An investigation of land-use impacts on water quality and algal communities in the Nottawasaga River and low-order streams of the Nottawasaga Valley Watershed

by

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Abstract

The Nottawasaga Valley Watershed (NVW) is a large catchment south of Georgian Bay that drains a primarily agricultural region including one of the largest wetland complexes in Southern Ontario, the Minesing Wetlands (MW). The MW are designated both provincially and internationally significant due to its large area and high biological diversity. Agricultural practices have been a large influence on the Nottawasaga River ecosystem throughout history, but intensification of agricultural demands have put stress on surface water quality across the river network. In order to understand how ecosystem health may be impacted from current agricultural land-use, baseline water quality and phytoplankton data were collected monthly (June-September 2014) over 15 sites across the Nottawasaga River continuum. In addition, first- and second-order streams directly influenced by agricultural land-use runoff were assessed for impacts to surface water quality and periphyton biomass across the NVW.

Water quality across the Nottawasaga River exhibited large amounts of variation, with Innisfil Creek having a disproportional influence on suspended sediment related impacts to water quality. Agricultural and urban land-use were positively correlated with many water quality parameters, whereas natural land-use features (i.e. forest, water and wetland land-use) were negatively correlated with many water quality parameters. Principal components analysis revealed that sites upstream of the MW exhibited higher values associated with nutrients and suspended sediments, while those downstream of it exhibited higher values associated with decreased riparian vegetation. Redundancy analysis reinforced that upstream sites are highly influenced by urban and agricultural land-use, linking these to impacted water quality. Algal

community structure and biomass were variable, but consistently were maintained at oligotrophic levels, dominated by pollution tolerant bioindicator taxa.

Water quality of the low order streams across the NVW was highly variable, with differences in multiple water quality parameters found even when agricultural type was the same. Many of the sites had high concentrations of nutrients linked with agricultural land-use, but limited statistically significant relationships could be attributed to the different agricultural types. Water quality variation was important in describing the variation in fatty acid composition of periphyton biomass, as described by redundancy analysis. Total chlorophyll content was directly linked with increases in certain fatty acid proportions, some of which can be classified as essential fatty acids.

Keywords: Land-use, water quality, phytoplankton communities, Nottawasaga River, fatty acids, periphyton

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List of Abbreviations

ALA	α-Linolenic acid
ANOVA	Analysis of Variance
BIO	Total Algal Biomass
CHL	Total Chlorophyll *a* Content
COND	Conductivity
df	Degrees freedom
DO	Dissolved Oxygen
EFA	Essential Fatty Acid
FA	Fatty Acid
FAME	Fatty Acid Methyl Ester
GC	Gas Chromatography
MUFA	Monounsaturated Fatty Acid
MW	Minesing Wetlands
N	Nitrogen
NR	Nottawasaga River
NVW	Nottawasaga Valley Watershed
OMAFR	Ontario Ministry of Agriculture, Food and Rural Affairs
OMNR	Ontario Ministry of Natural Resources
P	Phosphorus
PUFA	Polyunsaturated Fatty Acid
RDA	Redundancy Analysis
PCA	Principal Components Analysis
SAFA	Saturated Fatty Acid
SD	Standard Deviation
SE	Standard Error
SHAN	Shannon Diversity
TAN	Total Ammonia Nitrogen
TEMP	Temperature
TIOSS	Total Inorganic Suspended Solids
TN	Total Nitrogen
TNN	Total Nitrate Nitrogen
TOSS	Total Organic Suspended Solids
TP	Total Phosphorus
TSS	Total Suspended Solids
TURB	Turbidity
UOIT	University of Ontario Institute of Technology
WQ	Water quality

List of Taxonomic Abbreviations

ACM	*Achnanthidium*
ACS	*Achnanthes*
AMP	*Amphora*
BIR	*Biremis*
BRA	*Brachysira*
CAV	*Cavinula*
CHO	*Chlorophyceae*
CHR	*Chroococcus*
COE	*Cocconeis*
CRY	*Cryptomonas*
CYM	*Cymbella*
DES	*Desmodesmus*
DIA	*Diatoma*
DID	*Didymocystis*
ENC	*Encyonema*
EPI	*Encyonema*
EUC	*Eucapsis*
EUG	*Euglenoid*
FRA	*Fragilariforma*
FRG	*Fragilaria*
FRU	*Frustulia*
GEI	*Geissleria*
GOM	*Gomphonema*
GYR	*Gyrosigma*
KIR	*Kirchneriella*
KOB	*Kobayasiella*
MER	*Meridion*
MON	*Monoraphidium*
NAV	*Navicula*
NIT	*Nitszchia*
SCE	*Scenedesmus*
SUR	*Surirella*
SYN	*Synedra*

Chapter 1: General Introduction

Aquatic ecosystems are some of the most diverse systems in the world, with everything from the Great Lakes to the Amazon River contained under this general designation (Allan and Castillo 2007). Streams and rivers, also known as fluvial or lotic ecosystems, are some of the most variable due to flowing water, which can alter the physical and chemical environment throughout the entire length of the ecosystem's reach (Hudon *et al*. 1996). Fluvial ecosystems have a great deal of connectivity with their surrounding terrestrial landscape as overland flow drains into surface water from precipitation on the adjacent land. This water thus accumulates nutrients, organic matter, organisms and particulates that are washed from the landscape during these events and deposits them into the connected aquatic ecosystem. Fluvial ecosystems maintain connected over large distances due to the intricacies that occur within drainage networks. They contain a diverse range of small, headwater streams that are ground fed or drain a small portion of landscape, which continue to interconnect until reaching the junction of the main river branch, eventually draining into a lake or ocean. In the study of these systems, water quality is an essential factor for inclusion studying fluvial ecosystems, as it is the main driver of fluvial ecosystem biological processes.

There are a multitude of factors that influence water quality in aquatic systems, but water quality itself is characterized by the chemical, biological and physical parameters that can be measured in order to determine the ecological health of an aquatic ecosystem. Dissolved oxygen (DO) is one of these fundamental components of water quality due to its necessity in biological processes. Oxygenation of river systems is important for aquatic animals, such as

fish, aquatic invertebrates, shellfish and other gilled organisms which over prolonged periods of decreased oxygen, or hypoxia, can lead to large scale fatalities (Winn and Knott 1992). Biologically relevant concentrations of DO are not usually of concern in fluvial ecosystems due to the ability of oxygen to diffuse into flowing water via aeration, but there are natural and anthropogenic links to hypoxia and anoxic conditions (i.e. oxygen depletion) (Dodds 2006). Reductions in DO concentrations can occur from large scale inputs of organic matter, from naturally occurring photodegradation and autotrophic primary production or from anthropogenic inputs, particularly from agricultural land-use (Wilson and Xenopoulos 2013). Decreased DO concentrations that are linked with increased organic matter are connected with increased bacterial activity and degradation, which require oxygen in order to occur, depleting the surrounding water of DO. Anoxic conditions have also been linked with increased nutrient driven primary production and eutrophication in general, both of which are linked with increased anthropogenic land-use in a watershed (Cooper and Brush 1991).

Water chemistry of aquatic ecosystems dictates biotic and abiotic processes, including nutrient speciation. Nutrients (particularly nitrogen and phosphorus) are typically received in run-off from the surrounding environment and then processed along the river continuum. Water pH is one important aspect of water chemistry due to the fact that many biological organisms have a specific tolerance range of pH, as well as a the role that pH has in controlling ions in acidic or basic conditions (Camargo and Alonso 2006). The naturally occurring balance in most aquatic, known as the bicarbonate buffer system, is a series of reactions where CO_2, HCO_3^- and CO_3^- all buffer each other, preventing changes in pH (Allan and Castillo 2007). This prevents drastic changes from occurring, but shifts in this equilibrium can occur when there are

processes that disproportionately use components of this buffering system. The main group of organisms that utilize CO_2 are aquatic plants and algae via photosynthesis. In primary production, which can also use bicarbonate (HCO_3^-) as a source of inorganic carbon, this buffering capacity can dramatically shift to a more alkaline pH. Other natural sources of H^+ ions that augment pH levels can be from the release of ions from bedrock, acidic precipitation, organic acids released from decaying plant matter and most importantly, anthropogenic inputs (Allan and Castillo 2007).

Anthropogenic land-use, such as agriculture or urban development, can increase ion concentrations, including Mg^+, Ca^{2+} and Cl^-, as well as pH, which directly relate to conductivity of surface waters (Chessman *et al*. 1992). Conductivity is linked with pH due to it also affecting dissolved nutrients and ions, but it also positively correlated with chloride ions from agricultural dust-suppressants and road salt application (Sutcliffe and Carrick 1983, Porter-Goff *et al*. 2013). Many benthic diatoms and aquatic invertebrates have lethal thresholds of conductivity and chloride, making conductivity an important measure of anthropogenic land-use (Wallace and Biastoch 2016). Although these chemical parameters are important as a whole due to tolerance ranges for biological process and organisms, nutrients are more essential for primary producers, the base of aquatic food webs.

Nutrients used in primary production are some of the most important aspects of aquatic ecosystems because of their bottom-up control of the food web based in primary production, usually relating to algal growth (Reynolds and Descy 1996). It is well known that algae, similar to plants, have their overall growth restricted by a select few nutrients, deemed limiting nutrients (Dzialowski *et al*. 2005). Most prevalent and importantly, these nutrients are

the bioavailable forms of nitrogen (N) and phosphorus (P) (Schindler 1974), which will inherently dictate the primary production in most systems (Smith *et al*. 1999). Nitrogen has been shown to be an important limiting nutrient in many natural systems, dependant on the ratio between bioavailable forms of N and P, usually expressed as TN:TP (Chessman *et al*. 1992, Stelzer and Lamberti 2001) and dependant on the microbial community structure, as bacterial and algal nutritional needs vary with proportions (Sundareshwar *et al*. 2003). Alternatively, there is an equal proportion of systems with phosphorus limitations, with higher levels of nitrogen in comparison to phosphorus causing phosphorus limitations due to this important ratio (Yun and An 2016), as well as taxonomic differences in nutrient needs and exploitation (Stelzer and Lamberti 2001). The total concentration of both organic and inorganic sources of nitrogen in aquatic systems are important for determination of their potential to be in bioavailable forms (Camargo and Alonso 2006), but also for the determination of individual nitrogen sources.

Sources of inorganic nitrogen can be linked to ammonia nitrogen and nitrate nitrogen, which allows for the characterization of agriculturally linked non-point sources of nitrogen (Ding *et al*. 2015). Ammonia based nitrogen sources are strongly related to agricultural animals, such as livestock, and fertilizers, which are the two largest contributors in Canada to total nitrogen emissions (Vet *et al*. 2005). Nitrate is similar, but is found to be a larger contributor to total inorganic nitrogen sources, with natural nitrification processes oxidizing ammonium into nitrate (Camargo and Alonso 2006). It also has strong links to fertilizers and agriculture (Tesoriero *et al*. 2013) and can be found in high concentrations in waste water treatment plant effluent, which often runs directly into fluvial ecosystems (Dodds 2006). Not only are these nutrients of interest

due to their control of algal growth, but they can be toxic to fish (unionized ammonia and nitrite ions), nitrifying bacterial strains (unionized ammonia) and aquatic invertebrates (nitrate ions) (Anthonisen *et al*. 1976, Constable *et al*. 2003, Camargo *et al*. 2005), making them of higher interest and essential for inclusion in aquatic ecosystem study. These chemical parameters can also be linked with physical parameters, most importantly suspended sediments contained within the water column.

Fluvial ecosystems are differentiated from other aquatic systems due to their movement of water, which in turn allows for the transport of sediment and ultimately erosion to take place (Montgomery 2007). The movement of sediments and bed material occurs when there is sufficiently high discharge to initiate motion of particles, which is directly related to the size of particles and the velocity of water in the system, making each system unique with regards to its erosion and sedimentation characteristics (Allan and Castillo 2007). Both sedimentation and erosion are natural processes of moving waters, but are highly affected by changes in land-use from natural to anthropogenic. Increased erosion rates have been linked with agriculture directly, with rates of soil loss of ~1mm/year, compared to natural systems that maintain an equilibrium of soil production and losses (Montgomery 2007). These increased losses have been linked with the loss of riparian and natural vegetation cover, both of which play a key role in prevention of soil movement (Schlosser and Karr 1981a). Direct tillage of cropland that breaks down the complex root systems of the previous year, which act as a substitute for natural cover (Montgomery 2007) and increased compaction of soil in either cropland from heavy machinery or from cattle, both of which deteriorate macropores, changing the soil structure (Dunne *et al*. 2011) and influencing soil loss.

Urban land-use is also linked to increased erosion rates through the creation of flashy discharge regimes from decreased soil infiltration from increased impervious cover, thus creating large quantities of overland flow when precipitation events occur (Booth *et al*. 2002). This increased volume and flow is transported to fluvial systems, occurring at higher rates than naturally occurring events, increasing bank erosion (Booth *et al*. 2002). Increased sedimentation and turbidity of fluvial systems can have negative effects on benthic macroinvertebrates through alterations of these substrates (Richards and Bacon 1994) and decrease of algal biomass (Cloern 1987). Control of algal community structure, overall biomass and photosynthetic activity all are linked with light penetration, which can be impeded by increased turbidity from suspended sediments (Irigoien and Castel 1997, Izagirre *et al*. 2009). This control over periphyton (attached algae) and phytoplankton (free-floating algae) by multiple factors makes both forms of algae important bioindicators in aquatic ecosystems.

Aquatic algae can generally be broken down into two groups, phytoplankton, which are those species that live solely suspended in the water column, as well as the portion of benthic algae that become free floating due to disturbance, and periphyton, which is a biofilm that grows on aquatic substrates that includes benthic algae, bacteria, protozoa and fungi (DeForest *et al*. 2016). The study of algae in fluvial ecosystems has led to the understanding that light, temperature and nutrients are the most important limiting factors, making them similarly controlled in comparison to lake systems, but with the addition of discharge regime (Allan and Castillo 2007). Though challenging to study due to the inherent differences in river ecosystems and their surrounding area, algal community structure and overall production have been shown as indicators of river health and nutrient loading (Gallegos and Jordan 1997). This, as well as

short generation time, makes them an ideal candidate for analysis. Although phytoplankton are typically associated with lentic environments, they can be an important part of fluvial ecosystems because of the resuspension of periphytic algae into the water column, even if there are few specific free-floating species maintained within the natural biota (Peterson and Stevenson 1989). Phytoplankton community structure has been shown to be important in indicating organic pollution (Kelly and Whitton 1995), specifically linked to relevant ions (Na^+, Ca^+) (Hill *et al*. 2001), indicators of land-use (Knoll *et al*. 2003) and differences in disturbance regimes (Duong *et al*. 2007). These differences can be evaluated through trophic index information (Rawson 1956, Kelly 1998); simple statistical tests and the use of multivariate analyses, allowing multiple controlling factors to be correlated with highly variable environmental and biological data (Lavoie *et al*. 2011).

Similarly to phytoplankton, periphyton are also an important source of information and indicators of water quality in fluvial ecosystems. Periphyton, though not exclusively algae, share similar relationships to phytoplankton, attributed to the overlap in species that characterize either designation and algal growth frequently dominates periphyton communities. Periphyton share the same quick response time to physical and chemical variability of water quality with phytoplankton, which can alter algal species diversity, relative abundance and potential exclusion of algal functional groups related to water quality shifts (Lavoie *et al*. 2004). Their use also extends to long term nutrient trends linked to their overall community structure and trends in community structure, which can be determined when assessing sediment cores for bioindicator taxa or through long term studies of community structure dynamics (Peterson and Stevenson 1989, Birks *et al*. 1990) One important difference between phytoplankton and

periphyton is that periphyton maintain the same position in fluvial ecosystems due to their attached nature, representing the cumulative changes in water quality among sites, including nutrient type and amount. Both periphyton and phytoplankton can also be used in a more general sense to assess primary production in the form of chlorophyll-a levels (Knoll *et al*. 2003) and algal biomass (Taylor *et al*. 2004), in combination with community structure. These metrics allow for increased information gained from a single algal sample and may offer different information necessary to answer specific research questions.

Algal community structure can also be assessed through the use of fatty acid (FA) extraction for the creation of unique FA profiles. FA profiles are created to determine distribution of the proportions of FAs present in a sample. FAs are found in abundance in algae because they are important structural components of cell membranes, intermediates in cell signalling pathways, storage products of metabolic energy and comprise protective coverings in some species (Arts *et al*. 2009). Profiles can be influenced by not only species abundance, but more importantly by the nutrient and physical (e.g. light availability) conditions over time (Reitan *et al*. 1994, Silva *et al*. 2013). Individual and grouped classifications of FAs can be used in order to characterize specific groups of periphyton, such as increased proportions of palmitic acid (16:0), palmitoleic acid (16: 1ω7) and eicosapantanoic acid (18: 2ω6) as indicators of the dominance of diatoms in an algal community (Hill *et al*. 2011). This is also true for green algae and photosynthetic prokaryotes, which have specific groups of FAs that allow for determination of dominance in a periphyton community (Honeyfield and Maloney 2014). In comparison, proportions of saturated fatty acids (SAFA) and monounsaturated fatty acids (MUFA) will increase as light availability increases, whereas polyunsaturated fatty acid (PUFA) content will

decrease, which relates to the high proportion of PUFAs in photosynthetic membranes of chloroplasts (Hill *et al*. 2011). There are also links to changes in the ratio between SAFAs, MUFAs and PUFAs, with environmentally stressed conditions (e.g. nutrient limitation) leading to increased carbon storage in the form of SAFAs and MUFAs, thus diluting the proportion of PUFAs present (Hu *et al*. 2008). There have been limited studies on the relationships between land-use and proportions of FAs in algae and periphyton, though there is some information to suggest that the proportion of fatty acids (EFA) in periphyton biomass may become reduced when the percentage of intense land-use (i.e. urban and agricultural land-use) increases (Larson *et al*. 2013). This is hypothesized to be related to the increased concentrations of nutrients that are associated with intense land-uses, affecting the algal community and impacting water quality.

Degradation of water quality can occur from nutrient pollution, which in turn influences algal population and alters its structure, controlling many other interrelated processes and food web interactions. These alterations to fluvial ecosystems usually stem from anthropogenic land-uses, (e.g. agriculture and urbanization) and general changes that decrease or alter natural processes, usually in the form of increased nutrient loads. Eutrophication is the result of increased nutrient inputs to aquatic systems, with anthropogenic eutrophication usually causing detrimental changes to aquatic ecosystems, increasing productivity (Schindler 1974). Eutrophication most importantly stems from increases in concentrations of N and P, but may also be linked to other limiting nutrients, dependant on the system. Historically, eutrophication, or “aging” of an aquatic ecosystem, was thought to be a natural process, but in the past 50 years, has shifted to becoming a primary issue in both freshwater and coastal marine

ecosystems globally (Smith and Schindler 2009). As humans change and shape the world, they are impacting the global cycles of carbon, nitrogen, and phosphorus through the increased release of these nutrients into natural ecosystems (Vitousek *et al*. 1997). Eutrophication has been attributed to increased agricultural runoff, increased nutrient loads from urbanized areas and land-use changes from permeable to impermeable surfaces, increasing the contaminants in overland runoff (Vitousek, *et al*., 1997). Agriculture is a factor that, although it has been improved immensely since the Green Revolution, still releases limiting nutrients in great quantities, though in lesser amounts than historical concentrations (Tilman *et al*. 2001). Agricultural practices are becoming more intensified, still relying on large monocultures that must be supplied with excess amounts of N and P, as well as pesticides, in order to keep the high yields that are demanded out of today's farmers (Tilman 1999).

The Nottawasaga Valley Watershed: Current and Emerging Issues

The Nottawasaga River (NR) is located in the Nottawasaga Valley Watershed (NVW), which is located in south-central Ontario (Figure 1.1). The NVW drains 2903 km^2 of the Lake Huron sub-basin, which drains into Nottawasaga Bay of the larger Georgian Bay (Lake Huron). The NVW contains eight subwatersheds (Upper Nottawasaga River, Innisfil Creek, Boyne River, Middle Nottawasaga River, Mad River, Pine River, Willow Creek and Lower Nottawasaga River subwatersheds), which all drain into the Nottawasaga River. Land-use is dominated by agricultural land-use (Table 1.1), but is also characterized by urban, forested, barren and wetland land types. The NVW encompasses one of Southern Ontario's largest wetland complexes, the Minesing Wetlands (MW).

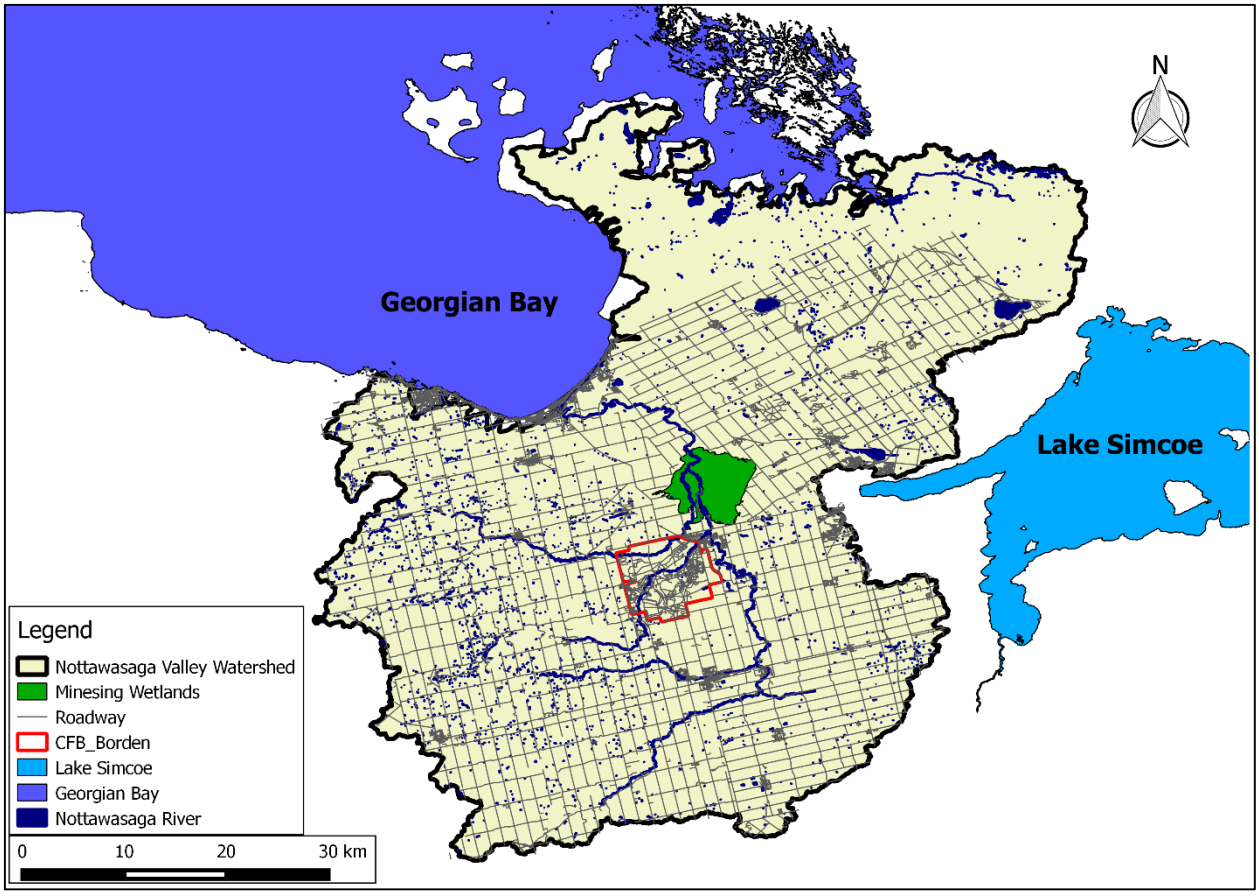

Figure 1.1: Map of the Nottawasaga Valley Watershed

Table 1.1: Summary of land-use breakdown in the Nottawasaga Valley Watershed by percent (%) cover of drainage area at each site.

Site	Urban (%)	Agriculture (%)	Forest (%)	Water (%)	Wetland (%)	Barren (%)
1	5.47	42.4	26.0	0.48	10.9	14.7
2	7.44	64.3	11.8	0.43	8.51	7.50
3	6.64	55.4	17.6	0.45	9.49	10.4
4	7.92	58.9	14.1	0.78	9.33	9.04
5	7.00	60.6	14.9	0.70	9.64	7.17
6	8.13	43.4	26.7	0.57	9.93	11.3
7	8.64	34.4	30.8	0.55	12.7	12.9
8	8.61	34.3	30.6	0.57	13.1	12.9
9	3.58	48.5	20.5	0.60	19.8	7.09
10	8.47	35.9	22.6	0.99	24.1	7.97
11	8.40	36.3	22.5	0.99	23.9	7.91
12	4.92	48.2	17.2	0.69	22.7	6.28
13	5.11	48.5	19.6	1.10	19.3	6.43
14	6.03	56.6	15.9	0.81	14.8	5.91
15	7.66	54.9	17.0	0.94	13.8	5.62

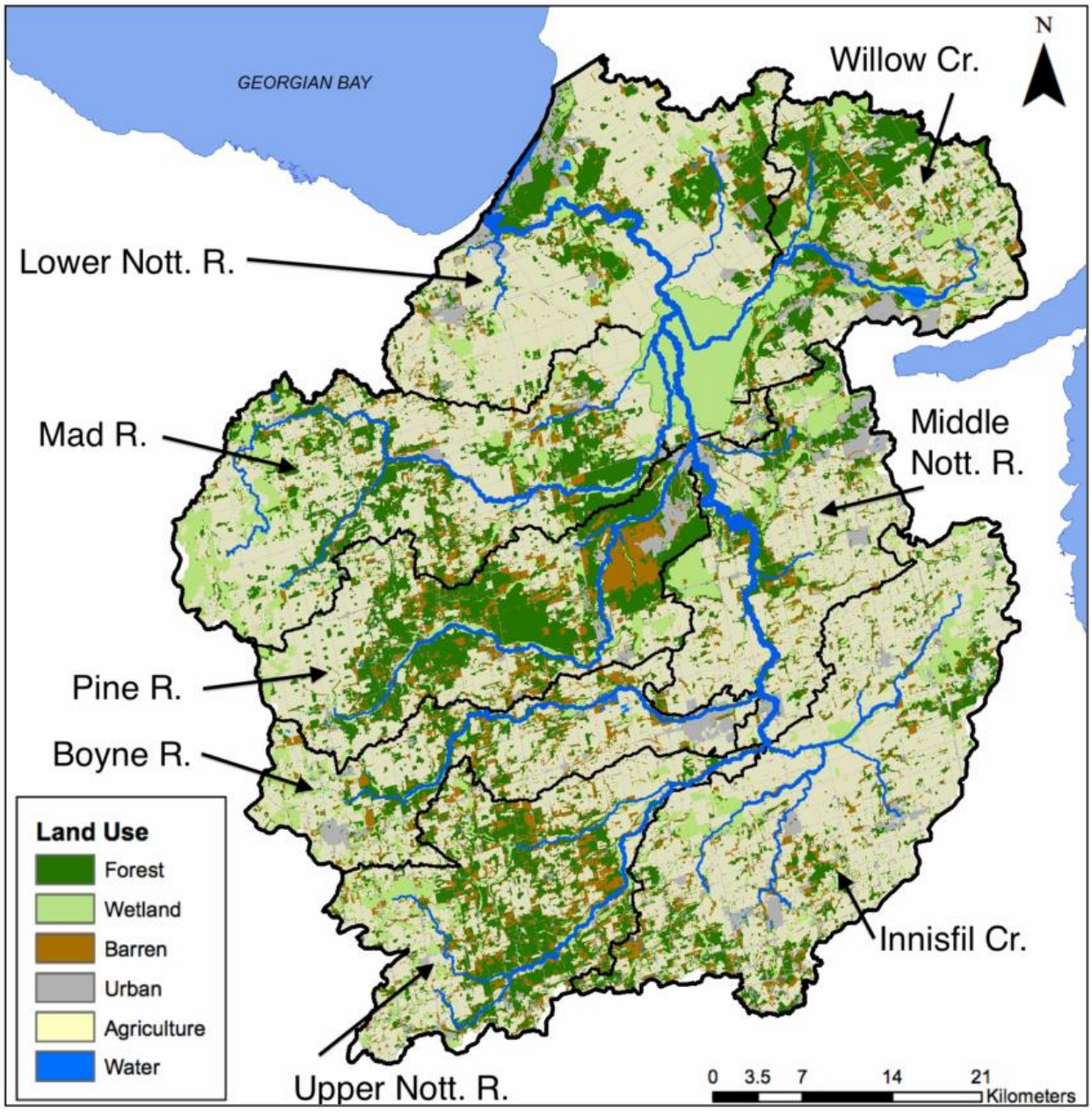

Figure 1.2: Land-use classifications in the Nottawasaga River Valley Watershed divided by subwatershed. Each colour represents a significant land-use type: forest (dark green), wetland (light green), barren (brown), urban (grey), mixed agriculture (biege) and water (blue).

The MW have been designated as an internationally significant natural area under the RAMSAR convention and is a provincially significant wetland (Parker and Dawson 1984) and Life Science Area of Natural and Scientific Interest designation by the Ontario Ministry of Natural Resources (OMNR) (Bowles *et al*. 2007). The MW consists of a mixture of swamp, marsh and fen wetland types, which contain many important features that increase its importance. Some of these features include sequestering of carbon in the fen/peatland area from decaying plant material (Gorham 1991) and the ability for phytoremediation of surface water from the marsh (Williams 2002). It spans 60 km^2 (6000 ha) throughout the Springwater, Clearview and Essa Townships (Figure. 2.3). The MW has been documented to contain 500 vascular plant species, forty-one butterfly species (including 5 provincially rare species), 46 dragonfly/damselfly species, 5 turtle species (3 of which are Species at Risk), 226 bird species and the bisecting NR acts as a migratory corridor for lake sturgeon (*Acipenser fulvescens*) , which are classified as "special concern" under the Species At Risk Act (Bowles *et al*. 2007, Brown *et al*. 2011).

Although the MW is an ecologically important region with important features worthy of preservation, being located within the NVW and its location make it a prime candidate for further human development, whether for agricultural, urban or resource extraction land-uses. It is difficult to determine the impact that this further development would have on the NVW because of the variability seen in previous studies, which could not be directly linked to agricultural, urban or natural land-use (Chow-Fraser 2006, Brown *et al*. 2011). Multiple factors are threatening the health of this system, most importantly the water quality and biological processes that depend on suitable water quality. Firstly, riparian habitat destruction both from agricultural and urban land-uses leads to increased bank erosion, especially with the fine

substrate that dominates the NVW. This will lead to increased sediment in the fluvial water column and increased movement of sediment from the river to Nottawasaga Bay. These increased suspended sediments and turbidity in the river can lead to increased water temperatures since there will be a decreased amount of light reaching areas of increased depth, increasing the temperature of shallow waters. Alterations in sediment loadings can also relate to channel widening, altering the natural flow of the river. With excess suspended solids comes the potential for excess phosphorus in the form of particulate P, whether it be from legacy P bound to sediment or from present-day agricultural sources, aluminum or iron-bound particulate P concentrations are readily available for algal growth (Boström *et al*. 1988, Reynolds and Davies 2001). Nutrients from land-use, particularly agricultural land, as well as inputs from Innisfil Creek, which has been given a surface water quality grade of "F" in previous years (Brown *et al*. 2011), will continue to alter primary production and overall instream water quality in the Nottawasaga River (NR), but limited information has been gathered about the current state and how this may be altered.

Not only have agricultural activities impacted water quality in the NR, but there is also the potential for increasing impacts from urban development. Areas along the NR, such as Midhurst, Simcoe County, Angus and Wasaga Beach, have been projected to have large increases in population over the next 10-20 years, with up to 120% population increases in the next 15 years (Brown *et al*. 2011). This population increase will also lead to increased removal of naturally occurring riparian habitat in favour of retaining walls, which are a poor substitute for the naturally riparian habitat. The Minesing Wetlands, although protected from development, is still under development pressure as the population of the area grows.

