUTROPHICATION

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ABSTRACT

EXAMINING THE EFFECTS OF RUNOFF ORIGINATING FROM BIOSOLIDS AMENDED SOIL PLOTS ON THE BIOGEOCHEMICAL NITROGEN CYCLE AND EUTROPHICATION

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One of the disposal methods for biosolids (nutrient rich organic matter that settles out of the wastewater during wastewater treatment process) is through application on agricultural fields as organic fertilizer. In order to determine the effects of runoff originating from biosolids treated fields on the nitrogen biogeochemical cycle and eutrophication of surface water, a lab-scale mesocosm experiment was carried out, simulating agricultural fields and thermally stratified water systems receiving agricultural runoff.

A significant difference was found between the effects of the runoff from unfertilized soil plots and plots fertilized with biosolids. The findings indicate that the majority of incoming nitrogen is either denitrified, lost to the sediment or is accumulated in the water column as nitrate. Further, it is hypothesised that the majority of incoming organic nitrogen was rapidly mineralized to ammonium in the hypolimnion, which has the potential to increase nitrogen bioavailability to primary producers in the epilimnion.

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LIST OF SYMBOLS AND ABBREVIATIONS

ANAMMOX	anaerobic ammonia oxidation
AOA	ammonia Oxidizing Archaea
AOB	ammonia Oxidizing Bacteria
CCME	Canadian Council of Ministers of the Environment
CO_2	carbon dioxide
DNRA	dissimilatory nitrate reduction to ammonium
DO	Dissolved oxygen
GC-ECD	gas chromatography coupled with an electron capture detector
KN	Kjeldahl Nitrogen
N	nitrogen
N_2	nitrogen gas
N_2O	nitrous oxide
NH_3	ammonia
NH_4^+	ammonium
NO_2^-	nitrite
NO_3^-	nitrate
OMAFRA	Ontario Ministry of Agriculture, Foods and Rural Affairs
P	phosphorus
TN	total nitrogen
TP	total phosphorus
USEPA	United States Environmental Protection Agency
UV-VIS	ultra violet/visible
WWTP	wastewater treatment plant

1. INTRODUCTION

Over seventy percent of the surface of our planet is covered in water. Ninety seven percent of the water is stored in the oceans. Glaciers and polar ice caps contain around 2.5%, 1.5% is stored as groundwater and less than 0.01% is found in the vapour form (Wright, 2008). Only 1.3% of the freshwater supply is found on the surface of the planet, in swamps, rivers and lakes (Shiklomanov, 1993).

Canada has a large number of lakes, with over 30,000 that have a surface area of 3 km^2 or more and 561 with surface area greater than 100 km^2 (Natural Resources Canada, 2010). The Great Lakes are Canada's most important water resource and the largest system of surface water on the planet, containing roughly 20% of the planet's freshwater supply (US EPA, 2011). Freshwater lake (lentic) systems represent a valuable water reserve that must be utilized in an environmentally sustainable manner.

In many regions in Canada, the task of wastewater treatment is allocated to the municipalities. Before municipal wastewater is returned to the environment, it is treated in a wastewater treatment plant (WWTP), where it undergoes a series of physical, chemical and biological treatments. One of the by-products of this treatment is sewage sludge.

There are inherent issues associated with the disposal of sewage sludge. One of the disposal options is to convert it (using stabilization procedures) into biosolids: nutrient rich organic materials which can be applied to agricultural fields as organic fertilizer. A major concern associated with the agricultural use of biosolids is that during heavy rain events the nutrient constituents can become washed off in the runoff and enter the nearby water systems. Once in the receiving water body, the runoff can cause nutrient pollution and eutrophication.

The overall quality of aquatic ecosystems can deteriorate as a response to nutrient pollution. Elevated concentration of nutrients in the watershed can cause an increase in the levels of primary productivity and changes in the community composition. Further, the quantity and relative proportion of limiting nutrients in the water can play a role in overproduction of photosynthetic algae, cyanobacteria and diatoms, shifts towards phytoplankton species which are inedible or toxic, and changes in plant distribution and growth (Smith, Tilman et al., 1998; Elser et al., 2000; Anderson et al., 2008). A number of studies confirmed that the most important

nutrients controlling the abundance and composition of phytoplankton in aquatic ecosystems are nitrogen (N) and phosphorus (P) (Schindler, 1977; Carpenter, 1996; Schindler, 2008).

Anthropogenic actions such as burning of fossil fuels and fertilizer use, contribute a significant amount of various reactive forms of N and P to terrestrial and aquatic systems (Vitousek, 1997). Since biosolids are rich in N and P there is a strong possibility that the runoff originating from the biosolids treated fields can also have an impact on the amount and proportion of nutrients in the receiving water system.

Currently, there is a shortage of scientific studies that examine the effect of biosolids runoff on eutrophication of waterbodies and biogeochemical cycles. As agricultural biosolids application is one of the disposal methods available to municipalities, there is an increasing need to study the possible impacts of biosolids runoff on various terrestrial and aquatic processes. The overall objective of this study was to determine the potential for biosolids to disrupt ecosystem function and nitrogen cycling. This study simulates a worst case scenario for biosolids runoff to aquatic systems. Biosolids were applied to soil lacking a vegetation buffer zone, at an application rate of 8 Mg ha^{-1}, and the maximum allowable agricultural soil slope in Ontario. Further, rainfall one week after application of biosolids and on subsequent runoff collection days mimicked 1 in 100 year rain storm events and runoff entering freshwater mesocosms contained 10% (by volume) water from soil fertilized with biosolids. This represents the extreme upper end of the spectrum for environmental relevance. Finally, the lake mesocosms lacked complex food-web interactions, including zooplankton grazers that might have dampened the response of algae to nutrients in biosolids runoff.

1.1 Problem Description

1.1.1 Biosolids

Stabilized sewage sludge (or biosolids) is a nutrient rich slurry originating from the primary and secondary treatments in the WWTP. There are multiple definitions of biosolids, but for purposes of this thesis I will use the definition used by the Canadian Council of Ministers of the Environment (CCME) (2010). Biosolids are defined as:

"Organic product obtained from the physico-chemical and/or biological treatment of wastewater. Biosolids result from primary wastewater treatment (primary biosolids), or from secondary wastewater treatment (secondary biosolids), and these two types of biosolids are often combined (mixed biosolids). These biosolids can be derived from the treatment of either municipal wastewater or industrial wastewater."

In order to understand the potential benefits and risks associated with agricultural use of biosolids, it is important to know how biosolids are produced. An overview of a typical water purification process is outlined in the following section.

1.1.2 Wastewater Treatment

In North America municipal wastewater from residential, industrial and commercial sources is treated in the Wastewater Treatment Plants before it is released back into the receiving water system. The wastewater treatment process can differ depending on municipality, but usually consists of four steps: preliminary, primary, secondary and, in some environmentally sensitive areas, tertiary treatments.

Preliminary treatment is the first step in the process and involves the removal of large particles and debris from the wastewater. In this step, raw sewage passes through large screens designed to trap larger objects and separate them from the liquid sewage.

Primary treatment consists of a series of large tanks called primary clarifiers. The water flow in these tanks is slow, which allows the organic matter to settle at the bottom. Fat and oily materials float to the top at which point they are removed. The treatment is essentially a large bucket that allows the solid material to settle at which point the overlying water is "poured off". This treatment is quite simple, but very cost-effective way of separating close to 50% of organic

matter out of the sewage water and reducing 20-30% of the biological oxygen demand from the water (Prescott et al., 1996; Wright, 2008). The settled solid material which is left behind makes up a portion of raw sludge.

Secondary treatment utilizes microorganisms (such as decomposers and detritus feeders) in order to reduce the levels of organic matter and nutrients (Wright, 2008). The process is carried out in large storage tanks with oxygen added to the slurry (using a trickling filter or activated sludge systems) in order to enhance the growth and respiration rates of aerobic microorganisms within. This process also reduces the numbers of bacterial pathogens in the water as many of them are unable to tolerate oxygen rich conditions (Prescott et al., 1996). After secondary treatment the waste activated sludge, containing bacterial biomass and the remaining organic chemicals sinks to the bottom of the tanks and is removed for further processing.

Tertiary treatment is a process which reduces the nutrient content of sewage water that has been subjected to primary and secondary treatments. The process is especially important in reducing the levels of nitrogen (by promoting denitrification) and phosphorus as well as heavy metals and persistent organic pollutants (Prescott et. al., 1996; Wright, 2008). One alternative to using live organisms to remove phosphorus is through the use of filter of lime during the secondary treatment or treating the sludge with ferric chloride. The former causes the phosphate to precipitate as calcium phosphate, while the latter produces water insoluble ferric phosphate (Schönberger, 1990; Wright, 2008). The precipitate is then removed by filtration or settling out. Phosphorus precipitation is of most importance when treated water is being discharged into the aquatic habitats that are P-limiting (such as many freshwater lakes in Canada) (Carpenter, 1998).

Particulate organic matter that settles during the primary and secondary treatments is removed and at this point is referred to as raw sludge. Raw sludge is considered to be a biologically hazardous material because it contains pathogens and toxic contaminants (Wright, 2008). Before raw sewage sludge can be disposed of, it has to be treated (stabilized), in order to reduce the levels of organic matter, water content and pathogenic bacteria.

1.1.3 Biosolids Production Methods

There are multiple biosolids production methods that effectively reduce the levels of contaminants in order to meet the regulatory guidelines for agricultural application (Oleszkiewicz & Mavinic, 2001). This section will present the most relevant processes to this discussion: anaerobic digestion, aerobic digestion and alkaline stabilization.

1.1.3.1 Anaerobic Digestion

Anaerobic digestion is the preferred method of biosolids production for many WWTPs. This process is carried out in large airtight tanks which contain raw sludge and a consortium of detritus feeding organisms (such as anaerobic bacteria). These microorganisms break down organic matter and other organic molecules in the slurry while at the same time increasing the solids content of the sludge (Oleszkiewicz & Mavinic, 2001). The process is usually carried out at mesophilic temperatures (36^{o}C), which increases the efficiency of organic matter breakdown.

In general, anaerobic digestion process can be divided into two steps: hydrogen and acetate production from hydrolysed organic substances and methane generation produced from acetic acid and hydrogen (Oleszkiewicz & Mavinic, 2001). The end result of this process is biogas with high proportion of methane and carbon dioxide (typically 60% methane and 40% carbon dioxide) (Wang et al., 2008) and liquid slurry. In some WWTPs methane which is produced in the tanks is used for power generation (Rickerson, 2006).

In the past anaerobic digestion could take up to six weeks to complete (Wright, 2008), however when anaerobic digestion is combined with additional treatments, such as application of a thermophilic aerobic treatment (at 56^{o}C) and hydroxide hydrolysis, the production time can be reduced to around 30 days (Oleszkiewicz & Mavinic, 2001). In addition, combining anaerobic digestion with aerobic treatment or pasteurization (at 70^{o}C for 30 minutes) is an effective way of lowering the counts of many pathogenic bacteria and viruses (Mavinic et al., 1995; Oleszkiewicz & Mavinic, 2001).

1.1.3.2 Aerobic digestion

Aerobic digestion is a biological process that uses bacteria to break down the organic matter in raw sludge in the presence of oxygen. Aerobic oxidation process can cause an increase in the

temperature in the tanks, which results in thermal processing of the sludge as well which is an effective way of decreasing the counts of pathogenic organisms (Layden, 2007). Typical suggested temperature for the process is 55 °C for 20 hours (Piterina et al., 2010). At the end of the process, a major portion of organic matter is oxidized to carbon dioxide (which can reduce the mass and volume of sludge). The main advantage of using this technology is that it allows for short sludge residence time in the tanks and fast degradation rate (Kelly & Mavinic, 1993).

One of the shortcomings of this technology is that aerobic digestion requires an input of energy in order to increase the oxygen flow into the system, which can increase the operating costs of the plant using this type of biosolids production. As a result this technology is usually used by the medium and small-sized WWTPs (Liu et al., 2010) which typically have a low wastewater input and where the use of anaerobic digestion is not economically viable.

1.1.3.3 Alkaline Stabilization

Alkaline stabilization is a biosolids production process that involves an addition of alkaline substances (i.e. CaO or KOH) which raises the pH of the slurry and since the reaction is exothermic, produces heat within the reaction tanks (Krach et al., 2008). The lowest requirement for alkali stabilization is a pH of 12 for 2 hours. In many cases the higher quality of biosolids (in terms of pathogen and organic matter content) is achieved only when the pH of the mixture is maintained at or above 12 for at least 72 hours, with temperatures of 52 ^{o}C for the first 12 hours (Spellman, 1997). The increased pH and heat production effectively kill harmful bacteria in the slurry (Farrell, 1974) and reduce the production of offensive odours.

The use of alkali stabilization is considered to be a cost-effective and time saving process when compared to alternative biosolids production methods such as composting and anaerobic digestion. One of the major drawbacks of alkali stabilization is that if the chemicals are not properly mixed with the sewage sludge, the pH can later decrease allowing microorganisms to repopulate the treated sludge. In addition, since the end product can have a high pH, soil testing has to be carried out in order to make sure that the pH of the soil does not increase beyond normal alkalinity post fertilization. High pH in the soil can increase solubility (Ni and Cu) and oxidation states (Cr) of heavy metals, which can affect their fate in the soil and toxicity (Ščančar et al., 2001; Yang et al., 2008).

1.1.4 Disposal

Biosolids produced by the WWTPs can vary in their chemical composition depending on the differences in stabilization processes, spatial variability between plants as well as the nature of sewer systems (combined sewers versus separate storm water and wastewater sewers). The composition can vary even if production procedures are similar due to the differences in wastewater sources and chemical composition on a particular day. In order to assess the impacts of biosolids on nitrogen cycling and eutrophication in aquatic systems, it is necessary to understand the nature of these materials. The constituents of the biosolids include essential nutrients; however they can also be rich in many potentially hazardous chemical substances such as heavy metals, pharmaceuticals, organic pollutants and pathogens. These materials can have important individual and cumulative impacts on receiving waters and biogeochemical cycles.

In the past, municipalities in Canada and the US disposed of raw sludge by releasing it into the nearby water body. Because disposal of sewage in this way can have persistent impacts on the receiving water (such as potential to cause an increase in toxins and pathogens) (Bothner et al., 1994), this practice is prohibited in the United States and is being phased out in Canada.

As of 2001, approximately 388,700 dry tonnes of biosolids are produced in Canada every year (CH2MHill Canada, 2001). As human population grows there is an increasing pressure on the WWTP to find viable disposal routes for biosolids. Disposal options can be quite expensive and account for as much as 50% of the overall cost associated with operation of a WWTP (Spinosa & Vesilind, 2001). As of 2001, around 43% of biosolids are being land applied, while 47% are incinerated and 4% are landfilled. The remaining biosolids are used for alternative purposes such as land reclamation (Apedaile, 2001).

1.1.4.1 Burial in a Landfill

One of the most widespread ways of biosolids disposal is through burial in landfills. This option is viable if the land space is available and the quantity of biosolids is relatively low. However in many cases in the US and Canada the practice of landfilling biosolids is being reduced because of increasing compliance costs, public opposition, leachate production and greenhouse gas emissions (USEPA, 1994; Wang et al., 2008). The two most common alternatives to placing biosolids in a landfill are incineration and land application.

1.1.4.2 Incineration

Incineration is considered a viable option for highly populated municipalities and areas where land application is difficult. The two technologies that are most widely used in the industry are fluidized bed combustion and multiple-hearth furnaces (Werther & Ogada, 1999). Since biosolids are high in moisture content, they are usually burned with a starter fuel (such as coal or gasoline), in order to sustain the combustion process.

Incineration reduces the volume of biosolids by evaporating the liquid content and oxidizing organic matter to CO_2. The physical by-product of the process is dry ash, which can be stored in a landfill or "ash lagoons" (ash storage ponds). In some cases biosolids ash can also be used in construction industry (such as during concrete production) (Tay & Show, 1997; Werther & Ogada, 1999).

Incineration of biosolids is less costly than storage of raw biosolids in a landfill, and there is a potential for power generation (Brown et al., 2003; Lundberg, 2008). However, there are a number of issues that can make incineration procedure a less appealing alternative. Incineration of biosolids produces greenhouse gas emissions (i.e. CO_2) and promotes volatilization of toxins (i.e. dioxins and furans) and heavy metals (i.e. Cu, Hg) (Barbosa et al., 2009; Wang et al., 2008). Disposal and storage of the leftover ash can also be an important consideration issue. Biosolids ash can contain high levels of toxins, which makes the storage option costly and potentially environmentally hazardous (Werther & Ogada, 1999).

1.1.4.3 Agricultural Application

Because biosolids have been shown to improve the physical properties and nutrient composition of soils, promote plant growth and provide a sustainable way of recycling nutrients (Logan & Harrison, 1995; Meyer et al., 2001; Rostagno & Sosebee, 2001), land application is seen as an attractive alternative when compared to other disposal methods (Wang et. al., 2008; Vasseur et al., 2000). In addition, land application may be more appealing to some municipalities because of lower costs associated with this practice, when compared to landfilling or incineration (Vasseur et al., 2000). In Canada, agricultural application of biosolids has been carried out for over 40 years (City of Toronto, 2010). The safety and environmental impact of this disposal

option however, continues to be a subject of some debate because of the nature of chemical constituents found in biosolids.

1.1.5 Constituents

In general, the exact composition of biosolids can vary depending on the WWTP. However, there are a number of substances which are usually present in biosolids regardless of location and production methods. Some constituents such as nitrogen, phosphorus and potassium make them ideal for agricultural use. However, since sewage water entering the WWTPs can originate from residential, industrial and medical facilities as well as surface (i.e. street) runoff, biosolids produced from this wastewater can have elevated levels of contaminants, such as heavy metals (Singh & Agrawal, 2008), organic pollutants (Harrison et al., 2006), pharmaceuticals and personal care products (Xia et al., 2005) and pathogens (Lewis & Gattie, 2002).

1.1.5.1 Nutrients

Nutrients which are commonly found in biosolids include nitrogen, phosphorus, potassium and trace elements such as calcium, copper, iron, magnesium, manganese, sulphur and zinc (Meyer et al., 2001; Rostagno & Sosebee, 2001; CWWA, 2003). This section will outline the three most important nutrients which are required for plant growth and development: nitrogen, phosphorus and potassium.

1.1.5.1.1 Nitrogen

Nitrogen is an important component of all living cells, because it contributes to the structural makeup of nucleotides and amino acids. As a result, it composes a major portion of plant and animal biomass and is a component of animal and human waste (i.e. urea).

Biosolids can contain high levels of nitrogen, in some cases comprising 2-6 % of biosolids (Sommers, 1977). Since agricultural application of biosolids is usually based on the plant nitrogen requirements, current production methods aim to increase the concentration of this element relative to all other constituents (Sommers, 1977; Wang et al., 2004). The two major nitrogen forms found in biosolids are organic nitrogen (i.e. urea) and ammonium. Ammonium can be rapidly incorporated into plant biomass, while organic nitrogen has to first be mineralized by bacteria to ammonium in the soil during decomposition. As a result biosolids production

methods also aim to increase the content of ammonium relative to organic nitrogen in biosolids which are used for agricultural application.

Apart from the beneficial effects in agriculture, nitrogen can have a number of negative effects on the receiving ecosystem. If the reactive nitrogen species escape through volatilization or runoff they can have negative effects on the nearby water systems. Elevated levels of ammonia in the aquatic systems can be toxic to fish and aquatic invertebrates and promote production of harmful algal blooms (USEPA, 1990). Drinking water that contains elevated levels of nitrate can be dangerous to human health. High dietary nitrate intake has been shown to cause methemoglobinemia (in newborns and the elderly) and increase the risk of developing some types of cancers (Weyer et al., 2001).

1.1.5.1.2 Phosphorus

Phosphorus is the tenth most abundant element in the Earth's crust and is present in many naturally occurring minerals. It is also an essential element in living cells, where it is a structural component of proteins, nucleic acids, phospholipid bilayers and adenosine triphosphate (ATP), (Ingall et al., 2010). ATP is an energy transfer molecule used for metabolic processes within cells of all living organisms (Knowles, 1980).

Compared to nitrogen the concentrations of phosphorus in biosolids are relatively high (N:P ratio as high as 1.5:1). Since the nutritional needs of plants are higher for nitrogen than phosphorus, application of biosolids based on the nitrogen requirements alone can cause phosphorus overfertilization (Gove et al., 2002). Phosphorus which is not used by plants can be retained in the soil (by incorporation in bacterial biomass and formation of insoluble compounds) or lost from the system in agricultural runoff.

The concentration of phosphorus in the runoff has been shown to fluctuate according to the chemical composition and pH of soils. For example, in alkaline soils (pH>7.3) phosphorus can react with calcium to form calcium phosphate dihydrate, octocalcium phosphate, and hydroxyapatite which are no longer bioavailable to plants and microorganisms (Westermann, 1992). In acidic soils (pH<5) phosphorus reacts with aluminum and iron compounds, producing crystalline forms which are insoluble in water.

Since phosphorus is the primary limiting nutrient in the freshwater aquatic systems (Schindler, 1978; Correll, 1999; Carpenter, 1998), the concentrations of this nutrient must be taken into account before biosolids application. Soil P testing is usually carried out in order to reduce the risk of overfertilization and P losses in the runoff.

1.1.5.1.3 Potassium

Potassium (K) is one of the essential nutrients required for proper functioning of all living cell. Plant cells contain high concentrations of potassium, where it plays a role in plant growth and protein synthesis (Leigh, et al., 1984). It is also important in photosynthesis, osmoregulation and proper functioning of many enzymes within plant cells (Amtmann et al., 2008).

Many soils around the world are deficient in potassium (Römheld & Kirkby, 2010), with highest concern given to sandy soils, which are prone to K leaching. In addition, agricultural practices are responsible for depleting K concentrations in the soil, since potassium which is stored in plant phytomass is commonly collected during the harvest of crops. The input of K through fertilizer use can be lower than the K losses out of the soil. Low potassium levels in the soil can therefore have an impact on soil fertility and crop yield.

Biosolids can contain a high percentage of biologically available potassium (Wen et al., 1997), however, the overall quantity of potassium relative to nitrogen in biosolids is relatively low (Singh & Agrawal, 2008) and does not usually satisfy the overall plant requirements for this element. Supplemental fertilization of potassium is sometimes suggested after long term biosolids application in some agricultural areas (Miah et al., 1999).

1.1.5.2 Heavy Metals

When present in low concentrations, heavy metals can be considered trace elements that are necessary for plant growth and development. However, when the levels of these chemicals are elevated they can act as toxins (Qi., 2011), disrupting the biochemical processes within plants and impacting growth and development. In addition, since heavy metals can be stored in plant tissues, they can potentially accumulate in the food-web (Sikora et al., 1980; McLaughlin et al., 1999). The rates of biosolids application can have an effect on the accumulation of heavy metals in the tissues of plants (Chang et al., 1984).

Heavy metals of concern are arsenic, cadmium, chromium, copper, lead, mercury, nickel and zinc (Qi., 2011), because they pose the greatest human and animal health risks. Exposure to heavy metals has been associated with various types of cancers, kidney damage as well as development of autoimmune disorders. In addition, since heavy metals have been shown to affect the microbial processes within the soil by inhibiting growth of some species of bacteria (Baath, 1989; Giller, 1999), they may potentially have an effect on the bacterial species involved in the biogeochemical cycling (Hu et al., 2003).

The concentration of heavy metals in biosolids is dependent on solubility and chemical fractionation of heavy metals in sewage. For example, copper and chromium have been shown to attach to organic matter and sulphides. Hydroxides and iron oxides can be carrier of Pb, Zn and Ni (Angelidis & Gibbs, 1989; Ščancar et al., 2000). The concentration of the heavy metals in biosolids can also depend on whether they precipitate with the organic matter during the primary and secondary processes. Karvelas et al., (2003) found that more than 70% of Mn and Cu accumulated in the sludge, while 47–63% of Cd, Cr, Pb, Fe, Ni and Zn remained in the treated effluent during the wastewater treatment.

Some heavy metals are able to leach from the soil in agricultural runoff and make their way into a nearby water body. Antonious et al., (2011) found that concentrations for all heavy metals tested (Cd, Cr, Ni, Pb, Zn, Cu and Mo) in runoff water collected from plots treated with biosolids were higher than the maximum allowed concentrations set up by the USEPA. However, once in the major water body, the runoff is expected to be diluted to the point where these concentrations are assumed to no longer pose a health concern (Antonious et al., 2011).

1.1.5.3 Organic Pollutants

Biosolids produced from wastewater can have elevated levels of persistent organic pollutants (POPs) such as polychlorinated biphenyls (PCBs), pesticides and polycyclic aromatic hydrocarbons (PAHs) (Harrison et al., 2006). Many of these pollutants are hydrophobic, so their removal from the wastewater is carried out through adsorption to the structural matrix of sewage sludge.

Once in the environment persistent organic pollutants can travel over large distances and bioaccumulate and magnify in the food-web. The most well documented effects have been on the

marine mammals and birds (Prest et al., 1970). Human health effects associated with ingestion of some POPs includes disruption of endocrine and immune systems. Chronic exposure to these chemicals has been linked to some types of cancers (Ritter, et al., 1995).

The exact values of organic pollutants in the biosolids can depend on the production methods as well as the sources of water (Rogers, 1996). However, studies estimate that the levels of PCBs can be as high as 1.7 mg/kg (dioxins and furans), chlorobenzenes up to 184 mg/kg (trichlorobenzene), and pesticides at levels of 564 mg kg^{-1} (DDT) (Harrison et al., 2006).

Since testing of biosolids for organic pollutants is currently not carried out in all countries and provinces there is an uncertainty associated with the amount of these chemicals making their way into the environment after land application of biosolids. Further, since POPs can accumulate in the environment, long term effects of repeat biosolids soil application on the pool of POPs is unknown.

1.1.5.4 Pharmaceuticals and Personal Care Products

Pharmaceuticals and Personal Care Products (PPCPs) can be excreted into wastewater directly or in human waste as parent compounds, conjugated compounds or metabolites (Xia et al., 2005). Land application of biosolids is an important source of PPCPs entering the environment (Xia et al., 2005). When in the environment these substances can exhibit negative endocrine disruptive and toxic effects on organisms in concentrations as low as μg kg^{-1} (Daughton & Ternes, 1999; Wilson et al., 2003).

When undergoing wastewater treatment PPCPs can be oxidized completely to CO_2, become partially oxidized, or go through the treatment unchanged. It is estimated that a high percentage of PPCPs (30-90%) can be removed from wastewater through the treatment process (Xia et al., 2005). Some PPCPs (such as nonylphenols) however, are not effectively removed and can accumulate in biosolids.

A large portion of PPCPs that are left in the sewage sludge are broken down further during stabilization process. The length of time designated for biosolids production can vary, but in many cases the process takes up to a month to complete. This time frame however, is insufficient for decomposition of many PPCPs as their half-lives can exceed 30 days. In addition, some

PPCPs have the ability to become absorbed into the biosolids matrix, which makes their breakdown more challenging.

There is limited information regarding the presence of PPCPs in biosolids. Studies by Berset et al., (2000) and Difrancesco et al., (2004) have shown that the values of fragrances ranged between 1.5 to 147 µg kg^{-1} (dry mass) in biosolids from US, Netherlands and Switzerland. Biosolids from some US states have been shown to contain nonylphenol polyexthoxylates (detergents) and nonylphenols concentrations of 981 mg kg^{-1} (dry mass) and 1380 mg kg^{-1} (dry mass) respectively (La Guardia et al., 2001; Keller et al., 2003). De Boer et al. (2003) and Golet et al. (2002) found that fluoroquinone (antibacterial agent) concentrations ranged from 1.4 to 2.4 mg kg^{-1} (dry mass) in biosolids in Switzerland.

1.1.5.5 Pathogens

Since wastewater can contain human waste from a variety of sources (residential, industrial and medical health care institutions), sewage sludge, produced in a WWTPs can contain elevated levels of pathogenic bacteria (i.e. fecal coliforms) (O'Connor et al., 2005). Of particular concern are microorganisms such as bacteria (*Escherichia coli*, *Listeria* spp, and *Helicobacter pylori*) viruses (coxsackievirus, echovirus, hepatitis A, rotavirus, and norovirus), as well as intestinal parasites (such as C*ryptosporidium, Cyclospora, Toxoplasma*, *Microsporidia* and *Giardia)* (USEPA, 2000). These organisms have the potential to cause the greatest human harm and therefore must be reduced to acceptable levels during the biosolids production.

In general, biosolids are separated into categories according to the acceptable pathogen levels. These classifications can differ depending on the jurisdiction, however, they are usually separated into biosolids that have no agricultural application restrictions (Type A) or biosolids that are subject to some restrictions (Type B). Type A biosolids are held to a very high standard in terms of pathogen counts. For example, when tested, these biosolids must show that *Salmonella* sp. are not detectable or presence of fecal coliforms does not exceed 1000 CFU g^{-1} (O'Connor et al., 2005). Class B biosolids are not required to meet the same standards and therefore can have a higher pathogen content (i.e. up to 2 x 10^6 fecal coliforms per gram of solid) (O'Connor et al., 2005). Consequently, Class B biosolids are subject to a number of application restrictions. For example, they are not to be used for agricultural purposes in some provinces

(such as in Nova Scotia), while in the US depending on the land use, public access to Class B land application sites can be restricted for up to one year (Lewis & Gattie, 2002).

1.1.6 Regulation of Agricultural Use of Biosolids

Since land application of biosolids can potentially have an impact on the receiving ecosystems, their application is regulated by government policies. The regulations usually focus on two forms of contaminants: heavy metals (i.e. copper lead, nickel, zinc, cadmium) and pathogen counts (such as fecal coliforms) (Lewis & Gattie, 2002). At this time the regulation of organic pollutants is not carried out in every region. However since the levels of these contaminants can be elevated in the raw sewage sludge, there is an increasing pressure on the government to regulate the permissible levels of these contaminants in biosolids used as organic fertilizer (O'Connor et al., 2005).

In the United States, land application of biosolids is regulated by the federal government by the 40 CFR Part 503 Regulations "Standards for the Use and Disposal of Sewage Sludge" (USEPA, 1994). The 503 Rule allows for long-term application of biosolids to agricultural land, provided that the soil accumulation of ten traces elements in biosolids (arsenic, cadmium, chromium, copper, lead, mercury, molybdenum, nickel, selenium, and zinc) does not exceed the ceiling concentration limits, or cause environmental or health problems (USEPA, 1994; Sukkariyah et al., 2007).

In Canada, there are no specific federal regulations concerning the use of biosolids (Jacques Whitford 2004). The federal *Guidelines for Effluent Quality and Wastewater Treatment at Federal Establishments* (1976) prohibit such actions as disposal of treated sludge into the receiving waters. However, these regulations only apply to the wastewater systems under the jurisdiction of federal government. If biosolids are manufactured into a fertilizer the *Federal Fertilizers Act* requires different standards for labelling registration and product quality (Jacques Whitford, 2004). On the provincial level, every province uses a different classification of biosolids based on parameters such as trace metals, pathogen counts, separation requirements and organic chemical compounds. In addition, most provinces have specific regulations concerning the application procedures (such as soil testing before application, distance from wells and maximum application rates).

In Ontario, land application of biosolids is regulated by the *Nutrient Management Act* (2002) and the Ontario Ministry of Environment's Guidelines for Utilization of Biosolids and Other Wastes on Agricultural Land (Jacques Whitford, 2004). As of January 1, 2011, Ontario is changing the way it is categorising the "non-agricultural source materials" (NASM). The new framework categorizes NASM into three categories (1, 2 and 3). These are based on the material quality, with biosolids falling into "category 3 material". These are further subcategorised depending on their metal, pathogen and odou, which ultimately determines the application rates, distances from wells and residential areas. Biosolids are also categorized based on the acceptable pathogen levels into CP1 and CP2. CP1 pathogen count must not exceed the levels of E. coli of 1,000 colony forming units g^{-1} dry weight or 100ml, Salmonella counts must be < 3 CFU or Most Probable Number (MPN) 4 g^{-1} or 100 ml, and Viable Helminth ova or total culturable enteric virus < 1 organism per 4g or 100 ml. Category CP2 biosolids have to meet the E.coli < 2x106 CFU/g of total solids dry weight standard (CCME, 2010).

The quality standards apply for eleven inorganic elements (arsenic, cadmium, cobalt, chromium, copper, mercury, molybdenum, nickel, lead, selenium and zinc), and soil testing for nutrients and trace elements is also required before the application of biosolids can commence (CCME, 2010).

1.1.7 Public Perception

Even though animal waste (such as manure) has been used as organic fertilizer for centuries, it is unclear whether the chemicals of concern in biosolids have the ability to accumulate in the soil, bioaccumulate in crops or be released into environment as a result of agricultural application. There is also some concern that biosolids can pose a direct danger to human health post application. Multiple health issues such as symptoms of burning eyes, respiratory problems and skin rashes have been reported by residents living in the vicinity of biosolids land application (Lewis et al., 2001). However, at this time there is not enough scientific evidence to support these claims.

1.1.8 Scope of Work and Objectives

Increased population, limited landfill availability and public perception of incineration are all important factors that increase the pressure on land application as a disposal strategy for municipal biosolids. Public perception and risks associated with land application of biosolids is

based on the fact that the composition of biosolids is quite complex, with some constituents which are known to negatively affect the ecosystem. The regulatory framework for biosolids can feed this concern as it is based on quantification of a small number of possible constituents without any validation that these materials cause no adverse health or ecological effects. To date, little research has been conducted to determine the likely environmental effects of land application of municipal biosolids. Chemistry and Biology department at Ryerson University has been working with the Ministry of the Environment on determining these effects on land and aquatic ecosystems. In the current phase of the research program, we are hoping to determine the impact on receiving water, particularly the potential for biosolids to contribute to eutrophication and alteration of aquatic ecosystem processes. This thesis will contribute to the growing body of research on ecological effects of biosolids application.

Two types of biosolids production methods were examined in this study. The first source was received from the city of Kitchener (Ontario), wastewater treatment plant. These biosolids were produced using anaerobic digestion. The second source of biosolids tested was received from the city of Guelph (Ontario), wastewater treatment facility. These biosolids were produced using the Lystek® production method, which involves addition of potassium hydroxide to the sewage sludge, followed by heating and mixing in order to breakdown the biomass (Lystek, 2011). Both types of biosolids contain high levels of nitrogen species and are suitable for agricultural application.

Once applied to the field, the nitrogen species are released into the soil altering the pool of nitrogen species within. An input of nitrogen into the ecosystem can have a subsequent effect on the nitrogen cycle. An overview of the nitrogen cycle is presented in the following section.

1.2 Fate of Nitrogen in the Environment

1.2.1 The Nitrogen Cycle

Nitrogen biogeochemical cycle is a process that converts the different species of nitrogen between their chemical forms in the atmospheric, aquatic and terrestrial systems. Under natural conditions nitrogen species are present in gaseous, mineral, organic and inorganic forms. Nitrogen can exist in many oxidation states, which makes it an important reactive element. It can therefore be used by a variety of organisms for cell structure and energy needs. An overview of the nitrogen cycle is presented in Figure 1.

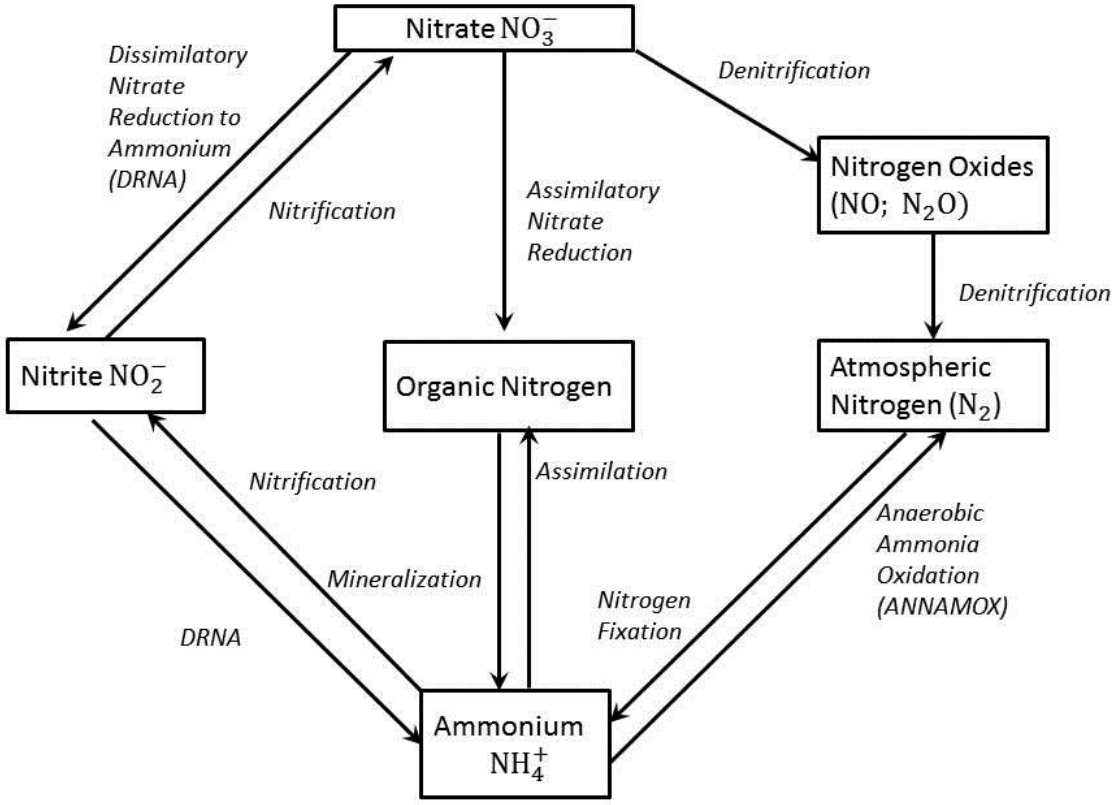

Figure 1. An overview of the nitrogen cycle in aquatic and terrestrial systems (modified from Painter, 1970).

1.2.1.1 Nitrogen in the Atmosphere

A major reservoir of molecular nitrogen is stored in the air as dinitrogen gas (N_2), where it makes up nearly 80% of all atmospheric gases. N_2 does not readily participate in chemical reactions and is considered to be a safe and relatively inert gas. The two nitrogen molecules are covalently bound by a triple bond, which is not easily broken under normal atmospheric conditions.

Other chemical species of nitrogen, found in the atmosphere include nitrous oxide (N_2O), nitrogen oxides (NO_x) and ammonia gas (NH_3). Nitrous oxide comprises almost 99% of the remaining atmospheric nitrogen species and contributes 0.0003% of the total atmospheric gases (Machefert et al., 2002). Unlike N_2, N_2O is a reactive greenhouse gas, which has been implicated in the stratospheric ozone layer depletion (Liikanen et al., 2002; Ravishankara et al., 2009).

The abundance of NO_x in the atmosphere has increased substantially due to anthropogenic actions such as burning of fossil fuels. These gasses can act as pollutant playing a role in formation of acid rain and increase in reactive nitrogen content of soil and water through dry and wet deposition (Logan, 1985).

Ammonia and ammonium ions are important components of the atmosphere. The major sources of this gas include burning of fossil fuels and volatilization after fertilizer application (Robertson &Vitousek, 2009). Ammonia is the third most abundant nitrogen gas after N_2 and N_2O, and is the only natural alkaline gas in the atmosphere (Schlesinger & Hartley, 1992). As a result, gaseous NH_3 can react with aerosols that contain sulphuric, hydrochloric or nitric acids (Asman et al, 1998) and plays a role in neutralization of acidity in the atmosphere (i.e. $2NH_3 + H_2SO_4 - (NH_4)_2SO_4$). NH_3 has an atmospheric residence time of around 10 days (Schlesinger & Hartley, 1992). It can then re-enter terrestrial and aquatic systems through dry and wet deposition.

1.2.1.2 Nitrogen fixation

Nitrogen fixation is a process which converts N_2 into more reactive forms such as NH_3, NO_x, and HNO_3. This process can happen naturally during lightning storms or through the metabolic activities of a small group of living organisms which are able to break the N_2 triple bond using nitrogenase enzyme. Nitrogenase contains a Mo-Fe active cite which can bind dinitrogen gas and reduce it to ammonia or ammonium ion (Howard & Rees, 1996). In the soil, diazotrophs (which

are usually associated with leguminous plants), such as bacteria of the genus *Rhizobium* and *Frankia* are able to convert N_2 into NH_3/ NH_4^+ (Igarashi & Seefeldt, 2003; Rees & Howard, 2000), which is immediately incorporated in the organisms' biomass. In aquatic ecosystems some species of *Cyanobacteria* are able to convert atmospheric nitrogen into NH_3/ NH_4^+, which is the major source of reactive nitrogen in some lakes, coastal and open ocean systems (Howarth et al., 1988; Vitousek, 1997; Zehr et al., 2001; Conley et al., 2009).

Anthropogenic actions play a significant role in the global balance of reactive nitrogen species. Industrial N-fixation (Haber-Bosch process) for fertilizer production, burning of fossil fuels, and agricultural manipulation (such as planting of clover and soybean crops in order to increase the nitrogen content of the soil) were instrumental in the increase in the abundance of reactive nitrogen and a shift in the equilibrium between nitrogen fixation and denitrification (Galloway et al., 1995; Howard & Rees, 1996; Smil, 1997).

1.2.1.3 Mineralization

Mineralisation is a biological process that converts organic nitrogen into inorganic forms (mainly NH_4^+) as a result of degradation or urea and catabolism of amino acids and nucleotides under aerobic or anaerobic conditions (Sahrawat, 2010). Mineralization is a part of decomposition process during which organic matter is oxidized to CO_2 by microorganisms. The nutrients that are released as a result of decomposition are incorporated into biomass of microorganisms (process of assimilation) while the remaining portion is mineralized (dissimilation) (Janssen, 1996). Mineralization of nitrogen therefore happens when the assimilated material contains nitrogen in the concentrations that are higher than the assimilatory needs of microorganisms (Hassink, 1994).

The process is carried out by a variety of organisms in both terrestrial and aquatic ecosystems and includes heterotrophic bacteria and fungi, as well as some species of phytoplankton (Van Breemen 1993; Vitousek et al., 1997). In some places such as the Baltic sea the organic matter mineralization can also be catabolized by solar-radiation induced photochemical reactions which can break down the dissolved organic matter in the water column to CO_2 and NH_4^+ (Vähätalo & Zepp, 2005).

The mineralization rates in the soil can depend on a number of factors including C:N ratio in the organic matter, temperature, activity of the microorganisms, as well as limiting nutrients (White & Reddy, 2000). In the aquatic sediments, factors such as the availability of organic matter, sediment redox potential, nutrient accumulation and burial rates are important in determining the rates of mineralization of organic nitrogen (Farías, 2003).

1.2.1.4 Nitrification

The process of nitrification is carried out by three groups of microorganisms: ammonia-oxidizing bacteria (AOB), ammonia-oxidizing archaea (AOA) and nitrite-oxidizing bacteria (NOB) in the soil and aquatic environments. Ammonia oxidizing bacteria include species of Beta-proteobacteria (such as *Nitrosospira* and *Nitrosomonas*) and Gamma-proteobacteria (such as *Nitrosococcus*), which are responsible for conversion of ammonia to nitrite in freshwater and marine environments respectively (Fortunato et al., 2009), using ammonia monooxygenase enzyme. Many archaea in the phylum *Crenarchaeota* are capable of performing ammonia oxidation similarly to AOB and are important in many marine environments (Könneke et al., 2005; Jin et al., 2011).

Conversion of nitrite to nitrate is primarily carried out by the NOB of the Alpha-proteobacteria subclass, genera: *Nitrobacter* and *Nitrospira* (Fortunato et. al., 2009). The process is catalyzed by the nitrite oxidoreductase enzyme (Risgaard-Petersen, 2003).

Nitrification process is carried out in two steps. In the first step ammonia is oxidized by AOB:

$$NH_4^+ + \frac{11}{2} O_2 \rightarrow NO_2^- + H_2O + 2H^+$$

In the second step NOB oxidize nitrite to nitrate:

$$NO_2^- + \frac{1}{2} O_2 \rightarrow NO_3^-$$

Nitrification usually takes place in the oxic layer of the sediment or within the top 15 cm of the soil and is influenced by a number of factors including: relative availability of NH_4^+, alkalinity (pH 8-8.5), light intensity and dissolved oxygen concentrations (Skadsen, 2002; Zhou, 2007).

1.2.1.5 Denitrification

Denitrification is a process which removes reactive nitrogen from the system by converting nitrate back to dinitrogen gas. The process is carried out by many species of bacteria, archea and fungi. Denitrifying bacteria include a diverse number of species including *Bacillus, Enterobacter*, *Micrococcus*, *Pseudomonas* and *Spirillum* (Zhou, 2007). Most of these species are facultative anaerobes that use denitrification as an alternative pathway to oxygen respiration (Zumft, 1997). The rates of denitrification can depend on multiple factors, but in general this process requires anoxic environments and a source of both organic matter and nitrate (Rabalais, 2002).

The overall denitrification reaction can be described as:

$2NO_3^- + 12\ H^+ + 10e^- \rightarrow N_2 + 6H_2O$

However, products which can form during the intermediate steps of denitrification are nitrite, nitric oxide and nitrous oxide.

Denitrification is an important environmental sink for reactive nitrogen. It is estimated that N-removal carried out by the watersheds globally can account for as much as 19.7 Tg N yr^{-1} and 5 Tg N yr^{-1} in estuaries (Seitzinger et al., 2006). In addition, up to 70% of reactive nitrogen is removed from lakes and rivers through denitrification (Seitzinger et al., 1985).

1.2.1.6 Anaerobic Ammonia Oxidation

Anaerobic ammonia oxidation (ANAMMOX) is a bacterial process which oxidizes ammonium and nitrite to dinitrogen gas under anoxic conditions (Strous et al., 1997). The most well studied species of bacteria that are capable of carrying out this process are of genus *Planctomyces* and *Pirellula*.

The overall ANAMMOX reaction is:

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

ANAMMOX activity is sensitive to increases in nitrite concentrations. The rate of reaction decreases when the concentrations of nitrite are greater than 0.1g L^{-1} (Strous et al., 1999). Aerobic conditions can also inhibit the reaction, instead favouring nitrification processes. An important feature of the anammox process is the slow growth rate of microorganisms, with a

doubling time of around 10 days, with reaction rates that are faster than the nitrification reaction carried out by *Nitrosomonas* (Jetten et al., 1998). In some marine environments, anaerobic ammonia oxidation is an important N removal process and can account for up to 60% of dinitrogen production (Thamdrup & Dalsgaard, 2003; Risgaard-Petersen et al., 2003).

1.2.1.7 Dissimilatory Nitrate Reduction to Ammonium

Dissimilatory nitrate reduction to ammonium (DNRA) is a process carried out by some heterotrophic bacteria in the terrestrial and aquatic systems. During this process NO_3 is converted back to NH_4^+ under anaerobic conditions (Brunet & Garcia-Gil, 1996; An & Gardner, 2002). The process is important in some environments because it increases the pool of bioavailable ammonia, which can further fuel primary production. It also favours nitrogen retention since it does not produce nitrogen gasses, which effectively remove the bioavailable nitrogen from the ecosystem (Scott et al., 2008).

Currently the mechanisms involved in DNRA are not well understood (Burgin & Hamilton, 2007), however it is favoured in conditions of high availability of organic matter, low availability of nitrate and relative abundance of reduced sulphur and iron species (King & Nedwell, 1985; Burgin & Hamilton, 2007).

1.2.2 Effect of Biosolids Application on the Nitrogen Cycle

Agricultural practices can have an important effect on the nitrogen cycle processes in the terrestrial and aquatic systems. An increase in the pool of bioavailable nitrogen as a result of fertilization can impact the rates of nitrification and denitrification in the soil and in the water systems receiving agricultural runoff. A presence of drainage network on the agricultural field can further affect the amount of nitrogen stored in the soil and in part determine the concentration of nitrogen in the runoff.

1.2.3 Fate of Biosolids Post Application

When used as organic fertilizer, biosolids can be surface applied or incorporated into the soil. In majority of cases, when they are applied to agricultural fields, biosolids are combined with the top 15-20 cm of the soil (Rostagno & Sosebee, 2001) before planting of crops. After biosolids are applied to the field the chemical composition of their constituents changes (Jaynes et al., 2003) as a result of multiple biological, chemical and physical processes within the soil (Gove et al., 2002). These processes ultimately determine whether the chemical substances that make up biosolids are retained in or removed from the field.

Living organism within the soil can be important in altering the composition of chemicals in the biosolids amended soils. As biosolids decompose their constituents can be transformed between the different forms by microorganisms, soil invertebrates and plants. For example, the concentrations of nutrients and heavy metals within the soil can change as a result of absorption by plants. Soil microbes are able to break down the organic matter and utilize the bioavailable nutrients for their growth and respiration needs. Of particular importance to this research, mineralization of organic matter will generate ammonium, nitrification will oxidize ammonium to nitrate and denitrification will convert mineral nitrogen to a gaseous form.

Chemical parameters such as pH, presence of chelating ions, porosity and soil composition can play an important role in determining the retention of chemical substances in the soil (Atalay et al., 2007). During biosolids breakdown, their chemical constituents can become sorbed onto clay particles, labile metals and organic matter (Gove et al., 2002). For example, iron, aluminum and calcium species have been shown to immobilize water soluble phosphorus by forming insoluble precipitates (Penn & Sims, 2002). The pH of soil-biosolids mixture can also play a role in

determining the fate of some biosolids constituents. For example, heavy metals have been shown to precipitate in soils that have a high pH in the top soil horizons (Fuller, 1990).

Physical parameters such as temperature, precipitation, wind patterns and soil properties can also contribute to the fate of the biosolids constituents. Under high UV light some substances will break down into their derivatives (i.e. persistent organic pollutants). Weather patterns can cause some chemicals within biosolids to volatilize and migrate to a different area (i.e. NH_4^+). In addition, particularly heavy rain events can cause some of the biosolids constituents to be washed off from the field in the runoff.

As biosolids are naturally rich in nitrogen, when applied to agricultural fields they can potentially have an impact on the nitrogen cycle by increasing the pool of reactive species and altering the rates of N-cycle processes. Most soils do not have readily available mineral forms that can bind nitrogen (Robertson &Vitousek, 2009). As a result, nitrogen does not form insoluble precipitates, but is continuously recycled within a system until it is removed by absorption into organic matter (temporary storage), denitrification (permanent removal) or runoff (export from the system).

1.2.4 Quantity and Bioavailability of Nutrients in the Runoff

1.2.4.1 Surface Runoff and Tile drainage

The increase in human population created a higher than ever demand for food production. As a result, many areas around the world that are suitable for crop growth have been converted for agricultural use. For instance, estimated 98% of prairies and forests in North America have been replaced with croplands (Blann, et al., 2009). One of the primary goals of agricultural production is maximizing crop yields by providing plants with suitable growing conditions.

Most plants do not grow well in wet, muddy soils. Particularly heavy rain seasons can increase the moisture content of agricultural fields, impacting crop production. Excessive surface water is usually removed from some fields with the use of pumps or construction of open ditches. The subsurface water is removed out of the soil with the use of tile drainage. Tile drainage is made up of underground “plastic pipe” networks, which collect the leachate water out the soil through small openings in the pipes (Spaling & Smit, 1995). The leachate then empties into a nearby water system (i.e. streams, rivers or lakes).

The addition of drain tile effectively lowers the water table, which allows plants to absorb more nutrients. It can further benefit the plants by promoting proper root development and improving physical condition of the soil (Blann, et al., 2009). The improved growing conditions translate into higher yields. It has been estimated that in some regions, tile drainage is an effective way of increasing crop yields by as high as 25% annually (Eidman, 1997).

Surface runoff does not usually enter the aquatic system directly, as most of the water collects on the surface of the field or percolates into deeper layers of the soil. Tile drainage is of particular interest to the eutrophication discussion, since it can contain a significantly higher runoff volume that can rapidly reach the nearby water systems (Mehnert et al., 2007). Subsurface drainage has been shown to increase the losses of nitrate and soluble contaminants through leaching from the soil (Blann, et al., 2009) and alter the nutrient composition of the receiving systems.

The chemical composition of the runoff can depend on several factors such as fertilization rate, types of crops planted and physical structure of the soil. In addition, weather patterns can be important consideration factors, as the majority of nutrient loss in the runoff happens during isolated heavy rain events (Hubbard et al., 1982).

1.2.4.2 Factors Determining the Quantity and Bioavailability of Nitrogen in the Runoff

Since nitrogen is one of the major limiting nutrients in the soil, in many areas around the world, fertilization is based on the nitrogen requirements of crops. In some cases the application rates exceed the requirements of plants. It is estimated that only 50% of the applied bioavailable nitrogen is absorbed into crop biomass (Dwivedi et al., 2007). The remaining nitrogen in stored in the pant-soil interface or is lost to the environment, where it is assimilated into the biomass of soil microorganisms or escapes through volatilization or leaching.

Particularly heavy rain events can cause a detachment of nutrient rich, low density particles from the soils (Atalay et al., 2007), which can then be carried off in the runoff. The soil particles can increase the overall nitrogen content of the runoff directly or by releasing the water soluble nitrogen fractions into the water. The proportion of nitrogen in the runoff from biosolids treated fields can depend on multiple factors including biosolids nitrogen species composition (Smith,

Woods et al., 1998a), ammonia volatilization (Robinson & Röper, 2003) and slope of the agricultural field (Chen et al., 2010).

Biosolids that are used as organic fertilizer usually contain concentrations of 2-6% total nitrogen (dry weight) (Smith, Woods et al., 1998a).The two environmentally important forms of nitrogen present in biosolids are reactive inorganic species (i.e. ammonium and nitrate) and organic species (i.e. urea) (Smith, Woods et al., 1998b). The exact proportion of nitrogen species is dependent on the production process. Anaerobic digestion produces biosolids that are high in ammonium (up to 15% of total nitrogen) relative to organic nitrogen (Smith & Tibbett, 2004). Composting on the other hand, has been shown to produce higher levels of nitrite and nitrate than anaerobic digestion.

The species composition of biosolids can determine concentration of nitrogen in the runoff. Organic nitrogen adsorbs to soil particles, which can prevent it from leaching in the runoff. Similarly, ammonium is cationic and can readily adsorb to clay particles within the soil. Nitrate on the other hand is anionic and soluble in water. Even though the two types of biosolids examined in this thesis do not contain elevated levels of nitrate initially, mineralization and nitrification processes within the soil can increase the pools of soluble nitrate increasing the potential for leaching and loss in the runoff.

Volatilization of ammonia usually occurs within the first few days after fertilizer application when the pH is high and NH_4^+ is abundant (Robertson &Vitousek, 2009). The exact amount of ammonia losses is site specific and dependent on factors such as biosolids nitrogen content and application rate, post application time and wind patterns at the application site. As a result, it is difficult to estimate the exact rates of volatilization in biosolids treated fields. The rates have been shown to vary from 4% over a 72 day period (Pu et al., 2010) to 32% over 24 hours (Donovan & Logan, 1983) to as high as 60% over a 5-day experimental period (Beauchamp et al., 1978). In some cases ammonia losses can be substantial. For example, Robinson & Röper (2003) found that the volatilization can represent losses of up to 12% of the total bioavailable nitrogen applied to the fields.

The slope of the agricultural field can play an important role in the concentration of nitrogen in the runoff. The increase in soil slope can alter the concentration of nitrogen in the runoff by increasing the overall quantity of the water (Fox et al., 1997; Chaplot et al., 2003). In addition,

under steep slope conditions, higher speed and force of the runoff water can increase the detachment and transport of soil particles (Torri & Poesen, 1992), which can subsequently increase the nutrient and organic matter concentrations in the runoff.

1.2.5 Effect of Quantity and Bioavailability of Nitrogen on N-cycling

Excessive input of nitrogen can have an effect on the rates of N-cycling within the ecosystem. At low loading rates the bioavailable nitrogen is incorporated into biomass of primary producers and other microorganisms. When these pools are saturated, the remaining nitrogen is converted between its forms in the N-cycle, or is retained by the system (i.e. denitrification) (Agren & Bosatta, 1988). Species composition of nitrogen can also impact the rates of N-cycling as buildup of substrates (i.e. ammonium and nitrite) can inhibit some bacterial N-cycle processes (i.e. nitrification) (Anthonisen et al., 1976).

1.2.5.1 Soil

In the soil, an increase in nitrogen content (such as N-fertilization) has been shown to impact the rates of nitrogen conversion between its forms. These impacts can be a result of increase in the substrate levels or by altering the physical conditions within the soil to favour or inhibit N-cycle processes.

1.2.5.1.1 Nitrification

The rates of nitrification are usually dependent on the quantity of nitrifying bacteria within the soil and the concentrations of NH_4^+ substrate (Sahrawat, 2010). Generally the increase in NH_4^+ increases the rates of nitrification in the soil. At elevated levels, however ammonia can inhibit nitrification in the soil. Anthonisen et al. (1976) found that NH_3 inhibited nitrification at concentrations from 2 mg L^{-1} to 150 mg L^{-1}. The effect is most likely due to the fact that elevated levels of ammonia and nitrite can inhibit the growth and respiration rates of *Nitrobacter* and *Nitrosomonas* species (Anthonisen et al., 1976). Similarly, Vadivelu et al. (2007) also found that free ammonia inhibits the biosynthesis of *Nitrobacter* at concentrations as low as 6 mg L^{-1}.

Application of biosolids that contain a high quantity of total nitrogen can also decrease nitrification rates. Ryan et al., 1973 found that during N overfertilization with biosolids (TN ≥940 ppm) the rates of nitrification were lower than during moderate TN input (TN=235 ppm). The effect is most likely due to the fact that nitrification process can decrease the pH of the soil

when most organic fertilizers are converted to nitrate (Tisdale & Nelson, 1970). In soils with pH<6 the rates of nitrification can be lower than soils with higher pH (>7.5) (Sahrawat, 1982; Kyveryga et al., 2004).

1.2.5.1.2 Denitrification

The rates of denitrification can be affected by N-input. In general agricultural soils that receive the highest rate of nitrogen input exhibit higher denitrification rates than soils which are not fertilized with nitrogen (Barton et al. 1999; Hofstra & Bouwman, 2005). In particular high levels of NO_3^- input and high organic carbon and moisture content are the factors which have been shown to positively affect the rates of denitrification (Cambardella et al., 1999).