Although development may not be directly in the MW, wetlands are highly connected with the river and surrounding area, making it important to increase our understanding on its effect on NR water quality, and conversely how the NR affects water quality as it passes through the wetlands. As previously stated, fluvial ecosystems are highly connected and not only is it important to look at the big picture when it comes to protecting the NR, but also understanding how the smaller headwater streams are being influenced by surrounding agricultural activity, where these impacts may be hard to determine directly in the NR's stem channel. Although they may not individually affect those areas at great distances downstream, the addition of many small inputs from each stream will have a large cumulative effect. Characterization of water quality and algal biomass of agricultural streams may give more insight into the true impact that agriculture can have on the base of food webs, rather than simply a source of nutrients.

Thesis Objectives

The first research objective of my thesis project was to examine water quality (both chemical and biological) along the main-stem branch of the NR, focusing on spatial (i.e. longitudinal) and temporal (i.e. monthly) trends throughout the upper, middle and lower reaches of the river continuum. With a large drainage system impacting the NR, important differences in land-use types, nutrient inputs and point-/non-point sources of nutrients need to be identified over the spatial scale that the NR encompasses, as well as the patterns that arise in agricultural practices, overland runoff and weather that effect the river temporally. These factors can all directly or indirectly affect the surface water chemistry, affecting biota such as phytoplankton: the main primary producers in the river. Thus, the second chapter of my thesis

focuses on water quality trends in the NR and their relation to and influence on phytoplankton community structure. As well, I want to determine if these relationships are related to temporal or spatial land-use types and gradients across the watershed. This information will improve knowledge gaps on baseline water quality trends in the NVW, and for the first time, describe the phytoplankton community composition and abundance in the NR. Ultimately, this knowledge will allow the Nottawasaga Valley Conservation Authority to target land-use management best practices and improve conservation efforts in the watershed.

To improve our understanding of the influence that certain land-use activities (i.e. non-point sources) impact water quality and nutrients in the NVW, my second research objective and third chapter exams first- and second-order streams across the NVW. These streams represent a variety of agricultural land-cover types (corn and soy cropland and pasture land) in order to assess how the run-off from these agricultural types affect total instream nutrients and the bioavailable portion of these nutrients via *in situ* periphyton growth assays. Periphyton growth characteristics and fatty acid composition in relation to study site was then examined to determine if agricultural cover type affects algal abundance and their nutritional quality as a food source for higher trophic levels in the NVW.

Chapter 2: Temporal variation in water quality and phytoplankton community structure along a longitudinal-spatial gradient in the Nottawasaga River

2.1 Introduction

Anthropogenic land-use, whether for agricultural or urban use, are two of the most important impacts to natural ecological and biological processes of aquatic systems (Smith 2003). Overland flow and groundwater of agriculturally dominated landscapes are impacted due to increased risk of surface water eutrophication (McDowell and Sharpley 2003), increases in nitrate concentrations in recharging groundwater (Tesoriero *et al*. 2013), increased algal biomass in streams (O'Brien and Wehr 2009) and overall impacts to water quality from large non-point sources of nutrients (Tran *et al*. 2010). These impacts have been seen both in close proximity to the affected area (Woodcock *et al*. 2006), as well at large distances from the point of impaction (Tran *et al*. 2010). With this in mind, it can often be difficult to distinguish sources of nutrients or other negatively impacting sources in a watershed. Not only is agricultural land-use a large source of negative impacts to natural biological processes, but most anthropogenic land-uses can also cause disruption.

Anthropogenic land-use has become more intensified over the last 100 years, mainly from increased technical developments in agricultural practices, but also increases in population size and increased large urban centres, leading to larger impacts to natural systems (Limburg and Schmidt 1990). Over this time, agricultural practices have changed from small farm sizes, to large, highly simplified and nutrient rich land areas that are typically a monoculture of vegetation (Tilman 1999). Urbanization has been linked with alterations of natural habitats, altered hydrological regimes and changes in nutrient cycles (Alberti *et al*. 2007). Landscapes alterations, especially related to increased urban area size, are occurring at

a rate that is approximately twice that of the respective areas population growth rate (Alberti *et al*. 2007). When defining urban land-use, one of the greatest alterations that can be made is through increased impervious surfaces. Impervious surfaces prevent the infiltration of water into the soil, stemming from rooftops, sidewalks, compacted soil and paved surfaces (Arnold and Gibbons 1996).

Impervious surfaces have negative effects on the hydrological cycle, increasing overland flow and volume of surface water runoff leading to increased erosion and decreased infiltration. This flow alteration decreases groundwater recharge, can cause drying of smaller, ground fed streams during low flow periods and disrupt natural flow regimes, leading to an increased number of peak discharge events. Additionally, receiving water temperature increases are linked with increased overland flow and low albedo values, absorbing a higher proportion of heat from the sun and transferring it to the water (Allan and Castillo 2007).

Primary production in many fluvial systems is dominated by algae, whether in the form of periphyton, algae that grow attached to aquatic macrophytes or other substrates (e.g. wood, rocks), or those that are suspended in the water column as free floating organisms, known as phytoplankton (Nõges *et al*. 1999, Knoll *et al*. 2003, Dodds 2006). Phytoplankton are excellent biondicators of water quality related to several factors. Algal growth is regulated by nutrient availability, especially nitrogen and phosphorus, and N:P ratios (Redfield 1958), as well as some instances of limitations from silica or iron (Van Donk and Kilham 1990) and light availability (Wall and Briand 1979). Quick response times, ranging from hours to weeks depending on environmental disturbance (e.g. nutrients toxic contaminants or physical disturbances) allow for rapid bioassessment of impacted areas, with changes in overall biomass, community

structure and specific species indicators all being important metrics to infer the type and magnitude of impact (Reynolds and Descy 1996).

Phytoplankton of fluvial ecosystems are less susceptible to light limitations due to their acclimation to turbulent and/or turbid environments, though it ultimately depends on the community composition (Reynolds and Padisak 1994). In particular, phytoplankton taxa have been used to assess the impact of agricultural pollution (Pitcairn *et al*. 2003, Lavoie *et al*. 2004), urban stormwater (Vincent and Kirkwood 2014), urban stream pollution (Bere and Tundisi 2011) and to assess impacts from changes in nutrient status (e.g. low nutrient vs. high nutrient) (Lavoie *et al*. 2014). These characteristics made phytoplankton an ideal group of organisms to assess nutrient impacts from land-use and trophic status along the upper, middle and lower NR reaches, including the longitudinal bisection of the Minesing Wetlands.

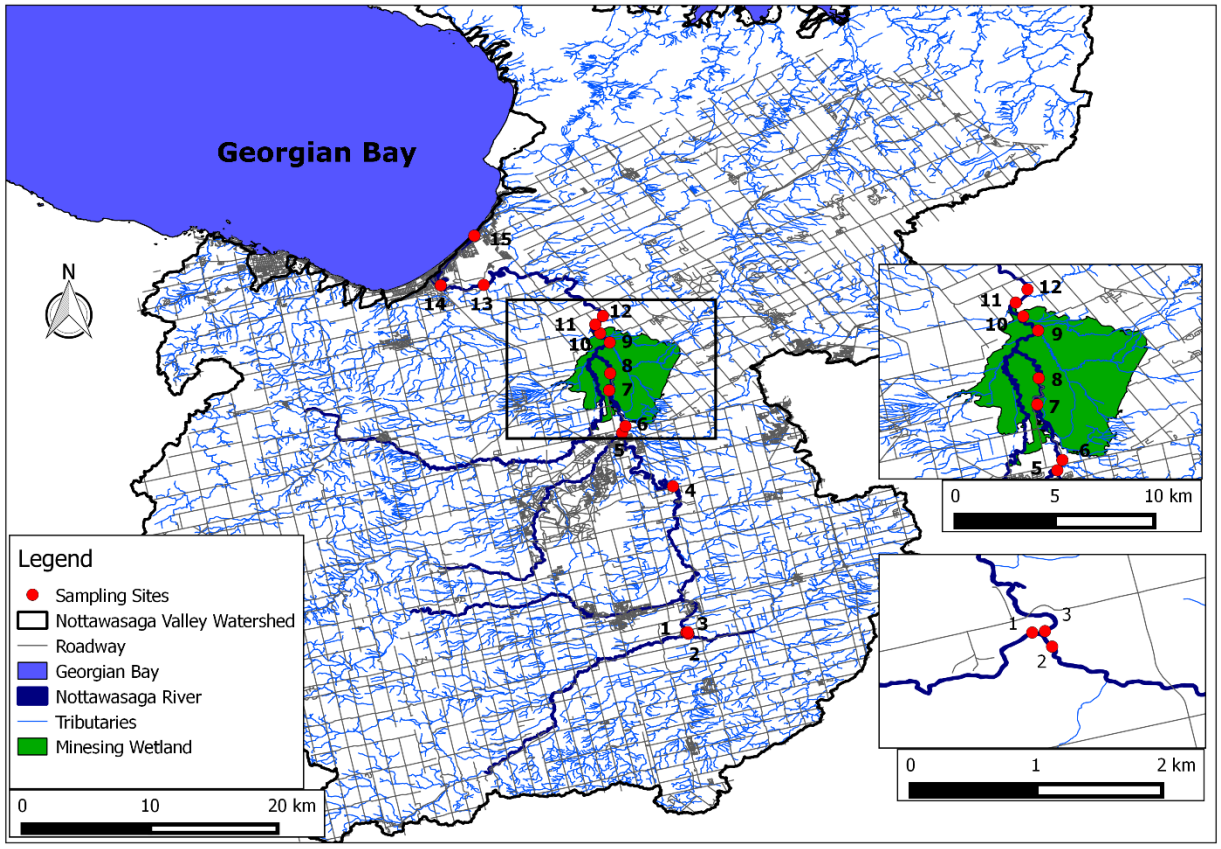

Figure 2.1: The Nottawasaga Valley Watershed with locations of sampling sites and significant features, such as Georgian Bay and the Minesing Wetlands.

Therefore, the main focus of this chapter was to evaluate the monthly water quality trends in the NR over a longitudinal flow gradient reflecting land-use in the NVW and how these trends relate to phytoplankton community structure. In order to achieve these research goals, it was important to address the following research objectives:

1. Characterize and quantify land-use in the NVW in order to determine relationships between land-use and water quality.
2. Determine how water quality is affected by the composition of land-use types across the drainage landscape.

3. Assess the NR for temporal variations in water quality as it relates to land-use.
4. Determine how land-use and associated water quality affects phytoplankton community structure, including taxonomic composition and biomass, along the NR continuum.
5. Assess the influence that the Minesing Wetlands have on water quality and phytoplankton community structure, including taxonomic composition and biomass.

It was anticipated that water quality would vary along the NR continuum, but with generally higher water quality occurring near the upper-reach sites due to less land-use impacts and drainage area, and lower water quality at lower-reach sites reflecting cumulative watershed drainage and associated land-use activities. For the first time, the phytoplankton community was documented in the NR and evaluated for impacts from changing water quality conditions. It was predicted that there would be shifts in the phytoplankton community from a higher proportion of taxa sensitive to pollution and nutrient enrichment to a higher proportion of taxa that were associated with degraded water quality and increased nutrients along the river continuum. This information will not only shed light on phytoplankton community dynamics in a river with variable turbidity and nutrients along its continuum, but also the occurrence of bioindicator taxa that could be monitored in future studies assessing water quality degradation in the NR ecosystem.

2.2 Methods

2.2.1 Study Site Selection

Sites selection was completed by our collaborators from McMaster University (Chow-Fraser, P) using ArcGIS 10.3 and orthophotos of the study area (Government of Canada 2009) in order to determine a robust, 15 site monitoring system along the main channel of the NR, ranging from the upper to lower NR. Monitoring sites were chosen in order to fill previous knowledge gaps determined by Chow-Fraser (2006), which was the rationale for fewer sites in the middle NR and a higher proportion of sites following the MW. As well, following the recommendation of Brown *et al.* (2011), four sites were established along the stretch of river that bisects the MW. All sites were field validated in order to adjust for river access and ease of sampling.

In total, 15 sampling sites, distributed along the upper, middle and lower reaches of the NR were selected for this study (Figure 2.1). To briefly summarize the locations of the NR sites: fourteen sites were located within the NR main channel, sites 1-3 were strategically grouped at the confluence of the upper NR and Innisfil Creek, with site 1 located in the upper NR, site 2 at the mouth of Innisfil Creek before it merges with the NR and site 3 was ~25 m downstream of the confluence between the upper NR and Innisfil Creek. Sites 4, 5 and 6 were located upstream of the MW in the middle NR, with site 5 located near the outflow of the Angus waste water treatment plant. Sites 7, 8, 9 and 10 were established throughout the MW to account for potential inflow impacts by the Mad River (site 9) and Willow Creek (site 10), as well as the wetlands themselves. Finally, in the lower NR, sites 11-15 were located at the exit of the MW (site 11), downstream of Marl Creek (site 12), in Jack's Lake (a widened area in the NR that is considered a lake due to its hydrological processes similar to a lake) (site 13), in the Wasaga

Beach urbanized area (site 14) and finally in the heavily anthropogenic influenced/channelized mouth of the NR before it exits into Nottawasaga Bay (site 15).

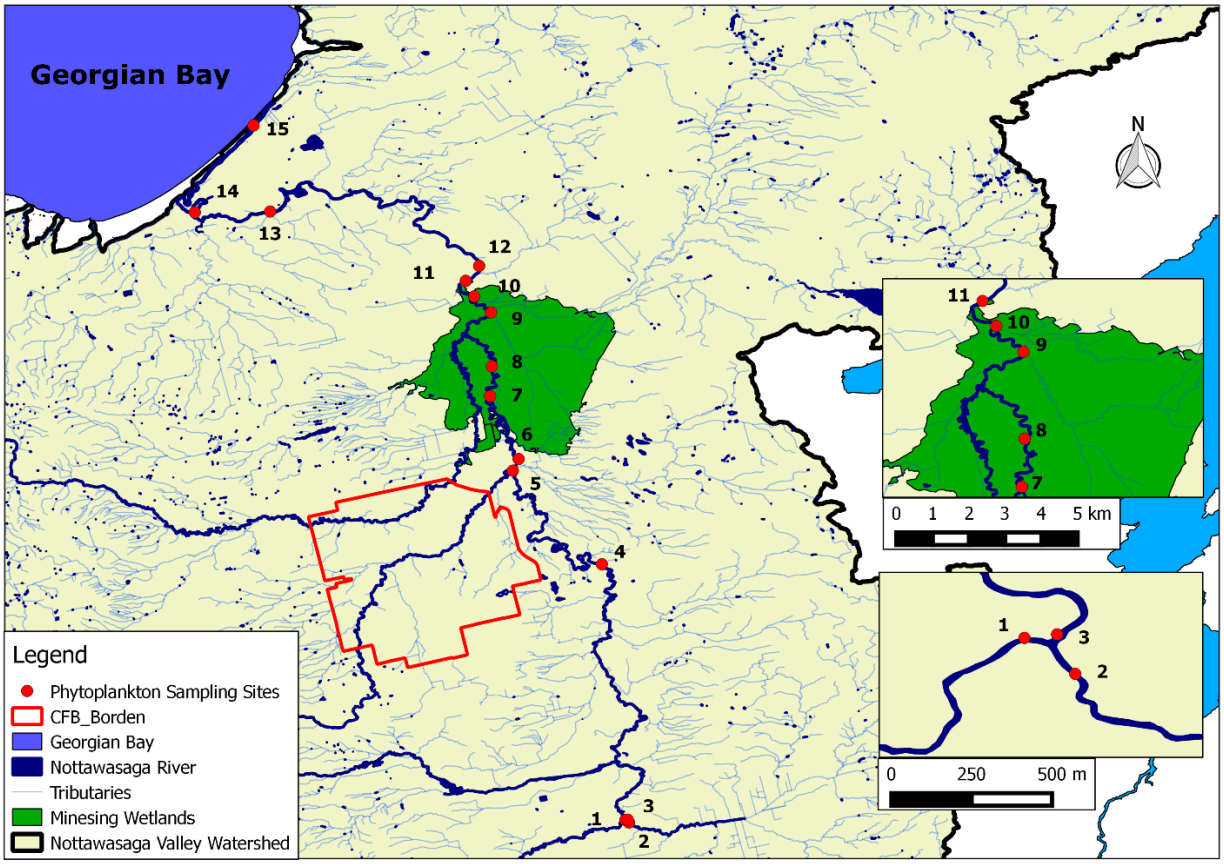

Figure 2.2: Detailed map of the Nottawasaga Valley Watershed illustrating the 15 sampling sites (red circles) sampled monthly between June and September 2014. Sites in close geographical proximity are enlarged for clarification. Significant features are shown, including the Minesing Wetlands and Canadian Forces Base Borden for context.

Table 2.2: Characterization of the Nottawasaga River study site locations. The highest percentage of agricultural land-use in the drainage area is highlighted in bold.

Site	Location in River Reach	Latitude/Longitude Coordinates	Dominant Land-Use Type	Percentage of Area Drained (%)
1	Middle	44.13654, -79.81033	Agriculture	42.4
2	Middle	44.13514, -79.80835	Agriculture	**64.3**
3	Middle	44.13668, -79.80906	Agriculture	55.4
4	Middle	44.27902, -79.82347	Agriculture	58.9
5	Middle	44.33117, -79.87307	Agriculture	60.6
6	Middle	44.33766, -79.86986	Agriculture	43.4
7	Minesing Wetlands	44.37266, -79.88573	Agriculture	34.4
8	Minesing Wetlands	44.3893, -79.88478	Agriculture	34.3
9	Minesing Wetlands	44.41936, -79.8852	Agriculture	48.5
10	Minesing Wetlands	44.4283, -79.89459	Agriculture	35.9
11	Lower	44.43695, -79.89935	Agriculture	36.3
12	Lower	44.44528, -79.89188	Agriculture	48.2
13	Lower	44.47557, -80.00843	Agriculture	48.5
14	Lower	44.4751, -80.0504	Agriculture	56.6
15	Lower	44.52355, -80.017610	Agriculture	54.9

2.2.2 Water Sampling Procedures

In order to capture both spatial and temporal trends, sampling occurred monthly from June-September of 2014 across all 15 sites. A Van Dorn water sampler was employed to collect river water samples at each site. Water samples were transferred to acid-washed 110mL and 200mL Corning™ snap-seal containers or 1000 mL Nalgene® bottles, which were both rinsed with source water as indicated by (Lind 1979) for determination of: total ammonia nitrogen (TAN), total nitrate nitrogen (TNN), total nitrogen (TN), total phosphorus (TP), total suspended chlorophyll-*a* (CHL), total organic suspended solids (TOSS), total inorganic suspended solids (TIOSS) and total suspended solids (TSS). All water samples were stored on icepacks in a cooler until they could be processed within hours of collection (TN, TNN, TAN), or placed in a freezer for storage until they could be processed in the lab (TP, CHL, TOSS, TSS). As well, in-field values for temperature (TEMP), conductivity (COND), pH and DO were obtained using a calibrated YSI multiparameter probe (YSI Inc., Yellowsprings, Ohio, USA). Turbidity (TURB) readings were also measured in triplicate with an in-field Turbidimeter (HACH®, Loveland, Colorado, USA). Water depth was measured using a demarcated anchor line, which ranged from 0.4-4.2m, and all water samples were taken at mid-depth against the current in order to ensure the sample was thoroughly mixed (Shelton 1997). These parameters are widely used as indicators of environmental health in many aquatic systems (del Giorgio *et al*. 1991).

2.2.4 Water Sample Processing.

TP concentrations were determined using the molybdenum blue method (Murphy and Riley 1962) after a potassium persulfate digestion in an autoclave for 50 minutes at 120°C/15

PSI. Absorbance values were then taken using a UV Spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and concentrations were obtained using a standard curve based on known phosphate concentrations. TN and TNN samples were analysed following the HACH® TNT 826 and cadmium reduction methods using a Spectrophotometer (HACH®, Loveland, Colorado, USA). TAN values were obtained after processing using the HACH® 8155 method and analysed in a colorimeter (HACH®, Loveland, Colorado, USA).

Glass fibre filters (0.45 µm pore size) were used to filter known volumes of sample water for both TSS and CHL and frozen at -20°C until analyses could be completed. TSS was calculated by weighing the desiccated filter before and after filtering and subtracting the filter weight, while TOSS was calculated through ashing of the filters in a muffle furnace for 1 h at 550°C to burn off any inorganics present. CHL filters were extracted in 90% reagent grade acetone in a freezer over a 24 h period. Following extraction, samples were acidified with 0.1 N hydrochloric acid and fluorescence was read with a flourometer (Turner Designs, San Jose, California, USA).

2.2.5 Phytoplankton Sampling and Analysis

Phytoplankton samples were taken at all sites concurrently with the water quality samples. Water collected for phytoplankton taxonomic analyses were place in 120 mL Qorpak™ glass bottles. Each sample was preserved with several drops of Lugol's Iodine solution and kept in the dark to prevent further growth. The samples were concentrated via sedimentation before analysis. In brief, a resuspended aliquot of 50 mL was transferred to a 50 mL graduated cylinder and placed in the dark for 60 minutes for acceptable settling to occur (Claessens and Prast 2007). The top 49mL was siphoned off and the final 1 mL was resuspended and transferred to a 1.5 mL eppendorf tube. Samples for identification and enumeration were

placed in a 0.098 mL PhycoTech (ID#615) nanoplankton chamber and visualized on an EVOS™ xlcore phase-contrast inverted light microscope. Using a single transect representing 40 fields of view, cells were identified to lowest possible taxonomic resolution, which was generally the genus level, but species level when possible. Genus level identification has been shown to adequately represent the relevant taxonomic specificity with regards to explanation of environmental variation in comparison to species level identification (Hill *et al*. 2001). Phytoplankton taxa were identified using the keys of Dillard (Dillard 2008), Biggs & Kilroy (Biggs and Kilroy 2000), Spaulding *et al* (Spaulding *et al*. 2010) and Bellinger & Sigee (Bellinger and Sigee 2010). In order to calculate algal biomass, individual cells were measured for standard length and width relationships that allowed for determination of overall phytoplankton biomass (Lund *et al*. 1958)

2.2.6 GIS Analysis of Watershed Features

All land-use information for the study area was determined using QGIS 2.4.0 (QGIS Development Team 2014). Land-use layers for the NVW were used to determine the percent cover of each of six land cover types in the catchment area of each site: urban, agricultural, forest, water and wetland for the drainage area of each site (Government of Canada 2009).

2.2.7 Data Analysis

All water quality and biological data, including taxa abundance, were *log* transformed in order to fulfil the assumptions of normality and homogeneity when completing statistical analyses. Analysis of variance (ANOVA) was used to assess differences in water quality and

biological data across spatial and temporal variation, with a 95% confidence interval. Kruskal-Wallis tests were employed in determination of detectable statistically significant ($p<0.05$) differences attributed to the MW. Principal components analysis (PCA) was completed in order to identify key temporal and spatial variations in biological and water quality data among the sampling sites. PCAs were performed on normalised (i.e. centre-standardized) general biological/chemical parameters and nutrients, to detect correlations between water quality parameters and determine consistency between sampling periods. Pearson correlation coefficient's were determined for significant relationships ($p<0.05$) between water quality variables and biological data, as well as between water quality data and individual taxa abundances. In order to assess the relative contribution of each land-use type on water quality variation, stepwise multiple regression procedures were used. As well, stepwise multiple regression was used in order to determine the contribution to variation that individual water quality parameters had on CHL. Redundancy analysis (RDA) was used to assess the spatial variation in water quality and biological variables that can be attributed to land-use and variations temporally. RDA was chosen as appropriate for the data set because of the linear response in the descriptive variables in relation to the explanatory variables, which was determined using detrended canonical analysis (DCA) in order to distinguish between unimodal or linear data. In order to determine appropriate variables to include in each RDA, variance inflation factors (VIF) were calculated (Pan *et al*. 1996). VIF values allow for the removal of descriptive variables that can cause over inflation of environmental variance explanation, with VIF values of >10 being removed from the analysis(Pan *et al*. 1996). Algal count and abundance information was square-root transformed before running the RDA, as it has been shown to

improve linearity of species-data, as well as retain a similar distance as raw-data when computing ecological distances, such as those found in an RDA (Legendre and Gallagher 2001). RDA was used to assess key spatial variations in the phytoplankton community that could be explained by the water quality parameters and land-use. Finally, Bray-Curtis dissimilarity cluster analysis was completed in order to determine the dissimilarity between sampling sites with regards to the phytoplankton community abundances. All statistical analyses were completed using RStudio 3.3.0 (R Development Core Team 2008).

2.3 Results

2.3.1 Summary of spatial and temporal trends of water quality in the Nottawasaga River

Water quality was found to be highly variable between the sampling sites and between the sampling months (Figures 2.3-2.15). The majority of water quality parameters varied over space and time, with statistically significant ($p<0.05$) differences between at least one of the sampling sites (DO, pH, COND, TURB, TAN, TSS and TOSS), as well as at least one of the sampling periods (DO, TEMP, TP, TN, CHL and BIO) (Table 2.3). DO was consistently lowest at site 13, located near Jack's Lake. Site 1 consistently had the highest pH, with sites above and contained within the MW having consistently higher pH than those that occur downstream. COND was significantly higher at site 2, the mouth of Innisfil Creek, than all other sites, as well as site 1 having a significantly lower COND than sites 4 and 5. Related parameters of TURB, TSS and TOSS showed some similarities, with site 2 having significantly higher TURB than the majority of the other sites, significantly higher TSS than sites 1, 4, 14 and 15, and significantly

higher TOSS than sites 1 and 15. Finally, significantly higher concentrations of TAN were found in site 14 than those in site 1, 3, 7, 9 and 11.

When looking at the difference between the sampling months, DO was found to be significantly lowest in July and highest in both June and August (Table 2.4). July was the warmest month, as well as having the highest concentrations of TAN and TP. September had significantly reduced levels of TAN and TP while also having significantly increased levels of CHL and BIO than June and August. TN can be characterized as being significantly higher in June and significantly reduced in September.

Table 2.3: Means (±SD) and results of one-way ANOVAs for detectable statistically significant (α=0.05) site differences of water quality. Data from all months (n=4) was pooled. Statistically significant p values are shown in bold.

	Site															
Parameter	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	***p***
DO (mg/L)	8.03 (0.30)	8.05 (0.54)	8.44 (0.59)	8.34 (0.75)	7.90 (0.39)	7.96 (0.29)	7.79 (0.53)	7.75 (0.56)	7.64 (0.78)	6.51 (1.11)	6.58 (0.71)	6.54 (0.79)	6.11 (1.47)	6.85 (0.94)	7.31 (1.10)	**<0.001**
pH	8.2 (0.04)	8.1 (0.06)	8.2 (0.05)	8.2 (0.07)	8.2 (0.07)	8.1 (0.09)	8.2 (0.03)	8.2 (0.03)	8.0 (0.09)	7.8 (0.15)	7.9 (0.12)	7.9 (0.09)	7.8 (0.14)	7.8 (0.16)	7.8 (0.17)	**<0.001**
COND (µs/cm)	517 (13.4)	668 (38.1)	563 (31.1)	582 (20.2)	579 (20.5)	543 (19.1)	543 (11.5)	547 (10.2)	532 (12.6)	538 (11.1)	544 (12.8)	542 (12.7)	531 (21.4)	531 (21.1)	540 (27.7)	**<0.001**
TEMP (C°)	18.9 (2.6)	19.7 (2.2)	18.7 (2.2)	20.1 (1.4)	20.8 (0.9)	19.9 (0.8	19.4 (1.6)	19.6 (1.6)	19.2 (0.9)	18.7 (0.5)	19.2 (0.2)	19.1 (0.2)	21.3 (1.5)	21.8 (1.7)	21.6 (1.8)	0.0527
TURB (NTU)	8.3 (0.91)	41.4 (2.08)	15.9 (0.64)	12.3 (0.41)	16.7 (0.48)	19.3 (1.29)	15.1 (0.56)	12.5 (0.45)	13.3 (0.67)	11.6 (0.74)	13.8 (0.67)	15.5 (1.15)	15.6 (0.49)	11.6 (0.17)	7.6 (0.41)	**<0.001**
TP (µg/L)	12.2 (0.7)	48.2 (2.3)	31.1 (2.6)	29.7 (2.5)	30.2 (2.3)	33.9 (3.5)	28.0 (1.9)	30.2 (2.0)	33.4 (2.3)	42.5 (3.3)	34.4 (3.5)	37.3 (3.5)	42.1 (4.4)	32.7 (1.9)	31.6 (1.7)	0.16
TAN (mn/L)	0.01 (0.01)	0.05 (0.01)	0.02 (0.01)	0.03 (0.01)	0.03 (0.00)	0.03 (0.01)	0.02 (0.01)	0.03 (0.02)	0.02 (0)	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)	0.04 (0.01)	0.08 (0.02)	0.04 (0.02)	**0.018**
TNN (mg/L)	0.06 (0.02)	0.04 (0.01)	0.04 (0.01)	0.24 (0.03)	0.08 (0.01)	0.20 (0.06)	0.12 (0.06)	0.15 (0.01)	0.04 (0.03)	0.06 (0.01)	0.08 (0.02)	0.04 (0.02)	0.06 (0.02)	0.09 (0.07)	0.03 (0.01)	0.0861
TN (mg/L)	1.90 (0.05)	1.88 (0.10)	1.63 (0.08)	2.38 (0.05)	2.29 (0.14)	2.17 (0.11)	2.22 (0.13)	2.20 (0.09)	1.89 (0.12)	2.04 (0.08)	1.90 (0.16)	1.73 (0.03)	1.70 (0.05)	1.78 (0.13)	1.91 (0.14)	0.0575
TSS (mg/L)	6.9 (0.5)	29.1 (8.1)	15.1 (1.7)	10.9 (1.4)	13.7 (0.7)	15.9 (1.9)	13.6 (1.2)	12.2 (1.3)	16.2 (1.7)	18.7 (0.9)	15.6 (1.5)	17.4 (1.7)	13.2 (1.2)	10.0 (0.6)	5.5 (0.7)	**<0.001**
TOSS (mg/L)	3.0 (0.3)	6.6 (0.9)	4.9 (0.5)	4.0 (0.5)	4.4 (0.3)	5.5 (0.7)	5.3 (0.8)	4.8 (0.5)	4.9 (0.4)	5.5 (0.4)	4.9 (1.0)	5.5 (0.6)	5.2 (1.1)	3.7 (0.6)	3.1 (0.6)	**<0.001**
CHL (µg/L)	1.54 (0.13)	4.43 (0.32)	2.90 (0.17)	2.75 (0.21)	3.73 (0.27)	2.80 (0.29)	3.33 (0.18)	3.09 (0.19)	2.85 (0.16)	2.70 (0.26)	2.63 (0.13)	2.61 (0.22)	3.92 (0.23)	3.39 (0.23)	3.57 (0.28)	0.302
BIO (µg/L)	193.6 (226.1)	230.1 (237.6)	192.7 (244.8)	128.9 (139.5)	136.6 (117.8)	199.3 (122.6)	198.8 (135.7)	193.6 (119.8)	204.5 (69.7)	212.3 (73.8)	179.1 (82.9)	164.8 (73.5)	172.4 (39.6)	122.3 (50.4)	107.3 (86.4)	0.916

Table 2.4: Means (±SD) and results of one-way ANOVAs for detectable statistically significant (α=0.05) monthly differences of water quality. Data from all sites (n=15) was pooled. Statistically significant p values are shown in bold.