1.2.5.1.3 Mineralization

Since biosolids are rich in organic nitrogen (approximately 80% of TN in biosolids is usually in organic form) (Sommers, 1977), long term conversion of organic nitrogen into inorganic forms can have an impact on the rates of nitrification and denitrification by increasing the pool of NH_4^+. Several studies indicate that mineralization rates can be as high as 90% in some soils (Pascual et al., 1998), which can have an effect on the concentrations and bioavailability of nitrogen species over time.

1.2.5.2 Aquatic Systems

Depending on the loading rate, community composition and lake size considerations, nitrogen that enters the system from external sources can accumulate in the lake or have a relatively quick turnaround time. The fate of nitrogen in aquatic systems usually follows a three stage process. In the first stage the nitrogen requirements of primary producers are satisfied, which is then followed by the saturation of microbial nitrogen requirements (such as heterotrophs). Once these pools are saturated the only available removal strategy is through denitrification (Bernot & Dodds, 2005).

1.2.5.2.1 Streams

In many agricultural areas, before nitrogen reaches a large water body (such as a lake), it travels through a series of streams and rivers. When in the stream a major portion of inorganic nitrogen is uptaken into the biomass of living organisms or is denitrified (Peterson et al., 2001).

The amount of nitrogen that subsequently enters the lake can depend on the residence time and the rates of external loading into the streams and rivers. If the added nitrogen exceeds the ability of the system to denitrify, the remaining portion can accumulate in the water column. In streams that receive high external input of nitrogen, NO_3^- is the dominant form of nitrogen export (Royer et al., 2006).

1.2.5.2.2 Nitrification

In the absence of buffer zones such as streams and rivers, lakes will receive all the nitrogen forms present in the runoff including organic nitrogen and ammonia. Nitrification is an important process in many aquatic systems because it reduces the levels of potentially toxic ammonia and increases the levels of nitrate, which is a substrate for denitrification. Initially the response to nitrogen loading can cause a linear increase in rates of nitrification (Peterson et al., 2001; Kemp & Dodds 2002), however rapid nitrification can cause a drop in the pH of the system, which shifts the NH_4^+/NH_3 equilibrium towards higher concentration of ammonium ions (Bernot & Dodds, 2005). Since ammonia and not ammonium is thought to be a substrate for nitrification (Strauss et al., 2002), low pH conditions can negatively impact the nitrification rates. This feedback mechanism however is not expected to be very prevalent during extensive phytoplankton blooms (fuelled by N-loading), since photosynthesis will ultimately cause the pH to increase. It is therefore expected that nitrification rates will increase linearly with low to moderate N-loading, but become inhibited at elevated levels (Bernot & Dodds, 2005).

1.2.5.2.3 Denitrification

Accumulation of free nitrate in the lake can negatively affect water quality. Denitrification is an important environmental sink of nitrogen, because it converts its reactive forms back into nitrous oxide and dinitrogen gas. Even though the processes is carried out in aquatic as well as terrestrial ecosystems, the overall denitrification rates carried out in soil are $1/10^{th}$ of the rates in the sediments of rivers, estuaries and lakes (Seitzinger, et al., 2006). The rates of denitrification can also be influenced by the physical conditions of the waterbody such as water temperature, concentration of dissolved oxygen and organic carbon (Nielsen et al., 1990). Denitrification rates are optimal under conditions of low levels of dissolved oxygen, moderate temperatures (22^oC) and highly available organic carbon (Pfenning & McMahon, 1996).

Generally speaking as the N-loading rates increase so does the rate of denitrification, mainly because it increases the pool of bioavailable nitrate for denitrifiers (DeLaune et al., 1991; Kemp and Dodds 2002; Seitzinger, et al., 2006). However, in some cases the increase in the NO_3^- concentrations and respiration rates can decrease the overall efficiency of the denitrification process (Laursen & Seitzinger , 2004, Mulholland et al. 2008; Gardner & McCarthy, 2009). As a result, a proportion of nitrate removed as a function of input will decrease, causing more nitrate to be retained in the system. Denitrification rates are therefore maximized at moderate external N-loading rates (Sloth et al., 1995) and are negatively impacted during times of high nitrate input.

1.3 Fate of Nutrients in Receiving Water

The concentration of nutrients in aquatic ecosystems can fluctuate due to natural events, and depend on factors such as geology, flood patterns, climate and biogeochemical processes (Olde Venterink et al., 2003**;** Conley et al. 2009). However, it is widely accepted that the increase in the levels of nutrients in many water systems over the past several decades is primarily a response to anthropogenic actions such as burning of fossil fuels, sewage runoff and agricultural practices (Carpenter et al., 1998; Vitousek et al., 1997; Rabalais et al., 2002; Galloway, 2004). If the nutrients accumulate in the receiving water body they can act as pollutants, causing eutrophication.

1.3.1 Eutrophication

Eutrophication is an increase in nutrient content of an aquatic ecosystem, which causes an upsurge of organic matter production and negatively affects water quality. Water can be contaminated directly when the levels of some nutrients reach toxic levels (i.e. nitrate and ammonia), or be polluted by the overgrowth of photosynthetic microorganisms, some of which are known to produce toxins (Anderson et al., 2008).

Elevated levels of nutrients can also play a role in changing the community composition of aquatic ecosystems. Relative bioavailability of certain nutrients such as carbon, nitrogen, phosphorus and silica can cause an overgrowth of primary producers and give a competitive advantage to photosynthetic microorganisms over aquatic plants (i.e. macrophytes) (Smith, 1983; Elser et al., 2000). Since macrophytes can form important habitats for fish and pelagic invertebrates, the overabundance of phytoplankton can influence food-web dynamics and decrease the diversity of aquatic communities (Carpenter et al., 1996; Correll, 1999).

1.3.2 Dissolved Oxygen

Eutrophication can also have a substantial effect on dissolved oxygen concentrations in the water. Growth of primary producers can persist until the levels reach the carrying capacity of the system. When the nutrients (such as N, P and Si) necessary for cell formation and growth are used up, colonies of primary producers undergo a collapse. Dead cells are then transported to the bottom layer of the water body where they are decomposed by the detritus feeders. During the decay of organic matter, dissolved oxygen is depleted at a very rapid rate, which in turn causes

production of oxygen poor "dead zones". Dissolved oxygen is important in sustaining the lives of fish and aquatic invertebrates (Diaz & Rosenberg, 1995), and in general, most oxygen respiring organisms cannot survive if levels of oxygen fall below 2 mg L^{-1} (Diaz, 2001).

The two major factors producing hypoxia (low oxygen concentrations in the water) and anoxia (oxygen free environments) are bioavailability of organic matter and water column stratification (Diaz, 2001). Organic matter can enter the water directly (i.e. through runoff) or be produced as a result of phytoplankton die-off. Stratification can further exacerbate the problem. In some cases the oxygen poor water remains trapped on the bottom of the aquatic ecosystem and does not readily intermix with the oxygen rich layer above.

1.3.3 Thermal Stratification

Thermal stratification is a common characteristic of many lakes in North America. It is usually established twice a year: once during the warm summer months and once in the winter. Thermal stratification is formed as a result of differences in water densities at different temperatures. In the summer, the top layer of the lake (epilimnion) warms up, causing the warm water remain on top while colder water remains at the bottom layer (hypolimnion). Since water density is highest at $4^{o}C$, during cold months of the year the warmer water sinks to the bottom of the lake while the colder water remains on top.

The two zones are usually separated by a thin layer of thermocline which establishes a set of different environmental conditions such as nutrient composition, oxygen levels, pH and community structure (Dake & Harleman, 1969; Özkundakci et al., 2010). During warm seasons the oxygen rich waters at the top of the lake do not intermix with the oxygen poor water found at the bottom, which can therefore create hypoxic or anoxic zones (Hussainy, 1967). The two layers can also differ in nutrient composition. In eutrophic lakes with seasonally anoxic hypolimnion, bioavailable nutrients such as soluble reactive phosphate and ammonium-nitrogen can be released from the sediment into the water column (Beutel, 2001; Özkundakci et al., 2010). In some cases the internal releases can even exceed nutrient input from external sources (Özkundakci et al., 2010). The released nutrients are then recycled in the hypolimnion by the microorganisms living in the benthic zone or make their way into the epilimnion, fuelling the primary productivity in the photic zone. Burger and his colleagues (2007) found that the release of these nutrients is highest in the summer and may be dependent on high level of organic matter

content in the sediment. This is consistent with the fact that most algal blooms happen during the warm summer months when the organic matter is most abundant.

1.3.4 Primary Productivity

One of the most important consequences of eutrophication is that it stimulates an overproduction of phytoplankton in the water. When present in high numbers these organisms can negatively influence water quality (by producing offensive odours and increasing turbidity) or be hazardous to the aquatic life. Of particular importance to this discussion are nuisance organisms which are responsible for the production of phytoplankton blooms: algae, cyanobacteria and diatoms.

Algae are eukaryotic photosynthetic organisms which can be unicellular or multicellular. They vary in size from nanometers in diameter to over fifty meters in length (such as some species of Kelp). The major difference between algae and plants is that algae do not possess certain external features such as leaves and roots. When limiting nutrients are readily available algae are able to outcompete other microorganisms (in terms of overall abundance) by utilizing these nutrients more efficiently (Anderson et al., 2008).

Cyanobacteria are a group of photosynthetic prokaryotic organisms which are a natural component of many freshwater and marine ecosystems. They are unicellular, but can group together forming dense mats floating on the surface or within the water column. Public health concern over cyanobacteria blooms is associated with their ability to negatively affect the quality of water giving it bad taste and odour. In addition, some species are capable of producing cyanotoxins, which can be dangerous to human health (Humpage et al., 1993; Pitois et al., 2001).

One of the metabolic processes carried out by some species of cyanobacteria is N_2 fixation. By using the enzyme nitrogenase, some species of *Lyngbya*, *Anabaena*, *Nostoc* and *Ossilatoria* can convert N_2 into its more bioavailable form, NH_4^+ (Stewart, 1973; Bryceson et al., 1981; Phlips, et al., 1992). Lakes that have a low N:P ratio are usually dominated by N-fixing cyanobacteria species (Smith, 1983) .

Diatoms are unicellular photosynthetic organisms, which are a major group of algae. The main differentiating feature of diatoms is that they use silica as the material for their frustules, rather than producing cell wall composed of cellulose. Species like *Synedra* are commonly found in

many lakes and oceans and are important in the world silica cycle (Zakharova, et al., 2010). In aquatic conditions where silica is more abundant diatoms have a competitive advantage over other species of algae (Brzezinski, 1985), which allows them to dominate the community composition of phytoplankton in the water column.

1.4 Nutrient Limitation

A nutrient which is in short supply relative to other nutrients in the ecosystem is called a limiting nutrient (Correll, 1999). The concept of nutrient limitation in aquatic ecosystems is based on the fact that since some essential nutrients are relatively less abundant, the concentration of these nutrients will ultimately control the growth and abundance of photosynthetic organisms. By restricting the loading rates of these key nutrients it is possible to reduce the rates of phytoplankton growth in many aquatic systems (Smith, Tilman et al., 1998). A number of studies have successfully confirmed that the most important nutrients that control the abundance and species composition of phytoplankton in many aquatic ecosystems are nitrogen and phosphorus (Carpenter et al., 1996; Smith, Tilman et al., 1998; Elmgren & Larsson, 2001; Schindler et al., 2008).

The relative proportion of limiting nutrients in lentic ecosystems can play a role in the determining the community composition and the overall levels of phytoplankton production. The most commonly accepted indicator for optimal phytoplankton growth conditions is the Redfield ratio. Redfield ratio is a proportion of carbon: nitrogen: phosphorus which was found to be constant in marine phytoplankton with a molar ratio of 106:16:1 (Redfield, 1958). The phytoplankton production as a response to the ratio of these nutrients in the water however, does not always apply to freshwater lakes. Lake manipulation experiments in the Experimental Lakes Area (ELA) in Northern Ontario show that nitrogen fixation processes carried out by cyanobacteria can increase the levels of nitrogen above the Redfield ratio in lakes that were fertilized with N and P at a ratio of 16:1 (Schindler et al., 2008). In addition, fertilization of lakes with inorganic N to P ratio of 27:1 showed that phytoplankton blooms were produced in proportion to the P supply (Schindler et al., 2008), which indicative of phosphorus limitation. When the lakes were fertilized with N:P ratios of 12.5:1 and 9:1, it produced large algal blooms proportional to the rates of P, but the lake was mostly dominated by N-fixing cyanobacteria

species (Schindler, 1977). Nitrogen fixation is a major process contributing to the P limitation of many lakes, as it can add bioavailable nitrogen to the systems with low N:P ratios.

Estuaries and marine ecosystems that have been heavily loaded with nutrients can display P limitation, N limitation and co-limitation (Paerl et al., 2006; Conley et al. 2009). In some marine ecosystems such as the Baltic Sea, cyanobacteria are responsible for fixation of 2 to 4 X 10^5 ton of nitrogen each summer (Elmgren & Larsson, 2001). This is indicative of N-limited ecosystems that have a relative abundance of bioavailable phosphorus.

Even though multiple studies show that phosphorus is the limiting nutrient in most freshwater ecosystems and nitrogen is the limiting nutrient in many marine ecosystems, reduction in the levels of both nutrients is necessary in order to decrease the rates of eutrophication. If the excessive nitrogen inputs are not controlled they can eventually migrate into the coastal and marine ecosystems (National Academy of Sciences, 2000), where nitrogen can stimulate an overgrowth of primary producers, further polluting these regions. This is particularly evident in places like the Gulf of Mexico where eutrophication of the region is attributed to an increase in N-loading (Burkart & James, 1999).

The Mississippi River system is responsible for the major portion of the flux of water into the Gulf of Mexico. Since the majority of the Mississipi River basin is used as cropland (Turner and Rabalais, 1994), it is estimated that the input of nitrate into the Gulf tripled since the 1950s mainly due to agricultural food production (Rabalais et al., 2002). Overall, the Mississippi River brings in around 1.6 X 10^6 tons of nitrogen, 0.1 X 10^6 of phosphorus and 2.1 X 10^6 tons of silica per year (Rabalais et al., 2002). This increase in the nutrient content has been implicated in production of "dead zones" in the Northern Gulf of Mexico where an estimated 20,700 km^2 area of the bottom water becomes hypoxic in the terminal region of the river in mid-summer as a result of nutrient enrichment (Rabalais et al., 2002).

1.5 Research Rationale and Approach

As the application of treated sludge for agricultural purposes is one of the preferred disposal options in many regions in Canada, there is an increasing need for a better understanding of the consequences of this practice. There is a general lack of information concerning the effect of biosolids runoff on eutrophication in aquatic ecosystems, including the relative importance of nitrogen loading as a potential driver of eutrophication. Further, there is a need to understand how nitrogen transformations will affect the retention of nitrogen in these systems, as this will have implications for export of nitrogen to coastal systems.

Previous scientific studies often focus on either the concentration of nutrients in the agricultural runoff or the concentration of nutrients in the nutrient loading in aquatic systems. This study is novel in that it simulates both runoff from fields amended with biosolids and the subsequent response in the freshwater systems as a result of the runoff addition. This study attempts to limit the numbers of variables such as atmospheric deposition, complex predator-prey interactions and historical data of open lake systems in North America by using small scale mesocosm experiments and simulating a worst case scenario for biosolids application. By focusing only on the parameters which have been shown to be relevant to the eutrophication and biogeochemical nutrient cycle discussions we can assess whether the effects of biosolids runoff on eutrophication and the nitrogen cycle are statistically different from the effects of runoff from unfertilized soil. In addition, due to their complex composition (in terms of forms of nitrogen, phosphorus, and their relative bioavailability) it is speculated that the effects of the runoff originating from the biosolids amended field on the aquatic ecosystem should be different from the impact on aquatic systems receiving inorganic fertilizer.

The overall objective of this study is to determine the effects of biosolids runoff on the nitrogen cycle and eutrophication of the receiving freshwater systems. This study focused on two types of biosolids: produced by anaerobic digestion and chemical (alkaline) stabilization. One of the major differences between the biosolids tested in the study was their pH. Alkaline stabilized biosolids usually have a higher pH than the anaerobically digested biosolids since the chemicals used during stabilization (such as NaOH) increase the alkalinity of the resulting slurry.

It is hypothesised that the chemical composition of the two types of biosolids is different, so it is expected that they may have a different effect on the nitrogen biogeochemical cycle as well as eutrophication.

General hypotheses that were addressed in this research are:

1. Runoff originating from soil with biosolids application will have an impact on the nitrogen cycle in the freshwater mesocosms compared with runoff originating from reference soil.
2. Runoff originating from soil with biosolids application will contribute more strongly to eutrophication of receiving water than runoff originating from reference soil
3. Runoff originating from soil with biosolids runoff will have a different impact on nitrogen cycling and eutrophication in the water column than equivalent quantities of nitrogen and phosphorus loaded to mesocosms in inorganic form (NH_4^+ and PO_4^{3-}), as the forms and relative bioavailability of nutrients will differ.

In order to test these hypotheses a small-scale, mesocosm type lab experiment was conducted. Agricultural plots (soil boxes) were constructed and filled with artificial soil. The soil was either left untreated or was fertilized with biosolids. Rain events were then carried out at set intervals on four time points over the duration of the experiment. The runoff was added to freshwater mesocosms (10% v/v) simulating runoff flowing into a lentic ecosystem. The concentrations of nutrients were determined in the incoming runoff and in the freshwater mesocosms at five sampling events over 32 day experiment. Fifteen separate freshwater mesocosms were fertilized with inorganic N and P (and N+P), equivalent to the N and P concentrations in the biosolids runoff and sampled at the same time as the runoff treated freshwater mesocosms. The changes in the species composition over time indicated the overall transformation of the incoming nitrogen species. The effect on eutrophication was determined using two indicators of eutrophication: changes in the organic nitrogen and dissolved organic carbon over time. The increase in these nutrients would provide an indication of primary productivity happening in the mesocosms over time.

2. MATERIALS AND METHODS

2.1 Soil Troughs Setup

Nine soil boxes (clear polyethylene-lined wooden troughs, 1 m in length, 0.35 m wide and 0.40 cm deep, constructed by Sonja Gebert, a previous graduate student in this program) were setup on a 9% slope, which is the maximum slope allowed in biosolids application in Ontario (OMAFRA, 2009). The internal portion of each box was fitted with clear polyethylene liner (Film-Guard, 3.0 m x 2.0 m). Soil boxes were also equipped with a 1.15 m long and 15 cm diameter plastic weeping tile (mesh size= 0.1 cm), set up at the bottom of the box in order to simulate tile drainage, which was designed to let the runoff through but filter out large soil particles. The boxes were then filled with a shallow layer of gravel (bottom 20 cm) in order to facilitate percolation of water through the soil and to the tile drainage.

Reference soil was made up in accordance with the Environment Canada guidelines (2005). The composition of the soil was 70% silica sand, 20% kaolin clay and 10% peat moss by mass. An additional source of organic matter (< 1% w/w) was added to the soil in the form of regular garden soil (Berger, Canada). The garden soil was also intended to act as an inoculum for soil microorganisms. The soil was then mixed using a cement mixer and sprayed with water to achieve ~80% moisture (measured with a soil moisture probe Fujan E-Inginst Electron Co., China) in order to reduce dust and hydrate clay during mixing soil. The soil was then added to the boxes until they were full.

Two types of biosolids: Kitchener and Guelph were added to the soil mesocosms. Kitchener biosolids were produced in a municipal WWTP using anaerobic digestion production procedure, while Guelph biosolids were made using alkali addition, followed by thermal treatment and physical agitation (Lystek, 2007). The biosolids were added at 8 Mg ha^{-1} dry weight. As both biosolids were in a form of liquid slurry, % dry matter was determined to correct for necessary volume additions to achieve target load (Appendix B, Part B). The % dry mass was estimated by mass loss when a sample of biosolids was dried at 105°C for 10 hours. We then added 44.3 kg Kitchener biosolids and 27.9 kg Guelph biosolids in order to provide 0.288 kg dry weight in 0.36 m^2 (soil surface area in trough).

The biosolids were incorporated (with a metal shovel) into the top 15 cm of the soil of the corresponding randomized boxes in triplicates. Three remaining soil mesocosms were unaltered

and were designed to represent a reference soil runoff, in the absence of organic fertilizer. The soil in mesocosms was kept moist for the duration of the experiment (with addition of three liters of deionized water once a week), and was physically agitated with a hand shovel in order to achieve uniform consistency prior to rain simulation.

2.2 Freshwater Mesocosms

Forty five freshwater mesocosms (1.5 meters tall, 7.75 cm in diameter) were setup vertically. Each mesocosm was filled with 6 liters of dechlorinated municipal tap water. The amount of water was kept constant by addition of dechlorinated water to make up for water lost to evaporation. Out of forty five mesocosms, twenty seven received runoff from corresponding soil boxes, and fifteen were fertilized with inorganic N and P which correspond to the N and P concentrations found in the runoff.

2.2.1 Reference Sediment Preparation

Reference sediment was made up in accordance with the OECD Guideline (OECD, 1984) with a change in peat moss concentration from 10% to 2% dry weight in order to correspond to the low to moderate values of organic matter found in natural sediments (Suedel & Rodgers, 1993). We also changed a cellulose source from Urtica powder to finely ground and dried leaves of Sugar Maple tree (*Acer saccharum*) (Table 1).

Table 1. Percentage of dry constituents of the artificial sediment (OECD, 1984).

Constituent	Characteristics	% of dry sediment
Peat	Sphagnum moss peat (particle size ≤ 0.5 mm)	2 ± 0.5
Quartz sand	Grain size: ≤ 2 mm	76
Kaolinite clay	Kaolinite content ≥ 30%	22 ± 1
Dried Maple Leaves	Powdered leaves of *Acer saccharum* with alpha-cellulose (1 : 1 ratio)	0.4 - 0.5
Calcium carbonate	$CaCO_3$	0.05 – 1
Deionised Water	Conductivity ≤ 10 µS/cm, in addition to dry sediment	30 – 50

The peat was air-dried and grounded to a fine powder until no visible plant remains were detected. A suspension of the required amount of peat powder was prepared using deionised water (a water volume of 11.5 x dry weight of peat). The pH of this suspension was adjusted to 5.5 ± 0.5 with $CaCO_3$. The suspension was conditioned for three days with gentle stirring at room temperature. The pH was measured again and adjusted to 6.0 ± 0.5 with $CaCO_3$. Then all of the suspension was mixed in with the other dry constituents with deionised water added to obtain a homogeneous sediment. The pH was measured again (using a pH probe) and adjusted to 6.5 with $CaCO_3$. Thereafter, the quartz sand was mixed with the sediment. In addition, a source of lake sediment from a eutrophic pond (location) was added into the artificial sediments (<0.5% w/w) in order to inoculate artificial sediments and approximate the bacterial community structure of a real lake. After preparation, the sediments were added to the bottom 5 cm of each freshwater mesocosm.

2.2.2 Light Simulation

Two light banks were setup above the mesocosms with full spectrum fluorescent lighting (21 light bulbs per light bank), in order to approximate sunlight (T8 VitaLux bulbs, MT-DTC, USA, 121.9 cm in length). The light intensity at the water surface of the mesocosms was ~18, 000 lux. The lights were setup on a timer (Intermatic, Mexico), and provided 14 hour light: 10 hour dark cycles.

2.2.3 Addition of Phytoplankton

Eight strands of freshwater photosynthetic organisms were added to the mesocosms including algae: *Pseudokirchneriella subcapitata,* diatoms*: Navicula pelliculosa and Synedra* sp. and cyanobacteria: *Microcystis aeruginosa, Nostoc sp., Anabaena sp., Oscillatoria* and *Lynbyga sp.* 100mL of each phytoplankton species during their log growth phase were added to each mesocosm 1 week apart. All organisms were purchased from Ward's Scientific (St. Catharines, ON) and further sub-cultured at Ryerson University using protocols from Environment Canada (1992). Upon reaching the log growth phase, 100 mL of each species of phytoplankton were added to each mesocosm.

2.2.4 Simulation of Thermal Stratification

Five 90 liter, 30 cm tall storage bins (J. Terence Thompson, LLC, US) were setup underneath the columns with cold water circulating among them. The source of cold water was a freezer with two identical water pumps attached to the tubing, pumping water into and from the plastic cooling bins respectively. The water in the bins did not intermix with the water in the aquatic mesocosms; it did however create a temperature gradient (measured twice a day using an electronic thermometer (HANNA Instruments, Singapore) between the two different layers of the mesocosms, in order to simulate the hypolimnion and epilimnion layers of a stratified lake. The lower portion (70cm) of each column was covered with black plastic wrapping, in order to create a light gradient separating the photic and aphotic zones.

2.3 Addition of Runoff and Nutrient Loading

In order to simulate the effect of runoff entering the aquatic ecosystem we simulated rain events and collected the runoff from both surface and tile drainage of the soil mesocosms. Rain events were carried out on days 1, 8, 15 and 22. We simulated the "multi-annual extreme storm event for South Ontario" which was equivalent to 49.5 mm of rain occurring with a frequency of once in 100 years (Environment Canada, 2009), which is equivalent to adding approximately 18 L of water to each trough (calculations in the Appendix B, Part B). Distilled water was slowly added to each soil mesocosm over a 45 minute time period, and the runoff was collected from both surface and tile drainage. The runoff and leachate were analyzed for concentrations of ammonia, nitrate+nitrite and organic carbon within 5 hours of collection (note that the concentration of Kjeldahl nitrogen in the runoff was determined 3 months after the sampling procedure). The runoff from biosolids treated and reference soil was added to the randomized mesocosms in triplicates at 10% v/v concentration. The remaining mesocosms were fertilized with inorganic nitrogen and/or phosphorus corresponding to the concentrations of ammonia, nitrate and phosphorus found in the runoff. This was done in order to assess whether the effects of the biosolids runoff on the N-cycle and eutrophication will be the same as the effect of equivalent amount of N and P nutrients was added. The additional columns were set up in the following manner:

Table 2. Addition of inorganic N and P to the mesocosms

High N	Only the highest concentration of inorganic N equivalent was added to the mesocosms
High P	Only the highest concentration of inorganic P equivalent was added to the mesocosms
High N+High P	Highest concentrations of both N and P equivalent were added to the mesocosms
Low P	The runoff equivalent of the lowest P concentration in the runoff was added to the mesocosm
Low N+Low P	Lowest concentrations of both N and P equivalent were added to the mesocosms

2.4 Sampling Procedure

The aquatic mesocosms were sampled before the runoff addition and 3 days after each successive addition of the runoff (days 0, 4, 11, 18 and 32). The sampling procedure was carried out using a 60 mL syringe which was attached to a weighted tygon tube (WATTS, USA). The tube was slowly lowered into each mesocosm, to minimize intermixing between the layers. Two sets of samples were taken: one from the epilimnion and one from the hypolimnion. The samples were analysed for dissolved oxygen content, pH and electrical conductivity.

2.5 Sample filtering and Storage

The collected samples were then filtered using 0.22 µm pore-size filters (VWR, UK), (for ammonia, nitrate+nitrite and dissolved organic carbon analyses), or left unfiltered (for total Kjeldahl nitrogen and nitrous oxide analyses). The samples to be analysed for nutrient content were then stored in 50 mL conical tubes in the freezer at -20°C. The water samples for N_2O analyses were transferred to 60mL flint glass bottles in a manner that did not introduce any air bubbles. One "pellet" (~ 0.1 mg) of potassium hydroxide was added to each bottle in order to preserve the sample and to remove CO_2 which can interfere with N_2O analysis.

2.6. Chemical Composition and Analysis of Gases

2.6.1 Ammonium Assay

Ammonium analysis was carried out using a modified phenate method (Clesceri et al., 1999). Ammonium in the samples reacts with hypochlorite and indophenol reagents forming a blue

coloured mixture. The reaction is catalyzed by sodium nitroprusside. The intensity of the colour was analyzed using a UV-Vis spectrophotometer (Perkin Elmer Lambda 40, USA) at 640 nm.

2.6.2 Nitrite and Nitrate Assay

Nitrite and nitrate concentrations were analysed using the cadmium reduction method (Clesceri et al., 1999). Prepared samples were slowly processed passed through a cadmium column (copper-coated cadmium shavings) constructed by the researchers, which quantitatively reduces nitrate to nitrite, using a peristaltic pump. The nitrogen nitrite reacts with sulphanilamide and N-(1-napthyl)-ethylenediamine dihydrochloride creating a vibrant pink coloured diazo-dye. The concentrations were then determined using UV spectrophotometer (Perkin Elmer Lambda 40, USA) at 543nm.

2.6.3. Total Kjeldahl Nitrogen Assay

Total Kjeldahl Nitrogen concentrations were determined using a modified micro-Kjeldahl method (Clesceri et al., 1999). In the presence of potassium sulphate, cupric sulphate, sulphuric acid and heat organic nitrogen is quantitavely converted to ammonium. The reagent mixture was added to the unfiltered samples and digested using a micro-digestor apparatus (Labconco, USA). The sample was then added to the BUCHI distillation apparatus (BUCHI Labortechnik GmbH, Essen, Germany). Sodium hydroxide (45% w/w) was added to the solution after which point ammonia gas was distilled out and collected in the receiving flask containing 4% boric acid. The ammonia in the receiving vessel was then determined using the colorimetric phenate method for ammonia determination outlined previously (section 2.6.1). The accuracy of the process was verified by determining the concentrations of a known quantity of amino acid (L-alanine) in order to determine the % yield of N in samples.

2.6.4. Dissolved Organic Carbon Determination

Organic Carbon was determined using Shimadzu TOC-V Series analyzer. The TOC analyzer works by using high temperature combustion (680°C), with carrier gas being passed at a controlled rate of 150 mL/min through an oxidation catalyst-filled TC combustion tube. When the sample enters the combustion tube the total carbon is oxidized to CO_2. The carrier gas

carrying the combustion products from the combustion tube is cooled and dehumidified in the dehumidifier before passing via the halogen scrubber into the sample cell of the non-dispersive infrared detector (NDIR), which is the site of CO_2 detection. The NDIR signal forms a peak, and the data from the peak is compared to the calibration curve (which was created using standard solutions of total carbon).

Since total carbon is made up of organic carbon and inorganic carbon (i.e. carbonate and bicarbonate), the inorganic concentrations are determined by adding a small amount of hydrochloric acid to acidify the sample, which was then sparged with gas (compressed air). This converts all inorganic carbon in the sample to carbon dioxide and drives the CO2 out of the sample solution. By subtracting the concentration of inorganic carbon from the total carbon, the concentration of TOC is estimated.

Each filtered sample was measured twice with one water blank sample between the samples. The average concentration in the two measurements was recorded as the measured result.

2.6.5. Nitrous Oxide Measurement

A headspace was produced in the flint glass bottles bottle by inserting two 0.3 mm syringe needles (BD, US) and purging the liquid out of the bottle using a 10 mL syringe (BD, USA) filled with N_2 gas. The gasses within the water column were allowed to equilibrate for 1 hour before carrying out the detection.

The concentrations of N_2O in gas headspaces were measured by gas chromatography coupled with an electron capture detector (GC-ECD) (HP 5890, PerkinElmer Life and Analytical Sciences, Inc., Waltham, MA, U.S.A.) with GS-CarbonPlot column (30 m, 0.32 mm diameter, 3.00 mm film thickness) (Agilent Technologies, Santa Clara, CA). The ECD uses a beta emitter (^{63}Ni) in order to ionize the gasses and produce a current between a biased pair of electrodes. When molecules of N_2O gas passes by the detector, it reduces the current measured between electrodes.

The temperature settings were 375°C for the ECD, 35°C for the oven, and 185°C for the injector. Injections were performed using helium gas as a carrier with a split ratio of 0, at a flow rate of 30 mL min^{-1}. The injection volume was 100 µL, with standard gas analyzed after every 10 samples.

2.6.6. Dissolved Oxygen Determination

The concentrations of dissolved oxygen in the mesocosms were measured using a Clark-type oxygen microelectrode and picoammeter (Unisense A/S, Aarhus, Denmark). The microelectrode had a membrane diameter of 25 µm, which does not require stirring during measurements due to the small O_2 consumption by the electrode. The calibration was carried out using Millipore water which was first saturated with oxygen (by forcing air bubbles through the calibration chamber). The dissolved oxygen concentration was then measured at 0% saturation after "bubbling" the water with N_2 gas.

The samples to be measured for dissolved oxygen were gently transferred into 20 mL glass scintillation vials, which were filled from the bottom using a short piece of tygon tubing, and allowing 1.5 volumes to overflow the scintillation vial, in order to prevent reaeration of the sample during transfer. The measurements were done by immersing the tip of the electrode into each sample and taking a reading after 5 seconds.

2.6.7. pH Determination

The pH was determined using an electronic pH meter (OAKTON, Singapore). The analysis was carried out by placing the probe in the sample collection tubes and waiting for 20 seconds at which point the measurement was taken and recorded.

2.7 Statistical Analysis

Statistical analyses were performed using SYSTAT (2008) software for PC computers (Chicago, IL). Repeat measures analysis of variance (rmANOVA) was performed in order to determine the statistical difference between treatments, surface runoff and tile leachate, and between epilimnion and hypolimnion of freshwater mesocosms. Repeat measures ANOVA was also used to determine the statistical difference between concentrations of ammonium and nitrate in surface runoff and tile leachate as well as Biosolids compared to inorganic N and P amended analogs. In all cases, statistical differences were accepted when probability was less than 0.01.

3. RESULTS AND DISCUSSION

3.1 Nitrogen Mass Balance in the Soil

The concentration of Total Kjeldahl Nitrogen (TKN) was measured in the two types of biosolids, and was determined to be 26 g kg^{-1} (1.9 mol kg^{-1}) in Kitchener biosolids and 28 g kg^{-1} (2 mol kg^{-1}) in Guelph biosolids. The numbers are consistent with the concentrations expected in biosolids post anaerobic and Lystek digestion methods, which usually produce TN in the range of 2-6% per mass of biosolids (Sommers, 1977; Smith, Woods et al., 1998a; Singh et al., 2007).

The concentration of TKN in the reference soil was 1.7 mmol kg^{-1} on day 0 and 0.96 mmol kg^{-1} after 32 days (loss of 0.70 mmol kg^{-1}). Kitchener treatment had a soil + biosolids TKN concentration of 7.3 mmol kg^{-1} on day 0, and 3.5 mmol kg^{-1}on day 32 (loss of 3.7 mmol kg^{-1}). Guelph biosolids treatment had a TKN concentration of 8.7 mmol kg^{-1} on day 0 and 5.2 mmol kg^{-1} on day 32 (loss of 3.5 mmol kg^{-1}).

The loss of TKN in runoff over four rain events, calculated by multiplying the concentration of TKN in the runoff by the overall volume of runoff was 7.6 mmol, 74 mmol and 53 mmol from reference, Kitchener and Guelph soil boxes respectively. This represents a TKN loss of 16% for reference soil, 26% from Guelph and 32 % from Kitchener biosolids amended soil boxes.

The Nitrate (+ nitrite) loss in the runoff over the 4 rain events was 33.5 mmol from reference, 147 mmol from Kitchener and 141 mmol from Guelph treatments. This represents 77% of total nitrogen lost from reference, 64% from Kitchener and 70% from Guelph biosolids amended soil boxes.

From this data it was possible to estimate nitrogen losses from the soil boxes. The average amount of total nitrogen, ammonium, nitrite and nitrate species lost as well as the amount of nitrogen that was denitrified over the experiment is shown in Figure 2 (mmol m^{-2} d^{-1}). The rates of denitrification were low when compared to losses of nitrogen in the runoff. Reference soil boxes had denitrification rates of 233 µmol m^{-2} d^{-1}, Guelph biosolids treated soil boxes showed denitrification rate of 569 µmol m^{-2} d^{-1}, and Kitchener biosolids treated plots showed and average denitrification rate of 751 µmol m^{-2} d^{-1}.

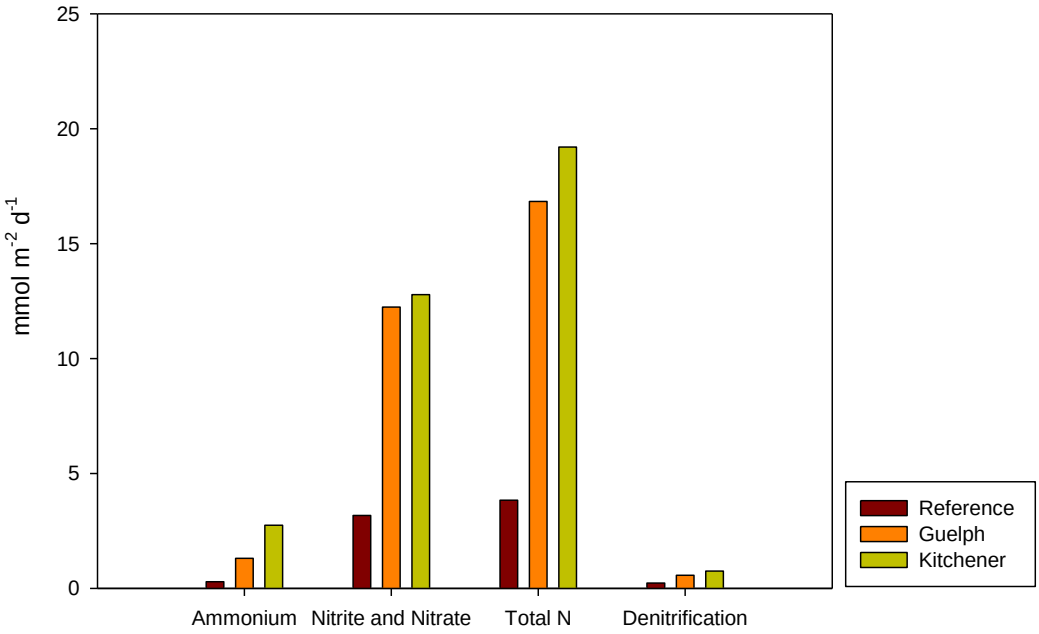

Figure 2. Average loss of ammonium, nitrite(+nitrate) and total nitrogen in the runoff and as a result of denitrification in soil boxes which were unfertilized or fertilized with Kitchener or Guelph biosolids (in mmol m^{-2} d^{-1}).

Denitrification rates in the soil plots used in this study were lower than those shown by previous research. In particular, Ryden (1983) recorded denitrification rates averaging 0.2 kg N ha^{-1} day^{-1} (equivalent to ~1.42 mmol m^{-2} d^{-1} in the current study) in soils subjected to an average of 250 kg N ha^{-1} a^{-1}. Similarly Barry et al. (1993) estimated denitrification rates of 62 kg N ha^{-1} yr^{-1} on a corn crop farm using mass balance determination of denitrification (as was done in this study). Barton et al. (1999) recorded average denitrification rates in agricultural soil of around 13 kg N ha yr^{-1}.

The discrepancy between the denitrification rates in this experiment and the results found by other studies could be explained by the fact that during simulated rain events the majority of nitrogen was lost in the runoff (as nitrate). As a result the pool of nitrate in the soil which would otherwise be available for denitrification was decreased. In addition, since the soil used in this study was artificially made, it had poor water retention. In general, soils with poor drainage show higher denitrification values than those with good drainage (Hofstra & Bouwman, 2005) as a

result of higher levels of nitrate available and anoxic conditions within the soil with high moisture content.

The levels of denitrification were higher in the biosolids treated soil boxes compared to the reference treatments. This is consistent with other agricultural studies. For example, Ryder (1982) showed that denitrification rates were higher in fertilized fields when compared to the unfertilized controls. Barton et al. (1999) showed that the rates of denitrification were dependent on the levels of nitrogen fertilization as well as the soil type (highest rates were found in irrigated loam soils). While Ryder (1982) and Barton et al. (1999) were not explicitly looking at biosolids as a nitrogen source, a stimulation of denitrification rates would still be expected when the total nitrogen in soil is increased by biosolids application as occurred here.

3.2 Concentration of Nutrients in the Runoff

There was a high variability of concentrations from the soil boxes that were subjected to the same treatment (unfertilized or fertilized with biosolids). The results in this research are consistent with Quilbé et al. (2005), who conducted a study focusing on the concentrations of nitrogen and phosphorus in the runoff from biosolids treated soil plots and also found a high variability among replicates.

The discrepancy was most likely due to the differences in the volume of runoff between the soil boxes as a result of variation in saturation points and inherent difficulties in attaining a uniform distribution and velocity of water drops onto the surface of the soil during the simulated rain events. In this experiment the runoff sample was collected within the first 30 minutes of starting the rain event, which produced a runoff volume that ranged from 1.5 L to 6.5 L.

3.2.1 Total Nitrogen

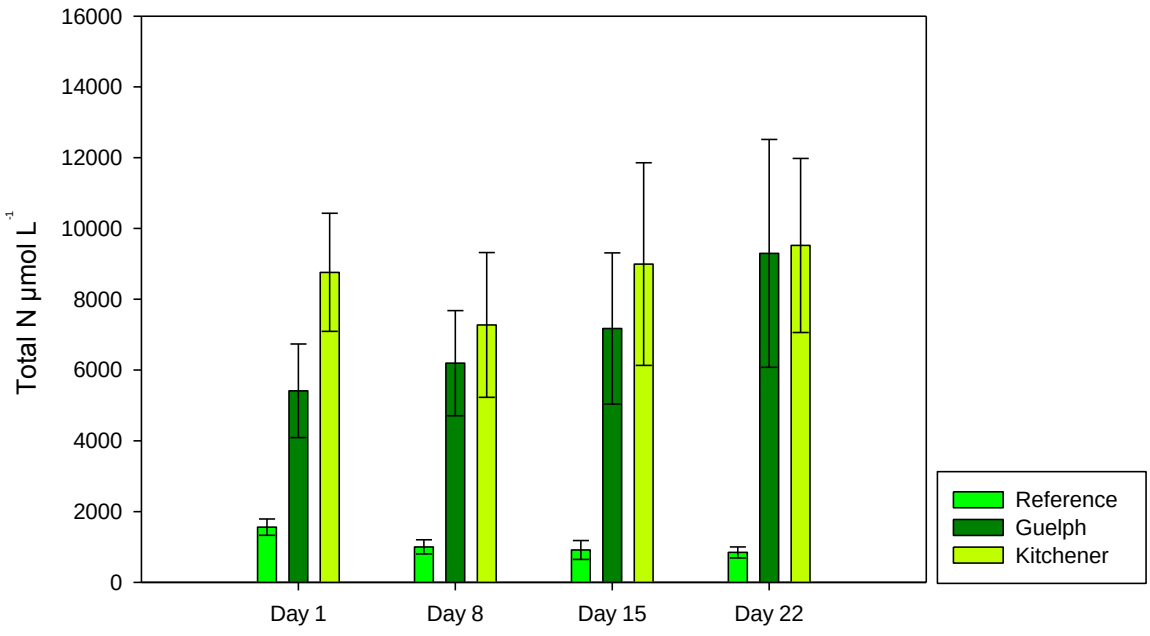

Figure 3. Concentration of TN in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes during four rain events on days 1, 8, 15 and 22.

There was a significant difference between the concentration of TN in the runoff from biosolids and reference treatments ($p<0.01$). The average concentrations of TN over the four rain simulations were highest in Kitchener biosolids runoff (8.6 mmol L^{-1}), followed by Guelph biosolids runoff (7.0 mmol L^{-1}) while reference runoff had an average of 1.1 mmol L^{-1}. In general the losses of TN in the runoff were higher in the Kitchener biosolids runoff than in Guelph runoff (Figure 3).

The TN concentrations in all treatments varied over time ($p<0.01$). Reference runoff concentrations increased slightly over the duration of the experiment. Biosolids treatments showed an increase in concentrations in both treatments, with highest concentrations of TN in the runoff on the last simulated rain event (Table 4 in Appendix A).

Total nitrogen concentrations in the runoff of this experiment were higher than those found by similar studies. In particular, Quilbé et al. (2005) found that the highest TN concentrations in biosolids treated plots averaged 18.2 mg L^{-1} (1.30 mmol L^{-1}), which is ~ 7 fold lower than the average TN concentrations in the Kitchener biosolids runoff. The discrepancy is most likely a result of differences in the initial concentration of nitrogen in biosolids, as well as the differences between soil types used. Biosolids applied in the Quilbé et al. (2005) study had a lower TN content (19 g kg^{-1}). In addition their soil contained a lower concentration of sand (37% vs. 70% in current study), and a higher concentration of clay (~24%) (soils with higher clay composition are known to better adsorb cationic chemical species such as ammonium). Ippolito et al. (2010) found that the TN concentrations from biosolids fields, varied according to application rates, while sandy soils are known for their poor ability to retain nutrients (such as NO_3^-) (Correa et al., 2005).

It is important to note that this study simulated a worst case scenario lacking a vegetative zone and simulating extreme rain events. A presence of a buffer zone has been shown to decrease the volume and concentration of nutrients in the runoff (Moss et al., 2006), while overall volume of the runoff can be affected by the incoming volume of water. In addition, the rain events were simulated manually (i.e. using a watering can), which could have played a role in the rate of particle detachment and overall concentration of nutrients in the produced runoff.

3.2.2 Ammonium

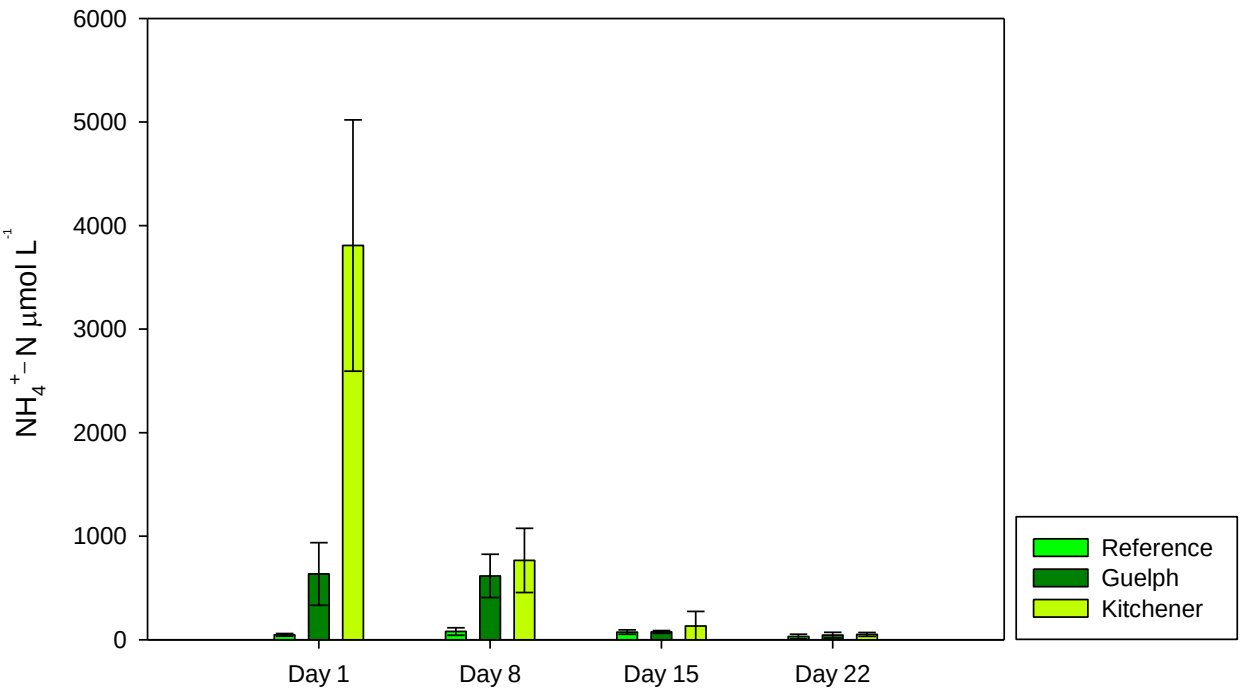

Figure 4. Concentration of NH_4^+-N in the runoff collected from reference, Guelph and Kitchener soil boxes during four rain events on days 1, 8, 15 and 22.

There was no statistical difference between the levels of ammonium nitrogen in the surface runoff and subsurface drain leachate at 0.01 confidence level (p= 0.074).

The concentration of ammonium was significantly different between biosolids and reference treatments (P<0.01). Table 5 in Appendix A outlines the average concentrations over the experimental period in all treatments. Reference runoff had an average NH_4^+-N concentration of 58 µmol L^{-1} over 4 rain events. Kitchener biosolids had average biosolids concentrations of 1.2 mmol L^{-1}, with the highest ammonium concentrations on the first rain event. Guelph biosolids runoff had an average of 0.34 mmol L^{-1}. Figure 4 further illustrates the changes in concentrations of ammonium over time.

The concentrations of ammonium nitrogen between biosolids treated and reference soil boxes were statistically different (p<0.01). The explanation for this difference is that the initial concentration of NH_4^+-N in the soil was higher in biosolids treatments, which translated into

higher losses in the runoff produced from those soil boxes. According to Gangbazo et al. (1995), in some soils receiving high fertilizer input, ammonium ions can be rapidly mobilized by runoff and leaching.

The concentrations of ammonium in the runoff of this experiment were higher than those found by similar studies. For example, Quilbé et al. (2005) found ammonium nitrogen concentrations of around 1.1 mg L^{-1} (78.6 µmol L^{-1}) which is ~3 fold lower than the average concentrations in the Guelph biosolids runoff. Similar to the TN explanation, the difference was most likely due to the soil composition, biosolids application rates and lack of vegetative buffer zone.

However, ammonium concentrations in the runoff of this study were similar to the ones shown by Mostaghimi et al. (1992) who estimated ammonium loss after application of inorganic fertilizer. After 147 kg ha^{-1} ammonium nitrate fertilization and 100 mm rain simulation, they produced ammonium concentrations of 12.8 mg L^{-1}.

The difference in ammonium concentration in the runoff between Kitchener and Guelph treatments could be partly attributed to the overall pH of the soil post application. Over time mineralization of organic nitrogen can produce ammonium, which is more likely to leach out of the soil. Mineralization process in the soil is optimized at pH 7.5<pH<8.5 (Amlinger et al. 2003). High pH can inhibit the rates of mineralization, which can therefore reduce the levels of NH_4^+ available for leaching. Since the pH of soil treated with Guelph biosolids was higher (pH 9.2) than Kitchener biosolids treated soil (pH 7.8), over time it is reasonable to expect a lower concentration of ammonium in the runoff from Guelph biosolids treated soil plots.

The concentrations of ammonium in the runoff decreased over four rain events in biosolids treated soil boxes. At the same time the concentration of nitrite and nitrate in the runoff increased (Figure 4 and Figure 5). This is consistent with the expectations. Nitrification processes within the soil oxidized a portion of ammonium to nitrate. Nitrate is more water soluble and as a result can be found in higher concentrations in the runoff than ammonia. Similar to this study Smith, Woods et al. (1998b) found that as time went on, the ammonia concentrations in their biosolids treated plots decreased and nitrate concentrations increased.

3.2.3 Nitrate + Nitrite

There was a significant difference between the nitrate (+ nitrite) concentrations in the runoff from the surface runoff and tile leachate in reference and biosolids treated soil boxes ($p<0.01$). These results are expected as nitrification rates are usually highest within the top 15 cm of the soil (Jurado-Guerra et al., 2006). A number of studies confirm this finding. The difference between surface and tile in terms of total N and nitrate was shown by Drury et al. (1996). Similarly, Downing et al. (1999) found that nitrate (+nitrite) losses were 2 fold higher in the subsurface drainage comparing to surface runoff.

In this study the difference between surface and tile is only evident in the last two runoff collection periods. The concentration between the samples in the first sampling period are not statistically significant ($p= 0.793$). As a result, the addition of runoff to the water columns was carried out in equivalent portions (50% surface runoff + 50% tile leaching). The results presented below are the average concentrations of pooled surface runoff and tile drainage (Figure 5).

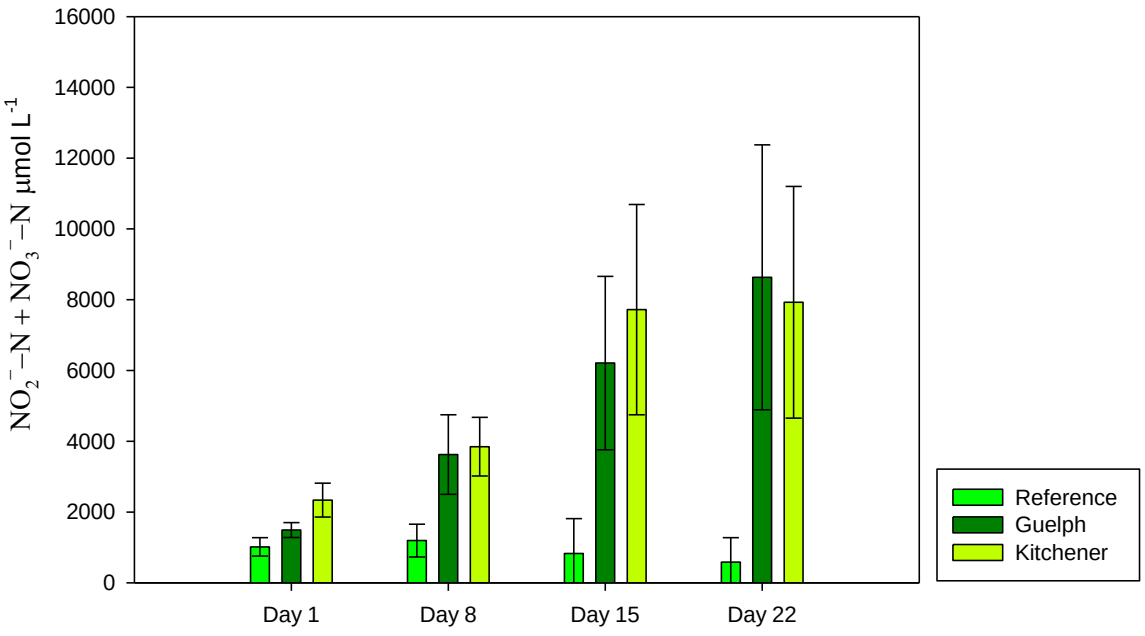

Figure 5. Concentration of NO_2^-+NO_3^- nitrogen in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes during four rain events on days 1, 8, 15 and 22.

The average concentration of nitrite+nitrate over 4 rain events in the reference runoff was 0.9 mmol L^{-1}. Kitchener biosolids had an average of 5.5 mmol $L^{-1}$and Guelph biosolids runoff had an average concentration of 5.0 mmol L^{-1}.

The concentrations of nitrate+nitrite decreased somewhat in the reference treatment, and increased substantially in the biosolids treatments over the four runoff collection periods (Table 6 in Appendix A). Overall there was around a 4 fold increase in the levels between days 1 and 22. There was no statistical difference between the two biosolids treatments ($p=0.267$). However, the concentrations of nitrate in Guelph biosolids runoff were higher than in Kitchener biosolids runoff on the last day of sampling. The apparent explanation for this discrepancy is that the rates of nitrification were different between two treatments. Nitrification in soils has also been shown to be dependent on the pH of the soil in fields fertilized with anhydrous ammonia (Kyveryga et al., 2004). Since Guelph biosolids had a relatively high pH, higher nitrification rates should be expected in the soil which was treated with these biosolids over time.

The concentrations of nitrate in the runoff were similar to the studies which measured the concentrations of this nutrient in tile drainage of agricultural fields. David et al. (1997) found runoff concentrations of 5-49 mg L^{-1} (up to 3.6 mmol L^{-1}) after fertilization with 197 kg N ha^{-1}. Cambardella et al. (1999) found nitrate concentrations in the tile drainage to be over 10 mg L^{-1} (714 µmol L^{-1}) at application rates of 51.3 kg ha^{-1} of N. Similarly, Baker & Johnson (1981) found runoff concentrations to average 20 mg L^{-1} nitrate after 100 kg N ha^{-1} fertilization rate.

The difference in concentrations of nitrite and nitrate between biosolids and reference treatments was expected. The overall concentration in the runoff was shown to be dependent on biosolids application rates in a previous study (Li, 1997), as a result of the higher quantity of nitrogen in the biosolids treatments when compared to the reference.

The increase of nitrate in the runoff over time could be attributed to mineralization of organic nitrogen to ammonia and subsequent nitrification of ammonium to nitrate in the soil and the water columns. Organic nitrogen can be rapidly mineralized in the soil increasing the pool of ammonia (Pascual et al., 1998). Since the ammonium can be rapidly nitrified to nitrate in soils that are aerated and have a high moisture content (Smith, Woods et al., 1998b) nitrification should play a significant role in the balance of nitrogen species in the runoff.

3.2.4 Organic Nitrogen

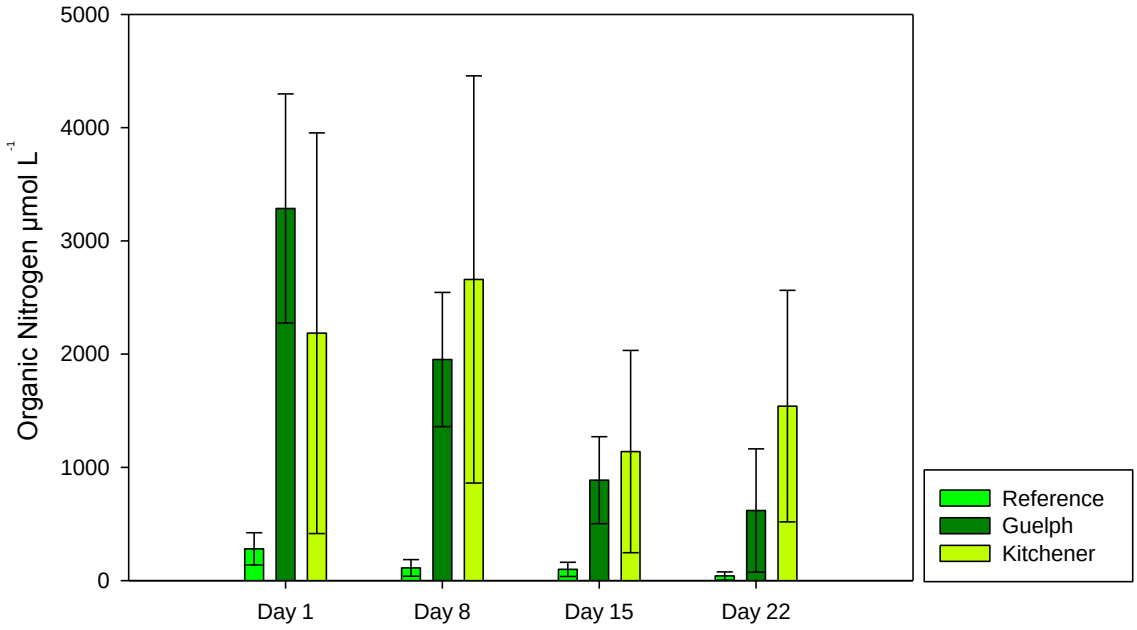

Figure 6. **Concentration of Organic Nitrogen in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes during four rain events on days 1, 8, 15 and 22.**

The levels of Organic Nitrogen (ON) between the surface runoff and tile leachate were not statistically different (p= 0.104).