	Month				
Parameter	**June**	**July**	**August**	**September**	***p* value**
DO	7.78 (0.95)	6.73 (1.31)	7.87 (0.47)	7.42 (0.75)	**<0.001**
pH	8.0 (0.16)	8.0 (0.28)	8.1 (0.12)	8.0 (0.18)	0.23
COND	569 (40.8)	559 (45.4)	534 (39.8)	550 (24.1)	0.06
TEMP	19.1 (1.1)	21.5 (1.8)	19.6 (1.1)	19.2 (1.2)	**<0.001**
TURB	12.27 (0.61)	15.14 (1.31)	15.59 (0.61)	18.48 (0.75)	0.28
TP	31.26 (1.68)	50.30 (4.04)	32.36 (2.21)	18.73 (2.32)	**<0.001**
TAN	0.03 (0.015)	0.03 (0.012)	0.03 (0.01)	0.03 (0.011)	0.93
TNN	0.08 (0.03)	0.15 (0.05)	0.07 (0.02)	0.06 (0.02)	0.06
TN	2.28 (0.17)	2.08 (0.09)	1.83 (0.05)	1.71 (0.07)	**<0.001**
TSS	14.36 (1.24)	15.02 (2.47)	12.47 (3.88)	15.24 (1.02)	0.62
TOSS	4.91 (0.6)	4.49 (0.43)	4.69 (0.84)	4.93 (0.69)	0.76
CHL	1.95 (0.11)	3.98 (0.29)	3.27 (0.24)	3.13 (0.21)	**<0.001**
BIO	140.0 (82.8)	169.7 (83.1)	95.1 (38.8)	298.1 (161.9)	**<0.001**

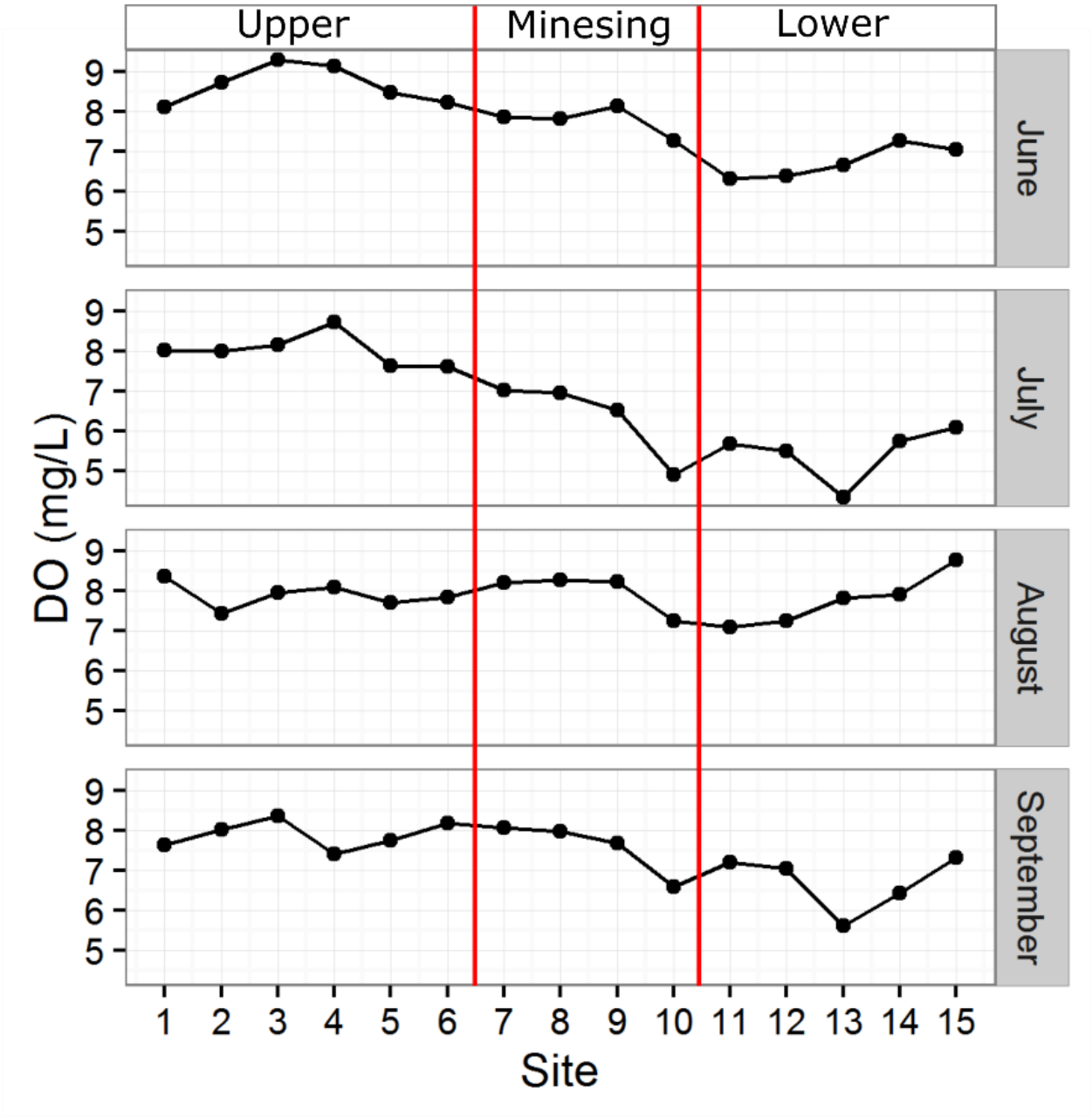

Figure 2.3: Variation in monthly (June-September) dissolved oxygen concentration (mg·L^{-1}) for each monitoring site. DO values are from individual YSI sonde measurements. Sites are divided into their locations relative to the Minesing Wetlands.

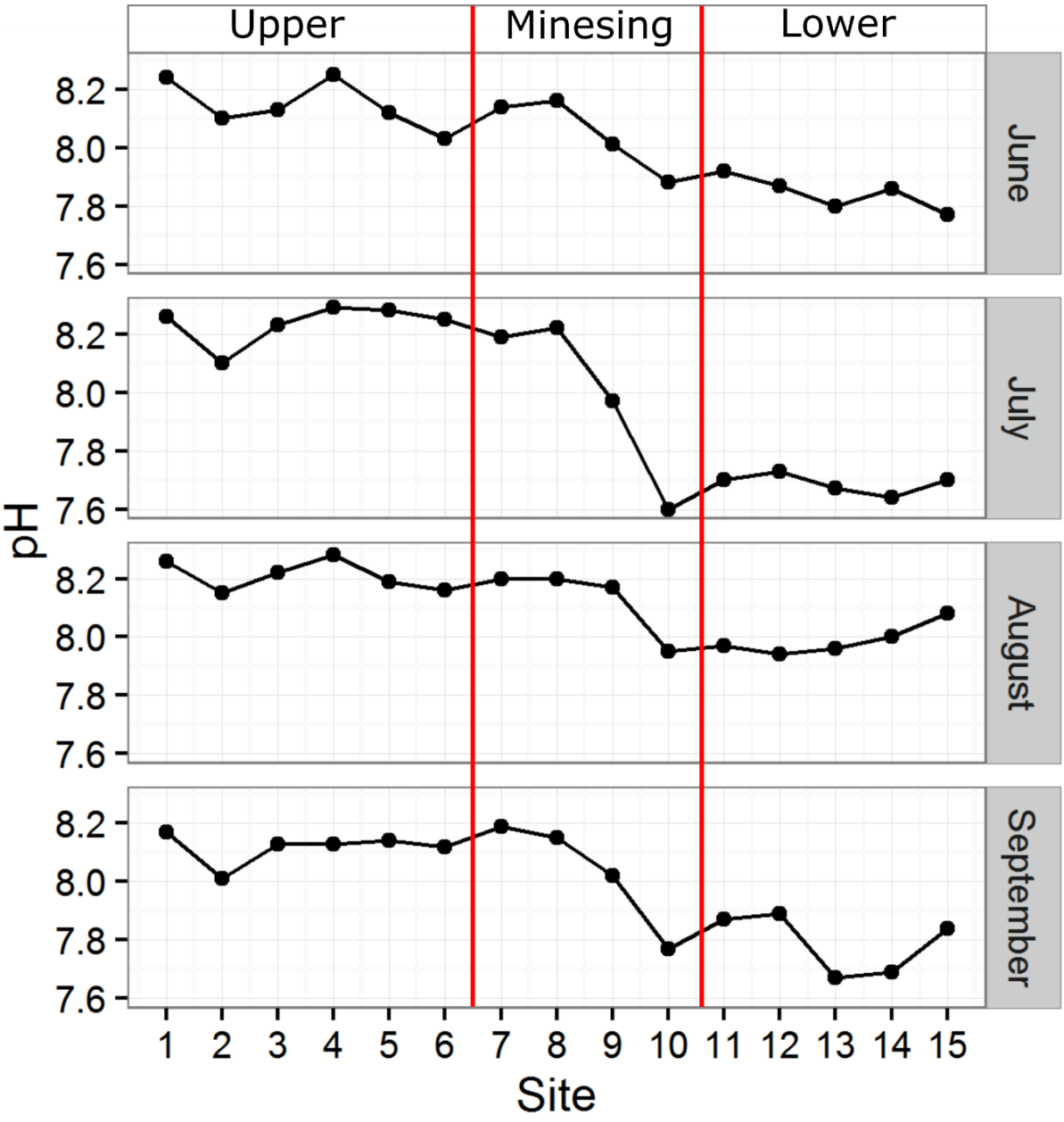

Figure 2.4: Variation in monthly (June-September) pH for each monitoring site. pH values are from individual YSI sonde measurements. Sites are divided into their locations relative to the Minesing Wetlands.

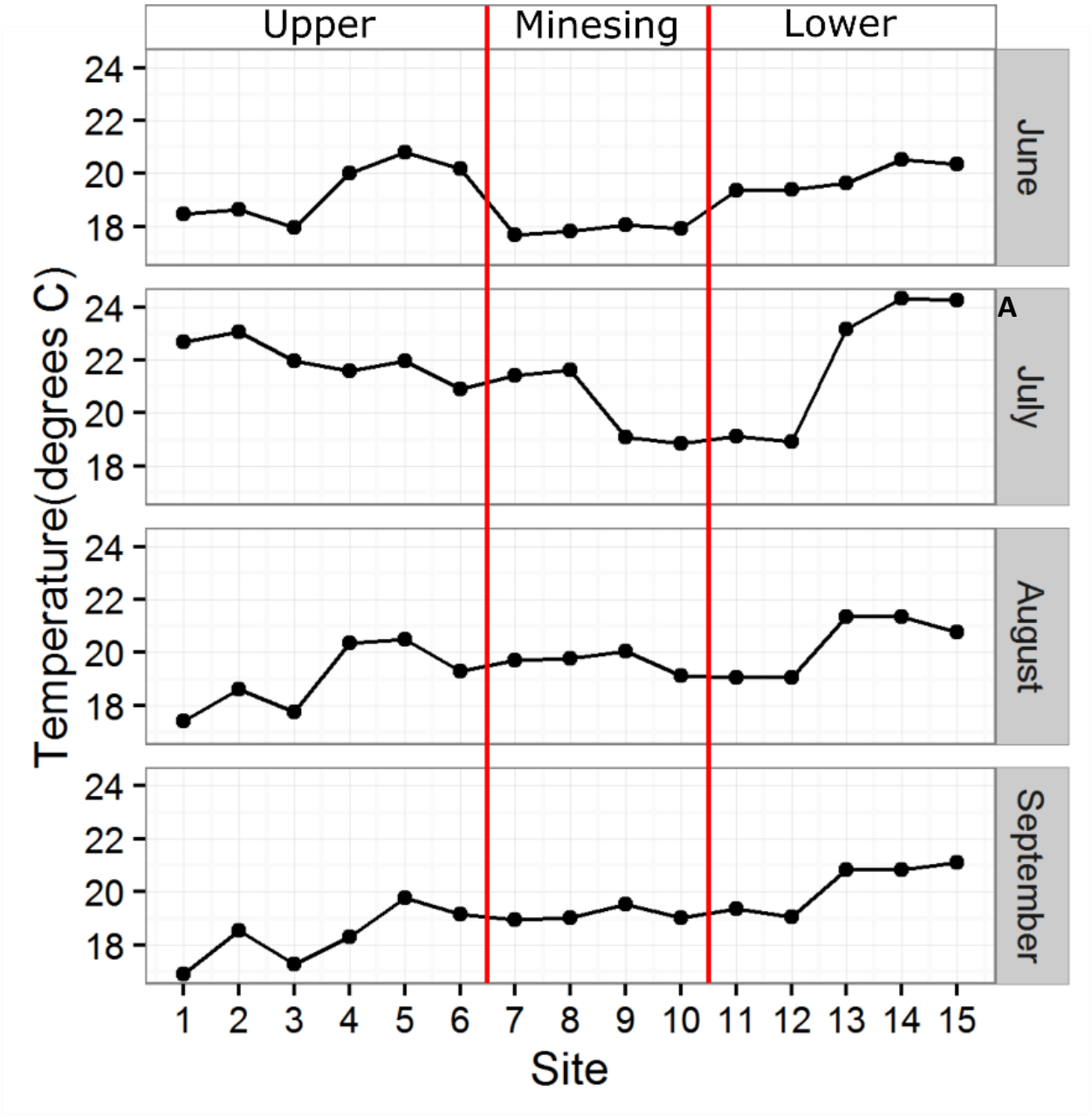

Figure 2.5: Variation in monthly (June-September) in water temperature (C°) for each monitoring site. Temperature values are from individual YSI sonde measurements. Sites are divided into their locations relative to the Minesing Wetlands.

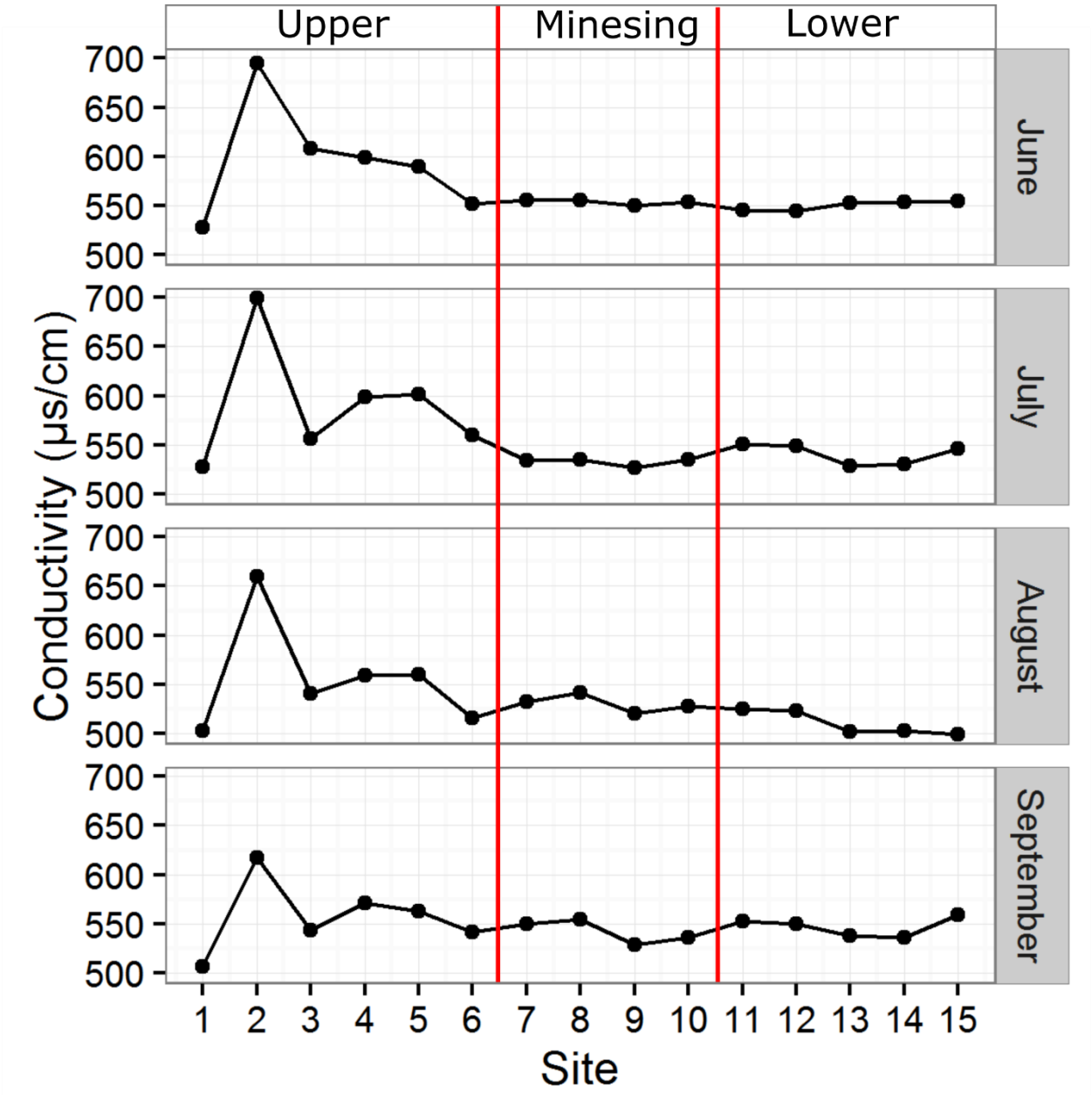

Figure 2.6: Variation in monthly (June-September) in water conductivity (µs·cm^{-1}) for each monitoring site. Conductivity values are from individual YSI sonde measurements. Sites are divided into their locations relative to the Minesing Wetlands.

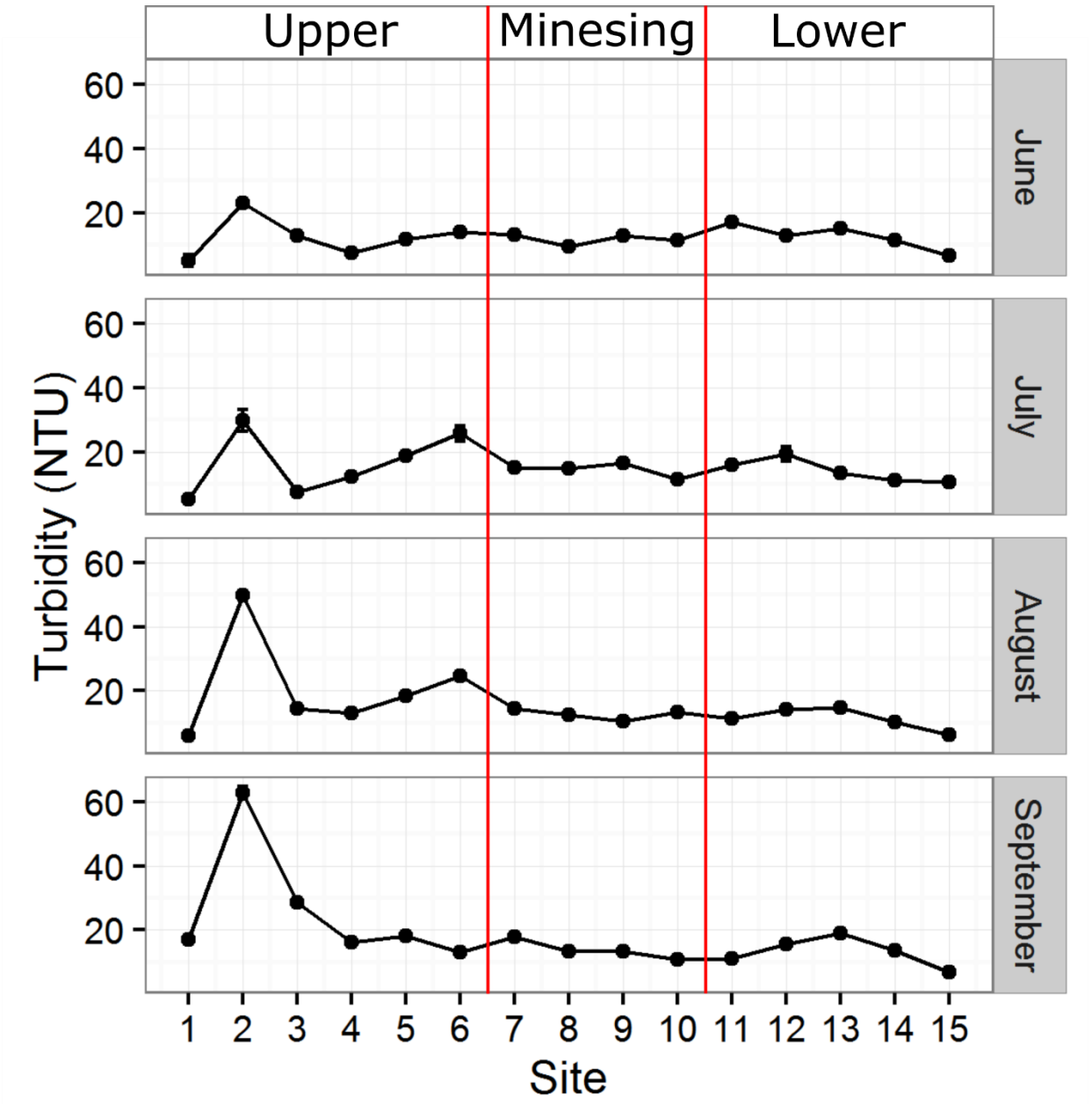

Figure 2.7: Variation in monthly (June-September) water turbidity (±SD) in Nephelometric Turbidity Units for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

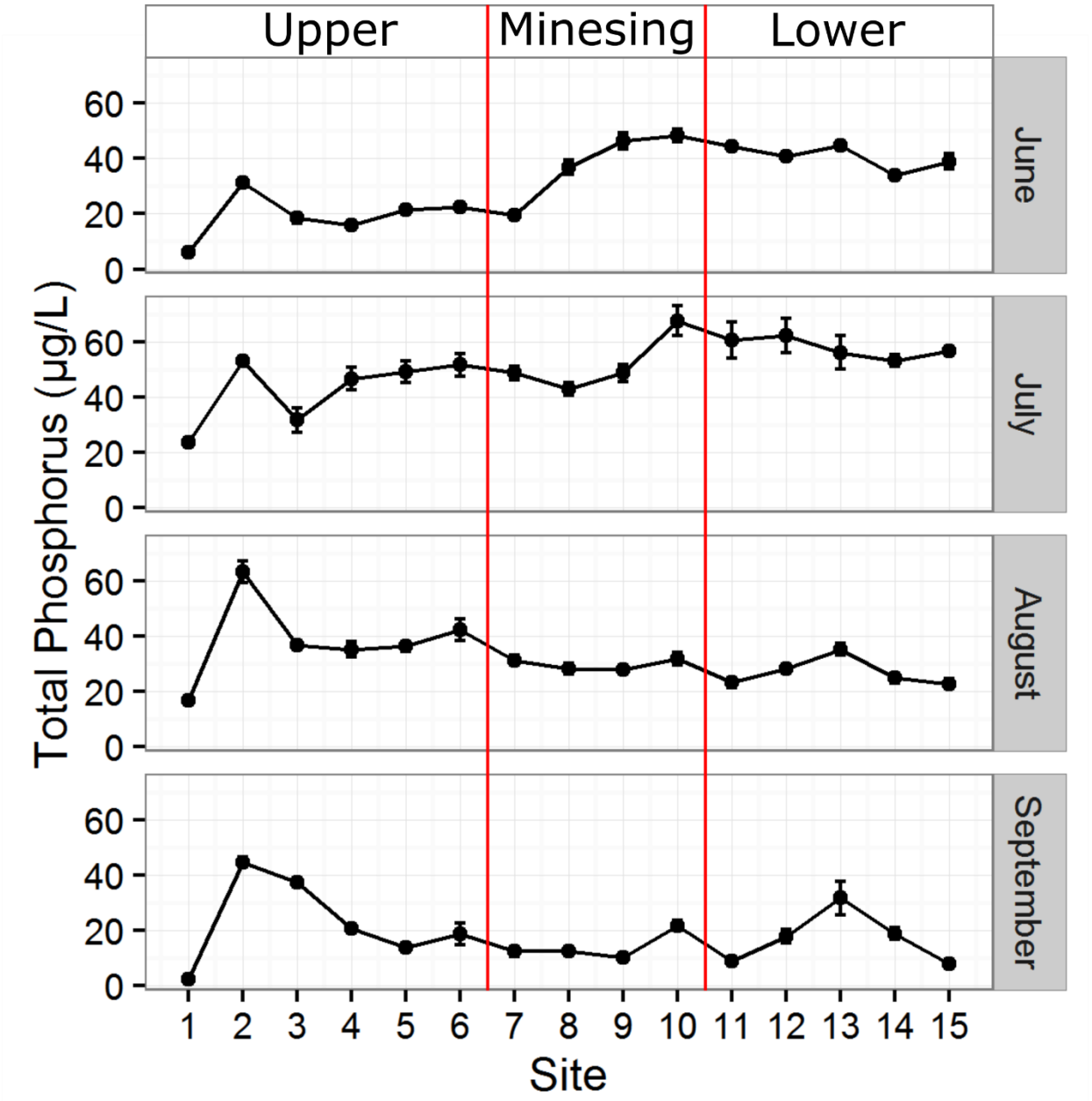

Figure 2.8: Variation in monthly (June-September) total phosphorus concentration (±SD) (µg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

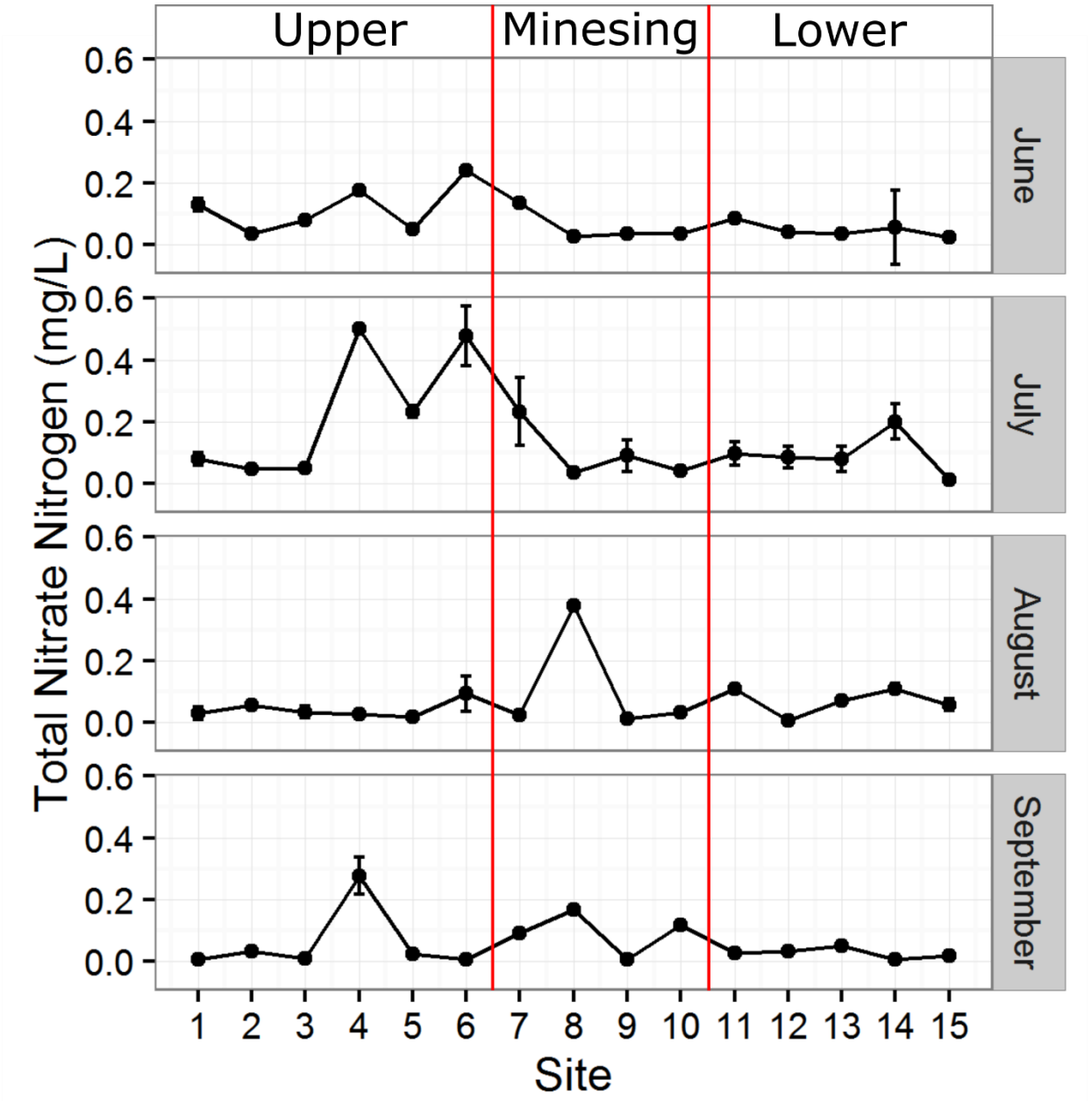

Figure 2.9: Variation in monthly (June-September) total nitrate nitrogen concentration (±SD) ($mg \cdot L^{-1}$) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

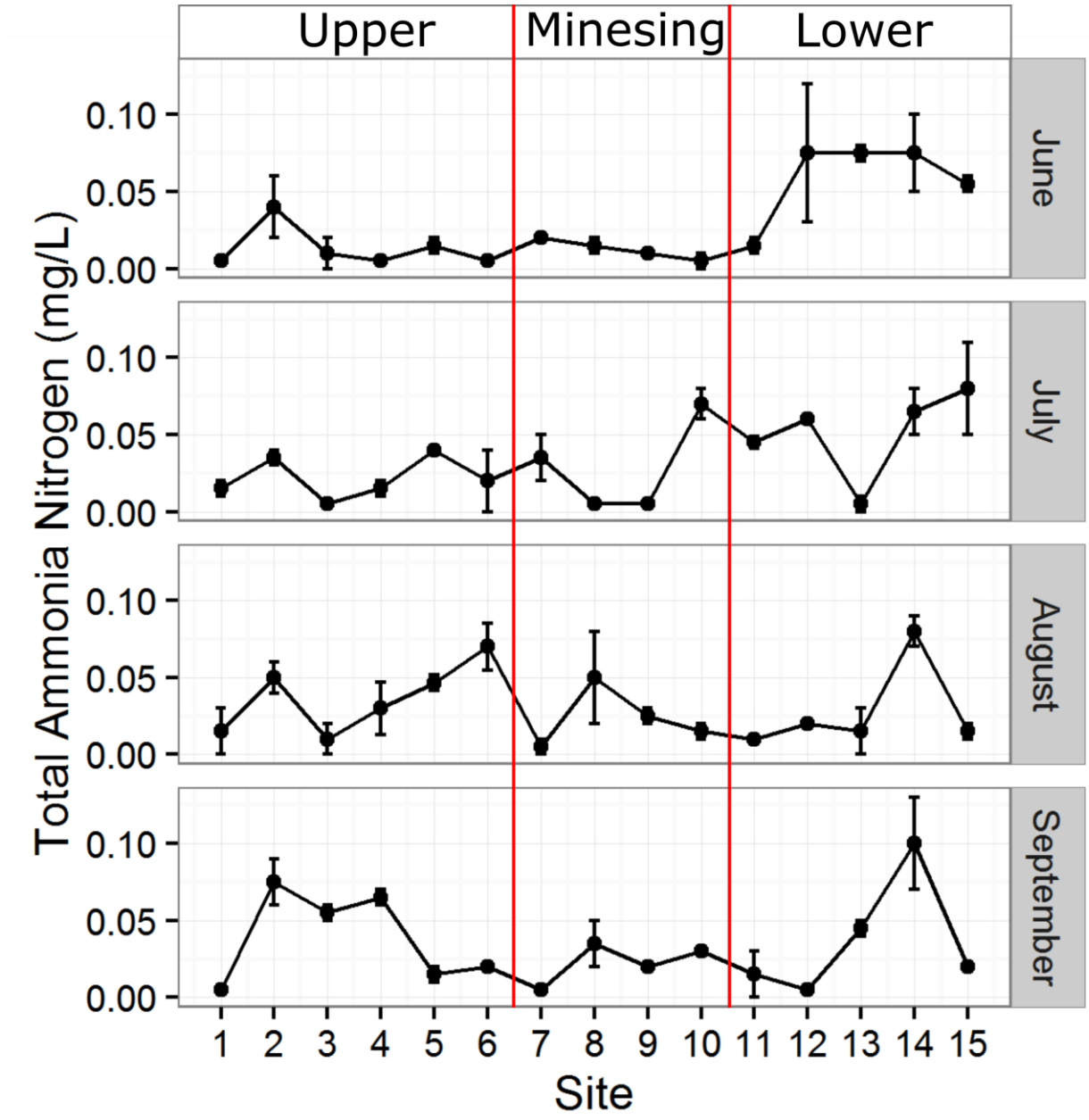

Figure 2.10: Variation in monthly (June-September) total ammonia nitrogen concentration (±SD) (mg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