The levels of organic nitrogen were statistically different between reference and biosolids treatments ($p<0.01$). The average concentrations over four sampling events were 0.13 mmol L^{-1}, 2.0 mmol L^{-1} and 1.7 mmol L^{-1} in reference, Kitchener and Guelph treatments respectively (Table 7 in Appendix A).

The concentrations changed as time progressed ($p<0.01$) in all treatments (Figure 6). The overall trend was a general decrease in concentrations of ON. Initially the concentration of ON in the runoff was highest in the Guelph biosolids treatment, however the concentrations of Kitchener treatment were higher than Guelph starting on day 8 simulated rain event. However the concentrations between the biosolids alone were not statistically different (p=0.325).

The difference in concentration of ON in reference and biosolids treatments is most likely a result of difference in initial concentration of organic matter in the soil boxes. Over time,

however, the organic nitrogen concentrations decreased most likely as a result of loss in the runoff and decomposition of organic matter. Mineralization of ON in biosolids incorporated into the soil has been shown to be as high as 38% over 6 months (He et al., 2003).

The rates of mineralization can decrease the overall pool of organic nitrogen in the soil. Different pools of organic nitrogen have been shown to have different fates in the soil. For example, biosolids produced by anaerobic digestion contain at least three pools of organic nitrogen (Smith, Woods et al., 1998b). The first pool is rapidly mineralized and nitrified to nitrate, a second pool which had slower mineralization rates than the first, and third, had very slow mineralization rates, longer than 30 days incubation period.

3.2.5 Dissolved Organic Carbon

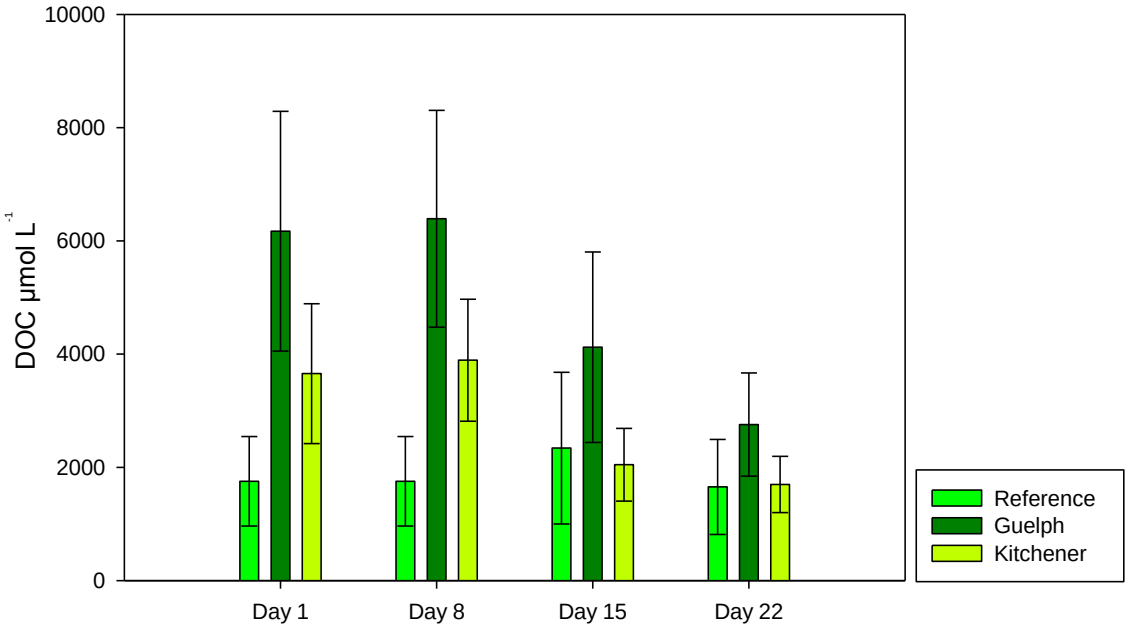

Figure 7. Concentration of Dissolved Organic Carbon in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes during four rain events on days 1, 8, 15 and 22.

There was no statistical difference between the concentrations of DOC in in the surface runoff and tile leachate at 0.01 significance level (p=0.022).

A significant difference was found between reference and biosolids amended soil boxes ($p < 0.01$) (Figure 7). The average concentration of DOC in the runoff was 1.9 mmol L^{-1}, 2.8 mmol L^{-1} and 4.9 mmol L^{-1} in reference, Kitchener and Guelph treatments respectively (see Table 8 in Appendix A).

The concentrations of DOC in the runoff were different as time progressed ($p<0.01$) (Figure 7). Highest concentrations were seen on day 15 in reference treatment (2.3 mmol L^{-1}). Highest concentrations of DOC in Guelph biosolids treatment was found on day 8 (6.4 mmol L^{-1}). Kitchener biosolids runoff was also highest on day 8 (3.9 mmol L^{-1}). The DOC concentration in biosolids treatment decreased after peaking on day 8.

The results are consistent with the expectations. Biosolids treated fields had a concentration of organic matter which were higher than the reference soil. In addition the simulated tile drainage

system in this study encouraged a high runoff volume subsequently increasing its organic carbon content (Dalzell et al., 2007). Royer et al. (2006) for example, reported concentrations ranging from 1 to 14 mg L^{-1} of organic carbon in tile drainage originating from agricultural fields. These numbers are similar to the results on the last two sampling days of the biosolids runoff and concentrations that are found in the reference runoff for the majority of the experiment.

There was a significant difference between the concentrations of DOC in the runoff of biosolids treatments ($p<0.01$). The difference could be attributed to the change in the pH as a result of biosolids application. Guelph Biosolids treated soil had a higher pH (9.2) than the reference and Kitchener counterparts (pH 7.3), probably as a result of alkali substances used during stabilization of Guelph biosolids. Soils with high pH have the potential to have up to 4 times higher concentrations of DOC than soils that are slightly acidic (Andersson et al., 1999).

It is important to study the DOC biogeochemistry because it is related to cycling of other nutrients in agricultural watersheds (Hedin et al., 1998). Of particular importance to this study is the effect of DOC on the nitrogen biogeochemical cycle. Since dissolved organic nitrogen makes up a portion of dissolved organic matter, it can be a carrier of organic nitrogen in the runoff. This portion of nitrogen can be mineralized increasing the levels of ammonium in the water column and contributing to the transformation of nitrogen species in the water.

The increase in DOC in the water can also change the bacterial trophic structure in the water column (Bott, 1984). This change can alter the chemical and physical parameters (such as decrease DO levels) in the water, which can indirectly affect various N-cycle processes (i.e. nitrification).

3.2.6 Expected Effect of Runoff on the Aquatic Mesocosms

The response of aquatic ecosystems to nitrogen loading can depend on the overall amount of nitrogen added to the system and nitrogen speciation. The runoff data indicates that the concentration of nitrogen species entering the receiving water was high in the biosolids treatments, which has the potential to alter the nitrogen balance within the freshwater columns and affect the processes within. In particular, elevated concentrations of NH_4^+-N during the first rain event has the potential to increase primary productivity within water columns. Input of nitrate has the potential to alter the rates of denitrification (by increasing the quantity of substrate) and in some cases phytoplankton production. Finally, organic nitrogen can be rapidly converted to ammonium in the hypolimnion, which can potentially increase the bioavailable pool of ammonium, further fueling phytoplankton growth. The input of organic carbon can alter the concentrations of DOC in the columns contributing to decrease in oxygen levels in the hypolimnion. A substantial source of organic carbon to the hypolimnion may also be supplied by primary producers if the colonies of phytoplankton collapse due to nutrient limitation or as a result of an increase in pH in the epilimnion.

3.3 Freshwater mesocosms, pH and Dissolved Oxygen

3.3.1 Stratification and Presence of Phytoplankton

A temperature gradient of 10^{o}C between the epilimnion and hypolimnion of the freshwater mesocosms was established within the first 2 days of the mesocosms setup. The photic layer had an average temperature of 22 ± 2 ^{o}C while the aphotic layer of the mesocosms had an average temperature of 10 ± 2 ^{o}C. The difference between the two layers is consistent with the temperature gradient in many thermally stratified lakes in Canada during the warm summer months (Atlas of Alberta Lakes, 2005).

The phytoplankton growth within the freshwater mesocosms was evident within the first week post runoff addition. As time progressed the phytoplankton production was evident upon visual examination in the photic layer of the mesocosms fertilized with biosolids runoff, reference soil runoff or inorganic fertilizer and was absent in the control mesocosms (Figures 8, 9, 10, and 11).

Figure 8. Freshwater Mesocosms on Day 0.

Figure 9. Freshwater Mesocosms on Day 10.

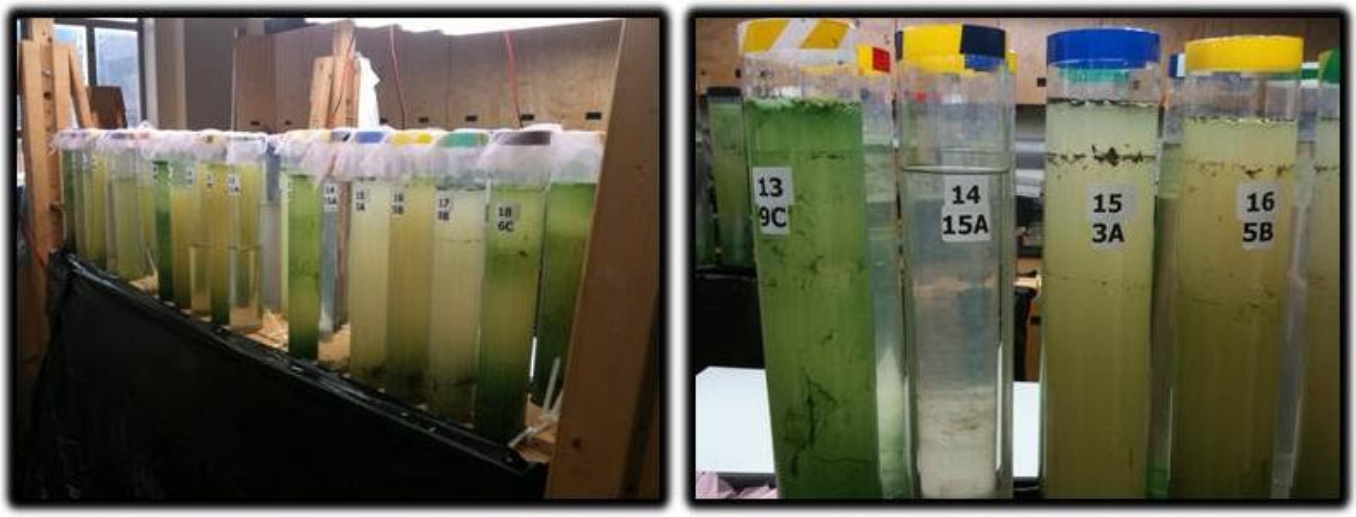

Figure 10. Freshwater Mesocosms on Day 17 (mesocosm #14 is a control blank).

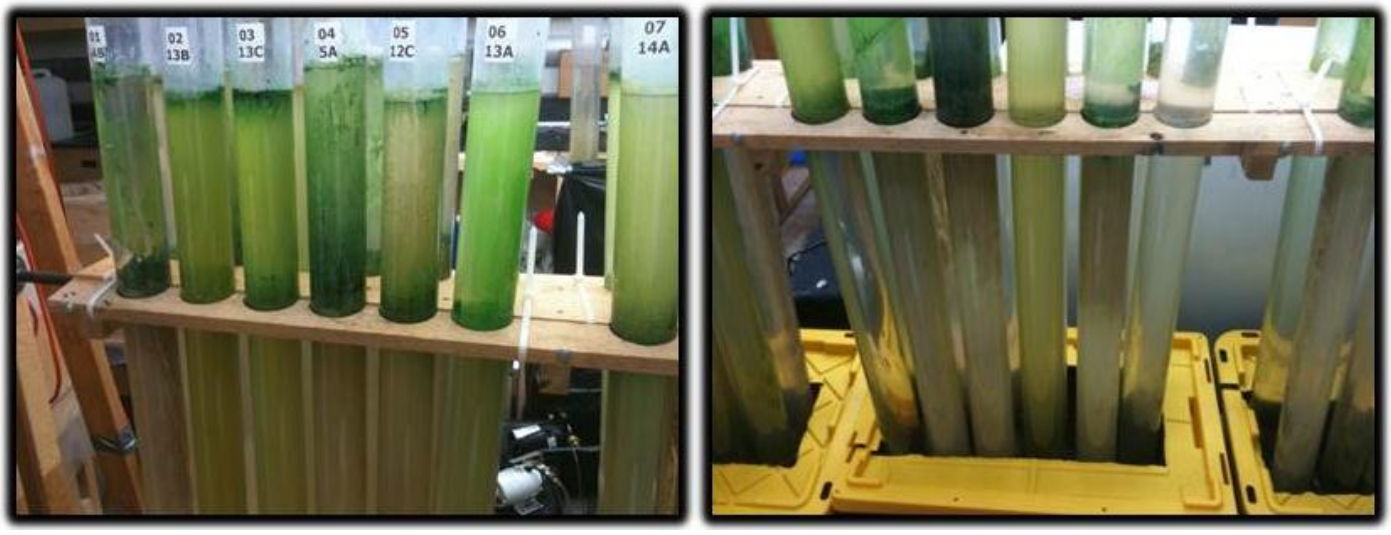

Figure 11. Freshwater Mesocosms on Day 32.

3.3.2 Dissolved Oxygen and pH

Dissolved Oxygen and pH measurements were carried out in collaboration with Aslam Hanief (Master of Molecular Science candidate, Ryerson University) on all sample collection days.

The levels of Dissolved Oxygen (DO) and pH were found to be related (the pH increased as DO increased). This result is most likely a reflection in concentrations of carbonic acid (H_2CO_3) within water columns. In the water, carbonic acid can be further broken up into H^+ and HCO_3^-. Since CO_2 is a substrate in the photosynthesis reaction, during times of elevated primary production, the concentrations of carbonic acid decrease. Reduction in concentration of carbonic acid therefore indirectly increases the pH of the system. Similarly, when photosynthesis is not carried out, the levels of carbonic acid remain the same or decrease which can lower the pH in the hypolimnion. As a result, the pH in the photic zone of many aquatic systems is basic, while the oxygen poor zones are somewhat more acidic. This section will combine the results of DO and pH since both are related to the primary productivity discussion.

A significant difference was found in the concentrations of dissolved oxygen and pH between Control, Reference and Biosolids treated mesocosms as well as the concentrations in the inorganic N and P loaded mesocosms ($p<0.01$) (Figures 12, 13, 14 and 15). Similarly the concentrations were different between all treatments as time progressed (i.e. significant time x treatment interaction) ($p<0.01$). These results were expected, as the levels of primary productivity varied among treatments due to the differences in quantity and bioavailability of nutrients.

There was a statistical difference in DO and pH levels between the epilimnia and hypolimnia in the freshwater mesocosms ($p<0.01$). The results matched the predicted expectations. The DO concentrations and pH in the epilimnion increased and remained high over the duration of the experiment as a result of photosynthesis, while the hypolimnion DO levels decreased as a result of organic matter decomposition.

3.3.2.1 Epilimnion

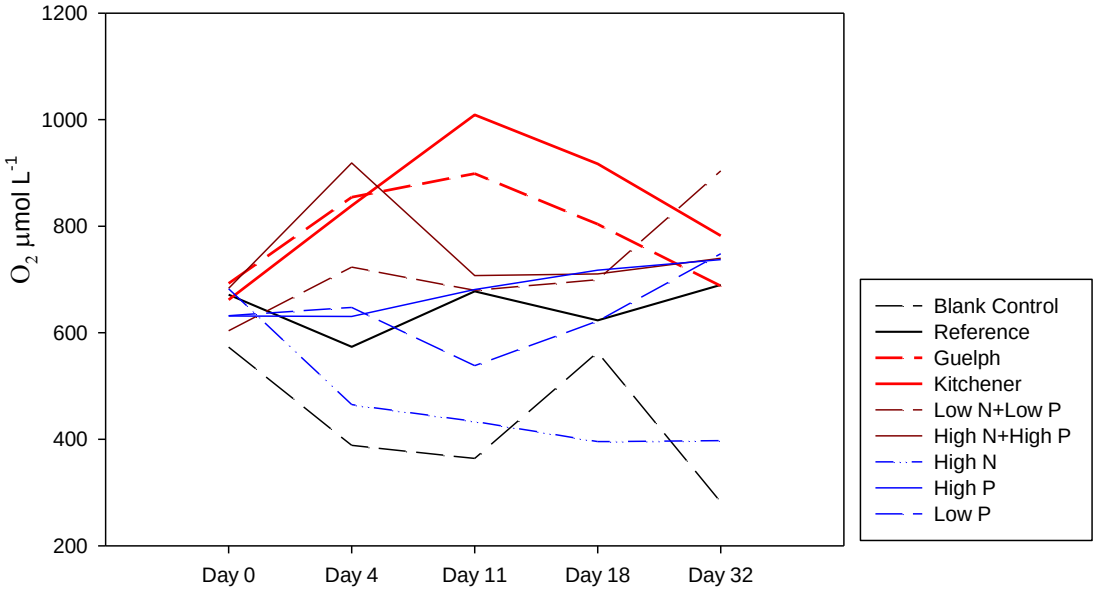

Figure 12. Concentration of Dissolved Oxygen in the epilimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

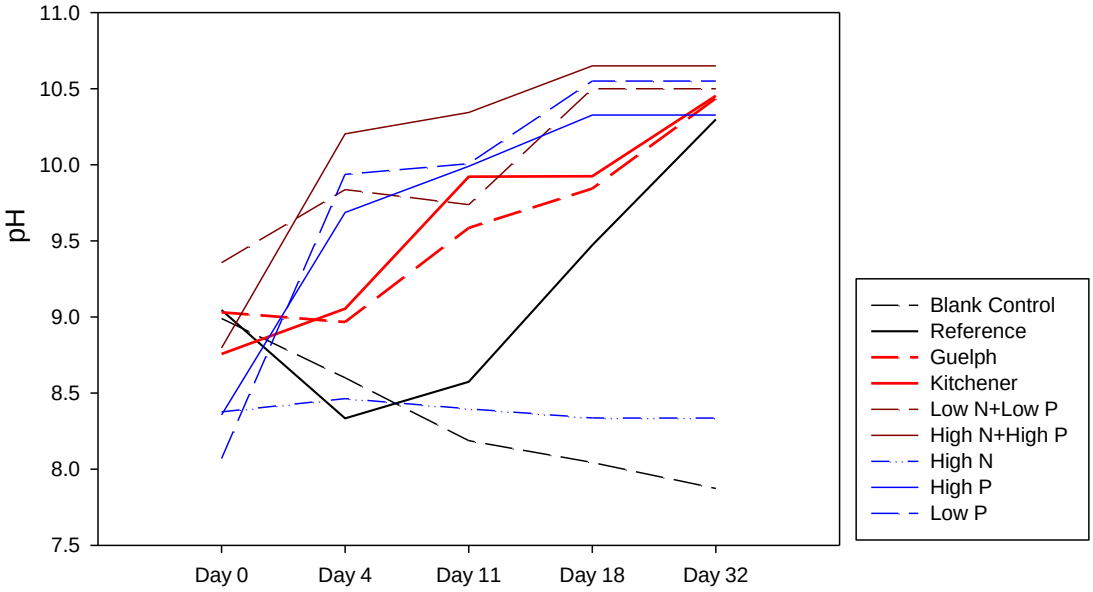

Figure 13. pH in the epilimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.3.2.1.1 Control, Reference and Biosolids Amended Mesocosms

The levels of dissolved oxygen in the epilimnia of the reference treatment fluctuated, but had an average between 573 µmol L^{-1} and 689 µmol L^{-1} over the five sample collection periods. The average DO concentrations in the biosolids runoff treated columns, showed an increase in the O_2 with highest levels in the Kitchener mesocosms (1.01 mmol L^{-1}) and 899 µmol L^{-1} in Guelph biosolids treatment.

As time progressed the DO and pH levels changed as well. Initially the levels of DO in the epilimnion increased after the addition of the runoff to biosolids treatments, peaking on day 11 and decreasing by day 32. The pH in the epilimnion continued to increase, to around 10.5 in biosolids treated mesocosms, while in the reference mesocosms the pH initially decreased, but increased to about the same levels by day 32.

The results indicate that the water in the columns was supersaturated with DO (water is saturated when DO concentrations reach 273 µmol O_2 L^{-1} at 22°C). The elevated concentration of DO in the photic zone was consistent with the fact that the rates of photosynthesis were high as a result of phytoplankton growth. In addition, elevated levels of DO are expected in narrow cylindrical water columns as the rates of convection between the air and water are low. In a real lake, convective mixing allows for more rapid re-equilibration across the air-water interface.

The levels of DO increased more in the biosolids treatments than the reference runoff, presumably because reference lacked the nutrients to support the same levels of primary productivity. The increase in pH in the reference mesocosm is somewhat surprising. The proposed explanation is that the increase in the pH was a result of presence of alkaline substances in the reference runoff, which was preventing the pH from dropping, as it did in blank control mesocosms.

The decrease in oxygen levels could also be related to the increasing pH in the epilimnion. At high pH (above 9.5), the levels of primary production decreased, as reflected by the decline of oxygen concentrations later in the experiment. The photosynthesis rates can be affected by low levels of dissolved inorganic carbon and high pH in lentic systems (Hein, 1997; Invers et al., 1997).

3.3.2.1.2 Inorganic N and P Amended Mesocosms

The concentration of DO in the epilimnion of all treatments except for High N increased three days after the addition of nutrients, decreased on day 11 and increased again on the last sampling day. DO concentrations in the High N treatment rapidly decreased and remained low for the duration of the experiment. Similarly, The pH of all inorganic nutrient amendments, except for High N treatment increased to around 10.5 over 32 days. High N treatment remained the same with an average pH of 8.5.

In general, the DO concentrations in inorganically amended mesocosms were lower than in mesocosms treated with biosolids runoff. In addition, there was a greater fluctuation in the levels of DO. The changes in the DO concentrations were most likely due to decrease in bioavailable nutrients in the water, which could cause the colonies of phytoplankton to collapse, decreasing the rates of photosynthesis. This is particularly evident on day 11, when the levels of oxygen dropped in all inorganic nutrient mesocosms except for High P treatment.

Inorganic N and P amended mesocosms had a somewhat faster increase in pH than the biosolids treatments. The pH increase in the biosolids treatments was most likely dampened by some other underlying mechanism (such as buffering) which was present in the incoming runoff. In addition, the growth of phytoplankton has been associated with increase in the dissolved organic carbon which has been shown to increase the alkalinity of some water systems (Kim & Lee, 2009). Even though phytoplankton enumeration has not the focus of this part of the study, the growth of primary producers responded more quickly to the inorganic N and P treatments (Aslam Hanief, chlorophyll data). It is therefore hypothesised that the pH increase in the epilimnion is a response to the increase in DOC production by the higher numbers of phytoplankton in the inorganic N and P treated mesocosms.

3.3.2.2 Hypolimnion

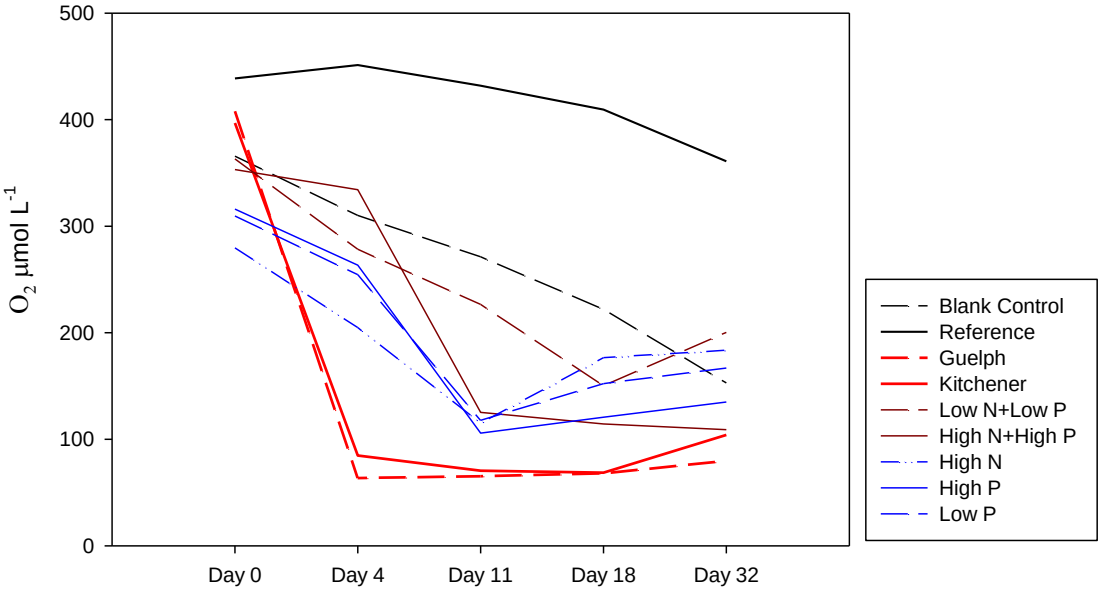

Figure 14. Concentrations of Dissolved Oxygen in the hypolimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

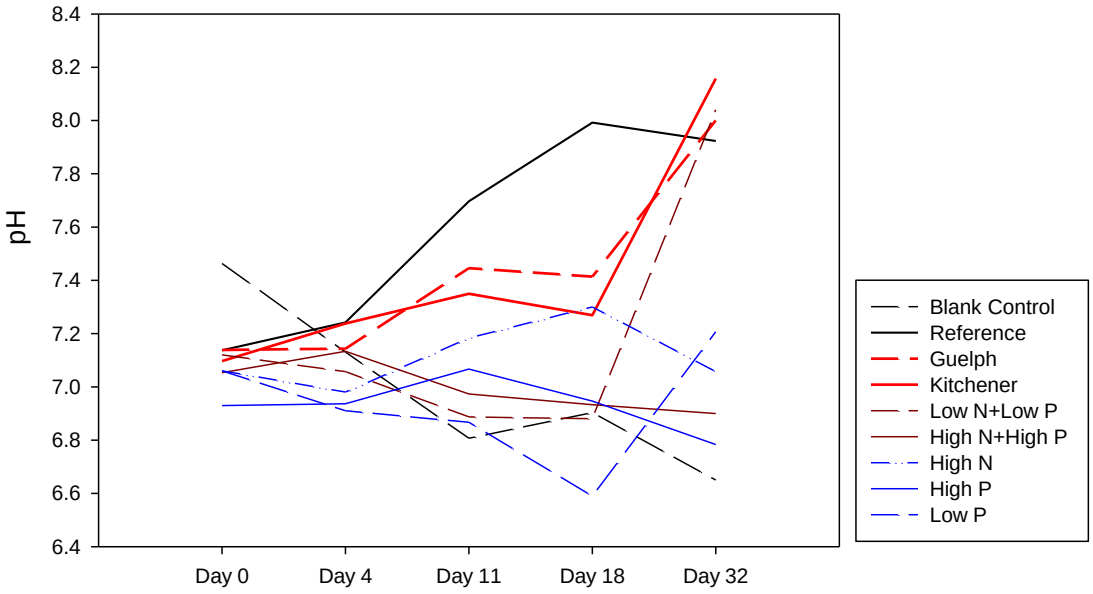

Figure 15. pH in the hypolimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.3.2.2.1 Control, Reference and Biosolids Amended Mesocosms

The DO levels in the hypolimnion rapidly decreased in the biosolids treated mesocosms to levels below 100 µmol L^{-1} and remained low for the duration of the experiment (Figure 11). The concentrations in blank control and reference treatments did not follow the same pattern. Reference treatment did not decrease to the same levels, but instead had average concentrations of around 430 µmol L^{-1} DO. Control treatment decreased at a faster rate and dropped to lower levels (less than 100 µmol L^{-1}) than reference treatment.

The pH of the biosolids treatment increased slowly until day 18 and more rapidly over the 2 weeks between day 18 and day 32 (highest pH 8.2). Reference mesocosms increased to comparable levels; however the overall rate of increase was higher. Blank control mesocosm dropped in pH over the duration of the experiment, with lowest pH of 6.6.

The increase in pH in the treatments is somewhat puzzling, but could have happened as a result of increase in DOC within the water column, which could have potentially dampened the pH drop in the mesocosms.