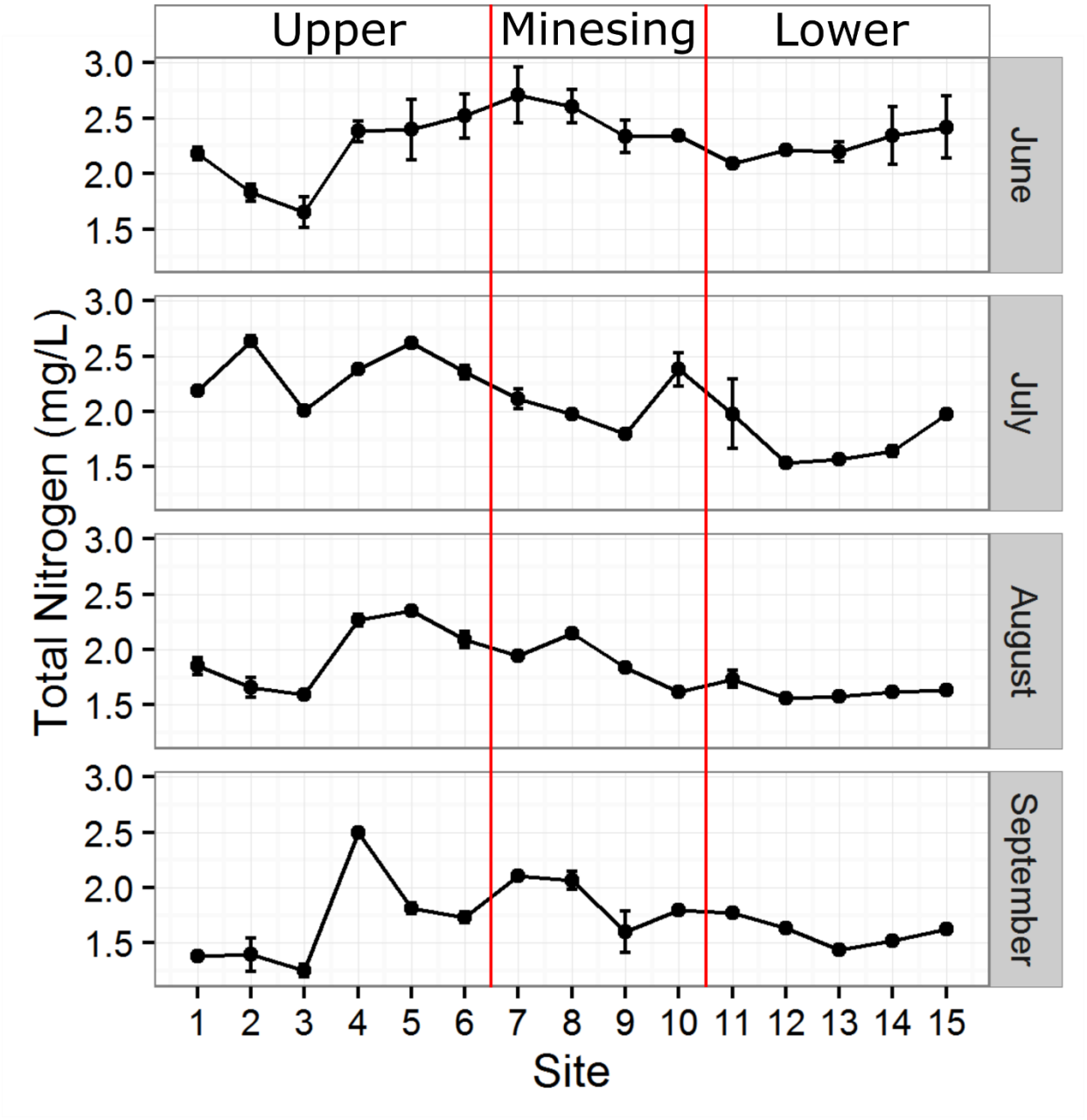

Figure 2.11: Variation in monthly (June-September) total nitrogen concentration (±SD) (mg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

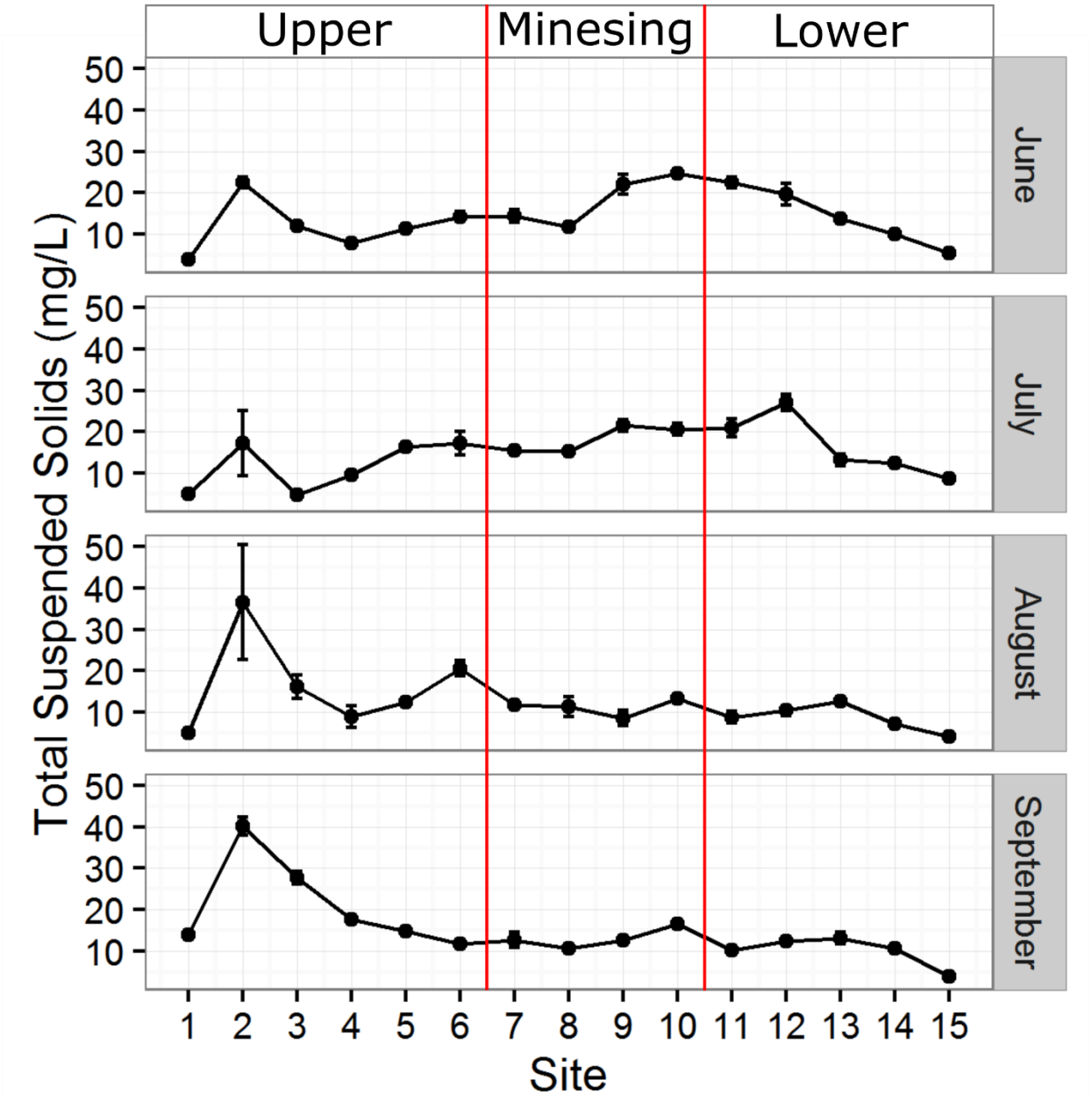

Figure 2.12: Variation in monthly (June-September) total suspended solids concentration (±SD) (mg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

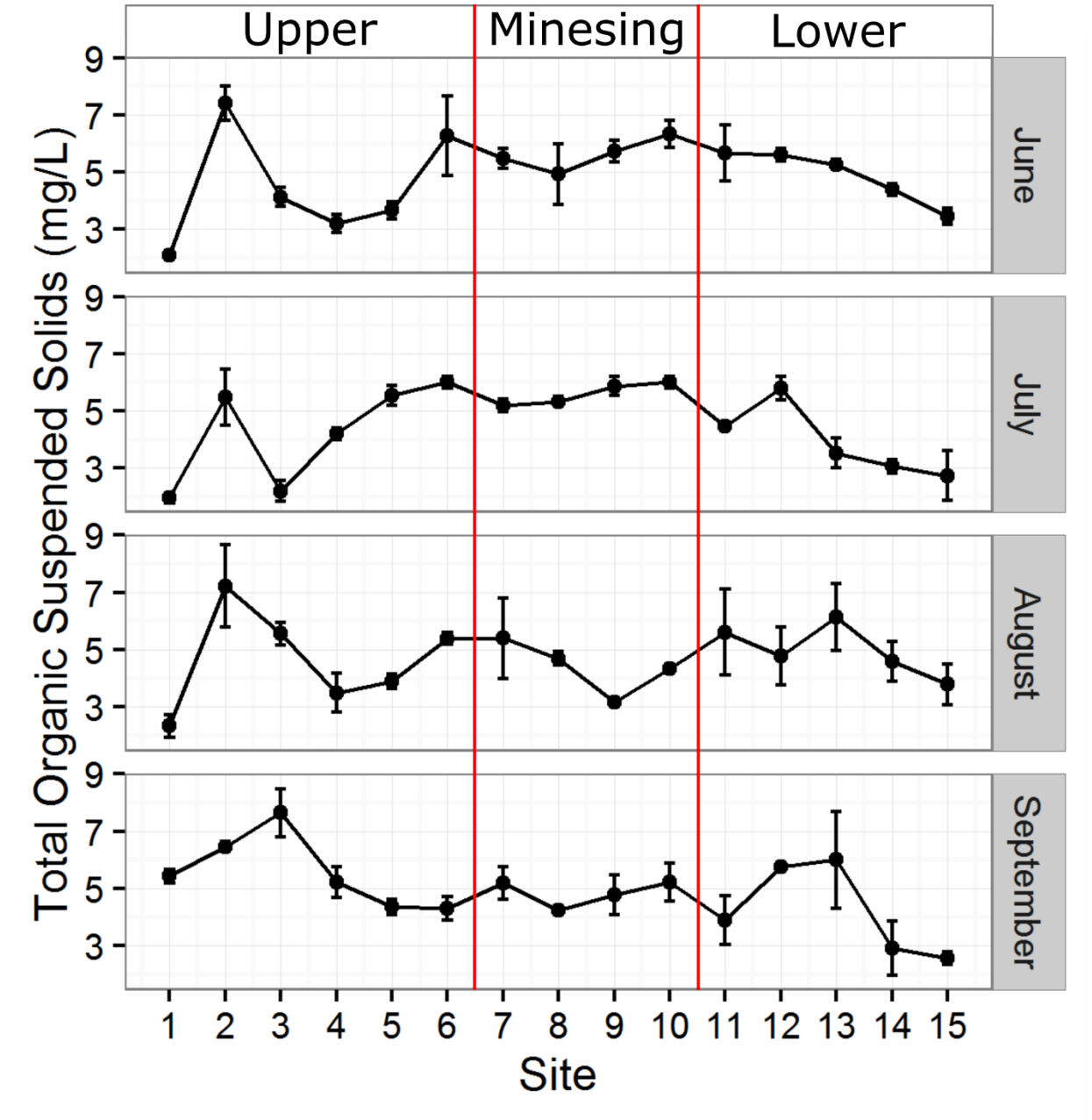

Figure 2.13: Variation in monthly (June-September) total organic suspended solids concentration (±SD) (mg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

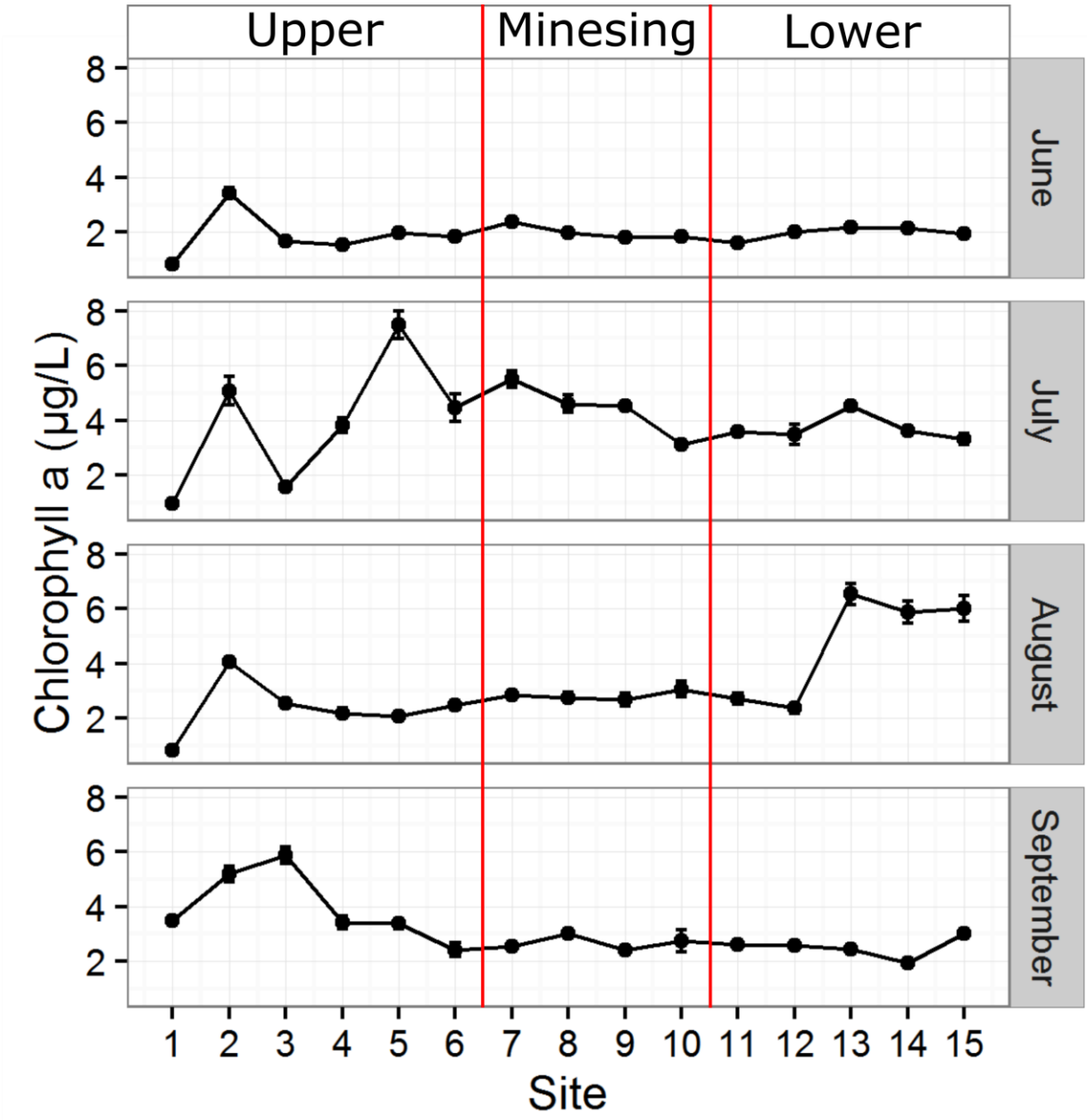

Figure 2.14: Variation in monthly (June-September) total chlorophyll a concentration (±SD) (µg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

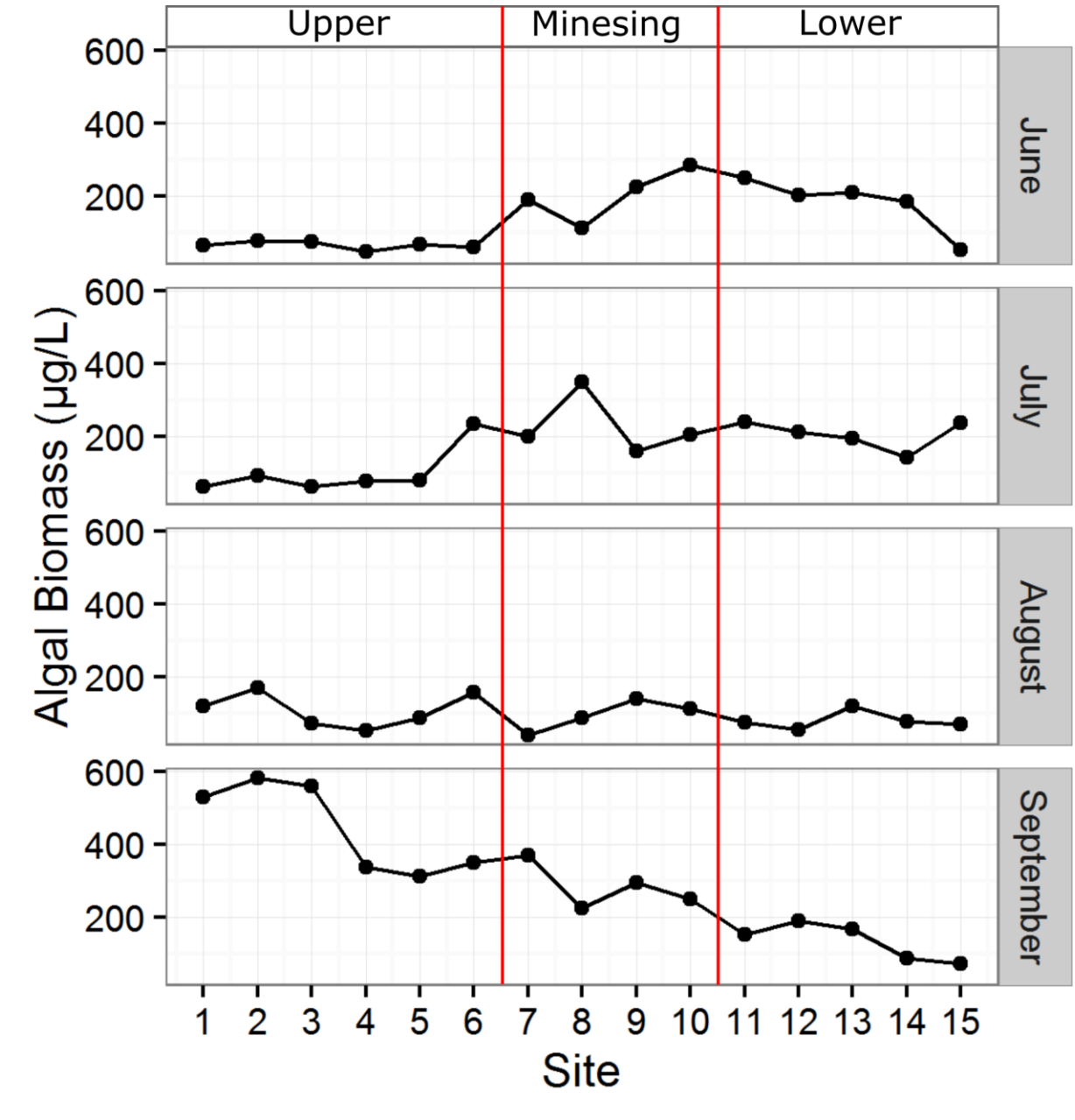

Figure 2.15: Variation in monthly (June-September) total suspended algal biomass concentration (µg·L^{-1}) for each monitoring site. Sites are divided into their locations relative to the Minesing Wetlands.

2.3.2 Summary of water quality variation linked to the Minesing Wetlands

In order to determine if the MW were having any detectable effects on the water quality parameters, sites were grouped into three distinct units: upper Nottawasaga (sites 1-6), Minesing (sites 7-10) and lower Nottawasaga (sites 11-15). These three groups were then averaged for their sites, as well as over the total sampling period in order to prevent any temporal patterns causing interactions.

Kruskal-Wallis tests revealed that there was statistically significant ($p<0.05$) differences in these three distinct sections of the Nottawasaga River for: DO, pH and COND over the pooled sampling period (Table 2.5).

Table 2.5: Means (±SD) and results of Kruskal-Wallis tests for detectable statistically significant (α=0.05) differences of water quality related to location of sites grouped into lower Nottawasaga River (n=24), within the Minesing Wetlands (n=16) and upper Nottawasaga River (n=20) sites.

	Location			
Parameter	**Upper**	**Minesing**	**Lower**	***p* value**
DO (mg/L)	8.12 (0.49)	7.78 (1.31)	6.68 (1.01)	**<0.001**
pH	8.2 (0.08)	7.9 (0.44)	7.8 (0.13)	**<0.001**
COND (µs/cm)	575 (53)	540 (12)	537 (19)	**<0.001**
TEMP (C°)	19.7 (1.76)	19.2 (1.14)	20.6 (1.67)	0.0553
TURB (NTU)	18.97 (1.13)	16.22 (0.62)	12.83 (0.69)	0.453
TP (µg/L)	30.89 (2.46)	22.77 (2.44)	35.62 (3.15)	0.62
TAN (mg/L)	0.03 (0.01)	0.02 (0.01)	0.04 (0.02)	0.0734
TNN (mg/L)	0.11 (0.03)	0.04 (0.03)	0.06 (0.04)	0.668
TN (mg/L)	2.04 (0.09)	1.14 (0.11)	1.80 (0.23)	0.0253
TSS (mg/L)	15.28 (3.53)	15.19 (1.31)	12.33 (1.21)	0.15
TOSS (mg/L)	4.73 (0.59)	3.34 (0.57)	4.50 (0.79)	0.244
CHL (µg/L)	3.02 (0.24)	1.85 (0.2)	3.22 (0.22)	0.843
BIO (µg/L)	180.2 (160.28)	107.8 (93.12)	149.2 (68.11)	0.102

2.3.3 Principal components analyses (PCA) on water quality

A PCA was performed on chemical, biological and nutrient parameters over the entire sampling period (June-September) (Figure 2.16). Even though there were significant differences found for multiple WQ parameters over the sampling period, a PCA was run on all of the data in order to compare the differences in the most important factors to describe the variation at each sites over the 4 month sampling period. Together, the first and second principle components explained 48.53% of the variation in the data set. The PCA biplots allowed for the visualization of the spatial and temporal differences that were evident in the determined statistically significant differences between sites with regards to their water quality parameters.

The sampling sites show fairly tight clustering between the sampling dates, although monthly variation is reflected in the biplot. Multiple water quality parameters also showed high levels of collinearity, such as suspended particle parameters (TOSS, TSS and TURB) and biological parameters (CHL and BIO). Site 1 (the “least impacted” site) was characterized by low values for COND, TSS, TOSS, TURB, CHL and BIO, while sites 2 and 3 (those directly in the outflow of Innisfil Creek) were characterized by high values of these parameters. Sites also demonstrated clear environmental characteristics when grouped into upper Nottawasaga, Minesing Wetlands and lower Nottawasaga sites, with the upper Nottawasaga sites showing the most variability between the groupings. The upper Nottawasaga sites (green) were positively associated with TOSS, TURB, TSS, CHL and BIO, while the lower Nottawasaga (red)

sites were positively associated with DO, pH, COND and TEMP. The sites located within the Minesing Wetlands (blue) show less variability, clustering around the origin of the biplot.

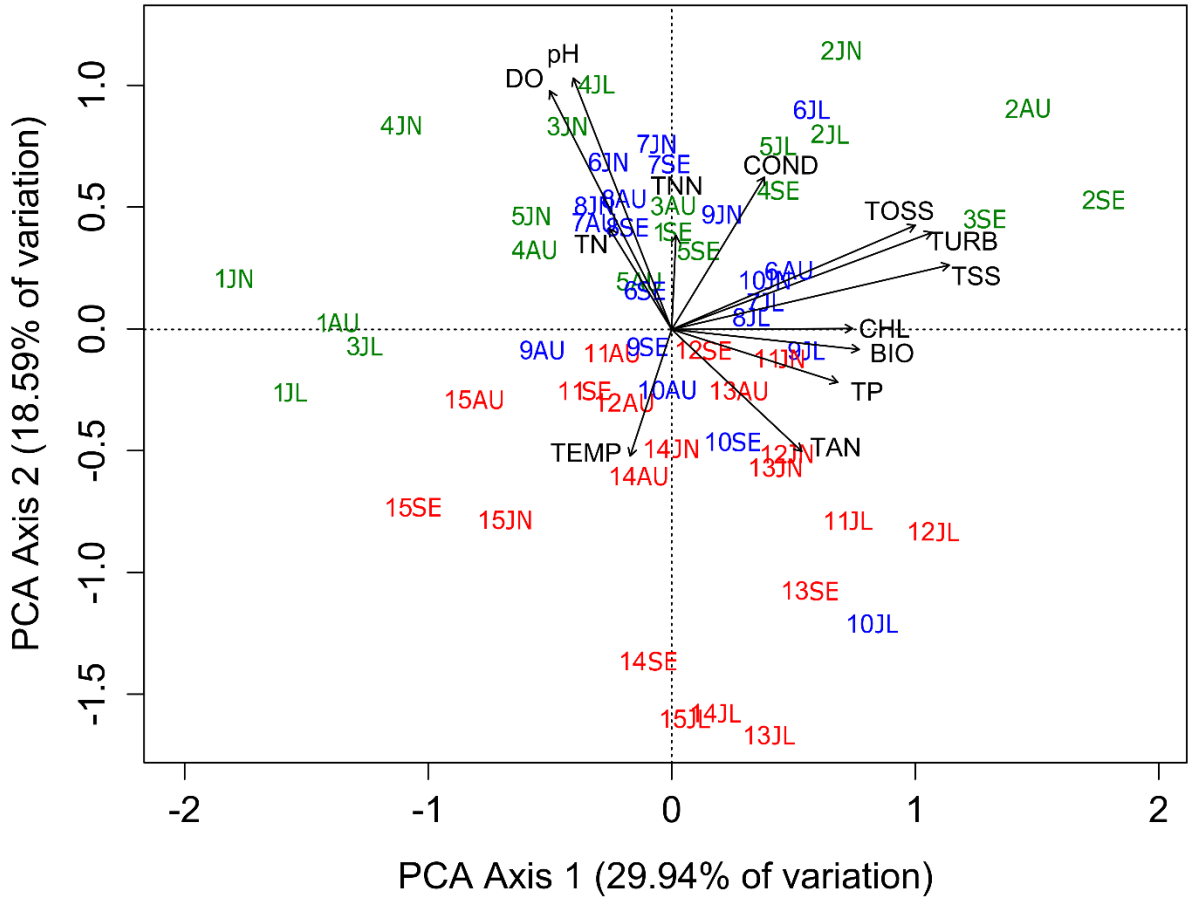

Figure 2.16: Principal components analysis biplot performed on all monthly normalised WQ parameter data for fifteen sites. Sites are colour coded for their grouped areas: upper Nottawasaga River (green), Minesing Wetlands (blue) and lower Nottawasaga River (black). Sampling period is denoted by two-letter month codes: June (JU), July (JL), August (AU) and September (SE)

2.3.4 Catchment land-use effects on water quality in the Nottawasaga River

Pearson correlation analyses were run on data sets from each month in order to identify differences in relationships between sampling months. During the June (Table 2.6) sampling period, COND and TEMP both increased, while BIO decreased as the percentage of agricultural land-use increased, COND and TEMP both decreased, while TN increased as the percentage of forest land-use increased, DO and pH both decreased, while TP increased as the percentage of water land-use increased, DO, pH and COND decreased, while TP, TSS and BIO all increased as the percentage of wetland land-use increased. Finally, pH and TNN increased, while TEMP, TP and TAN decreased as the percentage of barren land-use increased. There were no significant correlations between any of the water quality variables and the urban land-use percentage though it only covers minimal amounts of the landscape.

Table 2.6: Pearson correlation coefficient values for the June data set of all fifteen sites based on land-use cover type (percentage). Statistically significant ($p<0.05$) relationships are shown in bold.

WQ Parameter	Urban	Agriculture	Forest	Water	Wetland	Barren
Log DO	0.12	0.42	-0.14	**-0.69**	**-0.82**	0.41
Log pH	0.2	0.03	0.21	**-0.73**	**-0.67**	**0.74**
Log COND	0.19	**0.66**	**-0.59**	-0.37	**-0.52**	-0.17
Log TEMP	-0.05	**0.53**	**-0.52**	0.42	-0.14	**-0.53**
Log TURB	0.04	0.08	-0.19	-0.01	0.21	-0.34
Log TP	-0.03	-0.1	-0.15	**0.56**	**0.68**	**-0.70**
Log TAN	-0.38	0.31	-0.39	0.37	0.27	**-0.62**
Log TNN	0.29	-0.14	0.31	-0.28	-0.42	**0.54**
Log TN	0.26	-0.44	**0.53**	0.2	0.07	0.17
Log TSS	0.02	-0.2	-0.02	0.09	**0.54**	-0.32
Log TOSS	0.15	-0.19	0.06	0.06	0.39	-0.29
Log TIOSS	-0.07	-0.21	-0.03	0.06	0.48	-0.22
Log CHL	0.17	0.29	-0.29	-0.03	-0.05	-0.43
Log BIO	-0.22	**-0.51**	0.23	0.41	**0.85**	-0.27

During the July sampling period, similar correlations were seen to those captured in June, with a few differences (Table 2.7). Firstly, TN was positively correlated with urban land-use percentage, which differentiates it from June, whereas COND and BIO both followed the same relationship with agricultural land-use percentage as that found in June. COND decreased as forest land-use percentage increased, consistent with June, as well as the correlations between DO, pH and TP with water land-use percentage, DO, pH, TP, TSS and BIO with wetland land-use percentage and finally pH, TP and TAN with barren land-use percentage, which were all consistent with June, although all Pearson correlation values increased for those that were consistently statistically significant. Additional significant correlations were determined between TN and wetland land-use percentage, as well as DO and barren land-use percentage.

Table 2.7: Pearson correlation coefficient values for the July data set of all fifteen sites based on land-use cover type (percentage). Statistically significant ($p<0.05$) relationships are shown in bold.

WQ Parameter	Urban	Agriculture	Forest	Water	Wetland	Barren
Log DO	0.21	0.34	-0.05	**-0.78**	**-0.85**	**0.53**
Log pH	0.19	0.12	0.20	**-0.77**	**-0.80**	**0.69**
Log COND	0.24	**0.65**	**-0.59**	-0.32	-0.5	-0.18
Log TEMP	0.03	0.51	-0.25	-0.07	**-0.64**	0
Log TURB	0.16	0.14	-0.14	-0.08	0.02	-0.30
Log TP	0.19	-0.03	-0.22	**0.63**	**0.57**	**-0.71**
Log TAN	0.23	0.06	-0.27	0.43	0.35	**-0.52**
Log TNN	0.23	0.14	0	-0.09	-0.4	0.17
Log TN	**0.57**	0.18	-0.07	-0.33	**-0.55**	0.31
Log TSS	0.03	-0.25	0.05	0.25	**0.58**	-0.4
Log TOSS	0.21	-0.21	0.12	0	0.27	-0.16
Log TIOSS	-0.08	-0.27	0.04	0.32	**0.62**	-0.42
Log CHL	0.21	0.12	-0.09	0.15	0	-0.35
Log BIO	0.26	**-0.61**	0.49	0.42	0.56	-0.14

During the August sampling period (Table 2.8), fewer significant correlations were determined. Firstly, pH decreased as both water and wetland land-use percentage increased, but increased as barren percentage increased. TEMP also increased as water percentage increased, but decreased as barren increased and CHL increased as water percentage increased, but decreased as barren percentage increased.

Table 2.8: Pearson correlation coefficient values for the August data set of all fifteen sites based on land-use cover type (percentage). Statistically significant (p<0.05) relationships are shown in bold.

WQ Parameter	Urban	Agriculture	Forest	Water	Wetland	Barren
Log DO	-0.09	0.07	0.23	-0.24	-0.50	0.33
Log pH	0.13	0.18	0.15	**-0.72**	**-0.82**	**0.64**
Log COND	0.27	0.43	-0.39	-0.45	-0.41	-0.02
Log TEMP	-0.05	0.21	-0.21	**0.59**	0.13	**-0.57**
Log TURB	0.23	0.31	-0.26	-0.32	-0.29	-0.1
Log TP	0.22	0.43	-0.35	-0.25	-0.36	-0.17
Log TAN	0.06	0.33	-0.16	-0.21	-0.38	-0.10
Log TNN	0.38	-0.37	0.45	-0.02	-0.05	0.28
Log TN	0.34	-0.03	0.24	-0.28	-0.50	0.42
Log TSS	0.23	0.17	-0.12	-0.36	-0.24	0.03
Log TOSS	0.32	0.04	-0.09	0.05	0.04	-0.21
Log TIOSS	0.14	0.10	-0.12	-0.47	-0.34	0.23
Log CHL	0	0.25	-0.32	**0.54**	0.19	**-0.66**
Log BIO	-0.25	0.12	-0.06	-0.12	-0.04	-0.05

Table 2.9: Pearson correlation coefficient values for the September data set of all fifteen sites based on land-use cover type (percentage). Statistically significant (p<0.05) relationships are shown in bold.

WQ Parameters	Urban	Agriculture	Forest	Water	Wetland	Barren
Log DO	0.31	0.02	0.22	**-0.82**	**-0.60**	**0.55**
Log pH	0.24	-0.05	0.33	**-0.79**	**-0.66**	**0.74**
Log COND	0.41	0.50	-0.49	**-0.13**	-0.33	-0.30
Log TEMP	-0.06	0.11	-0.19	**0.67**	0.35	**-0.70**
Log TURB	-0.10	0.46	-0.35	**-0.57**	-0.47	0.11
Log TP	0.08	0.41	-0.46	0.02	-0.09	-0.38
Log TAN	0.01	**0.56**	**-0.53**	0.02	-0.29	-0.31
Log TNN	0.46	-0.14	0.09	0.14	-0.08	0.20
Log TN	**0.55**	-0.28	0.26	0.16	-0.02	0.18
Log TSS	-0.04	0.32	-0.30	-0.49	-0.31	0.16
Log TOSS	-0.20	0.05	-0.06	-0.41	-0.11	0.29
Log TIOSS	0.01	0.30	-0.36	-0.51	-0.40	0.21
Log CHL	0.14	0.44	-0.34	**-0.52**	**-0.56**	0.21
Log BIO	0.02	0.05	0.11	**-0.73**	-0.48	**0.60**

For the final sampling period in September, the overall correlations were more similar to those seen in June and July (Table 2.9). Statistically significant ($p<0.05$) positive correlations were determined between: TN and urban land-use percentage, TAN and agriculture land-use percentage, TEMP and water land –use percentage, as well as DO, pH and BIO with barren land-use percentage increases. Negative significant correlations were determined between: TAN and forest land-use percentage, DO, pH, TURB, CHL and BIO with water land-use percentage, DO, pH and CHL with wetland land-use percentage and TEMP with barren land-use percentage.

Pearson correlation analyses were also run on the *log* transformed annual data in order to determine correlations between land-use types and water quality variables that were consistent over the entire study period. There were significant correlations observed: pH, COND, TEMP, TURB and TAN all increased when the agricultural land-use % increased, pH increased and TAN decreased when the forest land-use percentage increased, DO, pH and COND all decreased as water land-use percentage increased, DO, pH and COND decreased when the wetland land-use percentage increased, DO and pH increased, while TEMP, TP, TAN and CHL decreased as barren land-use percentage increased and finally TNN and TN increased as urban land-use percentage increased.

Table 2.10: Pearson correlation coefficient values for annual pooled data for all fifteen sites based on land-use cover type (percentage). Statistically significant (p<0.05) relationships are shown in bold.

WQ Parameters	Agriculture	Forest	Water	Wetland	Barren	Urban
Log DO	0.21	0.02	**-0.58**	**-0.61**	**0.39**	0.14
Log pH	0.06	0.21	**-0.69**	**-0.68**	**0.65**	0.18
Log COND	**0.52**	**-0.48**	**-0.31**	**-0.42**	-0.14	0.24
Log TEMP	**0.28**	-0.23	**0.28**	-0.1	**-0.33**	-0.02
Log TURB	**0.25**	-0.23	**-0.26**	-0.16	-0.13	0.08
Log TP	0.13	-0.22	0.16	0.14	**-0.35**	0.06
Log TAN	**0.32**	**-0.34**	0.16	0	**-0.39**	-0.03
Log TNN	-0.08	0.16	-0.05	-0.23	0.24	**0.29**
Log TN	-0.10	0.18	-0.05	-0.21	0.21	**0.35**
Log TSS	0.01	-0.1	-0.13	0.14	-0.13	0.06
Log TOSS	-0.09	0.02	-0.06	0.16	-0.10	0.12
Log CHL	0.21	-0.20	0.09	-0.05	**-0.29**	0.10
Log BIO	-0.21	0.17	-0.01	0.19	0.04	-0.03

A redundancy analysis (RDA) was also completed in order to determine the proportion of the variation in water quality that could be attributed to each land-use type (Figure 2.17). The RDA had a total of 23.32% of the total variation in water quality constrained within the model, with 3 of the 4 first axes being statistically significant (p<0.05) following Monte-Carlo permutations (n=999). Water land-use percentage had a strong positive association with Axis 1, while agriculture and urban land-use percentage both exhibited weak negative associations with Axis 1. Axis 2 had a moderate positive association with agricultural land-use percentage with Axis 2, while forest land-use percentage had a moderate negative association with Axis 2.

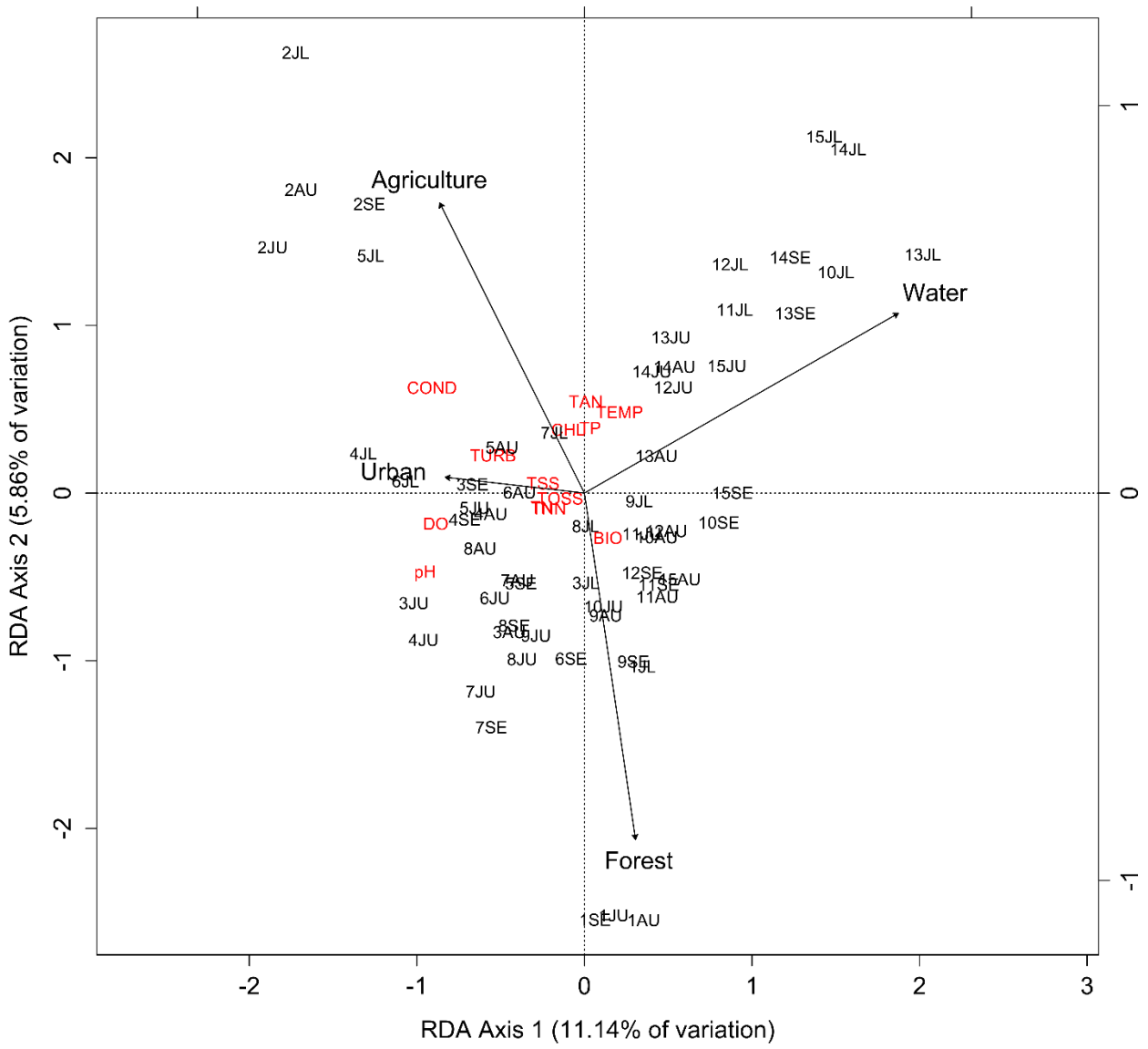

Figure 2.17: Redundancy analysis biplot of the variance in water quality explained by land-use percentage. Sampling period is denoted by two-letter month codes: June (JU), July (JL), August (AU) and September (SE). Monte Carlo Permutation test (n=999) determined that overall model to be statistically significant (p<0.05) at explaining relationships.

To better characterize the influence that multiple land-use types were having on individual water quality variables, multiple regression analyses were run on pooled water quality parameter data (Table 2.11). Water quality throughout the sampling period was highly variable, which is expressed by the fairly low r^2 values for many of the water quality parameters. Even with this large amount of variation, DO, pH and COND had statistically significant ($p<0.05$) negative relationships with percentage of wetland. Agricultural land-use appeared to be more influential overall, with statistically significant positive relation sups with COND, TEMP and TURB, while having a negative relationship with TOSS. Land-use as a predictor variable of water quality was improved when it was examined at the smaller scale of each sampling period individually (Table 2.13)

Water quality throughout the sampling period was heavily influenced by the percentage of land associated with wetlands, including being the sole significant predictor of BIO in June and TN, TSS, TEMP, and BIO in July (up to ~70% of the variation in BIO explained by percent land-use alone), as well as a partial predictor for several other parameters. Urban land-use percentage was the least influential, with none of the water quality parameters being significantly affected by percent urban land-use. Agricultural land-use, which dominates the watershed drainage area, is a significant influence to only COND from June-July (~25%). The best explained relationships were those for DO (68-81% of variation) and pH (73-83% of variation), both of which were negatively related to percent wetland and percent water (excluding August, which did not have significant explanation of DO by land-use), as well as pH being positively correlated with barren land-use percentage, excluding August. Forest land-use percentage was seen to only influence COND in July, with ~22% of the variation accounted for.

When comparing the sampling months, the earlier months (June-July) have a high proportion of nutrients (TP, TAN, TNN, TN) affected by land-use, in comparison to the later sampling months (August-September), which have no nutrients significantly influenced by the land-use type.

Table 2.11: Results of multiple linear regression modelling of pooled seasonal water quality and its relation to land-use practices. Only significant total models are included. Parameters that showed strong autocorrelation were removed from analysis.

Parameter	**Predictor** (Partial r^2)	**Sign of Coefficient**	**Regression r^2** (*p*-value)
DO	Wetland % (0.2348) Water % (0.1948)	- -	0.4296 (<0.001)
pH	Wetland % (0.2311) Water % (0.2141) Barren % (0.2052)	- - +	0.6504 (<0.001)
COND	Forest % (0.1551) Agriculture % (0.1487) Wetland % (0.1435)	- + -	0.4472 (<0.001)
TEMP	Water % (0.9292) Agriculture % (0.09035)	+ +	0.1833 (<0.001)
TURB	Water % (0.0595) Agriculture % (0.05754)	+ +	0.1167 (0.02907)
TP	Barren %	-	0.1208 (<0.001)
TAN	Barren % (0.0993) Forest % (0.0624)	- -	0.1617 (<0.001)
TOSS	Barren % (0.05834) Agriculture % (0.04772) Water % (0.04297)	- - -	0.1490 (0.02779)

Table 2.12: Results of multiple linear regression analyses, testing the ability of land-use to explain water quality parameters. Only significant ($p<0.05$) partial r^2 values for predictors and significant overall regression models included. Parameters that showed strong autocorrelation were removed from analysis.

Sampling Month	Parameter	**Predictor** (Partial r^2)	Sign of Coefficient	**Regression r^2** (*p*-value)
June	DO	Wetland % (0.4536) Water % (0.2638)	- -	0.7174 (<0.001)
	pH	Barren % (0.2928) Water % (0.2356) Wetland % (0.2075)	+ - -	0.7360 (<0.01)
	COND	Agriculture % (0.2227) Forest % (0.2103) Wetland % (0.1976)	- - -	0.6307 (<0.01)
	TP	Barren % (0.0.3487) Wetland % (0.3165)	- +	0.6652 (<0.01)
	TAN	Barren %	-	0.3879 (<0.02)
	TNN	Barren %	+	0.2965 (<0.04)
	BIO	Wetland %	+	0.7262 (<0.00001)
July	DO	Wetland %(0.4609) Water % (0.3554)	- -	0.8163 (<0.0001)
	pH	Wetland %(0.3471) Water % (0.2587) Barren % (0.2239)	- - +	0.8297 (<0.0002)
	COND	Forest % (0.2259) Agriculture % (0.2194) Wetland % (0.1941)	- - -	0.9394 (<0.01)
	TEMP	Wetland %	-	0.4108 (<0.02)
	TP	Barren % (0.3836) Wetland % (0.2093)	- +	0.5929 (<0.005)
	TN	Wetland %	-	0.3029 (<0.05)
	TSS	Wetland %	+	0.3337 (<0.05)
	BIO	Wetland %	+	0.3138 (<0.05)
August	pH	Wetland % (0.4450) Water % (0.2888)	- -	0.7339 (<0.001)
	TEMP	Water % (0.2257) Barren % (0.2037)	+ -	0.4294 (<0.05)
September	DO	Water % (0.5043) Wetland % (0.1850)	- -	0.6893 (<0.001)

	pH	Water % (0.3009) Barren % (0.2788) Wetland % (0.1848)	- + -	0.7646 (<0.001)
	TEMP	Barren % (0.3208) Water % (0.2739)	- +	0.5947 (<0.005)
	BIO	Water % (0.3809) Barren % (0.2014)	- +	0.5823 (<0.05)

2.3.5 Summary of spatial and temporal trends in phytoplankton community structure across the Nottawasaga River

Phytoplankton community structure was characterized for the NR over the sampling period. Absolute algal cell abundances are broken down by algal genus, allowing for visualization of the variability in the concentration of algal cells for each sampling site and date (Figures 2.18-2.21). For the months of June and July, there was an abrupt increase in the total number of individuals when comparing sites 1-5 to those further downstream, whereas there is a high proportion of variability in August, and a steady decline in abundance in September travelling downstream, although still having the highest algal biomass and abundance over the overall sampling period. There was also a high degree of variability in the individual taxa that were identified between each site and across the sampling months. There were in total 35 different genera identified from 4 different major algal groups, *Bacillariophyta* (diatoms), *Chlorophyta* (green algae), *Chryptophyta* (Cryptomonads) and *Euglenophyta* (Euglenoids). Phytoplankton community structure was dominated by *Bacillariophyta* for the majority of the sampling period (Figure 2.22-2.25), with increased abundances of *Cryptophyta, Chlorophyceae* and *Cyanophyta* during July.

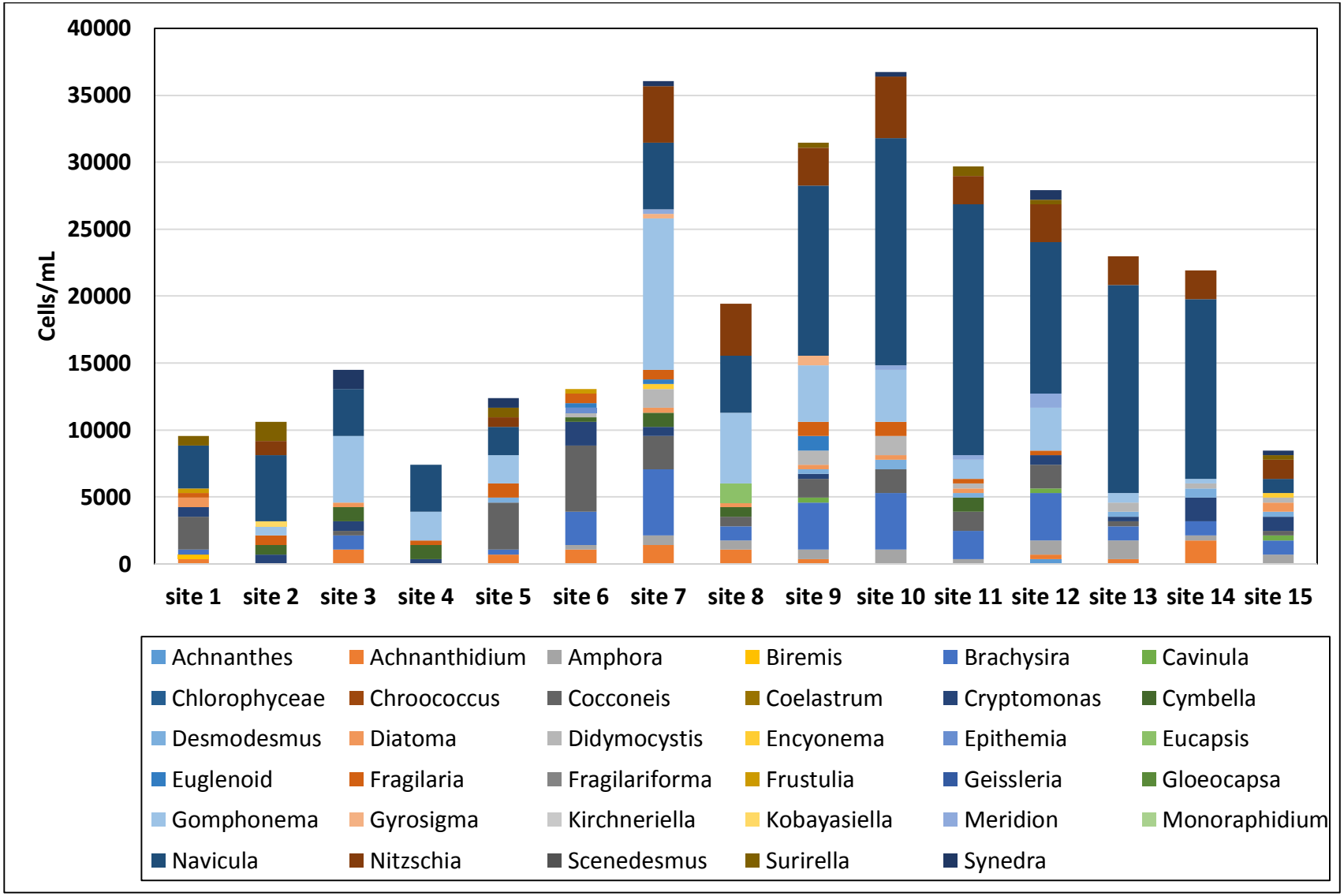

Figure 2.18: Variation of phytoplankton abundance at the studied sites of the Nottawasaga River during June, expressed as abundance (cells·mL^{-1}).

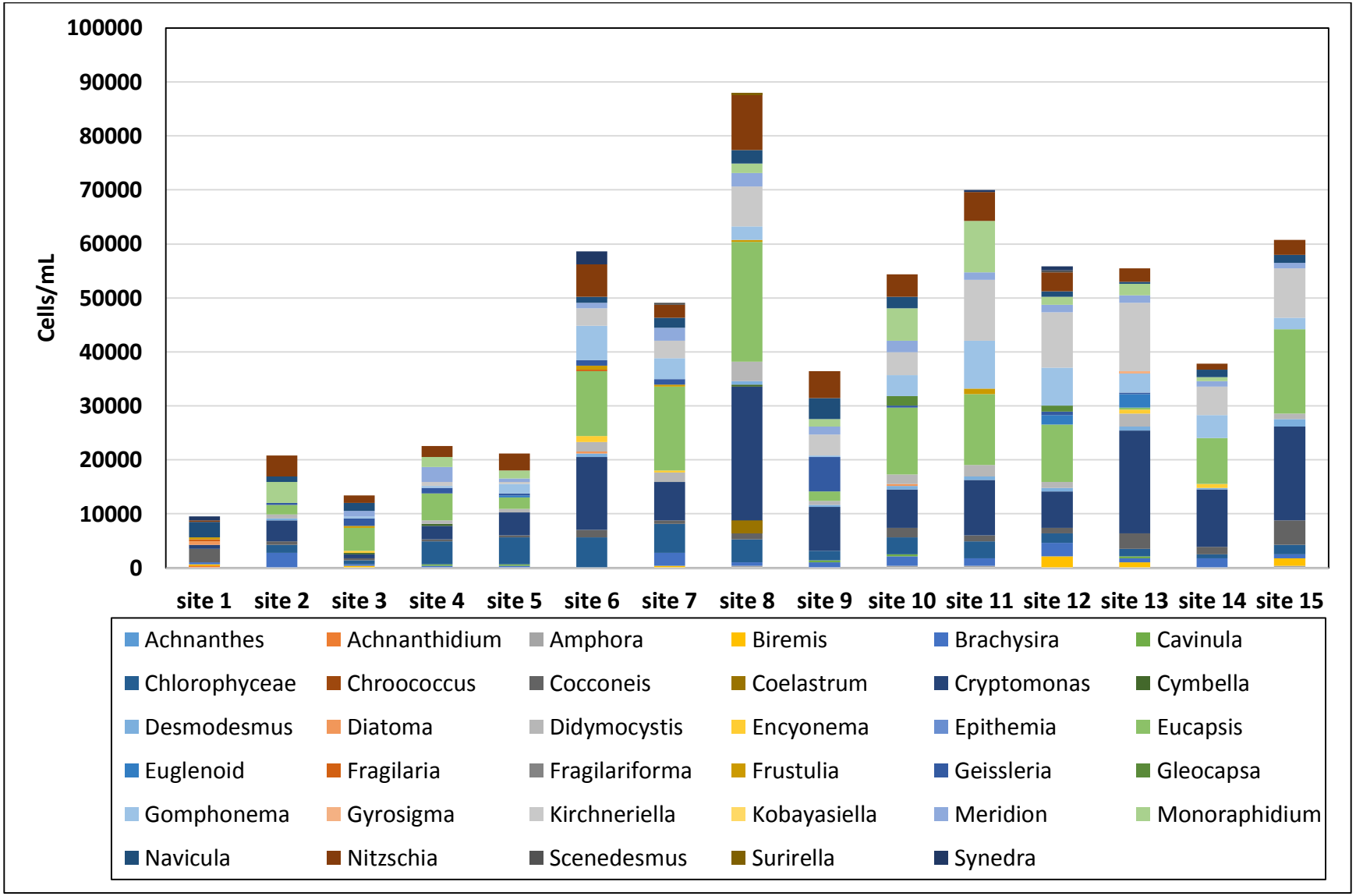

Figure 2.19: Variation of phytoplankton abundance at the studied sites of the Nottawasaga River during July, expressed as abundance (cells·mL^{-1}).

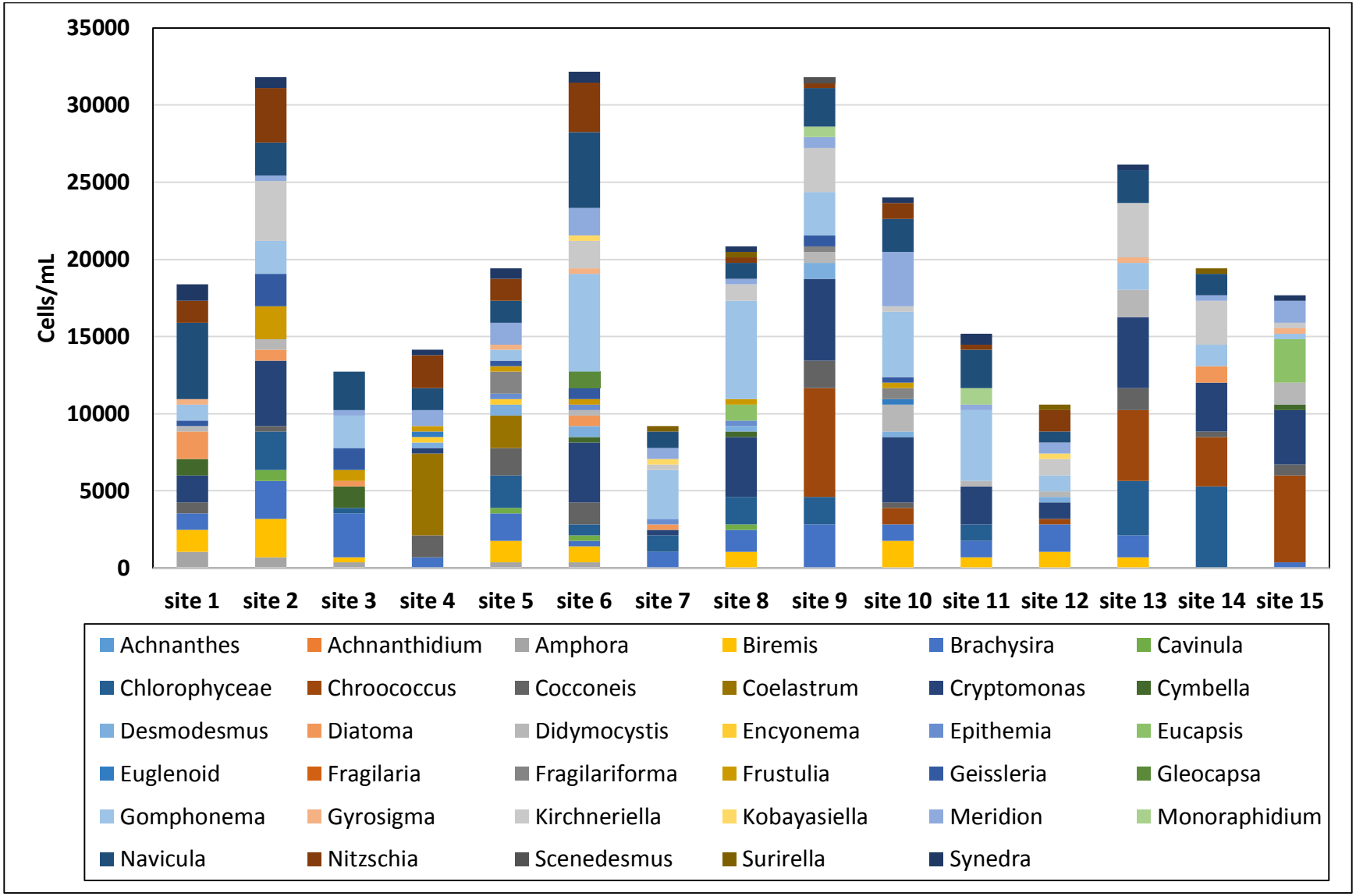

Figure 2.20: Variation of phytoplankton abundance at the studied sites of the Nottawasaga River during August, expressed as abundance (cells·mL^{-1}).

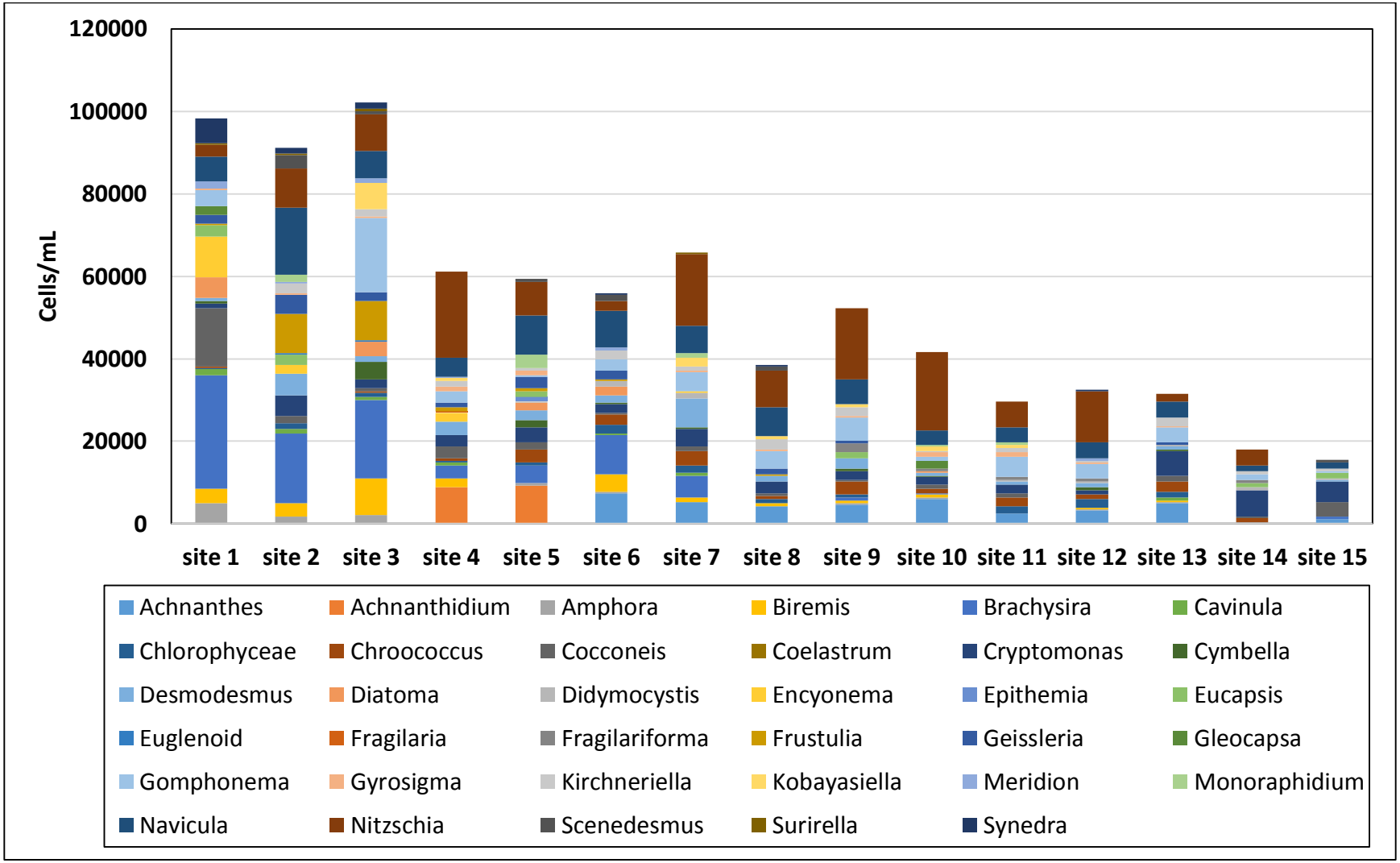

Figure 2.21: Variation of phytoplankton abundance at the studied sites of the Nottawasaga River during September, expressed as abundance (cells·mL^{-1}).

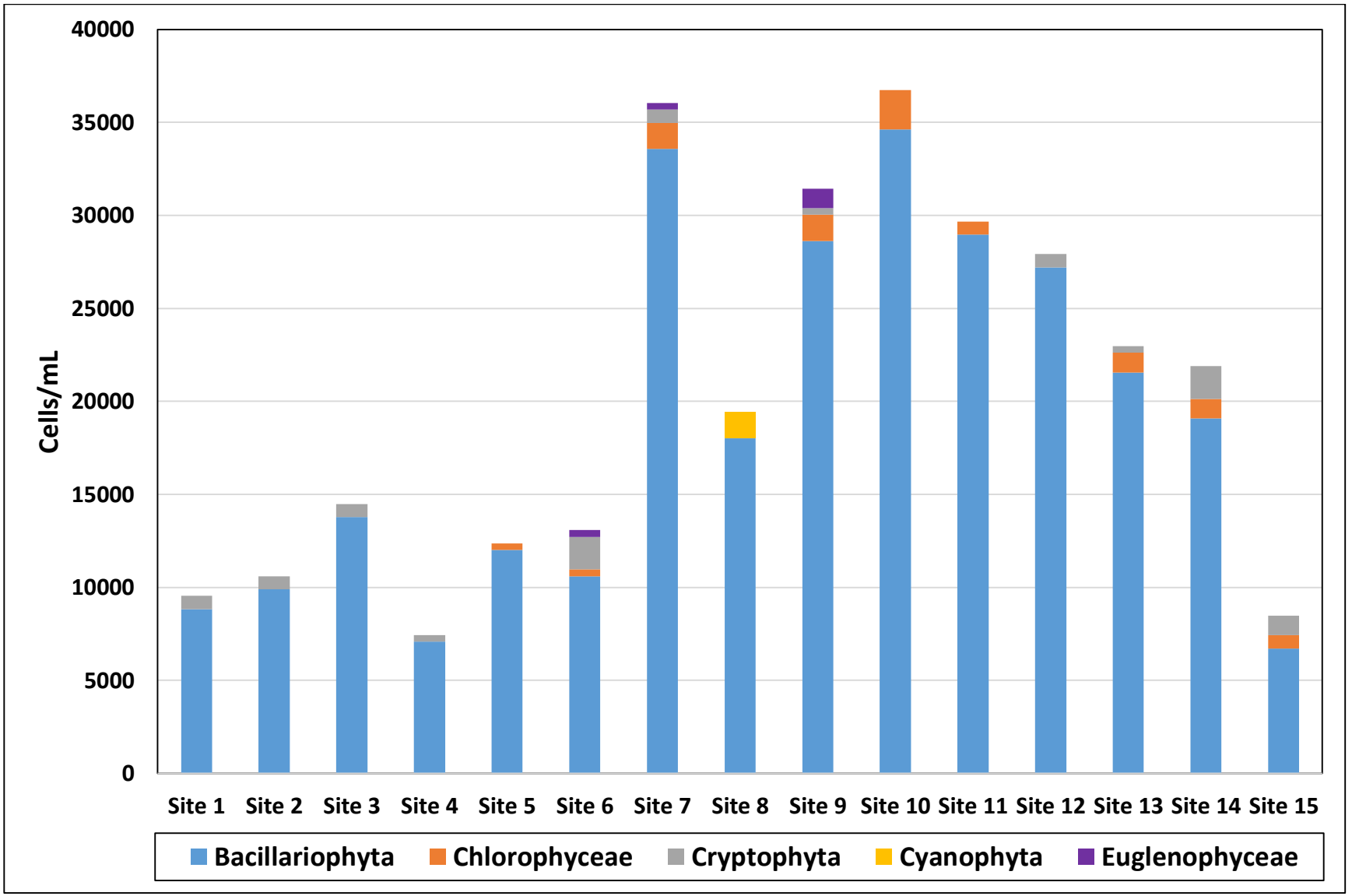

Figure 2.22: Variation of major phytoplankton group abundance at the studied sites of the Nottawasaga River during June, expressed as abundance (cells·mL^{-1}).