The decrease in DO in the hypolimnia of freshwater mesocosms in this study was similar to the concentrations found in small eutrophic thermally stratified lakes (Wilhelm & Adrian, 2008), which have been shown to have concentrations <2 mg L^{-1}, affecting up to 25% of the lake. A rapid decrease in the DO levels in the biosolids treatment was most likely a result of an increase in the organic matter content in the hypolimnion of the mesocosms caused by the addition of biosolids runoff. Breakdown of organic matter by the decomposing bacteria can cause the DO concentrations in the water to decrease (Beutel, 2001). In addition, DO is used up during nitrification of ammonium to nitrate. Since the concentration of organic matter and ammonium was lower in the reference runoff, the DO did not decrease as quickly and to the same levels as in the biosolids treated mesocosms.

3.3.2.2.2 Inorganic N and P Amended Mesocosms

The DO concentrations in the mesocosms loaded with inorganic forms of nitrogen and/or phosphorus did not decrease as quickly as in biosolids treatments. The effect is most likely due to the low levels of organic matter in the inorganic N and P mesocosms. Over time however, the levels of primary producers increased, increasing the pool of organic matter in the hypolimnion, causing the DO to drop to the levels similar to those in biosolids treatments.

The pH of the inorganically amended mesocosms did not increase to the same levels as in biosolids treated mesocosms and remained neutral for the major part of the experiment. Low N + Low P treatment and Low P treatments showed an increase in the pH between days 18 and 32, when the nutrient loading was stopped.

3.3.2.3 Biosolids and Inorganic N and P Loaded Analogs

The concentrations of inorganic N + P added to the mesocosms were analogous to the concentrations of these nutrients in the Kitchener (High N+High P) and Guelph (Low N+Low P) biosolids runoff. By comparing the effects of these nutrients alone or when mixed with other nutrients found in biosolids runoff it can be established whether the response is mostly due to the concentrations of limiting nutrients or other chemical constituents found in the biosolids runoff.

The DO concentrations were not significantly different between the Kitchener biosolids and High N+High P treatments ($p=0.562$), and Guelph and Low N+Low P treatments ($p=0.530$). This data implies that the effect of runoff addition on DO was primarily a response to the limiting nutrients added to the freshwater mesocosms.

3.3.2.4 Ecological significance

DO concentrations of below 2 mg L^{-1} are considered to be dangerous to most oxygen breathing organisms in the water. The decrease in DO concentrations in the freshwater mesocosms in this study was most likely a response to the organic matter concentrations. Organic matter which is produced as a result of overgrowth of primary producers can sink to the bottom, and use up available oxygen as it is decomposed (Blann et al., 2009).

The increase in the pH of the epilimnion has been shown to limit the amount of primary productivity in some lakes by negatively affecting photosynthesis rates (Humphrey, 1975). In addition, high pH has also been also shown to alter the community composition in some lakes, favouring cyanobacteria over algal species (Unrein, 2010). As a result, pH can indirectly affect water quality and trophic structures of lakes.

3.4 Concentration of Dissolved Organic Carbon, Organic Nitrogen, Ammonium and Nitrate in the Freshwater Mesocosms

The concentrations of nutrients in mesocosms that were subjected to the same treatment showed a high variability in nutrient levels. The variability was likely caused by the differences in the concentration of nitrogen in the runoff which was added to the mesocosms. Variability in nitrogen input has been shown to affect the rates and efficiency of N-cycling (Laursen et al., 2004). In addition, the rates of nutrient assimilation by primary producers can determine the overall levels of nutrients in the water column. In some instances the levels inorganic nutrients can fluctuate within water systems when they are sampled on a small scale (McCarthy & Goldman, 1979).

3.4.1 Dissolved Organic Carbon

The concentrations of dissolved organic carbon (DOC) were statistically different between all treatments ($p<0.01$). The concentrations were also different over time ($p<0.01$) (Figure 16). There were two major sources of DOC in the aquatic mesocosms. The first was from the runoff originating from soil boxes and second was internally generated from lysing of phytoplankton cells. The major sink of DOC in the water column was due to consumption by bacteria (through assimilation and respiration).

No statistical difference was found between the concentration of DOC in the epilimnia and the hypolimnia of freshwater mesocosms at 0.01 significance ($p= 0.058$). The explanation for this result is that the rate of organic matter breakdown by detritus bacteria was most likely uniform throughout the column, and thermal stratification did not play a significant role on the rates of organic carbon processing. Since there was no statistical difference between the mesocosms strata, the data presented in this section are pooled concentrations from the entire water columns.

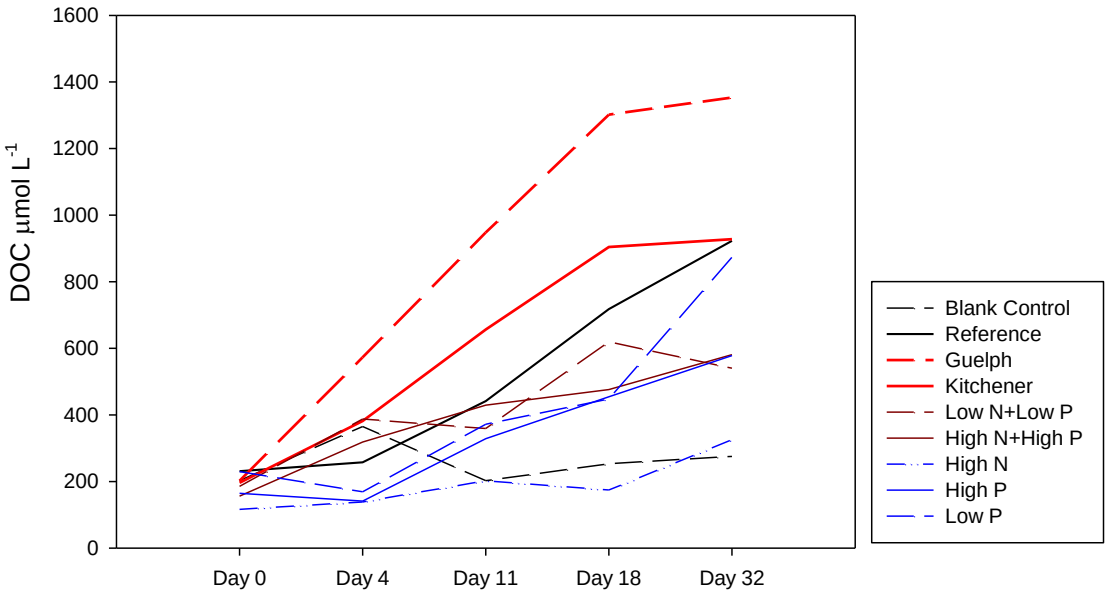

Figure 16. Concentrations of Dissolved Organic Carbon in the Control, Reference, Kitchener and Guelph biosolids and inorganic N and P loaded treatments mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.1.1 Control, Reference and Biosolids Mesocosms

The concentration of DOC in Control Blank mesocosms remained the same over the duration of the experiment, and had the highest value of 298 µmol L^{-1} on day 4. The concentrations in reference and biosolids treatments were highest on the last day of sampling with values of 841 µmol L^{-1}, 842 µmol L^{-1} and 1.34 mmol L^{-1} in reference, Kitchener and Guelph treatments respectively (Table 9 in Appendix A).

As time progressed there was an increase in the levels of DOC in the reference, Kitchener and Guelph biosolids. The increase was more pronounced during the four sampling periods and did not change very much between days 17 and 32 in the biosolids treatments.

The results are consistent with the expectations. Many lakes in Ontario have DOC concentrations between 1.8 and 5 mg L^{-1} (Dillon & Molot, 1997). In eutrophic lakes however, the concentrations of DOC can fluctuate between 12 and 14 mg L^{-1} (1000 µmol L^{-1}) (Sondergaard, et al. 1995). This study showed an increase in the DOC to eutrophic levels in biosolids treatments on the last two sampling days (Figure 16).The concentrations of DOC in the

reference runoff was similar to the Kitchener treatment on the last sampling day. It is therefore possible that the erosion of minerals from reference soil may have a similar impact on eutrophication (as measured by organic carbon content in the water system) as biosolids application when you consider that reference mesocosms were more similar to biosolids treatments than to inorganic nutrient loaded treatments (assuming all nutrients are bioavailable and should result in production of carbon).

3.4.1.2 Inorganic N and P Amended Mesocosms

The concentration of DOC in the inorganic N and P loaded mesocosms was lower than the biosolids, with the highest concentration in Low P mesocosm (553 µmol L^{-1}) (Table 9 in Appendix A). High P mesocosms had the highest levels of DOC on day 4 (425 µmol L^{-1}). The concentrations in the High N treatment were most similar to the blank control treatment, with highest concentrations on the final sampling day (369 µmol L^{-1}). DOC in High N+High P and Low N+Low P treatments increased as time progressed, with peak levels of 472 µmol L^{-1} and 485 µmol L^{-1} respectively.

The results indicate that the organic carbon production was as high or higher in mesocosms which were treated with P alone when compared to mesocosms with the addition of both nutrients. The effect was most likely due to the differences in the community of primary producers that were formed as a result of nutrient addition. Nitrogen poor environments (such as High P and Low P mesocosms) can offer a competitive advantage to nitrogen fixing cyanobacteria, which have faster growth rates than algae. In nitrogen rich environments, such as High N+High P and Low N+Low P treatments it is expected to see a community dominated by algae, which generally have slower growth rates. The concentration of DOC in these treatments is therefore a reflection of biomass produced by primary producers as a result of nutrient addition.

The increase in DOC is caused by a combination of the input of organic matter from agricultural runoff (Royer & David, 2005) as well as lysis and decomposition of phytoplankton cells within the water column (Mulholland & Hill, 1997). Over time the DOC in this study increased as a response to these processes.

3.4.1.3 Biosolids and Inorganic N and P Loaded Analogs

The concentrations of DOC were statistically different between the Kitchener Biosolids treatment and High N+High P mesocosms and Guelph vs. Low N+Low P treatments ($p<0.01$). This result meets the expectations, as the inorganic nutrient loaded mesocosms had a lower organic carbon input than the columns treated with biosolids.

3.4.1.4 Ecological Significance

DOC is a primary energy source for bacteria and is therefore important in supporting bacterial metabolism within the water column (Bott et al. 1984). One of the major sources of DOC in the water includes release of carbohydrates and amino acids during cell lysis with phytoplankton being one of the major sources of this material (Ittekkot, 1982; Mulholland & Hill, 1997).

Concentration of organic carbon in the ecosystem is also one of the measurements of eutrophication. In general the greater the amount of organic carbon in the system the more eutrophied the water system is (Nixon, 2009). The concentrations of dissolved carbon in the water can therefore be used as an indicator of water quality as it can provide an estimate of decomposing organic matter in the water. If present in elevated concentrations DOC in drinking water can be harmful to human health as it can react with chlorine during drinking water disinfection process and produce potentially harmful by-products (i.e. trihalomethanes) (Imai et al., 2003). In addition DOC originating from non-point sources can be a carrier of heavy metals and organic pollutants into the receiving water systems (Chiou et al., 1986; Schuster et al., 2008).

3.4.2 Organic Nitrogen

The concentration of organic nitrogen (ON) was statistically different between all treatments and as time progressed ($p<0.01$) (Figure 17 and Figure 18). There were two major sources of organic nitrogen in the freshwater mesocosms. The first was from the runoff originating from soil boxes and second from internal production of biomass (i.e. amino acids) as a result of primary productivity and bacterial growth. The major sinks of organic nitrogen were due to descending of organic matter from the epilimnia to the hypolimnia of freshwater mesocosms and mineralization to ammonium in the hypolimnion.

The concentration of organic nitrogen was significantly different between the epilimnia and hypolimnia of the freshwater mesocosms ($p<0.01$). This result is consistent with the expectations, since the biological processes happening in these layers are different.

3.4.2.1 Epilimnion

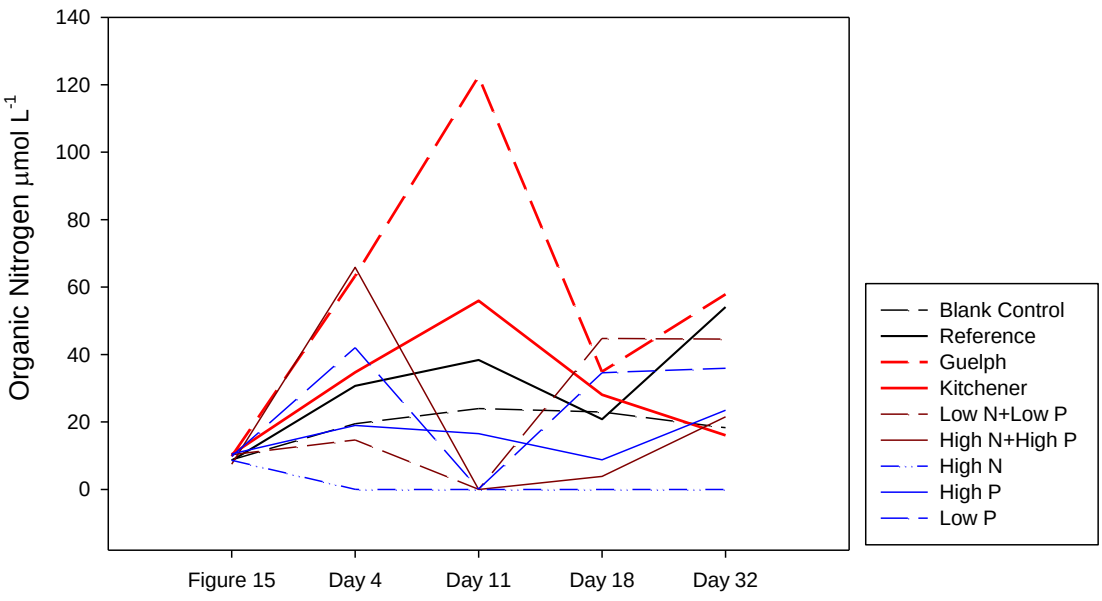

Figure 17. Concentrations of Organic Nitrogen in the epilimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.2.1.1 Control, Reference and Biosolids Amended Mesocosms

The concentrations of ON in control and reference treatments were low (<24 µmol L^{-1} and <54 µmol L^{-1}). The highest concentrations of ON in the biosolids mesocosms were 56 µmol L^{-1} in Kitchener and 122 µmol L^{-1} in Guelph treatments on day 11 (Figure 17).

Over time the concentrations of ON in the epilimnion remained roughly the same in control treatment, and fluctuated in reference and biosolids treatments. The concentration of organic nitrogen increased until day 11 and decreased on day 18. The concentration increased again in the reference and Guelph treatments on day 32. Kitchener treatment followed a similar trend, but did not increase on day 32. The average concentrations of all treatments are presented in Table 10 in Appendix A.

The results are consistent with the expectations. The pool of organic matter was increased directly by the addition of the runoff, and indirectly by the increase in primary productivity in the photic layer. As a result the concentrations of organic nitrogen also increased.

Unlike reference runoff, biosolids runoff contained high levels of organic matter, which most likely accumulated in the hypolimnion after runoff addition. The increase in nutrient loading also increased the levels of primary productivity in the photic zone of the columns. As the levels of nutrients needed for phytoplankton reproduction and growth decreased, the colonies of primary producers died off and descended to the bottom. The highest levels of primary productivity occurred on Day 11, which coincided with the highest levels of DO in the biosolids columns. A week later the ON levels decreased which is reflected in the DO concentrations as well.

The decrease in ON can also be attributed to the increase in the pH of the system. As pH increased, it could have limited the phytoplankton growth by inhibiting enzyme function, and negatively affecting the cellular reactions within phytoplankton cells. This could cause the cells to die and sink to the hypolimnion.

3.4.2.1.2 Inorganic N and P Amended Mesocosms

The concentration of ON in inorganic N and P treated mesocosms had the highest concentration in the High N+High P treatment (65 µmol L^{-1}), followed by Low N+Low P (44 µmol L^{-1}) Low P (42 µmol L^{-1}) and High P (42 µmol L^{-1}) treatments. The concentrations of ON

in the High N treatment decreased to below detection limits following N-loading. The ON in the remaining treatments fluctuated over time (see Table 10 in Appendix A).

The concentrations of ON in the inorganic nitrogen treated mesocosms decreased to below detection limits on day 11. The decrease was most likely a result of a drop in the concentration of phytoplankton biomass. As the primary producers died off they sank to the hypolimnion of the water columns. The High P treatment did not undergo the same collapse, with concentrations of ON remaining at 18 µmol L^{-1} on day 11. The concentrations of organic nitrogen in the epilimnion of inorganic N and P loaded mesocosms were most likely primarily controlled by the levels of bioavailable phosphorus in the water column.

The concentrations of ON in the columns can be used as indicators of primary productivity. The organic nitrogen concentrations increased most drastically in the High N High P treatment which is consistent with the expectations.

3.4.2.2 Hypolimnion

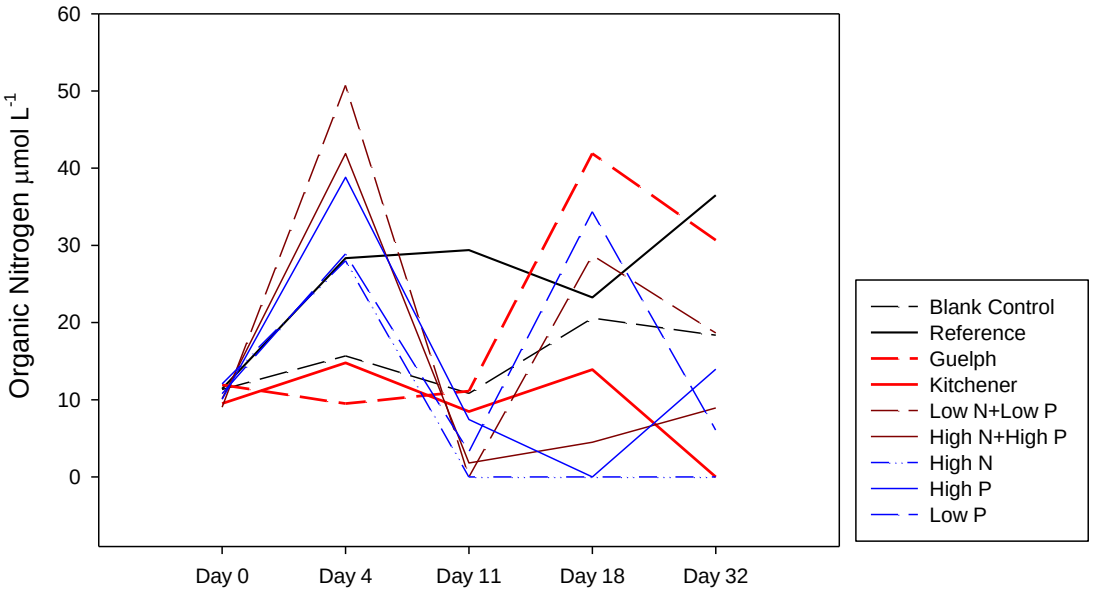

Figure 18. Concentrations of Organic Nitrogen in the hypolimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P loaded treatments mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.2.2.1 Control, Reference and Biosolids Amended Mesocosms

The highest concentration of organic nitrogen in the hypolimnion of the Blank Control was 21 µmol L^{-1}. Reference treatment peaked at 37 µmol L^{-1} on the final day of sampling, while biosolids treatment had highest concentrations of 15 µmol L^{-1} and 42 µmol L^{-1} in Kitchener and Guelph treatments respectively (Figure 18).

The concentrations of ON in the mesocosms fluctuated over time. Reference treatment increased as time went on, to highest concentrations on day 32. Similarly, Kitchener treatment increased until the final sampling period. Guelph biosolids increased until day 18 and decreased slightly on day 32.

Since a portion of the biosolids runoff contained elevated levels of organic nitrogen, it was expected to see higher levels of this nutrient in the hypolimnion as a result of organic matter settling. The result showed that the incoming organic nitrogen was rapidly mineralized to ammonium in the hypolimnion of the water columns, as indicated by a decrease in the levels of organic nitrogen and increase in the pool of ammonia over the same sampling periods. Similar to this study, Wilhelm & Adrian (2008) found that during stratification events the concentrations of inorganic nitrogen in the hypolimnion was up to 10 times higher than in epilimnion in a eutrophied lake that they studied.

3.4.2.2.2 Inorganic N and P Amended Mesocosms

Table 11 in the Appendix A outlines the concentrations of ON in inorganic N and P treated mesocosms over the duration of the experiment. The highest concentration of ON in the inorganic N and P loaded mesocosms was found in the High P treatment (44 µmol L^{-1}) on day 4. The concentration in the Low P treatment was 34 µmol L^{-1}. Low N + Low P and High N + High P treatments peaked at 39 µmol L^{-1} and 25 µmol L^{-1} respectively. High N treatment increased initially, but the levels decreased to below detection limits on day 11, and remained low for the remainder of the experiment.

3.4.2.3 Biosolids and Inorganic N and P Loaded Analogs

There was no statistical difference between the columns loaded with Kitchener biosolids and columns loaded with equivalent concentration of nitrogen and phosphorus (High N+High P) ($p=0.297$). However there was a statistical difference between Guelph biosolids and Low N+

Low P treatment ($p=0.145$). This finding indicates that the levels of organic N present in the columns were primarily a reflection of a balance between primary productivity in the photic zone and mineralization in the hypolimnion. In addition it implies that the additional input of organic matter from the runoff did not significantly affect the concentration of this nutrient in the columns.

3.4.3 Ammonium Nitrogen

The concentrations of ammonium in the water column were found to be statistically different between treatments ($p<0.01$). The concentrations were also different as time went on ($p<0.01$) (Figures 19 and 20). The major sources of ammonium included the input as a result of runoff addition and mineralization of organic nitrogen in the hypolimnia of freshwater mesocosms. The major sinks of ammonium were assimilation by primary producers and nitrification to nitrate.

The concentration of ammonium was different between epilimnion and hypolimnion ($p<0.01$). Overall, the concentrations of ammonium were higher in the hypolimnia of aquatic mesocosms, presumably as a result of rapid mineralization of organic matter within that layer. On the other hand, the concentrations were kept low in the epilimnia probably as a result of uptake by primary producers.

3.4.3.1 Epilimnion

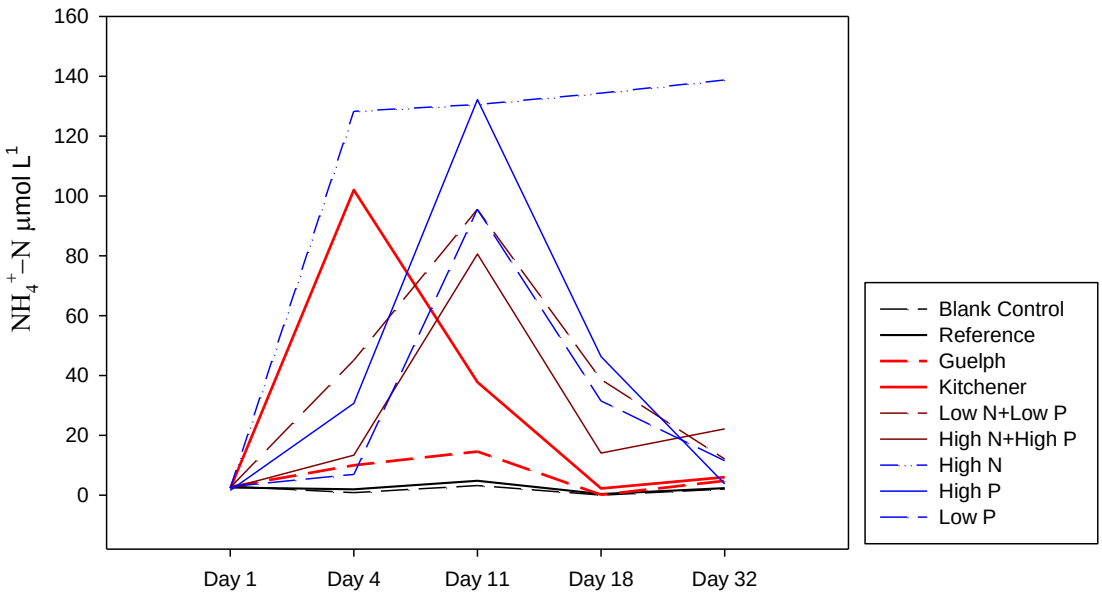

Figure 19. Concentrations of NH_{4+} nitrogen in the epilimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P loaded treatments mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.3.1.1 Control, Reference and Biosolids Amended Mesocosms

There was a significant difference between the ammonium nitrogen levels in control, reference and biosolids runoff treated mesocosms ($p<0.01$). Control treatment showed low levels of ammonia in the epilimnion (<4 µmol L^{-1}). The results were similar to reference treatment, which had ammonia concentrations < 5 µmol L^{-1} on five sampling days. The highest concentration of NH_4^+-N was found in the Kitchener biosolids treatment 3 day after first runoff addition with values of 102 µmol L^{-1}. The runoff from Guelph biosolids runoff reached highest levels on the third sampling period, with NH_4^+-N concentrations of 15 µmol L^{-1} (for complete data see Table 12 in Appendix A).

As time progressed, the concentration of ammonium in control and reference mesocosm remained low (most were <5 µmol L^{-1}), while the biosolids treatments increased after the first two rain events and then decreased on the subsequent sampling days.

The differences in concentration of ammonium in the mesocosms were correlated with the concentrations of ammonium in the incoming runoff. On days when the quantity of ammonium addition was higher, the amount remaining in the mesocosms three days after the addition was also elevated (Figure 19). As a general trend, however the NH_4^+-N levels decreased or remained low as time progressed which is consistent with the expectations, as the majority of ammonia in the epilimnion was either taken up by primary producers or nitrified.

3.4.3.1.2 Inorganic N and P Amended Mesocosms

The concentrations of ammonium nitrogen in inorganic nutrient loaded mesocosms were statistically different between all inorganic N and P treatments ($p<0.01$). The highest levels of ammonium in the columns of Low N + Low P, High N + High P, High P and Low P were 96 µmol L^{-1}, 81 µmol L^{-1}, 132 µmol L^{-1} and 101 µmol L^{-1} respectively on day 11.

The concentrations of ammonium in the columns were shown to be statistically different as time progressed ($p<0.01$). In all treatments except for High N, the concentrations peaked around day 11, and decreased until day 32 (Figure 19). High N treatment showed a steady increase in concentrations over the duration of the experiment, with highest levels on the last day of the experiment (139 µmol L^{-1}).

The majority of ammonium in the high N treatment accumulated in the water column, while ammonium concentrations in phosphorus only treatments were as high as or higher than the concentrations in biosolids mesocosms. These results point to possible phosphorus limitation in the mesocosms. When present without phosphorus, nitrogen alone was not being used by organisms and the nitrogen deficit in the phosphorus only columns was likely made up for by nitrogen fixation, carried out in the photic zone.

At some sampling points the concentrations of ammonium nitrogen in Low N+Low P were higher than the High N+High P treatments, however overall there was no statistical difference between the ammonium concentrations in these treatments ($p= 0.659$). The difference is most likely due to the nutrient uptake in the mesocosms. When present with higher concentrations of phosphorus the overall levels of ammonium are lower (since it is being uptaken by primary producers along with bioavailable phosphorus).

3.4.3.2 Hypolimnion

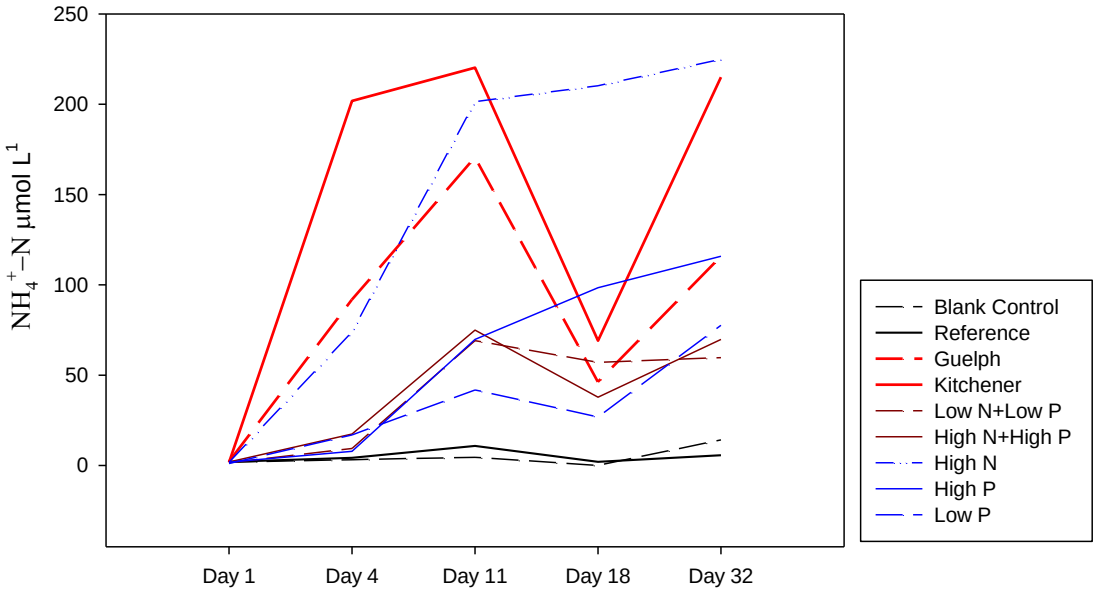

Figure 20. Concentrations of NH_{4+} nitrogen in the hypolimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P loaded treatments mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.3.2 .1 Control, Reference and Biosolids Amended Mesocosms

There was a significant difference between the levels of NH_4^+-N in control, reference, Guelph and Kitchener biosolids runoff amended mesocosms ($p<0.01$). The control and reference treatments in general had low concentrations of dissolved ammonium (<14 µmol L^{-1}). Biosolids treated mesocosms contained high concentrations of ammonia for most of the duration of the experiment. Kitchener biosolids mesocosms had the highest concentrations of 220 µmol L^{-1} on day 11, with concentrations on days 4, 11 and 32 over 200 µmol L^{-1}. Guelph mesocosms ammonium concentrations were highest on day 11 (171 µmol L^{-1}).

As time progressed the concentrations of the control and reference mesocosms were low, while those in biosolids treated mesocosms increased rapidly until sampling day 18, when the levels of ammonia dropped by around 30%. The levels increased again on the day 32 of sampling, by almost the same amount (Table 13 in Appendix A).

Average concentrations of ammonium nitrogen in the hypolimnion were a factor of 2 higher in reference, while in biosolids treatment, the concentration was as high as 5 and 14 times the levels found in the epilimnion. The difference between treatments is most likely a result of differences in loading rates and species composition in the incoming runoff. Biosolids runoff contained higher levels of ammonium, as well as organic nitrogen, which settled in the hypolimnion, where it was rapidly mineralized to ammonium. Mineralization rates were found to be high in the biosolids treated mesocosms, with majority of organic nitrogen mineralized 3 days after runoff addition.

Primary production occurring in the epilimnia served as an additional source of ammonium to the hypolimnia. It is hypothesised that the majority of the ammonium in the epilimnion was uptaken by the primary producers, which is consistent with the increase in organic matter in the photic layer, as a response to ammonia input. When the primary producers died they sank to the hypolimnion, where they were rapidly mineralized, increasing the pools of ammonia within. This effect is seen on days 11 and 32 in the biosolids treatments. The bioavailable ammonia was then either nitrified, or re-assimilated by the primary producers in the photic layer as seen on day 18 of sampling.

The ammonium accumulation in the hypolimnion is related to the low DO levels in that layer. Beutel (2001) for example showed that in eutrophic Walker Lake the rates of oxygen decrease were negatively correlated with the increase in ammonium nitrogen. Similarly, Hohener & Gachter (1994) showed that ammonium concentrations in a lake decreased from 6 to 13 mg-N m^{-2} d^{-1} to below 2, after the oxygenation of the hypolimnion in Lake Sempach, Switzerland. These effects are most likely a result of increase in the rates of nitrification when the DO oxygen levels in the water are restored, since nitrification has been shown to be negatively affected by low oxygen conditions (Zhou, 2007).

3.4.3.2 .2 Inorganic N and P Amended Mesocosms

The concentrations of ammonium in inorganically loaded mesocosms were highest in the High N loaded mesocosms (225 µmol L^{-1} on day 32). The concentration of High N+High P and Low N+Low P treatments were 78 µmol $L^{-1}$and 75 µmol L^{-1}, while the High P and Low P ammonium concentrations were 98 µmol $L^{-1}$and 83 µmol L^{-1} respectively.

Over time, the concentrations in High N treatment increased substantially. The concentrations in the Nitrogen + Phosphorus treatments fluctuated with highest levels seen on the last day of sampling, in Low N+ Low P treatment and day 11 in High N+High P treatment. Phosphorus alone treatments fluctuated over time, with peak on day 18 and day 32 in High P and Low P treatments.

The levels of ammonium in inorganically treated mesocosms were generally lower than in biosolids treated columns. In addition, unlike in biosolids treatments, the difference between the levels of ammonia in the hypolimnion and epilimnion of inorganically treated mesocosms were not statistically different ($p=0.076$) when considering inorganic treatments alone. The difference in quantity and distribution of ammonium in the water columns can be attributed to the overall quantity and mineralization rates of organic nitrogen in the hypolimnion of biosolids treatments.

3.4.3.3 Biosolids and Inorganic N and P Loaded Analogs

The ammonium concentrations were different between the biosolids treated columns and columns treated with equivalent amount of inorganic N + P ($p<0.01$). The biggest difference was between Kitchener ammonium concentrations on day 4, which was nearly 10 fold higher than in the High N+High P treatment. The Low N+Low P treatment showed ammonium levels that were higher than peak Guelph levels, however the peak occurred on the same sampling day (day 11). The difference is most likely a result of rapid mineralization of organic nitrogen in the hypolimnion over time, which caused higher levels of ammonium accumulation. In addition the ammonium was not converted to nitrate, since the levels of DO were lower in the biosolids runoff treated mesocosms for the duration of the experiment.

3.4.3.4 Ecological Significance

The concentration of ammonia in the water of many lakes is of primary ecological importance as it can be toxic to freshwater organisms in concentrations as low as 30 μmol L^{-1}. The toxicity is dependent on the pH of the system (low pH increases toxicity) and temperature (colder temperatures increase toxicity) (Eddy, 2005).

In the water ammonia breaks apart into ammonium and hydroxide ion:

$$NH_3 + H_2O \Longleftrightarrow NH_4^+ + OH^-$$

When the pH of the system increases the balance is shifted to the left side of the equation which increases the pool of ammonia in the water. In this study the NH_4^+-N concentrations in the hypolimnia of some biosolids treated mesocosms increased to above 200 µmol L^{-1} and the pH increased to around 8 over 32 days. At these concentrations the NH_4^+-N can be toxic to aquatic invertebrates and fish (Eddy, 2005).

3.4.4 Nitrate+Nitrite Nitrogen

The concentrations of nitrate+nitrite were statistically different in control, reference and biosolids treatments as well as inorganic N and P loaded mesocosms ($p<0.01$). In addition, the concentrations in all treatments were different as time progressed ($p<0.01$) (Figures 21 and 22). The major sources of nitrate+nitrite were from addition of runoff from soil boxes as well as nitrification of ammonium in the water columns. The major losses of nitrate+nitrite were due to denitrification and assimilation into biomass of primary producers and bacteria.

There was no statistical difference between the epilimnia and hypolimnia in all treatments ($p=0.169$). The similarity between the two layers is most likely due to the fact that the uptake of nitrate by phytoplankton was similar to denitrification rates in the hypolimnia of freshwater mesocosms. In addition low DO concentrations in the hypolimnion could have played a role in keeping the concentrations similar, since nitrification rates can be negatively affected by low DO concentrations.

3.4.4.1 Epilimnion

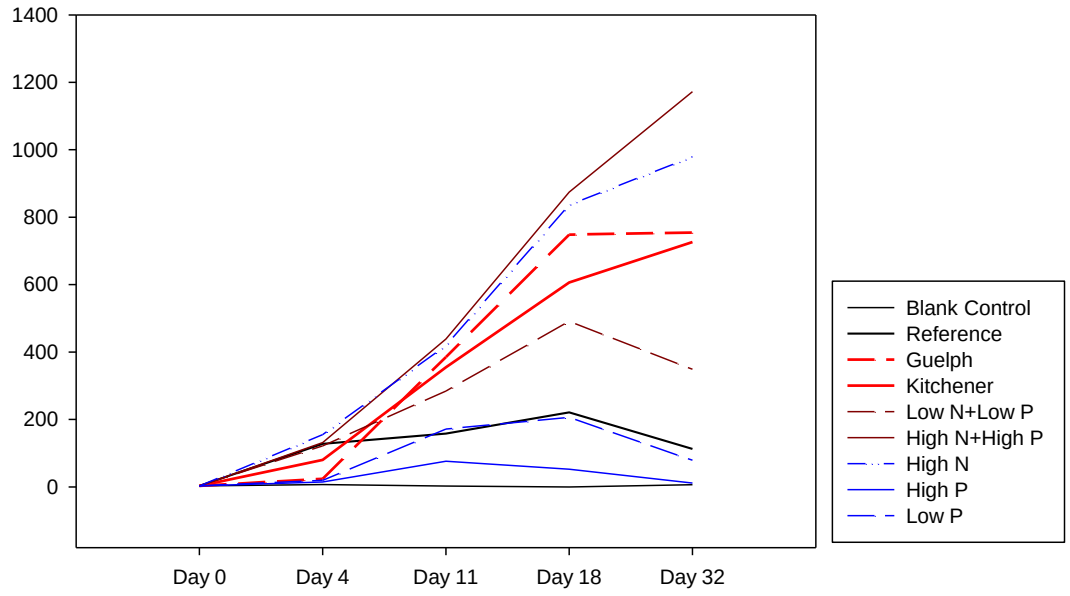

Figure 21. Concentrations of NO_2^-+NO_3^- nitrogen in the epilimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P loaded treatments mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.4.1.1 Control, Reference and Biosolids Amended Mesocosms

The concentrations of nitrite+nitrate over the duration of the experiment were different between control, reference and biosolids treatments. The concentrations in blank control mesocosms remained below 7 µmol L^{-1}, while the highest concentration in the reference treatment was 221 µmol L^{-1} on day 18. The concentrations in reference treatment increased steadily until Day 18, and decreased slightly on day 32. The concentration of nitrite+nitrate in biosolids treatments increased over 32 day period with highest concentrations of 727 µmol L^{-1} in Kitchener and 755 µmol L^{-1} in Guelph runoff treatments on the last day of sampling (Table 14 in Appendix A).

The concentrations of nitrate (+nitrite) in the biosolids treatments was 7 fold higher than reference on the final day of sampling. These results indicate that a portion of this nutrient was accumulated in the water. Accumulation of nitrogen after long term biosolids application has been shown by Tian et al. (2006). Biosolids were applied to as part of reclamation of a mining site at a rate of 28.2 dry Mg ha^{-1} annually for 31 years. The mean nitrate increase in the biosolids treated watershed were on average 12 fold higher than the control watersheds (0.18 mg L^{-1} in control and 2.23 mg L^{-1} in the biosolids amended watersheds). Mitchell et al. (2000) found that in the rivers receiving runoff from tile drainage the concentrations of nitrate can be as high as 10-15 mg L^{-1}.

3.4.4.1.2 Inorganic N and P Amended Mesocosms

The concentrations of nitrite+nitrate were highest in High N High P treatment (1.17 mmol L^{-1}), as well as High N treatment (979 µmol L^{-1}) on the last day of sampling. As time progressed the concentrations increased steadily in the High N+High P and High N treatments, in some cases doubling in concentration 3 days after the addition of the nutrients. Low N+Low P concentrations increased as time progressed until day 17, but decreased by 141 µmol L^{-1} between days 17 and 32. Phosphorus loaded mesocosms also showed a general increase in nitrite+nitrate concentrations as time progressed. The concentrations in Low P treatment increased to higher level than High P treatment, with highest increase seen on day 17 (206 µmol L^{-1}).

The elevated concentrations of nitrate+nitrite in the High N treated mesocosms are not surprising as the majority of nitrogen was not assimilated into the biomass of primary producers since the columns displayed P-limitation (as is indicated by the high concentrations of nitrogen

in the P alone treated mesocosms). The increase in the High N+High P treatment was somewhat puzzling, however could be explained by the fact that the majority of nitrate was not assimilated since the response of primary producers was mainly due to preferential assimilation of ammonium and phosphate. Similarly, since less ammonium was available in the Low N+Low P mesocosms, phytoplankton assimilated a larger portion of nitrate, which is indicated in the lower concentration of this nutrient in the mesocosms.

3.4.4.2 Hypolimnion

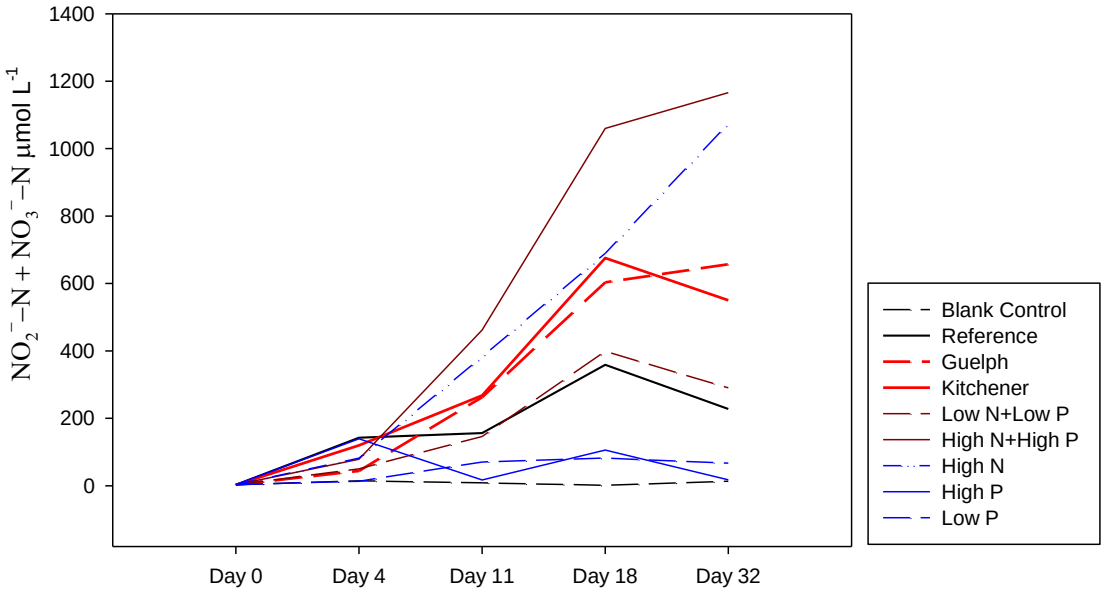

Figure 22. Concentrations of NO_2^-+NO_3^- nitrogen in the hypolimnia of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.4.2.1 Control, Reference and Biosolids Amended Mesocosms

The highest average concentrations of nitite +nitrate over the duration of the experiment were 15 µmol L^{-1} in control blank, 228 µmol L^{-1} in reference treatments, 657 µmol L^{-1} in Guelph and 676 µmol L^{-1} in Kitchener biosolids runoff treated mesocosms (Table 15in Appendix A).

Control mesocosms concentrations were below 14 µmol L^{-1} for duration of the experiment and the reference treatment had the highest value of 311 µmol L^{-1} on day 18, and decreased slightly on day 32. The concentration of nitrite + nitrate in biosolids treatments increased over the 32 day period with highest concentrations of 727 µmol L^{-1} in Kitchener and 755 µmol L^{-1} in Guelph runoff treatments on the last day of sampling.

3.4.4.2.2 Inorganic N and P Amended Mesocosms

The concentrations of nitrate+nitrite were found to be significantly different between the inorganic N and P treatments ($p<0.01$). The concentrations in the High N+High P and Low N +Low P treatments increased to 1.17 mmol L^{-1} and 399 µmol L^{-1} respectively over the duration of the experiment. High N treatment showed an increase to 1.07 mmol L^{-1} on the last day of sampling. The concentration in inorganic P only treatments showed a small increase in nitrite +nitrate levels with highest concentrations of 140 µmol L^{-1} in high P treatment and 82 µmol L^{-1} in the Low P mesocosms.

Nitrogen alone (High N) treatment showed a high degree of nitrate+nitrite accumulation in the system. This is consistent with the expectations as the mesocosms were P limited. In the absence of bioavailable P source the nitrate species were not assimilated by phytoplankton. The accumulation of nitrate was also a result of potentially lower denitrification rates in the inorganic N and P treated columns, since these systems were low in organic matter, which is necessary for denitrification to take place (Tomaszek & Czerwieniec, 2003).

The nitrite+nitrate levels of the inorganic P mesocosms were lower than the biosolids treatments; however the levels did increase relative to the control treatment. The increase in nitrogen suggests nitrogen fixation, as the species of cyanobacteria used in the experiment were capable of carrying out the process.

3.4.4.3 Biosolids and Inorganic N and P Loaded Analogs

There was a statistical difference between the concentrations of nitrate + nitrite in Kitchener biosolids and High N+High P loaded mesocosms ($p<0.01$). Similarly, the concentrations were statistically different between the Guelph and Low N+Low P loaded mesocosms ($p<0.01$). In general, inorganically loaded mesocosms with highest concentration showed higher concentrations of nitrate than Kitchener biosolids. The concentrations of Guelph biosolids treated

runoff were shown to be higher than the Low N+Low P treatment. The difference could be explained by the differences in denitrification rates between the biosolids treated mesocosms and inorganically loaded ones. It is hypothesised that at similar N and P loading rates, differences in organic matter will result in differences in denitrification rates. Therefore, in Kitchener mesocosms, loading of organic carbon along with N and P stimulated denitrification, reducing nitrate accumulation relative to mesocosms with comparable total N and P loading (inorganic forms), but without carbon loading. Similarly, loading of organic carbon to Guelph mesocosms stimulated denitrification resulting in less accumulation of nitrate relative to mesocosms with comparable total N and P loading (inorganic forms).

3.4.4.4 Ecological Significance

The concentrations in the biosolids treated mesocosms reached levels above the maximum allowable drinking water concentrations of 10 mg L^{-1} (714 µmol L^{-1}) NO_3^- N (Fan & Steinberg., 1996) in the epilimnion on the last day of sampling. Chronic exposure to concentrations of 10 mg L^{-1} or higher in drinking water has been associated with a risk of methemoglobinemia in infants and the elderly and carcinogenic risk due to formation of nitrosamino compounds (Selenka, 1980; Bouchard et al.,1992).

The concentrations of nitrate and nitrite increased even when the runoff loading was ceased (between days 17 and 32) in Kitchener and Guelph biosolids. The concentrations most likely increased as a result of higher rates of mineralization of organic nitrogen relative to nitrification.

Similar to findings of Syrett & Morris (1963) and McCarthy et al. (2007) this study implies that even though nitrate was more abundant, ammonium was most likely preferentially used by the phytoplankton in the water columns. We base this assumption on the following. On most rain events, the runoff was rich in both nitrate and organic nitrogen species, but not ammonium. The pool of ammonium that entered the water decreased rapidly and was low in the epilimnion for the majority of the experiment. The concentrations of nitrate on the other hand were quite high in both epilimnion and hypolimnion. The losses of nitrate were not reflected in the subsequent increase in organic nitrogen, which implies that the major form of nitrate loss was through denitrification and not uptake.

3.4.5 Total Nitrogen

The total nitrogen (TN) concentrations were found to be statistically different between treatments and over time ($p<0.01$) (Figure 23).

There was no statistical difference between the epilimnia and hypolimnia in the freshwater mesocosms ($p= 0.958$). This result is consistent with the expectations since the concentrations of some nitrogen species between the layers of thermally stratified lakes should be different, while the overall concentration of N input was the same throughout the water column. The results presented below are the average concentrations in the entire water columns over the duration of the experiment.

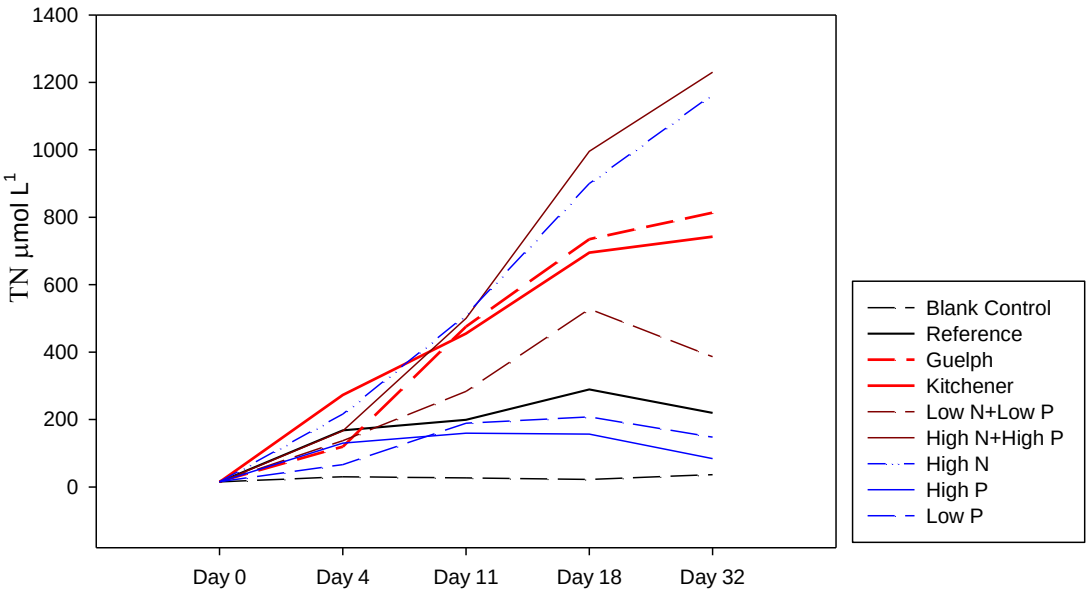

Figure 23.Concentrations of TN in Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

3.4.5.1 Control, Reference and Biosolids Amended Mesocosms

The concentrations of TN in the water columns increased in reference and biosolids and remained the same in blank control treatment as time progressed. Control treatment TN

concentrations remained under 37 µmol L^{-1} over the duration of the experiment. Concentrations in the reference columns increased until day 18 (289 µmol L^{-1}), but decreased to 220 µmol L^{-1} on the last sampling day. The concentrations in biosolids treatment increased to 742 µmol L^{-1} and 813 µmol L^{-1} in Kitchener and Guelph treatments respectively on the final day of sampling.

The concentrations of TN found in this study were similar to the concentrations in many lakes in Canada, which range between 2 to 10 mg L^{-1} (Atlas of Alberta Lakes, 2005) and can depend on the size of the lake and nutrient input.

The concentrations of TN increased over time, most likely as a result of nutrient addition to the mesocosms (Table 16 in Appendix A). The difference between TN in reference and biosolids treatments were therefore expected as a result of the differences in nitrogen content in the runoff. Jeppesen et al. (1998) also found that increasing the levels of incoming N also increases the amount of TN in lakes over time.

3.4.5.2 Inorganic N and P Amended Mesocosms

The concentrations of TN in mesocosm loaded with inorganic N have shown an increase over time with highest concentrations of 431 µmol L^{-1}, 443 µmol L^{-1} and 413 µmol L^{-1} in Low N+Low P, High N+High P and High N treatments. As expected the P alone loaded mesocosms did not show the same increase in TN as their biosolids and inorganic N treated counterparts. However, the concentrations were elevated on some sampling days, with values as high as 523 µmol L^{-1} (High P on day 11).

The difference between the biosolids and inorganic treatments was most likely due to the higher levels of ON in the biosolids runoff, which increased the pool of TN in the columns. The increase in the P alone treatments is somewhat surprising, but is plausible in some P limited systems capable of N-fixation.

3.4.4.3 Biosolids and Inorganic N and P Loaded Analogs

There was a statistical difference between the TN levels in Biosolids treatments and the corresponding High N+High P and Low N+Low P treatments ($p<0.01$). The implication here is that the overall effect on the nitrogen levels within the mesocosms was most likely not only the result of nitrogen and phosphorus loading, but interactions of other biosolids constituents with the microorganisms in the water column. The difference is most likely a result of different rates

of denitrification between the inorganic nutrients treated mesocosms and biosolids runoff treatments. In particular higher organic matter loading and micronutrients within biosolids seemed to have impacted the overall concentration of TN in the mesocosms.

3.4.4.4 Ecological Significance

Even though some lakes with high TN loading are able to resist change and have stable TN concentrations (James et al., 2011), long term effect of nitrogen loading can cause an accumulation of TN in the water column the water column when retention capacity is surpassed by loading.

Of additional importance to this discussion is the ratio of TN to TP in some lakes. The overall concentration of TN to TP has been used as an effective predictor of phytoplankton production in many aquatic systems (Guildford & Hecky, 2000). In addition, nutrient classification of many lakes is based on concentrations of TN:TP. Lakes with concentrations of TN of 0.65-1.2 mg L^{-1} and TP concentrations of 0.03 to 0.1 mg L^{-1} are considered to be eutrophic, while lakes with concentrations of TN <0.35 mg $L^{-1}$and TP <0.01 mg L^{-1} are usually oligotrophic (Smith, Tilman et al., 1998).

3.5 Fate of Nitrogen in the Mesocosms

3.5.1 Nitrogen Retention

Processes that determine the amount of nitrogen retained in the system include denitrification, sedimentation and uptake by primary producers (Saunders & Kalff, 2001). In this study nitrogen retention in the mesocosms was determined by calculating the amount of nitrogen added to the mesocosms (biosolids and inorganic N loaded) and subtracting the amount of nitrogen in the water columns on the last sampling day (percent retained in the column was then calculated by dividing the amount retained by total amount of nitrogen added and multiplying by 100).

In some lakes that receive high nutrient loading as much as 20% to 40% of the nitrogen is retained in the system (Jeppesen et al., 1998). The amount of retention relates to morphology (depth/retention time) (Seitzinger et al. 2006) as well as to N loading, with increased loading reducing N-retention efficiency (Howarth et al. 2006; Laursen & Seitzinger 2004). In this research the reference treatment retained 57% of the incoming nitrogen; Guelph treatment retained 71%, while Kitchener biosolids mesocosms retained 78%. Retention in the inorganic N loaded mesocosms was highest in the Low N+Low P (81%), and similar in High N+High P and High N treatments (54 % and 58% respectively) (Table 3). The greater efficiency in mesocosms with biosolids loading (relative to reference) was unexpected, but TN remained much higher in these mesocosms at the end of the experiment than in reference mesocosms. This experiment does demonstrate an ability of freshwater systems to have a major buffering impact on biosolids runoff, greatly reducing the export of nitrogen to marine systems where it could have a greater impact on eutrophication.

Table 3. TN retained in the water columns (%) over the duration of the experiment.

	Retained %	Added (mmol)	Total in Mesocosms on Final Sampling day (mmol)
Reference	57	2.6	1.1
Guelph	71	16.7	4.9
Kitchener	78	20.7	4.5
High N+High P	54	16.0	7.3
Low N+Low P	81	12.8	2.4
High N	58	16.0	6.6

The form that was most prevalent in the mesocosms at the end of the experimental period was nitrate+nitrite. Together, they comprised 77% of total nitrogen in the Reference columns; Guelph and Kitchener runoff treated columns contained NO_3^- + NO_2^- levels of 86% TN. High N+High P, Low N+Low P and High N treatments contained NO_3^- + NO_2^- concentrations of 95%, 83% and 88% TN respectively on the last day of sampling. Phosphorus alone treated mesocosms were had much lower concentrations of nitrate and nitrite, with 50% TN in Low P mesocosms and 14% in the high P treatment.

The fate of most incoming ammonium in the epilimnion of reference, Guelph and Kitchener mesocosms was most likely by assimilation into biomass of primary producers, as is indicated by the increase in the O_2 levels, pH and DOC and low levels of ammonium in the epilimnion over the duration of the experiment.

The pool of organic nitrogen in the epilimnia increased primarily as a result of runoff additions and phytoplankton growth. The mineralization rates in the epilimnia were low, presumably because any bioavailable NH_4^+, produced by mineralization was rapidly incorporated into the biomass of primary producers. Most of the organic N loss from epilimnion was a result of sinking of organic matter and sedimentation.

The pool of ammonium in the hypolimnion was most likely controlled by mineralization of organic nitrogen that settled from the epilimnion, since the input of ammonium in the runoff was low on most runoff collection days, and the concentrations of this organic nitrogen in the biosolids treated mesocosms was elevated for the major part of the experiment. In addition the levels of ammonium were lower in the inorganic N and P treated mesocosms (except for high N treatment), indicating that there was also an accumulation of ammonium in the hypolimnion of biosolids treatments over time as mineralization rates were apparently higher than the rates of nitrification (because of low DO in the hypolimnia).

Ammonium concentrations in the hypolimnion of Kitchener and Guelph treatments increased after the initial runoff addition and remained elevated for the duration of the experiment. The levels in the reference treatment mesocosms remained low. The difference between treatments is most likely a result of differences in rates and species composition in the incoming runoff. Biosolids runoff contained higher levels of organic nitrogen, which settled into hypolimnion, where it was rapidly mineralized to ammonium.

3.5.2 N_2O production

A portion of incoming nitrogen was lost to the environment as N_2O as a result of nitrification of ammonium and/or denitrification of nitrate and nitrite. In this study the concentrations within the water column were calculated using N_2O gas constant and subtracted from the equilibrium concentrations in the atmosphere.

A significant difference was found between the concentrations of N_2O in the water columns of all treatments ($p<0.01$) and the concentrations were different as time progressed ($p<0.01$) (Figure 24). There was an initial increase in the levels of N_2O in the control, reference and biosolids treated mesocosms, with concentrations as high as 34 and 44 µmol L^{-1} respectively on day 4.

The inorganic treatments did not follow the same trend as biosolids treatments, with highest concentrations occurring on day 18 of the experiment (~18 µmol L^{-1} in all treatments). In general, the concentration of N_2O in the inorganic N and P loaded mesocosms were also lower than biosolids and reference treatments.