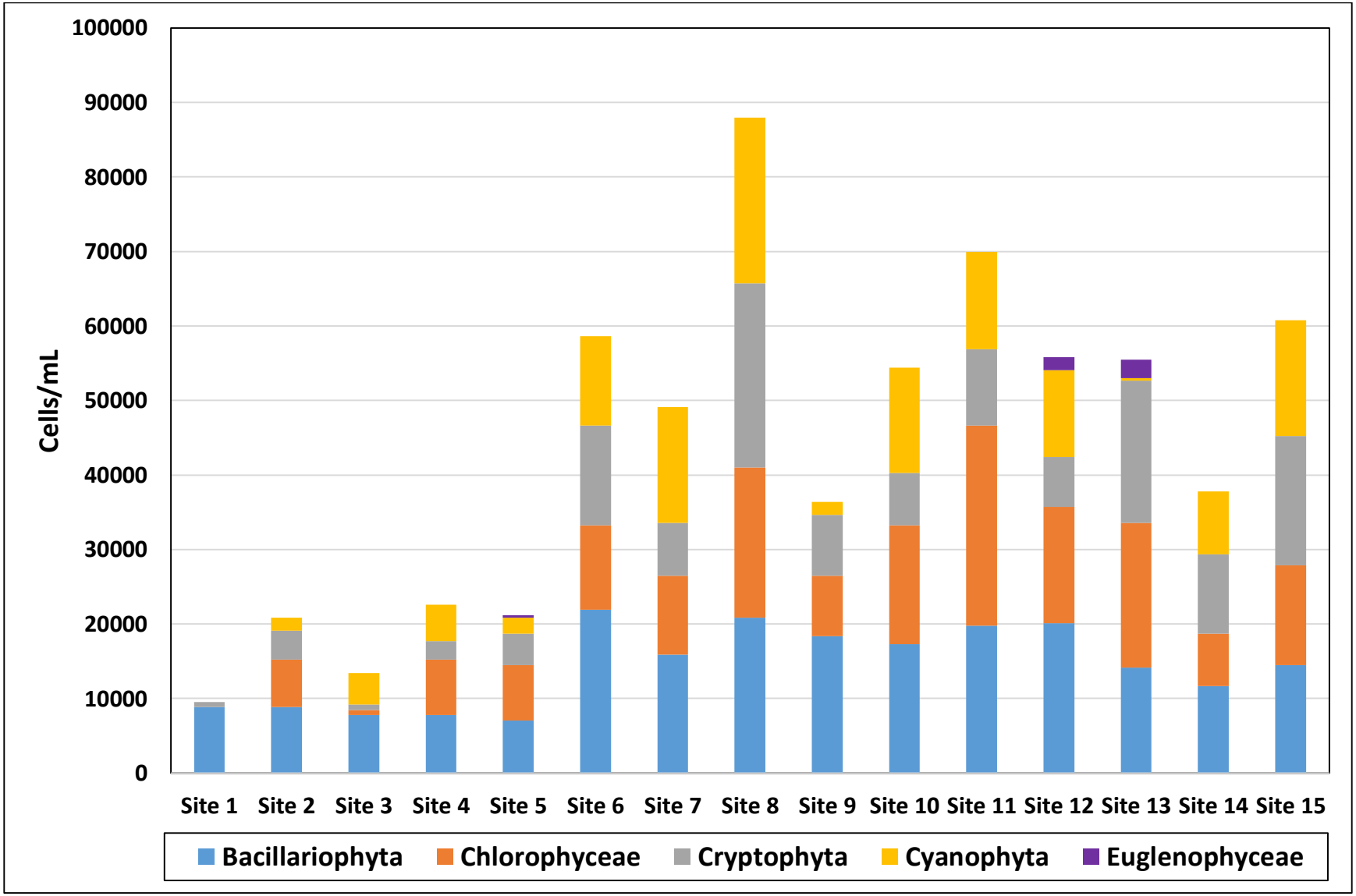

Figure 2.23: Variation of major phytoplankton group abundance at the studied sites of the Nottawasaga River during July, expressed as abundance (cells·mL^{-1}).

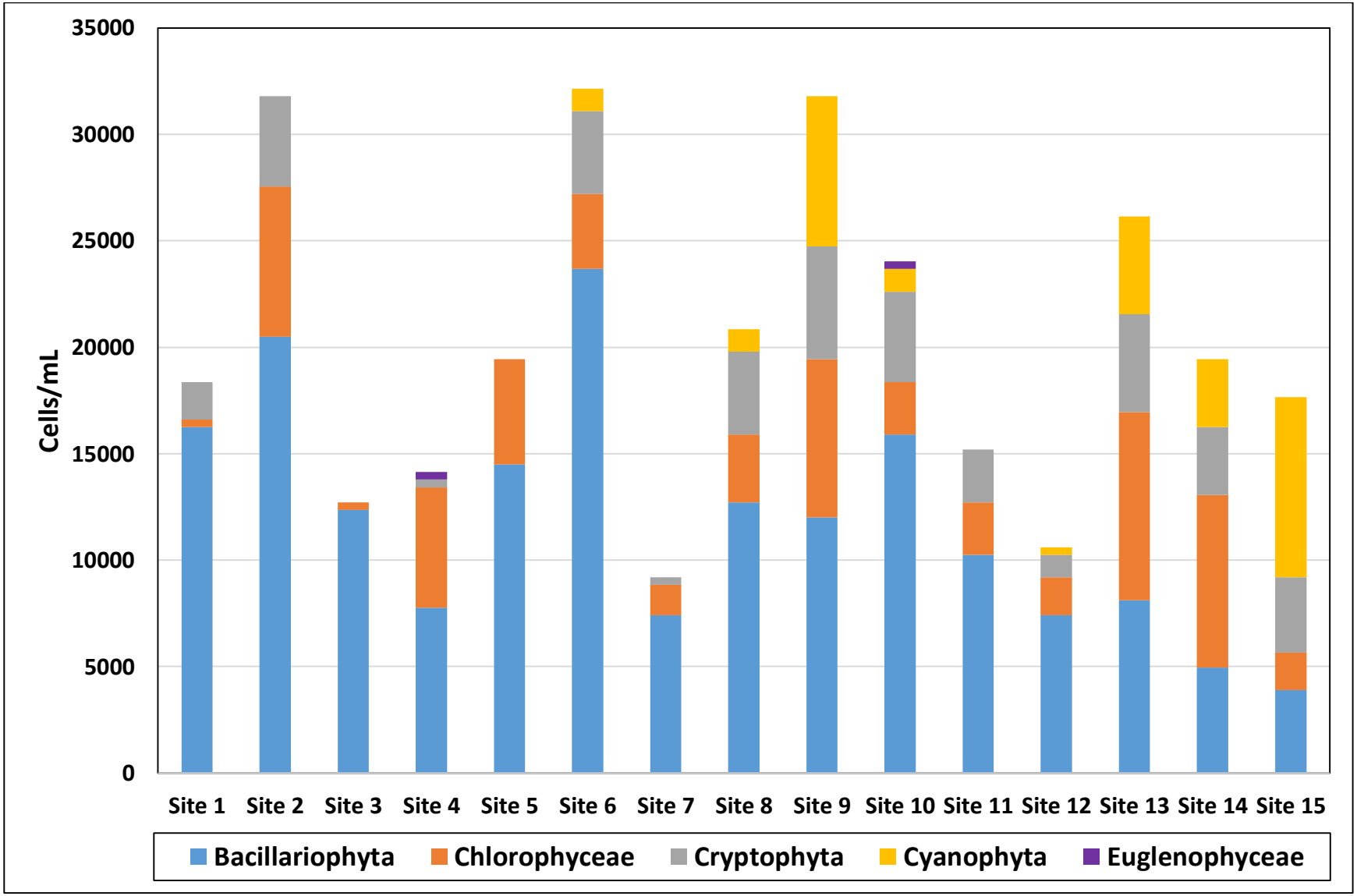

Figure 2.24: Variation of major phytoplankton group abundance at the studied sites of the Nottawasaga River during August, expressed as abundance (cells·mL^{-1}).

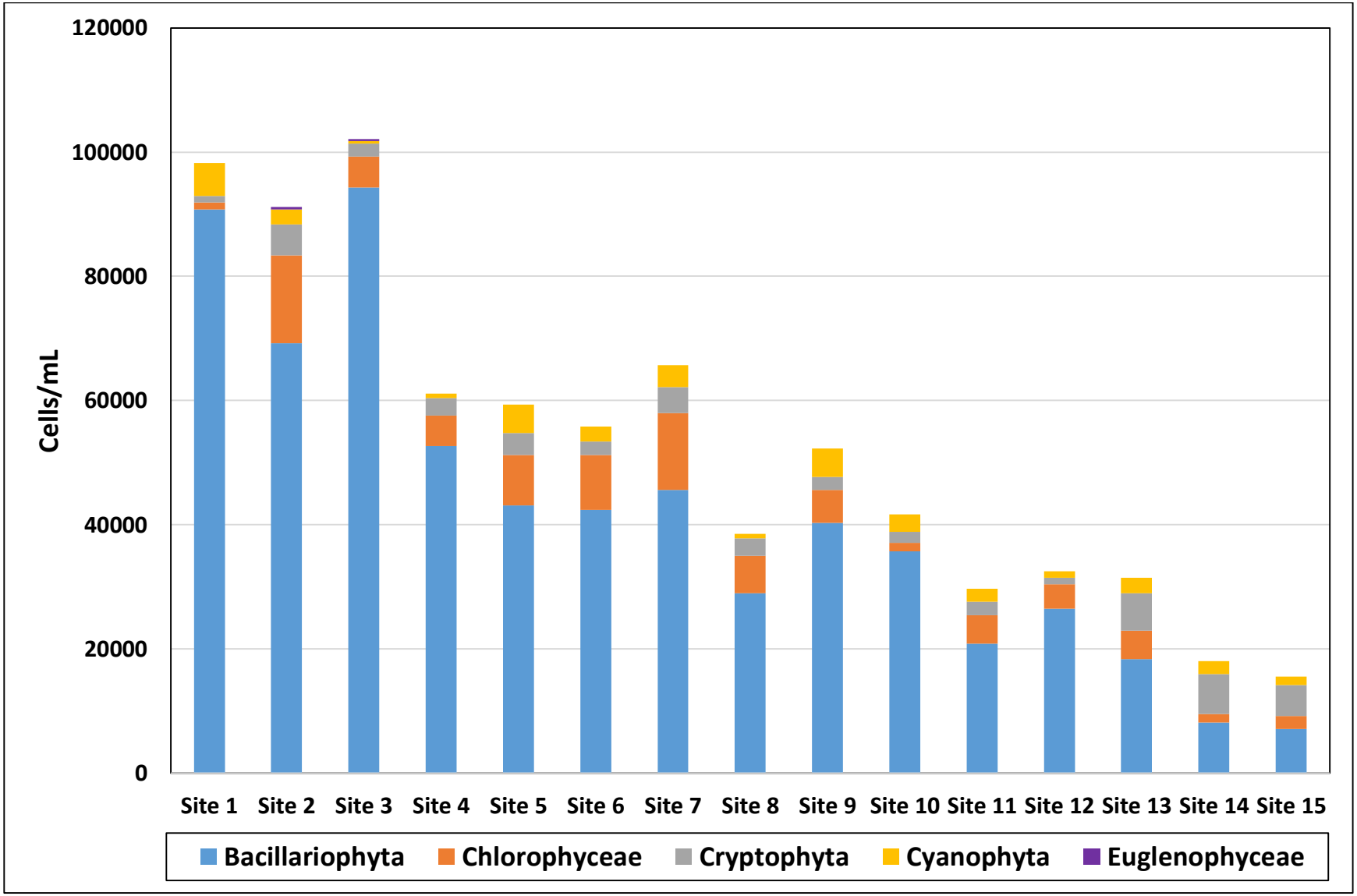

Figure 2.25: Variation of major phytoplankton group abundance at the studied sites of the Nottawasaga River during September, expressed as abundance (cells·mL^{-1}).

Additional characterization of the trends seen between the sites and months was visualized using Bray-Curtis dissimilarity cluster analysis, comparing the algal community composition at each site over the sampling period (Figure 2.26). Sampling sites that were proximal to each other showed few instances of clustering together, with sites having a minimum of ~20% dissimilarity. Sites 4 and 5 were the only sites that had a consistent cluster together, although it was only during the months of August and September. Site 1, which was thought to be distinct, was only unique during July, but was at minimum 50% dissimilar to the sites that it did cluster with on all other occasions. August and September also presented the highest amount of dissimilarity between the sites when compared to clustering in June and July.

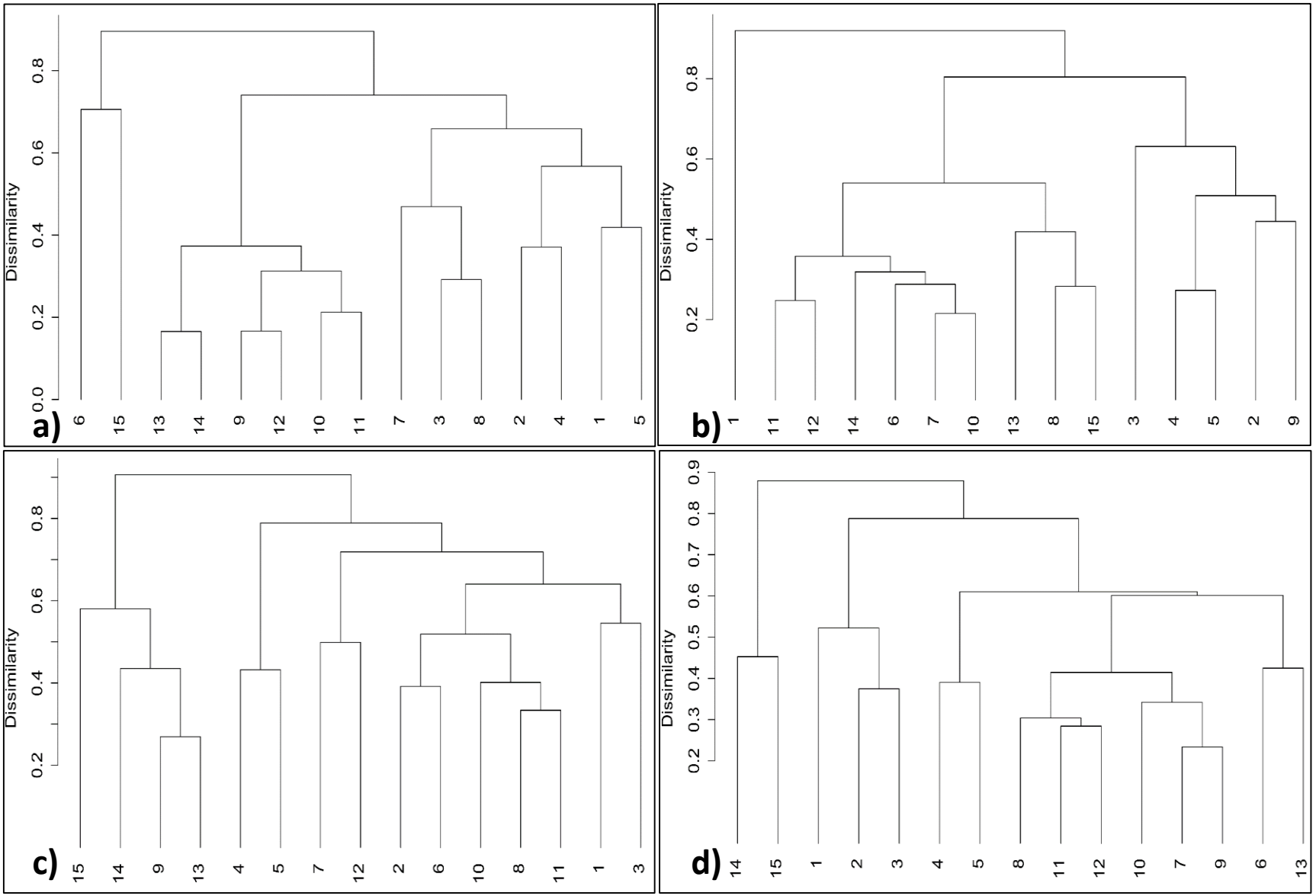

Figure 2.26: Results of Bray Curtis Dissimilarity cluster analysis between sampling sites for: a) June, b) July, c) August, and d) September.

2.3.6 Phytoplankton associations with water quality in the Nottawasaga River over spatial and temporal gradients

Before analysis was conducted on the individual algal taxa, multiple regression analyses were run on CHL in order to determine potential overall drivers of algal growth in the NR across the sampling period (Table 2.13). TP was the most dominant parameter at explaining the variance in CHL levels for June and July (30-42% of the variation explained), whereas it was not statistically significant during August and September. During these later months, TURB, pH and TEMP were all better predictors of CHL, whereas TP was not statistically significant in either of these months. When analysing pooled data for the entire study period, TURB and TN were the only statistically significant predictors of CHL, with TN having a statistically significant negative relationship and TURB having a statistically significant positive relationship.

Table 2.13: Results of multiple linear regression of *log* Chlorophyll *a* content across temporal scales. Data from all sites is pooled (n=15) for each sampling period.

Sampling Month	**Variable** (Partial R^2)	**Sign of Coefficient**	**Regression R^2** (*p*-value)
June	TP	+	0.3025 (0.03365)
July	TP (0.4235) TN (0.0458)	+ +	0.4693 (0.02235)
August	pH(0.2105) TEMP(0.3890)	- +	0.5995 (<0.005)
September	TURB(0.3305) TEMP (0.2693)	+ -	0.5997 (<0.001)

Table 2.14: Results of multiple linear regression of *log* Chlorophyll *a* content from pooled seasonal data (n=60).

Variable (Partial R^2)	**Sign of Coefficient**	**Regression R^2** (*p*-value)
TURB (0.2363) TN (0.0499)	+ -	0.2862 (<0.001)

In order to better understand how the water quality parameters were influencing individual algal taxa, RDAs were run for each sampling month, both for water quality parameters directly, as well as using land-use as a proxy for those factors that may have not been included in the water sampling regime and any interactions that may be linked to land-use differences directly (Figure 2.27 – 2.30 a) and b)). When comparing the variation explained by either water quality or land-use, water quality was better at explaining this variation than land-use type in all of the sampling periods, although there still remains a large proportion of the variation that is not explained by either of the sets of parameters used for the RDAs, with up to 59.70% and 72.78% respectively of the algal community variation remaining unexplained by water quality parameters and land-use using RDA axes 1 and 2. Through the large amounts of variability that was seen between the sites and sampling months, there were differences in the important controlling relationships for some of the taxa present. During the month of June, TN, TSS and TP showed the strongest positive relationships with *Gomphonema, Brachysira, Cocconeis and Nitszchia* abundances, while having strong negative relationships with COND and TEMP. The other strong trend was between high *Navicula* abundance and high TP and TURB, with low DO, pH and TNN. Land-use did not show as many strong trends, but *Navicula* abundance was positively related to percentage of water. During July, different relationships were exposed, with strong positive trends between *Gyrosigma, Coelastrum, Gleocapsa,* and *Epithemia* abundances with high TP/ TN, as well as low DO, pH and COND. *Navicula* were again positively linked to TP and TSS, which exhibited a similar relationship to *Synedra* abundance. Land-use was repeatedly not as informative as water quality in explaining the variation, but showed a different strong relationship between percentage of water and *Gyrosigma*

abundance. During August and September, TEMP explained a higher proportion of the variation, based on the lengths of the environmental arrows, but only had a strong positive relationship with *Chrococcus* abundance during August. August was further characterized by *Kirchneriella* and *Cryptomonas* abundances similarly positively related with TAN and TNN, while September had unique relationships of *Achnanthes* and *Nitszchia* abundances positively linked with TNN and TN, while *Brachysira* abundance was linked with COND. For the August and September land-use RDAs, no consistent relationships were exhibited, although the majority of the taxa were weakly negatively related to percentage of water.

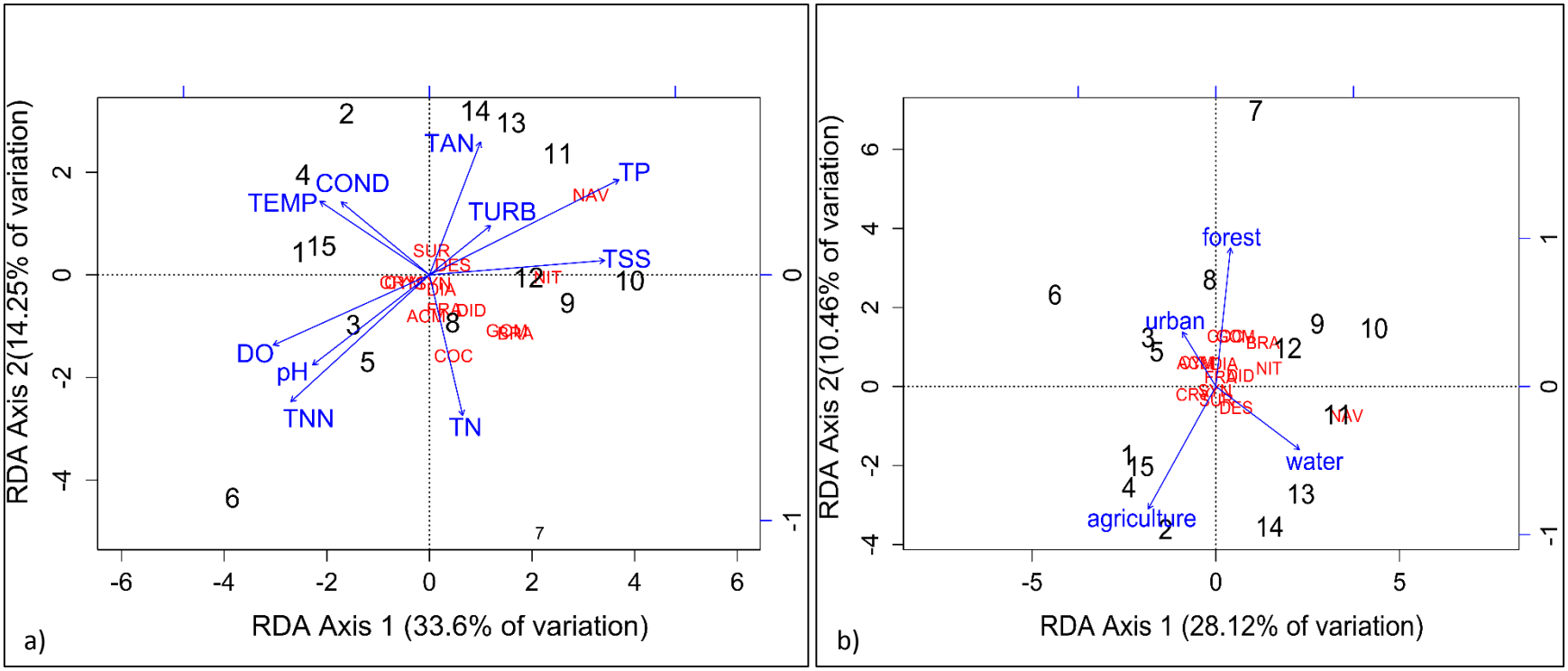

Figure 2.27: RDA ordination diagram of the 15 Nottawasaga River sites for the June data set with descriptive variables represented by arrows. a) RDA biplot visualization of the phytoplankton community structure and the variation explained by water quality parameters. The first two water quality RDA axes (both of which are significant) account for 48.89% of the variability in phytoplankton community structure. b) RDA biplot visualization of the phytoplankton community structure and the variation explained by land-use as a percentage of the total drainage area. The first two water quality RDA axes (both of which are significant) account for 38.50% of the variability in phytoplankton community structure.

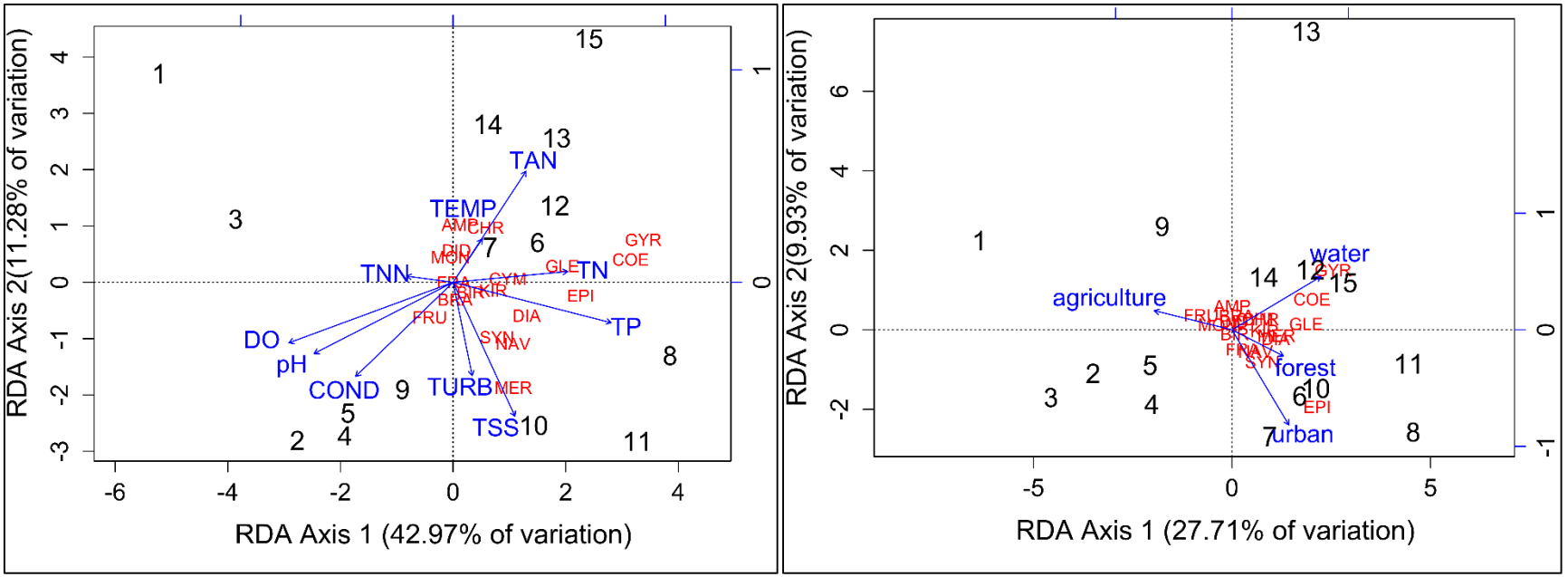

Figure 2.28: RDA ordination diagram of the 15 Nottawasaga River sites for the July data set with descriptive variables represented by arrows. a) RDA biplot visualization of the phytoplankton community structure and the variation explained by water quality parameters. The first two water quality RDA axes (both of which are significant) account for 54.25% of the variability in phytoplankton community structure. b) RDA biplot visualization of the phytoplankton community structure and the variation explained by land-use as a percentage of the total drainage area. The first two water quality RDA axes (both of which are significant) account for 37.64% of the variability in phytoplankton community structure.

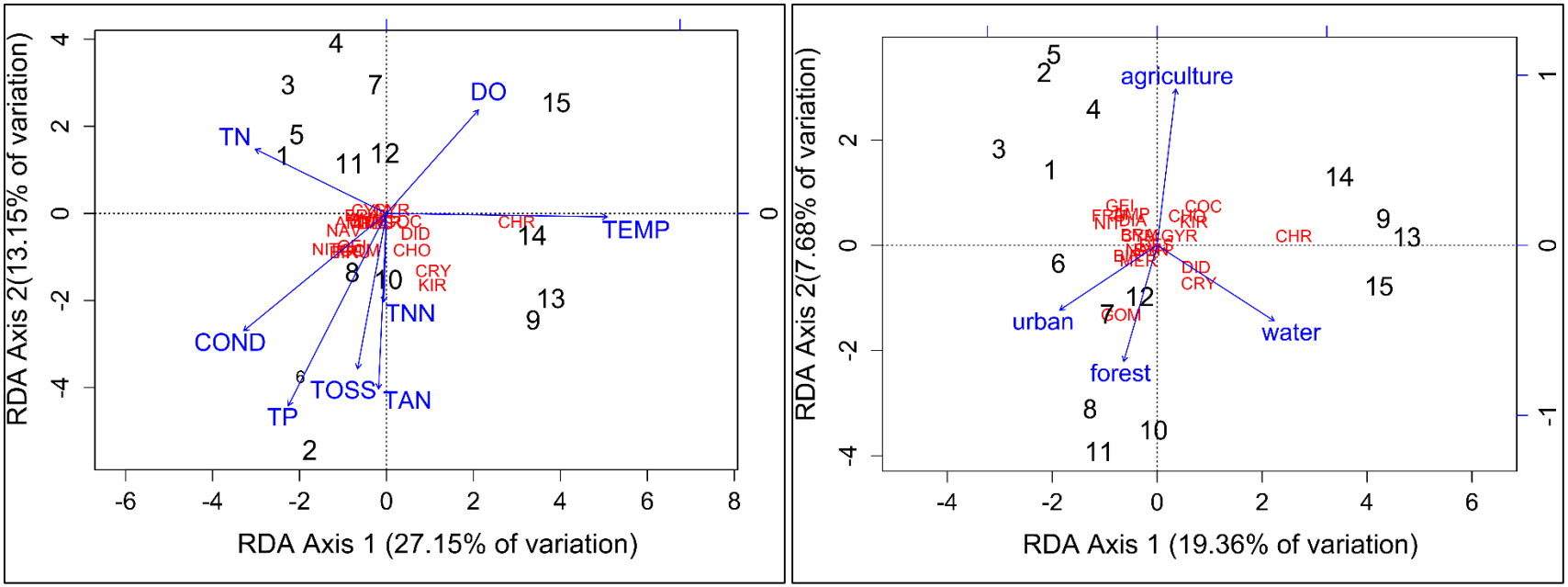

Figure 2.29: RDA ordination diagram of the 15 Nottawasaga River sites for the August data set with descriptive variables represented by arrows. a) RDA biplot visualization of the phytoplankton community structure and the variation explained by water quality parameters. The first two water quality RDA axes (both of which are significant) account for 40.30% of the variability in phytoplankton community structure. b) RDA biplot visualization of the phytoplankton community structure and the variation explained by land-use as a percentage of the total drainage area. The first two water quality RDA axes (both of which are significant) account for 27.20 % of the variability in phytoplankton community structure. Monte Carlo Permutation tests (n=999) determined the overall models to be statistically significant ($p<0.05$) at explaining relationships.

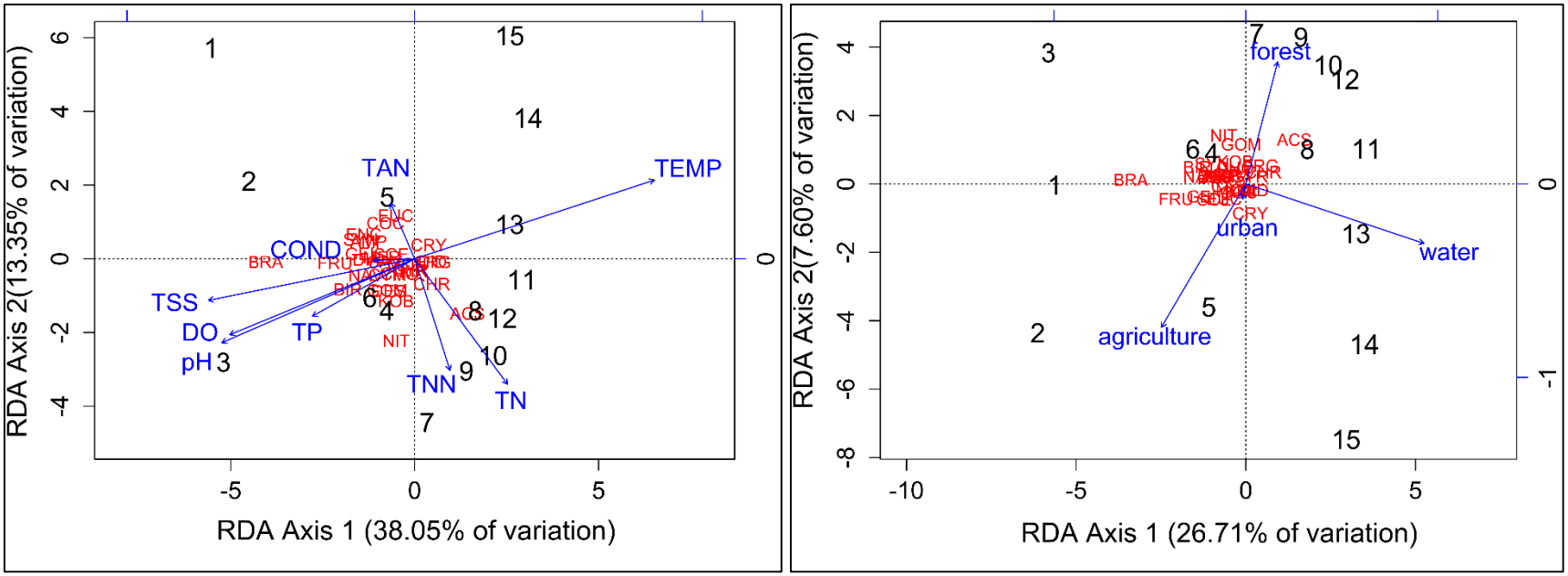

Figure 2.30: RDA ordination diagram of the 15 Nottawasaga River sites for the September data set with descriptive variables represented by arrows. a) RDA biplot visualization of the phytoplankton community structure and the variation explained by water quality parameters. The first two water quality RDA axes (both of which are significant) account for 51.40% of the variability in phytoplankton community structure. b) RDA biplot visualization of the phytoplankton community structure and the variation explained by land-use as a percentage of the total drainage area. The first two water quality RDA axes (both of which are significant) account for 34.31% of the variability in phytoplankton community structure. Monte Carlo Permutation tests (n=999) determined the overall models to be statistically significant ($p<0.05$) at explaining relationships.

Pearson correlation analysis was used to determine direct relationships between environmental variables, land-cover type and individual taxa over the sampling period. The results of the Pearson correlations between environmental variables and taxa are summarized in Table 2.15-2.18. Significant relationships were considered those with a $p \leq 0.05$, regardless of the r^2 coefficient.

Table 2.15: Statistically significant (p<0.05) Pearson correlation coefficient values for the June *log* transformed water quality and algal abundance data sets.

Genus	DO	pH	COND	TEMP	TURB	TP	TAN	TNN	TN	TSS	TOSS	TIOSS	CHL	BIO
SHAN														
CHL			0.60		0.73	0.55				0.55	0.72		-	
BIO	-0.64					0.62				0.67	0.55	0.68		-
AMP	-0.75	-0.71	-0.61			0.71								0.68
BIR					-0.59	-0.74				-0.59	-0.65	-0.53	-0.69	
BRA	-0.55		-0.73											0.62
COC			-0.59											
CYM		0.53												
DES		-0.68				0.56								
DIA				-0.62										
DID		-0.57												0.59
ENC														
EPI								0.68						
FRU						-0.62		0.67					-0.53	
GOM										0.58		0.64		0.52
KOB			0.83		0.52								0.64	
MER	-0.58											0.52		0.63
NAV								-0.65						0.68
NIT	-0.63	-0.55				0.76		-0.71			0.52S		0.54	0.73
SUR														

Table 2.16: Statistically significant (p<0.05) Pearson correlation coefficient values for the July *log* transformed water quality and algal abundance data sets.

Genus	DO	pH	COND	TEMP	TURB	TP	TAN	TNN	TN	TSS	TOSS	TIOSS	CHL	BIO
SHAN				-0.84						0.56	0.61	0.52		
CHL					0.80	0.64				0.69	0.75	0.65	-	
BIO	-0.61					0.63				0.61		0.63		-
ACS					-0.59	-0.75				-0.54	-0.54		-0.68	
ACM														0.59
BIR								-0.57						
COE	-0.60					0.72				0.62		0.64	0.62	0.90
CYM	-0.67	-0.70				0.66				0.55		0.60		0.78
DIA					0.63	0.67				0.70	0.74	0.66	0.68	0.70
EPI						0.64								0.61
FRU														
GEI				-0.59										
GLE	-0.60					0.65						0.52		0.73
GOM	-0.55													
GYR	-0.72	-0.61				0.71			-0.54	0.60		0.63		0.86
KIR														0.54
MER						0.56				0.62		0.63		
MON														
NAV					0.73	0.64				0.74	0.80	0.69	0.66	0.70
SYN					0.67	0.71				0.63	0.74	0.56	0.8S	0.52

Table 2.17: Statistically significant (p<0.05) Pearson correlation coefficient values for the August *log* transformed water quality and algal abundance data sets.

Genus	DO	pH	COND	TEMP	TURB	TP	TAN	TNN	TN	TSS	TOSS	TIOSS	CHL	BIO
SHAN					0.55					0.55		0.64		0.64
CHL		-0.54		0.69							0.51		-	
BIO														-
AMP				-0.61										
BIR	-0.58											0.53		
BRA							-0.56							
CAV			0.62		0.70	0.61	0.63			0.64		0.63		
CHR		-0.58		0.57					-0.55				0.64	
COC														0.54
COE									0.70					
CYM	0.52													
DES									0.55					
DID		-0.52							-0.54					0.56
ENC									0.70					
EPI									0.71					
EUC	0.56							0.56						
FRA														
FRU			0.74		0.69	0.75				0.73		0.75		
GEI				-0.56						0.54		0.62		0.67
KIR													0.60	
NAV												0.6		
NIT													-0.60	
SCE														
SUR														-0.52

Table 2.18: Statistically significant (p<0.05) Pearson correlation coefficient values for the September *log* transformed water quality and algal abundance data sets.

Genus	DO	pH	COND	TEMP	TURB	TP	TAN	TNN	TN	TSS	TOSS	TIOSS	CHL	BIO
SHAN	0.53				0.58					0.52	0.54			0.75
CHL	0.52			-0.63	0.68					0.66	0.58	0.62	-	0.64
BIO	0.60	0.73		-0.83	0.69					0.79	0.79	0.76	0.64	-
ACS							-0.53						-0.59	
ACM									0.53					
AMP					0.53			-0.55	-0.76	0.57		0.55		
BIR		0.52		-0.75						0.62	0.76	0.57		0.81
BRA	0.67	0.68		-0.60									0.63	0.75
CAV				-0.55	0.62					0.54	0.62			0.66
CHR													-0.57	
CRY				0.65			0.56							
CYM											0.54			
DES	0.58	0.68									0.57			0.71
DIA				-0.54										0.52
DID				0.59									-0.58	
ENC				-0.60										0.56
EUG					0.80	0.58			-0.52	0.74	0.57	0.71	0.86	0.52
FRA								0.77	0.63					
FRG														
FRU	0.61	0.61		-0.62	0.71					0.70		0.7	0.87	0.71
GEI		0.56			0.58					0.54		0.53	0.57	0.68
GOM			-0.70											
GYR											0.59			
KIR			0.54			0.68								
MER				-0.74							0.54		0.53	0.60
MON														
NAV	0.61	0.70		-0.55	0.70					0.70	0.64	0.68	0.52	0.87
NIT										0.67	0.59	0.69		0.58

Table 2.18 (cont.)

SCE	0.57	0.55							0.56	
SUR			-0.69	0.66		0.57	0.58	0.53	0.69	0.68
SYN			-0.73						0.59	0.60
CHO							0.56			0.54

Table 2.19: Pearson correlation coefficient values for the pooled, *log* transformed water quality and algal abundance data sets over the sampling period (June-September). Statistically significant (p<0.05) relationships are shown in bold.

Genus	DO	pH	COND	TEMP	TURB	TP	TAN	TNN	TN	TSS	TOSS
ACS	-0.08	-0.18	-0.08	-0.08	-0.04	**-0.42**	-0.11	-0.13	-0.22	-0.07	0.03
ACM	0.18	0.13	0.11	-0.18	-0.14	-0.18	0	0.07	**0.40**	-0.02	-0.04
AMP	-0.15	-0.20	-0.07	**-0.43**	0.24	0.03	0.24	**-0.39**	-0.07	**0.37**	**0.30**
BIR	-0.04	0.09	-0.19	-0.23	**0.25**	-0.15	0.05	-0.1	**-0.46**	0.13	0.06
BRA	0	0.07	-0.06	**-0.20**	0.22	0.12	-0.04	-0.2	-0.01	**0.33**	0.22
CAV	-0.11	0.02	0.09	-0.20	**0.42**	0.10	0.23	0.09	-0.06	**0.38**	**0.32**
CHR	0.09	-0.08	**-0.35**	-0.08	-0.02	**-0.44**	-0.04	-0.21	**-0.46**	-0.14	0.03
COC	-0.19	-0.10	-0.13	0.13	-0.01	-0.11	0.08	-0.03	0.11	0.03	-0.17
COE	0.04	0.24	0	0.14	0.05	0.11	-0.04	-0.14	0.14	-0.04	-0.07
CRY	**-0.34**	**-0.26**	-0.11	**0.36**	0.17	0.09	**0.27**	0.23	**-0.31**	0.02	-0.02
CYM	**0.35**	**0.30**	0.01	**-0.37**	0	**-0.30**	**-0.33**	-0.02	-0.16	0.01	0.15
DES	**-0.30**	**-0.29**	-0.05	-0.06	**0.33**	-0.02	0.16	-0.17	-0.15	**0.31**	0.15
DIA	0.19	0.23	-0.17	**-0.46**	-0.04	-0.19	-0.11	-0.15	-0.07	0.05	0.06
DID	**-0.38**	**-0.30**	-0.12	0.18	0.09	**0.34**	-0.06	0.02	0.05	0.14	0.16
ENC	-0.09	0.1	-0.01	0.03	0.21	-0.09	0.04	0.19	0.02	0.07	-0.03
EPI	0.18	0.21	-0.06	0.01	0.14	-0.04	-0.01	0.10	0.14	0.06	0.07
EUC	**-0.33**	-0.14	0.02	**0.39**	0.10	0.25	0.13	0.24	-0.04	0.09	-0.06
EUG	-0.17	-0.08	0.04	-0.06	0.24	0.24	-0.01	0.01	-0.06	**0.31**	0.19
FRA	0.21	0.16	0.21	-0.16	-0.11	-0.05	-0.20	0.22	**0.48**	0.11	0.08
FRG	-0.05	-0.16	-0.13	-0.03	-0.03	-0.24	0.01	-0.23	-0.21	-0.04	-0.11
FRU	0.20	**0.39**	0.08	-0.21	**0.42**	0.01	0.08	0.13	-0.14	**0.28**	0.13
GEI	-0.02	0.24	0.09	-0.14	**0.47**	0.09	0.04	0.09	-0.25	**0.34**	0.21
GLE	**-0.30**	-0.22	-0.20	-0.24	0.08	-0.07	0.17	-0.06	-0.12	0.24	0.19
GOM	-0.22	-0.14	-0.23	**-0.27**	0.14	0.05	0.02	0.05	-0.22	**0.32**	**0.32**
GYR	0.03	0	-0.19	**-0.28**	0.17	**-0.35**	-0.09	-0.12	-0.23	0.12	0.15
KIR	**-0.44**	**-0.31**	-0.21	**0.26**	**0.31**	0.24	**0.27**	0.16	**-0.43**	**0.26**	0.14
KOB	0.15	0.08	0	**-0.29**	0.19	-0.25	0.03	-0.05	-0.17	0.17	0.24
MER	**-0.28**	0.02	-0.22	0.08	0.23	**0.42**	0.09	0.16	-0.13	**0.26**	0.18
MON	**-0.40**	-0.23	0.15	0.19	0.23	**0.27**	0	0.1	-0.02	**0.27**	0.11
NAV	0.03	-0.02	-0.02	**-0.40**	0.12	-0.20	0.08	**-0.41**	-0.13	0.21	0.12
NIT	**-0.35**	-0.17	0.15	-0.12	**0.42**	0.19	0.24	-0.03	0.08	**0.49**	**0.28**
SCE	0.09	0.06	0.06	-0.12	0.25	-0.10	0.12	-0.12	**-0.32**	0.15	0.04
SUR	0.19	0.14	0.06	-0.23	0.12	-0.13	0.01	-0.13	-0.08	0.11	0.19
SYN	0.19	0.18	-0.05	**-0.34**	0.21	-0.06	0.02	-0.02	-0.17	0.14	0.12
CHO	-0.22	0.05	-0.01	**0.26**	**0.44**	0.25	0.11	**0.26**	-0.20	**0.28**	**0.26**

Phytoplankton abundance was highly variable, but there were statistically significant relationships that showed control over the algal community. These relationships included: positive relationship between *Cymbella* abundance and pH (June and July), positive relationship between *Didymocystis* abundance and pH (June and August), positive relationship between *Diatoma* abundance and TEMP (June and September), positive relationship between *Nitszchia* abundance and TOSS (June and September), positive relationship between *Navicula* abundance and BIO (June, July and September), positive relationship between *Brachysira* abundance and BIO (June and September), negative relationship between *Geissleria* abundance and TEMP (July and August), positive relationship between *Navicula* abundance and turbidity, TSS, TOSS, CHL and algal biomass (July and September), positive relationship between *Synedra* abundance and CHL and BIO (July and September), positive relationship between *Cavinula* abundance and TURB (August and September), positive relationship between *Frustulia* abundance and TURB (August and September), positive relationship between *Frustulia* and *Geissleria* abundances both with TSS and TIOSS (August and September) and a negative relationship between *Surirella* abundance and BIO (August and September). The remainder of the relationships were unique to the sampling period or were not consistently positive or negative.

2.4 Discussion

This chapter highlights the complexity and variability that can be associated with large lotic systems such as the NR. Initial characterizations of the NR's water quality showed that there were statistically significant differences in the DO, pH, COND, TURB, TAN, TSS and TOSS as the river travels from the upper to lower reaches of the river. Site 13 is situated in close proximity to an area of increased river width and depth, known as Jack's Lake. This area can

have the effect of slowing river water, reducing the aeration that directly can affect DO concentrations (Mallin *et al*. 2006). As well, site 13 is in close proximity to a potential point source of anthropogenic nutrients from an old infrastructure trailer park. Although it is difficult to directly pin-point what the source of this hypoxia is linked to using the data collected, there is the potential for an increase in the sediment oxygen demand in this location. River widening can lead to reduced water velocity, causing nutrient and sediment settling, which has been linked with increases in microbial sediment oxygen demand (MacPherson 2003, Matlock *et al*. 2003). These factors combined could lead to biologically important low DO concentrations.

When examining the decrease in pH in upstream sites to downstream sites, this trend can be spatially linked to sites above, in and below the MW. There is a steep decline in the pH before the NR exits the wetlands, which could either be attributed to wetland processes (e.g. decomposition), or to inputs from the Giffin Drain. The Giffin Drain is a man-made agricultural ditch which drains cropland and travels through a mixed-cedar swamp before entering the NR near site 10 and the exit of the MW. The Giffin drain was studied for its ability to decrease agriculturally relevant nutrients (e.g. N and P), but its effect on pH was not determined (Rutledge *et al*. 2015). Due to the open drainage ditch design of the Giffin Drain, there is the potential for it to have an effect on lowering pH due to microbial decomposition processes in the sediment (Qiu *et al*. 2013). Low soil pH could reduce water pH as it travels through the drain due to residence times in the 4-5 day range and interactions occurring at the soil/ water interface (Rutledge *et al*. 2015).

Alternatively, the MW receives both ground water and surface water via the NR, which flows through the wetlands, as well as flooding across the landscape and allowing for multiple

wetland types to occur (Spoelstra and Post 2012). These wetland classifications can have the effect of decreasing pH levels due to organic matter decomposition in areas of slow moving water, areas that contain high organic matter (e.g. peat, decaying vegetation) and have high amounts of bacterial decomposition (Vitt *et al*. 1995). Wetland types that are contained within the MW, specifically fen and bog classifications, are both classified as peatlands, which are areas that have high amounts of organic matter build up (i.e. peat) that becomes increasingly acidic as it continues to accumulate (Damman 1986, Bridgham *et al*. 1996). Limnogenous peatlands, or those fed by rivers and lakes, have a continuous source of water, therefore making their pH closer to circumneutral, but their interactions with the river water directly will ultimately cause the effect of decreased pH for water returning to the river, which may be occurring in the NR.

The high COND at site 2 at the mouth of Innisfil Creek, as well as significantly low levels at site 1 (before the confluence of the upper NR and Innisfil Creek), indicates that Innisfil Creek is the leading contributor to the high COND and high TURB, TSS and TOSS at downstream sites, although these parameters are not significantly higher than those found within the MW sites. The high values of sediment related water quality parameters may be linked with the high percentage of agricultural land-use contained within Innisfil Creek, the highest of all the drainage areas (64.3%). This was consistent with the significant seasonal correlation between percentage of agricultural land-use and TURB (r^2=0.25, $p<0.05$). In comparison to historical data during base-flow conditions and storm-flow conditions at the same sampling site, high TSS concentrations (~35 mg/L) were comparable to this study, but even higher concentrations during storm events (~75 mg/L) were recorded (Chow-Fraser 2006). This information further

confirms that Innisfil Creek is a long-term contributor to increased suspended sediment loads. As well, the proportion of inorganic (e.g. sediment, silt and river bed material) suspended solids that comprises the TSS is more than three-times the proportion of organic suspended solids at site 2 (77.17% vs. 22.83%), leading to the hypothesis that there is a high wash load (i.e. particulates that are washed into the river from overland flow), which is comprised of very small particles of silt and clay attributed to agricultural land-use (Allan and Castillo 2007).

The comparably high levels of TSS, TOSS and TURB at site 10 may be attributed to the Giffin Drain or the inputs coming from Willow Creek, which enters the NR upstream of site 10. In the case of Willow Creek, there are decreases in riparian vegetation, decreases in vegetation associated with the drainage area overall and unnatural channel morphology, which combine to increase bank erosion, sediment deposition and increased transport of suspended sediments (Schlosser and Karr 1981b).

Finally, TAN was highly variable across each site and each month, but considerably elevated concentrations occurred at sites 2 and 14, although still below the Provincial Water Quality Objective (PWQO) (Ministry of Environment and Energy 1994). This can indicate impacts from a variety of land-use activities, including urban and agricultural sources of nutrients (Rabalais 2001). Since both of these sites are highly influenced by both urban and agricultural land-use, there are links to multiple sources of TAN inputs into the NR, causing these increased concentrations.

Water quality parameters that varied significantly by sampling date have a high probability of being affected by climactic factors (e.g. precipitation, flow regime), as well as anthropogenic influenced nutrient cycles (Winter and Duthie 1998), many of which are beyond

the scope of this study. In this study, land-use/cover percentages were used as a proxy for non-point sources of most water quality parameters such as DO, TEMP, and pH. However, there are potential difficulties in determining specific land-use effects when there are equal mixtures of each type, rather than at the extremes of either agricultural-dominated or urban- dominated land-use in the watershed (Winter and Duthie 1998, Jarvie *et al*. 2008, Neal *et al*. 2008, O'Brien and Wehr 2009). With this study, there was only limited amounts of water quality variation that could be attributed to land-use, as seen in the RDA and linear regression analyses that were only able to link certain water quality parameters with land-use, even though the percentage of land characterized by agricultural use is relatively high. In studies that look directly at single land-use types or intensity of a land-use type, a more precise understanding of single influences can be determined (Buck *et al*. 2004, Herzon and Helenius 2008, Wallace and Biastoch 2016).

As previously discussed, there are differences in water quality that have the potential to be attributed to the MW, though it is difficult to attribute 100% of this variation solely to the wetlands due to multiple tributaries entering the NR throughout MW. The MW appear to be having a significant impact on decreasing COND, but this may be attributed more to a dilution effect of the suspended sediments that are inflowing from Innisfil Creek, as COND measurements, as well as TURB and TSS values, have a fairly steady decline as they travel further downstream from Innisfil Creek. The DO concentrations may be affected by slowing of water as it spreads out over the wetted wetlands area and from increased biochemical oxygen demand through increased heterotrophic processes (MacPherson 2003). Though these may be a side effect of wetland process, the Giffin Drain may also be having an impact on these values, which was discussed previously.

PCA, multiple regressions, Pearson correlation analyses and RDAs indicated that most water quality parameters were influenced by agricultural land-use across the river catchment. Overall, agricultural land-use percentage had the highest negative impact on water quality, with increases in COND, TEMP, TURB and TAN all significantly correlated, as well as the indirect impact that the Innisfil Creek has on the system that is likely due to agricultural land. Elevated levels of nutrients and other water quality parameters have been shown to be influenced by agricultural land, specifically row crops, as the majority of the agricultural land can be classified as such in the NVW (Withers and Lord 2002, Montgomery 2007). In this system, there is a significant correlation between TP concentrations and CHL content, whereas TN has only select correlations with individual taxa, rather than total algal biomass. Therefore, these results infer that the NR is a phosphorus limited ecosystem.

Identification of this system as being P limited also stems from the extremely high levels of TN that are found across the Nottawasaga River (1.2-2.6mg/L), which would classify it as eutrophic when using TN alone (Smith *et al*. 1999). In general, the ideal TN:TP ratio for algae is averaged at 16:1, with ratios exceeding this ratio generally thought to be P limiting the algal growth (Redfield 1958), although concentrations of TP were consistently above the PWQO (30 µg/L) for all sites that were influenced by Innisfil Creek. Although concentrations of total phosphorus are high, TP may be limiting this system due to its high positive correlation with TSS, linking most of the phosphorus with particulate phosphorus which in many cases is not bioavailable for phytoplankton (Logan 1982, Neal *et al*. 2006). Non-point sources of N in aquatic systems are strongly linked to croplands, but also forested land when comparing yearly discharge to surface waters (Carpenter *et al*. 1998). This may explain why the Site 1 had

comparable concentrations to other sites since it was influenced by both agricultural and forested land cover. These high levels of both N and P in the NR may also stem from legacy nutrients previously applied to agricultural fields. Over long time periods, nutrients can leech into groundwater and sediments, slowly being released to overland flow and into rivers, causing high background levels of these nutrients, regardless of current agricultural practices (Puckett *et al*. 2011, Tesoriero *et al*. 2013, Sharpley *et al*. 2014). With respect to statistically significant correlations found between TP concentrations and CHL concentration (i.e. a proxy for algal biomass), this is a common trend in lotic and lentic systems (Chow-Fraser 1999, McNair and Chow-Fraser 2003, Dodds *et al*. 2006).

When comparing the overall effects to individual phytoplankton taxa, there was limited spatial and temporal variability. The majority of the samples were dominated by *Navicula* (60%) in June, *Cryptomonas* (46.67%) in July, *Gomphonema* (26.67%) in August and finally *Nitszchia* (46.67%) in September. These taxa were dominated by specific species in most cases, *Navicula* (*N. gregaria)*, *Gomphonema* (*G. parvulum*) and *Nitszchia* (*N. acicularis).* These species have been identified as favouring very high concentrations of nutrients, scoring high on the nutrient sensitivity aspect of the Diatom Trophic Index (Kelly *et al*. 2001). To further reinforce that the majority of nutrients and water quality degradation is stemming from Innisfil Creek, there were higher proportions of sensitive taxa (e.g. *Brachysira, Cymbella*) found at site 1. Similarly, cluster analysis only distinctly clustered site 1 on its own during July. We can infer that although the water quality parameters at site 1 are significantly less impacted than most other sites, it is still nutrient enriched. Individual algal taxa were driven by TP and TN most often throughout the sampling period, with TP being the biggest positive driver of many algal species abundances.

Inverse relationships were also seen with some taxa that were indicators of either nutrient enrichment or of sensitivity (e.g. inverse relationship between high *Frustulia* abundance and low *Navicula/Nitszchia* abundance).

Although there is variability between lotic systems, there are areas of comparison that can be made when assessing river health. In general, the overall phytoplankton biomass, as determined through CHL, was relatively low (<10 μg/L) in comparison to other systems, although similar TP and TN concentrations were determined (Jones 2001). In a similar study in Argentina, ranges of CHL (0.8-7.9 μg/L) were determined for the Lower Lujan River, which had comparatively high TSS, increased TN concentrations and increased TP concentrations, although had a much wider channel width (O'Farrell *et al*. 2002). The mean CHL in rivers across Ontario and Quebec was found to be in the same range as well (6.62 μg/L) (Basu and Pick, 1996) . If looking to classify the NR with regards to trophic classification, CHL, TN and TP can all be used as boundaries for classification into oligotrophic, mesotrophic or eutrophic (i.e. low, medium and high nutrient input) (Dodds *et al*. 1998, Smith *et al*. 1999). Using either of these classification systems, CHL concentrations are in the oligotrophic range (<10 μg/L), TN is in the eutrophic range (2500 μg/L) and TP is in the mesotrophic range (60 μg/L). This places the NR in a fragile state, with increases in TP able to potentially dramatically shift the river into a eutrophic state, not only for TP, but also from control over increasing CHL, since the river may be phosphorus limited.

It is hypothesized that algal biomass (i.e. benthic algae, periphyton and phytoplankton) may be controlled more importantly by light penetration than nutrients (Cloern 1987, Dokulil 1994, Irigoien and Castel 1997). The combination of fine substrate (i.e. silt, sand and clay)

(Brown *et al*. 2011), as well as high suspended sediments already in the water column, may be limiting light penetration to areas of algal inhabitancy. We found no direct negative influence or threshold level controlling algal growth directly when looking at the parameters that were included in this study, but the variable nature of the system, as well as lack of information for direct measures of light, making a direct cause difficult to determine (Cloern 1987).

Not only can the overall CHL content be compared, but community structure can be related to other systems. Lotic systems tend to be dominated by diatoms in most cases, usually accounting for > 50% of the overall abundance of phytoplankton (Descy 1993, Schmidt 1994, O'Farrell *et al*. 2002), which is the case in the NR. Diatoms are the dominant group in most cases due to their silica structure which may protect them in the turbulent environment in many lotic systems. There is very little difference in the proportion of genera that are pollution tolerant (e.g. *Cymbella, Gomphonema, Scenedesmus, Navicula, Cryptomonas* and *Nitzschia*) across the NR, with the majority of all identified species being pollution tolerant, which is consistent with nutrient pollution occurring throughout the NR (Del-Giorgio *et al*. 1991). The variability in water quality does not appear to cause decreases in diversity across the NR, with on average 14 genera found at each site. With CHL and species composition in mind, it appears that the NR is impacted by anthropogenic nutrients, which are causing a community structure shift to more tolerant algal species, but there are limitations on the overall abundance most likely linked with P limitation.

Chapter 3: The effect of agricultural land-use type on water quality and periphyton of low order streams of the Nottawasaga Valley Watershed, Ontario, Canada

3.1 Introduction

Agriculture has been established as one of the largest contributors to eutrophication of both freshwater and marine ecosystems (Smith 2003). Many important nutrients have had their natural cycles altered through agricultural practices, mainly due to intensified use of carbon, nitrogen and phosphorus (Vitousek *et al*. 1997). In aquatic ecosystems, N and P concentrations have profound influences on primary productivity and many other processes that are directly and indirectly effected by increases or decreases in the base of the food web (Schindler 1974). Some of these alterations that can occur are differences in fatty acid (FA) content, proportions, and taxonomic distribution.

Primary production is the main source of many FAs, which in the case of aquatic ecosystems, stems from algal growth. FAs in algae are used for many biological processes that are important for cell functions, as well as growth. Algal photosynthesis relies heavily on FAs, due to the high proportion of glycolipids that are predominantly located within photosynthetic membranes (Guschina and Harwood 2009). Glycolipids are also an important component of thylakoid membranes, composing up to 55% of the total thylakoid lipid content (Guschina and Harwood 2009). These glycolipids are usually composed of polyunsaturated fatty acids (PUFA), such as the essential fatty acid (EFA) α-linolenic acid (ALA; C18:3n-3), as well as other constituents that vary in their composition dependant on taxonomic differences. Another related component of algal FA composition are phospholipids, which are also found in the photosynthetic components of the cell, most importantly the extra-chloroplast membrane

(Guschina and Harwood 2009). The last important group of FAs present in large quantities in algal cells are nonpolar triacylglycerols, which are used primarily as storage products (Guschina and Harwood 2009). They are mostly synthesized using light reactions, being stored in cytosolic lipid bodies, utilized as energy reserves during dark conditions (Thompson 1996). All of these important lipids become altered in their saturation and abundance dependant on temperature, which allows for proper functioning of the membrane bilayer under environmentally diverse conditions (Harwood 1998).

Some species of algae can alter their lipid composition by as much as 120% when large variations (e.g. 25 to 10˚C) in temperature are introduced over short periods of time (12h) (Jiang and Gao 2004), showing the plasticity algae have for alterations in temperature. Light intensity and availability will also alter lipid compositions, with large decreases in FA unsaturation linked with increases in light intensity, stemming from alterations in the chloroplast lipid content (Thompson 1996). Although these alterations in lipids can occur in individual cells, there are also differences in FA proportions between taxonomic groups. Phylogenetic relationships have been found to be the major driver of FA profiles, but it is difficult to differentiate those patterns from environmental conditions or from phylogeny alone (Galloway and Winder 2015).

FAs are one of several important food web components to consider when assessing food web dynamics. Since periphyton are the base of the aquatic food web, they provide higher trophic levels with food energy and a variety of nutritional constituents reflecting food quality (Torres-Ruiz *et al*. 2007). Periphyton are important food sources for many organisms, such as aquatic macroinvertebrate grazers (Torres-Ruiz *et al*. 2007) and zooplankton grazers (Brett and

Müller-Navarra 1997, Gulati and DeMott 1997). Energy transfer between trophic levels, most importantly between algae and grazers, is important because of the variability that exists and limited information on factors controlling the conversion of plant biomass to herbivore biomass (Brett and Müller-Navarra 1997). There are also complex relationships that cannot be directly related to food availability and herbivore populations, unlike higher trophic levels where predator-prey models usually are predicted by availability of prey, making this transfer of energy an important part of many food webs. FAs are the most important factor when discussing the quality of algal biomass as a food source for these higher trophic levels.

FAs can be broken down into three important classifications when discussing food quality: saturated fatty acids (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA). SAFAs and MUFAs are both of less importance when expressed as food quality due to their large quantities in algae and their ability to be synthesized from other constituents when ingested by other organisms (Brett and Müller-Navarra 1997). They are important in membrane function and fluidity, but more so in their use as a form of energy storage (Brett and Müller-Navarra 1997), whereas PUFAs are important in maintaining membrane fluidity, are found in photosynthetic membranes, such as chloroplasts. In many cases are classified as essential fatty acids (EFA) due to their limited quantity and difficulties in synthesis in many organisms (Hill *et al*. 2011). There are many EFAs that have been established, with linoleic acid (C18:2ω6), α-linolenic acid (C18:3ω3), arachidonic acid (C20:4ω6), eicosapentanoic acid (C20:5 ω3) and docosahexanoic acid (C22:2 ω6) being characterized as some of the most important (Kainz *et al*. 2004). These EFA PUFAs are found primarily in photosynthetic and cytoplasmic membranes, contained within galactolipids (Hill *et al*. 2011).