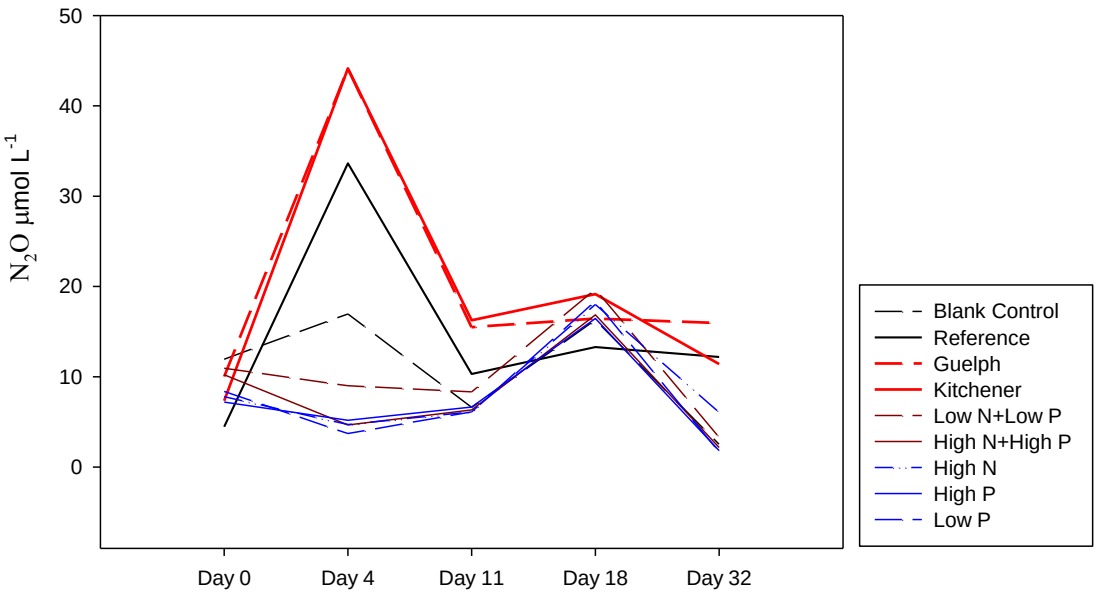

Figure 24. Concentrations of N_2O in the water column of Control, Reference, Kitchener and Guelph biosolids and inorganic N and P amended mesocosms over five sampling periods on day 0, day 4, day 11, day 18 and day 32.

The concentrations of N_2O in mesocosms were very high (>90 nmol L^{-1} which is the average concentrations found in some shallow eutrophic lakes) (Mengis et al., 1997). It is hypothesised that the results shown by this study are a reflection of the high concentration of incoming nitrogen in the runoff from reference and biosolids treatments.The rates of N_2O production in aquatic systems have been positively correlated with the rates of N loading. For example, the concentrations of waterbodies that have been enriched with N, such as a result of being downstream from wastewater plants have been shown to have high N_2O emissions (up to 32,600 μg-N m^{-2} d^{-1}) (McMahon & Dennehy, 1999).

In addition, it has been shown that the concentrations of N_2O increase with decreasing DO levels in the hypolimnion (Mengis et al., 1997) probably as a result of loss during nitrification of ammonium. In this study the increase in N_2O in biosolids concentrations were correlated to the rapid decrease of DO in the hypolimnion of the mesocosms. The levels of N_2O in the water column also declined after sampling day 11. The spikes on day 4 were correlated with the highest ammonium input and high DO concentrations in the hypolimnia. It is therefore hypothesised that in biosolids and reference treatments the initial increase in N_2O production was due to nitrification.

Even though there are a number of differences between mesocosms used in this study and real lake systems (such as convection and gas exchange rates), this study showed that the concentrations of N_2O in columns receiving high nutrient loading can be substantial. Since denitrification is usually highest in stratified water systems with high temperature gradient (as was simulated in the current study) (Bosch et al., 2009), it was shown that a large portion of nitrogen did not get fully converted to N_2 gas but instead escaped as N_2O. The concentrations of N_2O in the atmosphere have been shown to increase at a rate of ~0.3% per year (Khalil & Rasmussen, 1992). This study indicates that highly eutrophic lentic systems can potentially be important sources of N_2O production.

4. CONCLUSIONS AND RECOMMENDATIONS

4.1 Effect on the System

This study was performed in order to determine the effects of the runoff originating from biosolids amended fields on simulated aquatic systems. The hypotheses tested were as follows.

Runoff originating from soil with biosolids application will have an impact on the nitrogen cycle in the freshwater mesocosms compared with runoff originating from reference soil.

The overall concentration and speciation of nitrogen in the runoff originating from biosolids treated plots was different than in the reference soil. Consequently, the addition of the runoff from two sources potentially caused a different effect on the N-cycle within the water columns. Mesocosms showed that the majority of incoming nitrogen was retained by the system. The major portion of incoming ammonium was either taken up by primary producers or denitrified. Since there was a net loss of all nitrogen species over the duration of the experiment, it was not clear whether absorption or nitrification was the prevalent sink for incoming ammonium. This study did show an overall increase in nitrate concentrations, with higher nitrate levels in biosolids runoff treated mesocosms than in reference.

Runoff originating from soil with biosolids application will contribute more strongly to eutrophication of receiving water than runoff originating from reference soil.

According to Nixon (2009), eutrophication should be viewed as an increase in organic matter of the aquatic ecosystem rather than simple nutrient pollution problem. As such, the overall impact on the system can therefore be measured by the overall quantity of organic matter in the lake as a result of external carbon loading or an increase in primary productivity and decomposition within the ecosystem. In this study the concentrations of organic matter were controlled by a combination of the organic carbon rich runoff addition as well as by primary productivity within the system which was caused by nutrient loading.

Further, McCarthy et al. (2007) hypothesise that even when nitrate is available in the system, the growth of cyanobacteria can be nitrogen limited, since they preferentially absorb ammonia over nitrate. It is possible that the phytoplankton growth was ammonium limited over the experimental period. Over time, however, an increase in the ammonia concentration due to

mineralization can further alter the community composition possibly fueling the rates of primary productivity.

Even though the aquatic systems in this study could be considered hypereutrophic (based on organic C and N concentrations), we did not see a strong response in the levels of primary productivity as measured by chlorophyll data (Aslam Hanief, chlorophyll data).

Runoff originating from soil with biosolids runoff will have a different impact on nitrogen cycling and eutrophication in the water column than equivalent quantity of inorganic nitrogen and phosphorus loaded mesocosms, as the forms and relative bioavailability of nutrients will differ.

For most nutrients tested, the effect of biosolids runoff addition was statistically different from the inorganic N +P analogs (ammonium, nitrate, total nitrogen, DOC). The implication here is that for the parameters tested the columns responded differently to the addition of biosolids constituents than the columns which were fertilized with inorganic forms only. The results were expected as the biosolids runoff contained higher levels of organic carbon and nitrogen, which are known to stimulate the receiving waters by increasing the overall concentration of nitrogen by mineralization. In addition organic carbon has been shown to affect the rates of denitrification in some water systems.

In some cases the inorganic analogs had greater indication of eutrophication (if measured by the amount of primary productivity in the system), which suggests that nitrogen from biosolids may not always be in bioavailable forms.

There was no statistical difference in the effect of organic fertilizer and inorganic nutrients on concentrations of TN and organic nitrogen. The most likely explanation is that the levels of ON were kept low in the biosolids amended water columns as a result or rapid mineralization. As a result the two sets of columns had a similar concentration of ON for the duration of experiment.

The concentration in the high N treatments was shown to remain high in ammonium, nitrate+nitrite, while the levels of ON and DOC were generally low. At the same time inorganic P only treated mesocosms showed an increase in the levels of nitrogen species even though no nitrogen was added to the system. This indicates P limitation in the water columns, as the bioavailable nitrogen accumulated in the columns in the absence of phosphorus.

One of the symptoms of eutrophication is decrease in the DO levels in the hypolimnion of stratified lakes (Beutel, 2001). This study successfully simulated the production of anoxic environment in the hypolimnion (most likely as a result of organic matter decomposition).

4.2 Future Studies

Mesocosm type experiments simulating aquatic environments have been shown to be effective in some situations, where experimentation on a real lake is impossible or difficult. Gerhart & Likens (1975), for example have successfully shown that nutrient limitation with N and P was primarily responsible for phytoplankton growth using mesocosms simulating lake environments. The pattern of nutrient limitation found in these studies has been found to be similar to those found in real lakes (Elser, 1990).

However, since mesocosm-type experiments are carried out under controlled environments, they may overlook the importance of multiple factors that affect the interactions within real lake ecosystems. In particular, factors such as atmospheric nutrient deposition, water currents, variation in temperature and water levels, size of the lake, weather patterns and community composition are very difficult to simulate in the laboratory conditions. It is also difficult to approximate the community composition found in real lake systems, since some organisms can be difficult to include in the mesocosm experiments due to their size (i.e. large fish) or habitat requirements (i.e. wide range of habitats). Future studies, focusing on field experiments are necessary in order to determine the real-life effect of biosolids runoff on lakes.

This study limited the variables determining the amount of nutrients in lakes to one application rate and one concentration of nutrients added to mesocosms. In reality, the application rates can differ depending on the agricultural field, and concentration of nutrients entering the water systems can depend on the rain intensity, distance from the lake and size of the lake. Future studies simulating a set of different variables may be useful in estimating the effect of biosolids runoff on eutrophication and biogeochemical cycles.

Finally, a study involving more complex food web interactions is necessary in order to assess the potential impact of biosolids runoff on aquatic systems. The interactions between different trophic levels are known to be important in predicting the response of primary producers to nutrient loading (Carpenter et al., 1985). The variability in algal blooms can sometimes be

controlled by top-down control of primary productivity (Bio et al., 2008) which can have important impacts on the nitrogen cycle and eutrophication.

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APPENDIX A. Concentrations of Nutrients in the Runoff and in the Freshwater Mesocosms

Part A: Average Concentrations of Nutrients in the Runoff

Table 4. Average concentrations of TN in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes over four rain events on days 1, 8, 15 and 22 (mmol L^{-1} ± Standard Deviation).

	Day 1		Day 8		Day 15		Day 22	
Reference	1.6	±0.2	1.0	±0.2	0.90	±0.2	0.80	±0.1
Kitchener	8.8	±1.5	7.3	±1.9	9.0	±2.6	9.5	±2.3
Guelph	5.4	±1.2	6.2	±1.4	7.2	±1.9	9.3	±2.9

Table 5. Average concentrations of NH_4^+ nitrogen in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes over four rain events on days 1, 8, 15 and 22 (µmol L^{-1} ± Standard Deviation).

	Day 1		Day 8		Day 15		Day 22	
Reference	47	±11	79	±33	74	±19	30	±20
Kitchener	3807	±1107	766	±283	132	±128	50	±17
Guelph	635	±275	616	±190	75	±13	44	±25

Table 6. Average concentrations of NO_2^-+NO_3^- nitrogen in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes over four rain events on days 1, 8, 15 and 22 (mmol L-1 ± Standard Deviation).

	Day 1		Day 8		Day 15		Day 22	
Reference	1.2	±0.2	0.8	±0.2	0.7	±0.2	0.8	±0.1
Kitchener	2.3	±0.4	3.8	±0.8	7.7	±2.7	7.9	±3.0
Guelph	1.5	±0.2	3.6	±1.0	6.2	±2.2	8.6	±3.4

Table 7. Average concentrations of Organic Nitrogen in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes over four rain events on days 1, 8, 15 and 22 (mmol L^{-1} ± Standard Deviation).

	Day 1		Day 8		Day 15		Day 22	
Reference	0.28	±0.13	0.11	±0.07	0.10	±0.06	0.04	±0.03
Kitchener	2.6	±1.9	2.7	±1.6	1.1	±0.8	1.5	±0.9
Guelph	3.3	±0.9	2.0	±0.5	0.9	±0.4	0.6	±0.5

Table 8. Average concentrations of Dissolved Organic Carbon in the runoff collected from reference, Guelph and Kitchener biosolids treated soil boxes over four rain events on days 1, 8, 15 and 22 (mmol L^{-1} ± Standard Deviation).

	Day 1		Day 8		Day 15		Day 22	
Reference	1.8	±0.7	1.8	±0.7	2.3	±1.2	1.7	±0.8
Kitchener	3.7	±1.1	3.9	±0.1	2.1	±0.6	1.7	±0.5
Guelph	6.2	±1.9	6.4	±1.8	4.1	±1.5	2.8	±0.8

Part B: Average Concentrations of Dissolved Organic Carbon and Nitrogen Species in the Freshwater Mesocosms

Table 9. Average concentration of Dissolved Organic Carbon in the Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0	Day 4	Day 11	Day 18	Day 32
Control	218 ±63	298 ±151	204 ±59	298 ±59	275 ±48
Reference	242 ±71	323 ±104	536 ±130	674 ±145	842 ±150
Kitchener	225 ±65	453 ±124	696 ±135	820 ±100	843 ±141
Guelph	221 ±63	615 ±197	1051 ±197	1228 ±192	1344 ±152
L (N) L(P)	239 ±24	280 ±89	324 ±106	475 ±67	485 ±103
H (N) H (P)	178 ±60	245 ±33	382 ±51	460 ±91	472 ±47
H (N)	131 ±12	140 ±81	252 ±134	229 ±24	370 ±87
H (P)	180 ±66	189 ±30	426 ±181	399 ±82	402 ±52
L (P)	202 ±67	164 ±38	315 ±49	364 ±49	553 ±38

Table 10. Average concentration of Organic Nitrogen in the epilimnia of Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0	Day 4	Day 11	Day 18	Day 32
Control	9 ±6	19 ±8	24 ±6	23 ±10	18 ±3
Reference	9 ±4	31 ±10	38 ±13	21 ±4	54 ±24
Kitchener	10 ±3	35 ±19	56 ±33	28 ±18	16 ±7
Guelph	10 ±3	63 ±19	123 ±51	35 ±18	58 ±16
L (N) L(P)	10 ±3	10 ±8	0 0	45 ±11	45 ±6
H (N) H (P)	7 ±1	66 ±24	0 0	4 ±5	22 ±16
H (N)	9 ±5	0 0	0 0	0 0	0 0
H (P)	11 ±2	24 ±8	18 ±26	13 ±10	28 ±12
L (P)	10 ±2	42 ±13	2 ±2	35 ±16	36 ±20

Table 11. Average concentration of Organic Nitrogen in the hypolimnia of Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0	Day 4	Day 11	Day 18	Day 32
Control	11 ±1	16 ±2	11 ±3	21 ±10	18 ±2
Reference	11 ±4	28 ±12	29 ±12	23 ±6	36 ±20
Kitchener	10 ±2	15 ±20	8 ±18	14 ±27	0 0
Guelph	12 ±3	10 ±12	11 ±17	42 ±42	35 ±31
L (N) L(P)	9 ±2	39 ±9	0 0	29 ±5	19 ±16
H (N) H (P)	10 ±2	25 ±5	2 ±3	4 ±6	9 ±8
H (N)	12 ±4	28 ±19	0 0	0 0	0 0
H (P)	10 ±2	44 ±20	12 ±6	1 ±2	19 ±10
L (P)	11 ±3	34 ±18	5 ±7	34 ±8	7 ±12

Table 12. Average concentration of NH_4^+ nitrogen in the epilimnia of Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0	Day 4	Day 11	Day 18	Day 32
Control	3 ±1	1 ±1	3 ±1	0 0	2 0.5
Reference	3 ±1	2 ±0.4	5 ±2	0.4 ±0.9	2 ±1
Kitchener	2 ±1	102 ±47	38 ±50	2 6	6 ±2
Guelph	3 ±1	10 ±8	15 ±13	0 0	5 ±1
L (N) L(P)	3 0	7 ±3	96 ±24	32 ±16	11 ±6
H (N) H (P)	2 ±1	13 ±3	81 ±5	14 ±3	22 ±11
H (N)	2 ±1	128 ±46	131 ±54	134 ±64	139 ±44
H (P)	2 ±1	31 ±20	132 ±6	46 ±13	4 ±2
L (P)	3 ±0.5	12 ±3	101 ±24	37 ±16	16 ±6

Table 13. Average concentration of NH_4^+ nitrogen in the hypolimnia of Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0		Day 4		Day 11		Day 18		Day 32	
Control	1.9	±0.4	3.2	±0.3	5	±2	0	0	14	±2
Reference	2.0	±0.4	4.2	±0.4	11	±5	2	±2	6	±5
Kitchener	1.6	±0.8	201	±70	220	±74	69	±20	215	±68
Guelph	2.2	±0.8	92	±49	171	±47	46	±18	116	±63
L (N) L(P)	1.1	±0.3	17	±10	42	±9	27	±15	78	±17
H (N) H (P)	1.9	±0.7	17	±3	75	±23	38	±9	70	±12
H (N)	1.8	±0.9	74	±47	201	±40	210	±52	225	±20
H (P)	1.9	±0.7	8	±5	70	±22	98	±8	88	±7
L (P)	1.1	±0.3	22	±10	47	±9	32	±15	83	±17

Table 14. Average concentration of NO_2^-+NO_3^- nitrogen in the epilimnia of Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0		Day 4		Day 11		Day 18		Day 32	
Control	2.2	±0.4	7	±6	3	±3	0	0	7	±5
Reference	3.1	±0.4	128	±32	158	±22	221	±48	113	±60
Kitchener	2.9	±0.5	80	±38	355	±202	607	±60	727	±210
Guelph	3.1	±0.4	24	±19	385	±113	749	±96	755	±232
L (N) L(P)	3.3	±0.2	122	±25	285	±21	491	±28	349	±33
H (N) H (P)	2.6	±0.4	131	±23	439	±19	874	±93	1172	±27
H (N)	2.7	±0.3	155	±28	420	±134	836	±68	979	±136
H (P)	3.1	±0.2	14	±9	76	±55	52	±60	12	±12
L (P)	3.1	±0.1	20	±16	172	±21	206	±53	79	±16

Table 15. Average concentration NO_2^-+NO_3^- nitrogen in the hypolimnia of Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0		Day 4		Day 11		Day 18		Day 32	
Control	3.2	±0.4	15	±5	9	±7	1	±1	13	±14
Reference	2.9	±0.5	143	±22	156	±44	311	±65	228	±29
Kitchener	2.7	±0.4	120	±45	268	±93	676	±196	550	±180
Guelph	3.2	±0.5	44	±17	263	±79	603	±182	657	±222
L (N) L(P)	2.7	±0.4	50	±16	146	±53	399	±62	290	±52
H (N) H (P)	3.2	±0.3	79	±9	462	±49	1060	±204	1166	±175
H (N)	3.4	±0.3	82	±25	383	±65	689	±59	1074	±83
H (P)	3.4	±0.5	140	±19	17	±1	106	±122	18	±19
L (P)	3.2	±0.5	13	±2	71	±5	82	±48	67	±24

Table 16. Average concentration of Total Nitrogen in Control, Reference, Kitchener, Guelph, Low N+Low P, High N+High P, High N, High P and Low P treatments on five sampling events on day 0, day 4, day 11, day 18 and day 32 (µmol L^{-1} ± Standard Deviation).

	Day 0		Day 4		Day 11		Day 18		Day 32	
Control	15	±3	30	±6	27	±7	22	±11	36	±14
Reference	15	±4	168	±28	199	±39	289	±76	220	±73
Kitchener	15	±3	273	±95	455	±163	695	±146	742	±185
Guelph	16	±3	120	±95	476	±162	735	±146	813	±185
L (N) L(P)	292	±15	172	±58	289	±83	331	±37	431	±67
H (N) H (P)	200	±90	171	±52	336	±34	443	±160	364	±62
H (N)	146	±13	142	±51	303	±194	283	±42	414	±12
H (P)	196	±72	236	±36	523	±335	344	±130	226	±36
L (P)	174	±92	160	±36	258	±24	279	±15	233	±32

APPENDIX B. Biosolids Application Rates and Amount of Water Added During Simulated Rainfall

Part A: Determining Application Rates for Biosolids (Wet Mass)

Soil Surface area of the trough: 36 cm x 100 cm = 0.36 m^2

Application Rate: 8 dry ton per hectare = 8000 dry kg per 10000 m^2

Let x represent the amount of dry mass biosolids to be land-applied to the trough:

8000 kg(dry)/ 10000 m^2 = x kg(Dry) / 0.36 m^2

x = 0.288kg of dry biosolids to be applied to each trough

1. Guelph Biosolids solid concentration was 3.09% (i.e. 30.9 g dry weight / kg wet weight biosolids)

To provide 288 g dry weight: need X g wet weight

30.9 g dry weight: 1000 g wet weight = 288 g dry weight/X g wet weight

X = 9320 g or 9.32 kg wet weight of Guelph biosolids applied per trough

2. Kitchener Biosolids solid concentration was 1.47% (i.e. 14.7 g dry weight / kg wet weight biosolids)

In order to provide 288 g dry weight: need X g wet weight

14.7 g dry weight: 1000 g wet weight = 288 g dry weight: X g wet weight

X = 19591.5 g or 19.56 kg wet weight of Kitchener biosolids applied per trough

Part B: Simulated Rainfall Quantity Calculations

Multi-annual extreme storm event for South Ontario = 49.5mm of rain

49.5mm = 4.95cm

Area of each trough = 3600 cm^2

Amount of water to be added per trough: 4.95 cm x 36 cm x 100 cm = 17820 cm^3

Let X represent the amount of water per trough in Liters, 1L =1000 cm^3,

X = 17820 cm^3/1000 cm^3L^{-1} = 17.82 L

APPENDIX C: Nutrient Assay Analysis

1. Ammonium Nitrogen

1.1 Reagent preparation

Phenol solution: A phenol solution was prepared weekly by diluting 11.1 ml liquefied phenol (89%) with 95% v/v ethyl alcohol to 100 mL.

Sodium nitroprusside: a 0.5% w/v solution was made by dissolving 0.5 g of sodium nitroprusside in 100 mL deionized water.

Alkaline citrate: The solution was made by dissolving 200 g trisodium citrate and 10 g sodium hydroxide in deionized water and diluting it to 1000 mL.

Oxidizing solution: 100 mL alkaline citrate solution was mixed with 25 mL of sodium hypochlorite (commercial bleach ~5%).

Stock solution: A stock solution was prepared by dissolving 3.189g anhydrous NH_4Cl (1 mg ml^{-1} nitrogen) in 1L water.

1.2 Procedure

Standards: Standards were prepared each testing period using ammonium chloride stock solution. Six standards were prepared for the runoff samples (0.05 mg L^{-1}, 0.1 mg L^{-1}, 0.2 mg L^{-1}, 0.25 mg L^{-1} and 0.3 mg L^{-1}). Four standards were prepared for the mesocosm samples (0.02 mg L^{-1}, 0.075 mg L^{-1}, 0.1 mg L^{-1} and 0.2 mg L^{-1}). A blank sample was also made using deionized water. The standards were treated in exactly the same way as samples.

Samples: Frozen runoff and water samples were thawed and diluted according to the requirements (in order to fit the standard range). 5 mL of the sample was then transferred to a glass test tube, followed by the addition of 0.4 mL of phenol/sodium nitroprusside solution and 1mL oxidizing solution. The samples were allowed to develop for 1 hour under low light conditions at room temperature (22 ºC to 27ºC). UV-Vis spectrometer analysis was carried out at 640 nm on all samples and standards.

Calculations: A standard curve was prepared by plotting absorbance reading of standards against ammonia concentration of standards. Sample concentrations are then calculated by comparing sample absorbance with the standard curve.

2. Nitrate + Nitrite

2.1 Apparatus

Principle: In the presence of cadmium (Cd) nitrate (NO_3^-) in the water sample is almost quantitatively reduced to nitrite (NO_2^-). This method uses commercially available Cd granules treated with copper sulphate ($CuSO_4$) and packed in a column.

Preparation of the reduction column: A cadmium column was constructed using a copper cylinder with dimensions length 15 cm and a 5mm diameter. Both ends of the column were wrapped in steel wool and fitted with metal bolts (in order to prevent Cu-Cd granule leaks) as well as plastic aquarium tube adapters to connect the column to the external setup. Preliminary washing of stock Cu-Cd granule was conducted by rinsing 20g of granules with 6N HCl followed by deionized water. The granules were then rinsed with 50ml 2% CuSO4 (5g CuSO45H2O diluted to 250ml water) for 5 minutes, until blue colour faded. Successive 50ml 2% CuSO4 washings were repeated until a brown colloidal precipitate formed. A water wash was then performed using deionized water in order to remove the precipitated Cu. The granules were then packed into the column using a thin metal rod and a plastic tube filled with buffer (13g NH4Cl and 1.7g disodium ethylenediamine tetraacetate dissolved in 1L with 8.5 pH adjustment with concentrated NH4OH before dilution). The column was then stored in a plastic case filled with the buffer solution until further use.

2.2 Reagents Preparation

Colour reagent: A colour reagent was made by dissolving 1.0g sulphanilamide with 80ml water and 10ml 85% phosphoric acid. Once the solid has been dissolved, 0.1g N-(1-naphthyl)-ethylenediamine dihydrochloride was added and the whole solution was diluted with water to 100ml. The solution is light sensitive it was therefore stored in a light-proof brown plastic bottle.

Ammonium chloride-EDTA solution: The buffer solution was made by dissolving 13 g NH_4Cl and 1.7 g disodium ethylenediamine tetraacetate in 900 mL water and then adjusting the pH to 8.5 with concentrated NH_4OH and dilute to 1 L.

Stock solution: A stock solution was prepared by dissolving 7.22 g of KNO_3 (previously dried in an oven at 105ºC for 24 hours) in 1L water and diluting the solution 100 fold to give a 10 mg L^{-1} working stock solution.

2.3 Procedure

Standards: Standards were prepared each testing period using potassium nitrate (KNO_3) solution. Five standards were prepared for the runoff samples (0.125 mg L^{-1}, 0.3 mg L^{-1}, 0.5 mg L^{-1}, 0.75 mg L^{-1} and 1 mg L^{-1}). Four standards were prepared for the mesocosm samples (0.05 mg L^{-1}, 0.1 mg L^{-1}, 0.2 mg L^{-1} and 0.3 mg L^{-1}). A blank sample was also made using deionized water (0 mg L^{-1}). The standards were treated exactly the same way as the samples.

Samples: Frozen runoff and water samples were thawed and diluted according to the requirements (in order to fit within the standard range). 5 mL of the sample (or standard) was then mixed with 15 mL of buffer solution in 50 mL conical tubes.

The cadmium column was connected to a peristaltic pump with buffer filled tubes. Air bubbles were prevented from entering the initial column/pump hook-up by connecting the pump tubes while the column is still stored in the buffer. The column is then activated for experimentation by running 100 mL mixture of 25% 1.0mg/L nitrate standard and 75% buffer solution through the column.

Upon activation of the column the samples were processed through the column at the flow rate of 0.02 mL s^{-1}. The first 10 mL of the sample which was passed through the column was discarded, and then the subsequent 5 mL were collected in glass test tubes. 0.4 mL of the colour reagent was then added to the 5 mL sample. A pink colour developed after the first 10 minutes and the samples were analysed within the next 2 hours. A UV-Vis spectrophotometer was used to identify the colour development at 543nm.

Calculations: A standard curve was prepared by plotting absorbance reading of standards against nitrate concentration of standards. Sample concentrations are then calculated by comparing sample absorbance with the standard curve.

3. Kjeldahl Nitrogen

2.1 Apparatus

Principle: In the presence of sulfuric acid, catalyst and heat organic nitrogen present in the sample is converted to $(NH_4)_2SO_4$. In the presence of highly concentrated NaOH (45%) the ammonium in the sample is converted to free ammonia ($(NH_4)_2SO_4 + 2NaOH \rightarrow Na_2SO_4(aq) + 2H2O(l) + 2NH3(g)$), which can be boiled out of the sample and collected in a collection vessel containing boric acid (4%). Ammonia reacts with boric acid to form ammonium and tetrahydroxyborate ($B(OH)_4^-$).

Digestion reagent: 134 g of K_2SO_4 and 7.3 g $CuSO_4$ was dissolved in 800 mL water. Carefully 134 mL concentrated H_2SO_4 was added. When it cooled to room temperature, the solution was diluted to 1 L with water. The solution was kept at temperature close to 20°C to prevent crystallization.

Stock solution: A stock solution was prepared by dissolving 0.943 of $(NH_4)_2SO_4$ (previously dried in an oven at 105ºC for 2 hours) in 1L to give a 100 mg L^{-1} N stock solution.

2.3 Procedure

Standards: Standards were prepared each testing period using ammonium sulfate solution. Five standards were prepared for the runoff samples (1 mg L^{-1}, 2 mg L^{-1}, 3 mg L^{-1}, 4 mg L^{-1}). Four standards were prepared for the mesocosm samples (0.3 mg L^{-1}, 0.5 mg L^{-1}, 1 mg L^{-1} and 2 mg L^{-1}). A blank sample was also made using deionized water (0 mg L^{-1}). The standards did not undergo digestion, but were distilled directly using the distillation apparatus.

Samples: Frozen runoff and water samples were thawed and added to Kjeldahl flasks with a capacity of 100 mL in a semi-micro-kjeldahl digestion apparatus equipped with heating elements, to accommodate Kjeldahl flasks and a suction outlet to vent fumes. The heating elements provided the temperature range of 375 to 385°C. 10 mL of digestion reagent was added to kjeldahl flask containing 10 mL sample. The heating unit was set to medium heat settings while being set under the fume hood. The solution is boiled until it becomes transparent and pale green and copious fumes are observed. Each heating unit is then turned up to its maximum setting and digested for an additional 30 min. Cool. Quantitatively transfer digested sample by diluting and rinsing several times into micro-kjeldahl distillation. The ammonia is then distilled

in the distillation apparatus and the concentration of ammonium is determined using manual phenate method outlined earlier.

Calculations: A standard curve was prepared by plotting absorbance reading of standards against nitrate concentration of standards. Sample concentrations are then calculated by comparing sample absorbance with the standard curve.