Conditions that increase the production of photosynthetic membranes generally increase the EFA concentrations in algal cells (Guschina and Harwood 2009), while environmental conditions that increase the storage of fixed carbon in the form of SAFAs will reduce the proportion of EFAs (Hu *et al*. 2008). Relationships between water quality and EFAs have also been established. Both decreases in light availability and decreased P concentrations increased the proportion of PUFAs, whereas increased light and P concentrations increased the proportion of SAFAs and MUFAs, decreasing the food quality of these algae (Hill *et al*. 2011). In agriculturally influenced streams, it is unclear how these two factors that usually have a positive correlation with agricultural land-use will effect overall FA composition of periphyton due to their inverse influences (Larson *et al*. 2013).

The purpose of this chapter was to determine the influence that agricultural land-use type had on small streams exclusively draining agricultural land in the NVW. To assess this, the following objectives were outlined:

1. Determine the effect that different agricultural land-use types had on water quality of representative first- and second-order streams that exclusively drain small, single crop type agricultural lands.
2. Determine temporal and spatial patterns of water quality across these stream systems.
3. Assess how agricultural land-use type and water quality influence periphyton growth.
4. Determine the relationship between agricultural land-cover type and periphyton community structure and food quality through analyses of fatty acid content

It was anticipated that water quality would vary across the sites in the NVW because of the small size of the streams chosen, but would be distinguished by their nutrient content

related to the type of agricultural land-use drained. Water quality related to agriculturally relevant nutrients, such as nitrogen and phosphorus concentrations, would be increased at the sites that drained soy and corn row crops and would be only slightly elevated at the pasture sites due to the lack of potential influence from fertilizer additions. Periphyton growth was also hypothesized be elevated in these streams and should be directly related to elevated nitrogen and phosphorus concentrations. Algal FA profiling was conducted for the first time in the NVW in order give an alternative to direct species identification. This was chosen in order determine the effect that agricultural land-use type can have on FA content, proportions and overall profiles in periphyton due to their importance in the food web. Similar species can have alterations in FA proportions depending on environmental conditions, which allows for important information on differences in FAs in these types of ecosystems. This information will not only shed light onto the effects that agricultural land-use in the NVW can have directly on first- and second-order streams, but also how this may influence important food web dynamics at larger scales. This information may also be used for monitoring of agriculturally fed streams and their food quality for consumers in further studies of these inconspicuous ecosystems and their effect on the larger drainage network.

3.2 Methods

3.2.1 Study Site Selection

Sampling sites were identified using digital orthophotos of the study area (Government of Canada 2009) using ArcGIS 10.3 in order to initially identify first- and second-order streams for easy road access, as well as draining only a single crop type. Over 50 potential stream sites were chosen as candidates and due to crop rotations and lack of current land-use data, it was

necessary to field validate each site in order to determine its feasibility. In total, 6 monitoring sites were strategically chosen in first- or second-order streams, two of which drained corn crop agricultural land exclusively, two drained soy bean crop agricultural land exclusively and two that exclusively drained cattle pasture. Either first- or second-order streams were allowed in the study after previous research had shown that there was no significant difference in water chemistry between either type of stream, as long as it is only draining the single crop type (Dieleman and Chow-fraser 2012). Site choice was also limited by variability in first- and second- order streams and prolonged periods of low water depth over dry periods in the summer months.

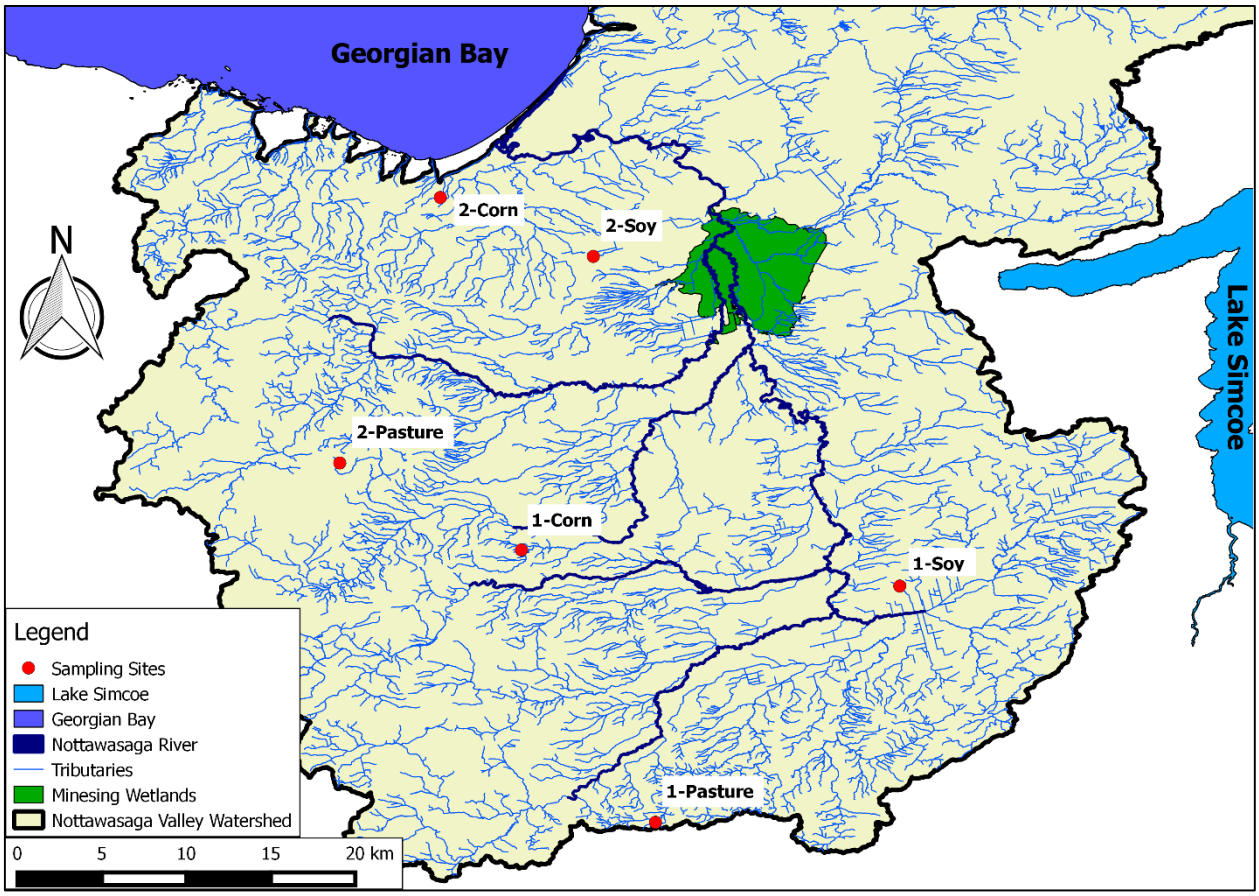

Figure 3.1: Overview map of the Nottawasaga River Valley Watershed and the location of the 6 sampling sites.

Table 3.1: Coordinates of the six sampling sites, subwatershed location and agricultural type drained.

Site	Subwatershed	Latitude/Longitude coordinates (decimal degrees)
1-Pasture	Innisfil Creek	43.98174,-79.94254
2-Pasture	Pine River	44.18246,-80.04137
1-Soy	Innisfil Creek	44.15583,-79.76237
2-Soy	Lower Nottawasaga River	44.39875,-79.98836
1-Corn	Pine River	44.24654,-80.17541
2-Corn	Blue Mountain Watershed	44.44218,-80.10118

3.2.2 Water Sampling and Processing Procedures

In order to capture trends within the agriculturally relevant growing season, sampling occurred initially on the last week of June and then on a 4-week basis to the last week of August. This time period was determined to be sufficient to allow proper colonization and growth of periphyton. Water quality was obtained at all three of the sampling dates, but periphyton samples were only collected at the end of the 4-week growth period for July and August rod deployments (see periphyton growth-assay below). Water samples were obtained using acid-washed 110mL and 200mL Corning™ snap-seal containers or 1000 mL Nalgene® bottles. These samples were used for the determination of: total ammonia nitrogen (TAN), total nitrate nitrogen (TNN) and total phosphorus (TP). After collection occurred, all samples were stored on icepacks in a cooler until they could be processed, or placed in a freezer for storage until processed in the lab. As well, in-field values for temperature (TEMP), conductivity (COND), pH and dissolved oxygen (DO) were obtained using a calibrated YSI multiparameter probe (YSI Inc., Yellowsprings, Ohio, USA) and turbidity (TURB) readings were measured in triplicate with an in-field Turbidimeter (HACH®, Loveland, Colorado, USA). TP, TNN and TAN measurements were performance following the methods described in Chapter 2.

3.2.3 Periphyton Growth Assays and Analyses

To directly compare algal growth between land-use types, periphyton growth assays using artificial substrates were employed. Algal growth assays using artificial substrates are ideal for *in situ* algal growth experiments because they reflect the colonization and growth of native algae over a defined period of time and environmental conditions across sites. In contrast, if algal communities were sampled from natural substrates at each site, the

periphyton community age and colonization history would be unknown. This would make it difficult to compare communities between sites if the algal communities reflected different stages of succession.

Following the protocol of McNair and Chow-Fraser (2003), clear acrylic rods (50cm x ¼ inch) served as artificial growth substrate (Figure 3.2). The rods were scored at 5cm intervals and deployed at each. This protocol was ideal for the small streams in this study, which were characterized by soft sediment and shallow depths. The rods provided a hard substrate for algal attachment and due to the variable nature of these streams, provide a continuously submerged portion of substrate even at low water levels. All sites had any aquatic or overhanging vegetation removed in order to prevent differences in light exposure, which could alter periphyton growth characteristics (Hill *et al*. 2011). Before deployment, rods were cleaned with 90% isopropanol and handled with Nitril™ gloves in order to prevent contamination of the rods and to allow for uniform periphyton growth (McNair and Chow-Fraser 2003). A minimum of 3 replicate rods were deployed at each site in a grid pattern, at a minimum of 50 cm distance from adjacent rods in order to allow for replication and prevention of the interaction of periphyton between each rod. After the 4-week incubation period, rods were removed and a minimum of 5cm length was scrapped into a 120 mL specimen cup using a small metal chisel and divided into sub-samples for ash free dry mass (AFDM), periphyton chlorophyll a (CHL) and FA analyses, respectively. CHL analyses were performance using the 90% acetone method described in Kirkwood *et al*. (1999), whereas periphyton AFDM was determined following the methods of (Bourassa and Cattaneo 1998).

Figure 3.2: Representative picture of algal assay acrylic rod placement. Rod length was dependant on water depth.

Periphyton samples for FA analysis were stored at -20°C until analyses could be completed. Before FA quantification, fatty acid methyl esterification was completed using a method adapted from O'Fallen *et al* (2007). Ten mL of each sample was lyophilised to remove excess moisture and prepare the samples for esterification. After lyophilisation, each sample underwent extraction of the fatty acids using methanol, potassium hydroxide (KOH) and sulfuric acid (H_2SO_4). HPLC grade hexane solvent was added to each sample after fatty acid extraction, as well as a C19 internal standard before gas chromatography (GC) analysis could occur for comparison purposes.

The fatty acid composition of each sample was determined by capillary GC using a Varian CP-3900, VF-23ms, 30m X 0.25mm X 0.25µm capillary column (Varian Inc.). The initial oven temperature was 140°C, increased to 240°C at a rate of 25°C/min and held for 5 minutes. Helium was used as the carrier gas at a flow rate of 2 mL/min. Split ratio was maintained at 10:1. Fatty acids were identified by comparing their retention times with Supelco FAME Mix C8-C23 standard. This standard was chosen due to containing short- and long-chain SAFAs, MUFAs and PUFAs in specific proportions, which allowed for the identification of the proportion of each sample that could be identified as each FA (O'Fallon *et al*. 2007).

3.2.4 Data Analysis

All water quality and biological data were *log* transformed in order to fulfil the assumptions of normality and homogeneity when completing statistical analyses. Due to the small sampling size for each crop type (n=2), analysis of variance (ANOVA) was used to assess differences in water quality by pooling the data from all sites during each sampling month and pooling each site over all the sampling period, using a 95% confidence interval. In order to

prevent pseudoreplication, each month was considered to be its own independent experiment. FA profiles were analysed for differences related to individual FAs and grouped FA classification at each site pooled across months, individual FAs pooled by sites across months and from pooled data for crop type for individual FAs and grouped FA classification to determine significant differences. Principal components analysis (PCA) was performed to identify key temporal and spatial variations in biological and water quality data among the sampling sites. PCA was also run on the FA proportion information, with FAs as the descriptive variables for the sites. Redundancy analysis (RDA) was used to assess the impact that crop type was having on the relationships between water quality and FA proportions. RDA was chosen as appropriate for the data set because of the linear response in the descriptive variables in relation to the explanatory variables, which was determined using detrended canonical analysis (DCA) in order to distinguish between unimodal or linear data. In order to determine appropriate variables to include in each RDA, variance inflation factors (VIF) were calculated (Pan *et al*. 1996). VIF values allow for the removal of descriptive variables that can cause over inflation of environmental variance explanation, with VIF values of >10 being removed from the analysis. Pearson correlation coefficient's were determined for significant relationships ($p<0.05$) between water quality variables and biological data sets. Multiple stepwise regressions were used in order to determine relationships between FA proportions and biological data. Finally, Bray-Curtis dissimilarity cluster analysis was completed in order to determine the dissimilarity between sampling sites with regards to the FA profile relative proportions. All statistical analyses were completed using RStudio 3.3.0 (R Development Core Team 2008).

3.3 Results

3.3.1 Summary of spatial and temporal trends of water quality first- and second-order streams of the Nottawasaga Valley Watershed

Water quality was found to be highly variable between the sampling sites and between the sampling months. This was evident in the differences between each sampling site, regardless if sites were consistent for agricultural land-use (Figures 3.3-3.12). Using the ANOVA results (Table 3.2), it was found that there were statistically significant ($p<0.05$) differences in DO, pH, COND, TNN and TAN across the sampling sites when pooled over the sampling period. Due to small sample sizes, Post-Hoc testing was not able to be used to determine significant differences. With regards to differences that can be related to sampling period (i.e. pooled sites), there were no statistically significant differences found for water quality or algal metrics (CHL and AFDM), which can be related to the large amounts of variability that occurred between the individual sites themselves (Table 3.3). Although there were no statistically significant differences, TURB was extremely high in August, which could be attributed to disturbance of the sediment, rather than actual effects from the site. Finally, the only statistically significant difference that was related to crop type was COND, which was significantly low at the corn cropland drained sites compared to the pasture and soy bean agricultural types (Table 3.4).

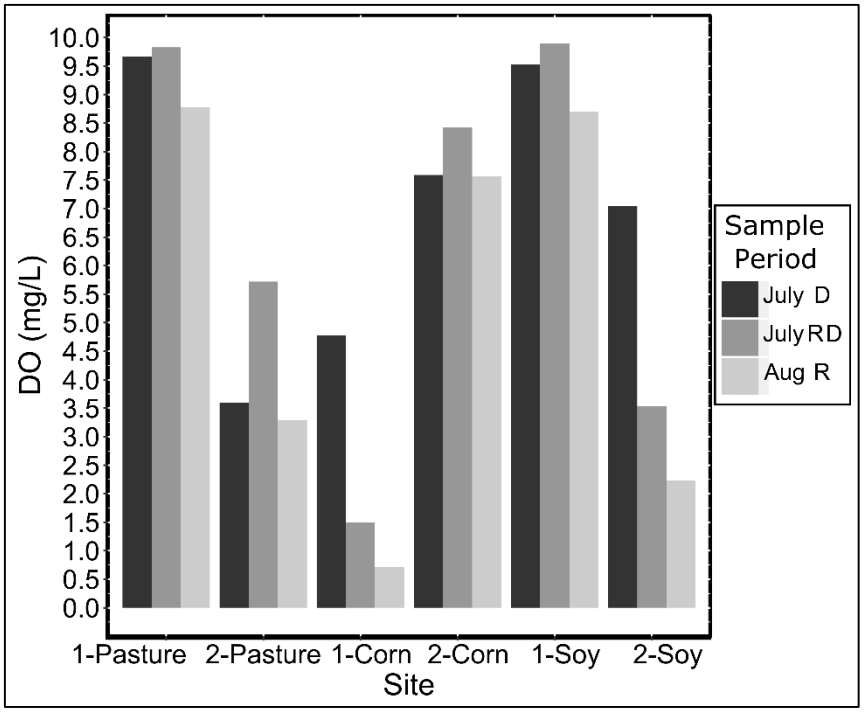

Figure 3.3: Variation in dissolved oxygen concentration in $mg \cdot L^{-1}$ across the sampling periods. Designations for sampling period are as follows: D= algal assay deployment period, RD=algal assay deployment and retrieval period, and R=algal assay retrieval period. DO values are from individual YSI sonde measurements.

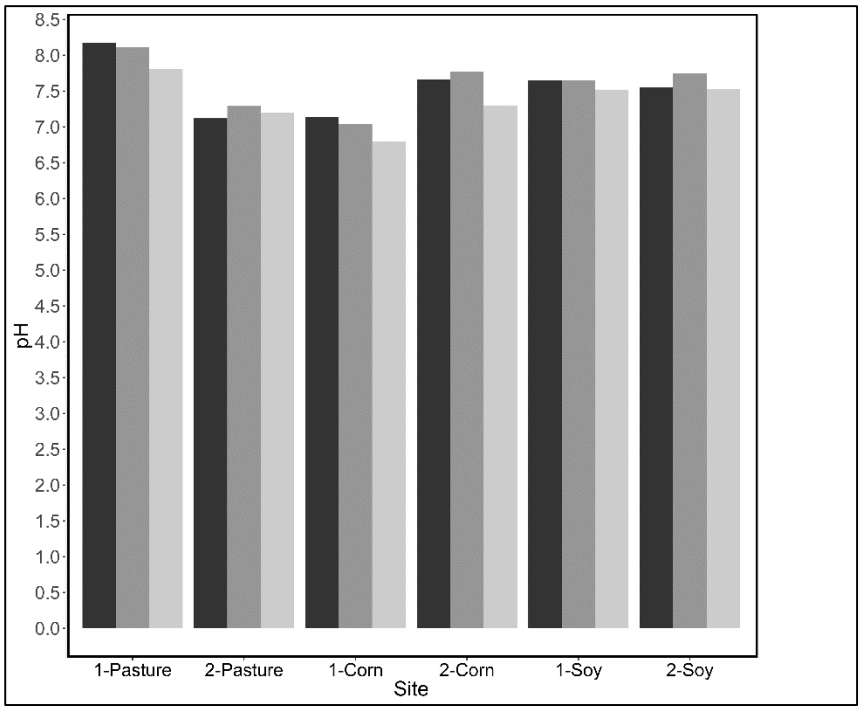

Figure 3.4: Variation in pH across the sampling period. pH values are from individual YSI sonde measurements.

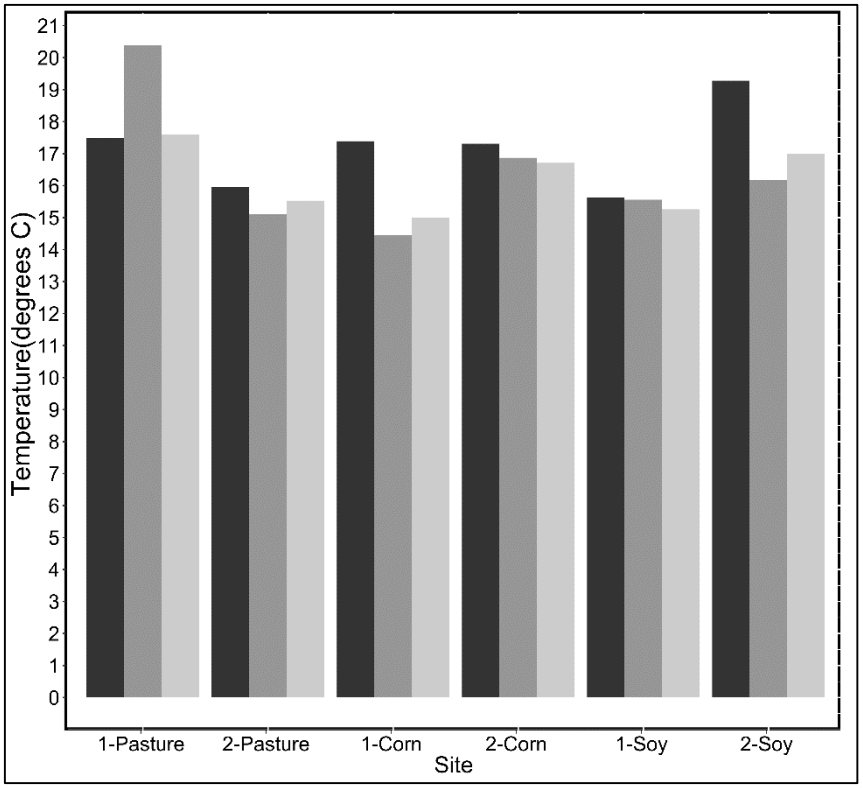

Figure 3.5: Variation in water temperature in degrees C° across the sampling period. TEMP values are from individual YSI sonde measurements.

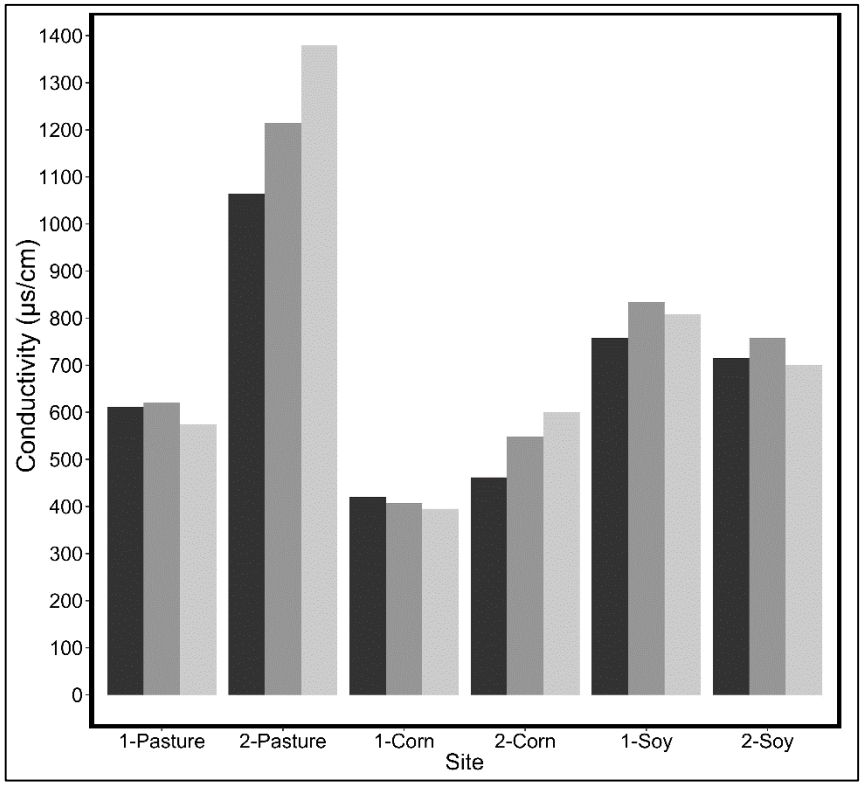

Figure 3.6: Variation in water conductivity in µs·cm^{-1} across the sampling period. COND values are from individual YSI sonde measurements.

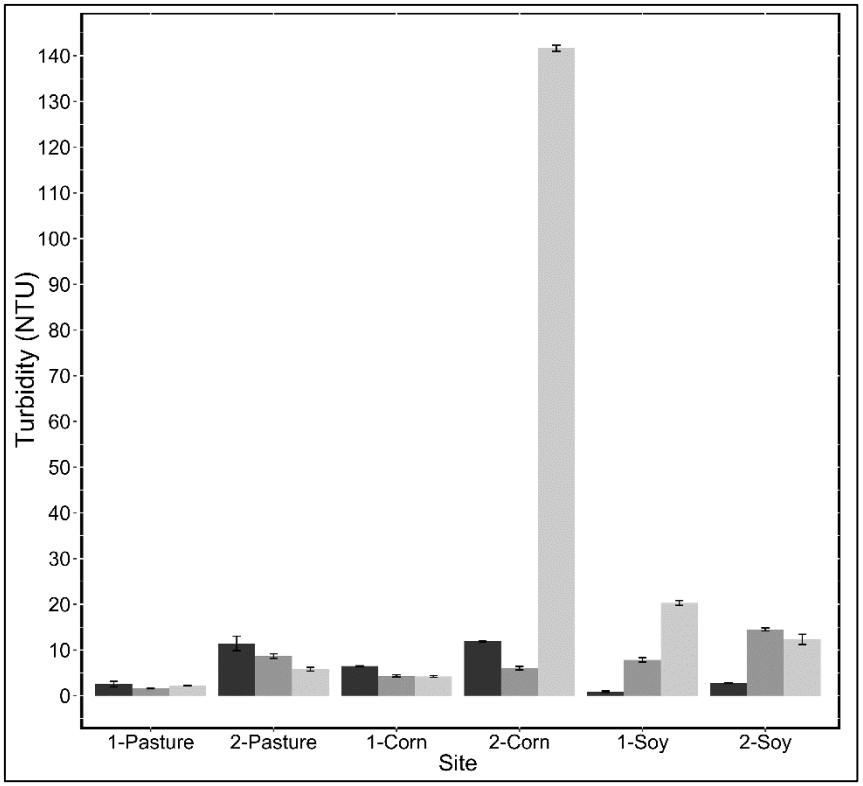

Figure 3.7: Variation in water turbidity (±SE) in Nephelometric Turbidity Units across the sampling period.

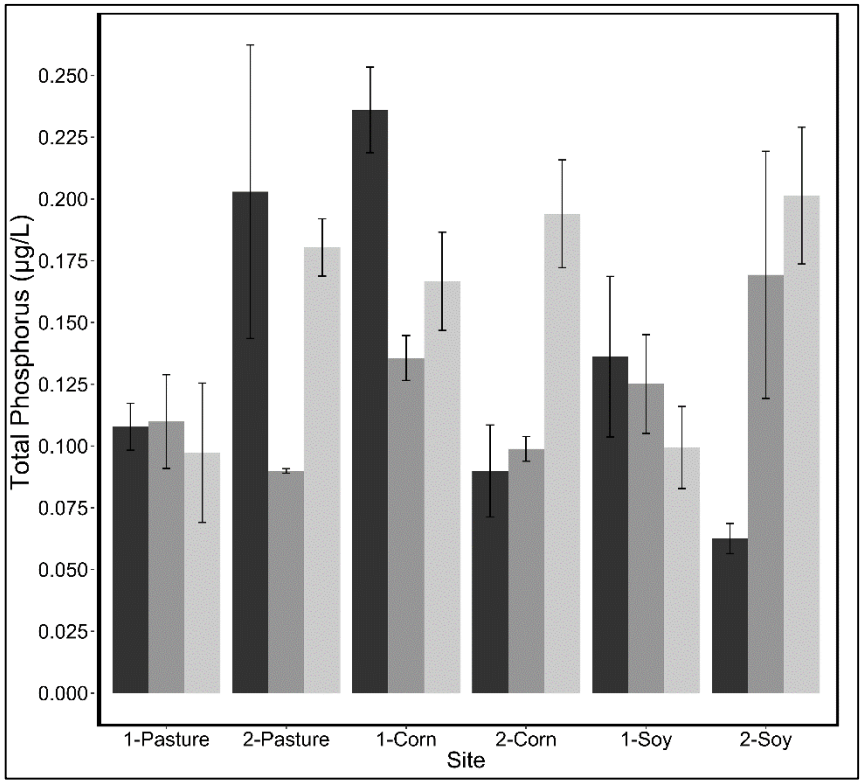

Figure 3.8: Variation in total phosphorus concentration (±SE) in $\mu g \cdot L^{-1}$ across the sampling period.

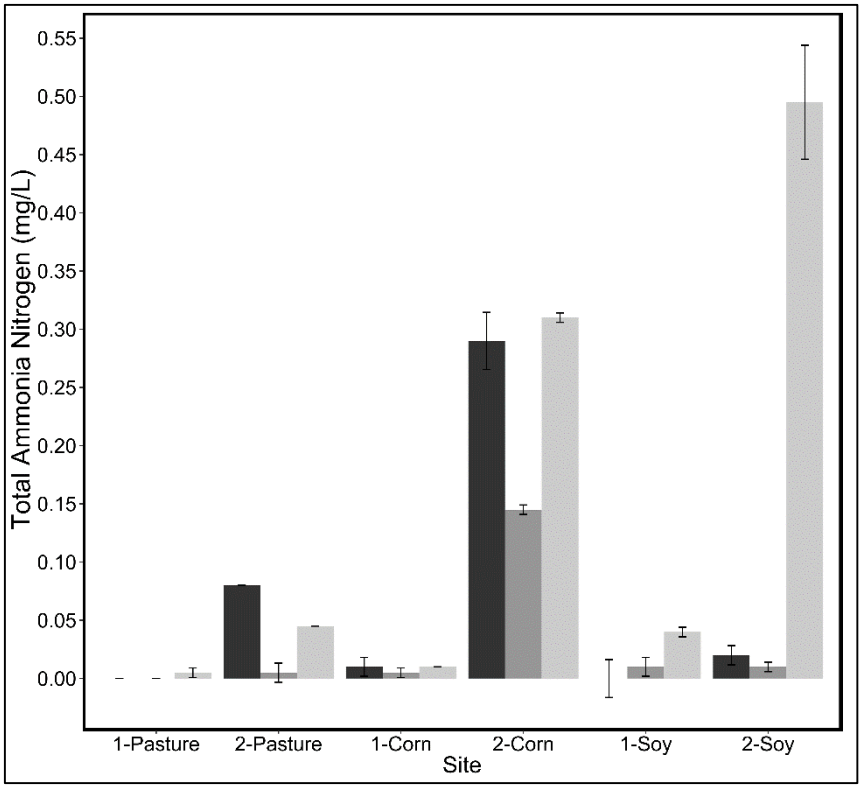

Figure 3.9: Variation in total ammonia nitrogen concentration (±SE) in mg·L^{-1} across the sampling period.

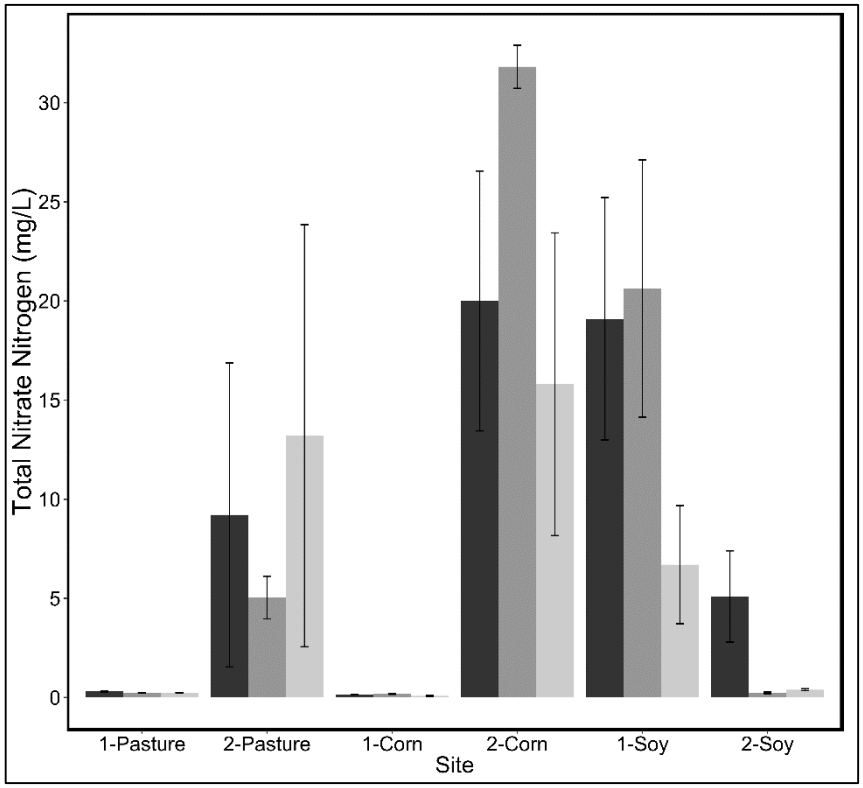

Figure 3.10: Variation in total nitrate nitrogen concentration (±SE) in mg·L^{-1} across the sampling period.

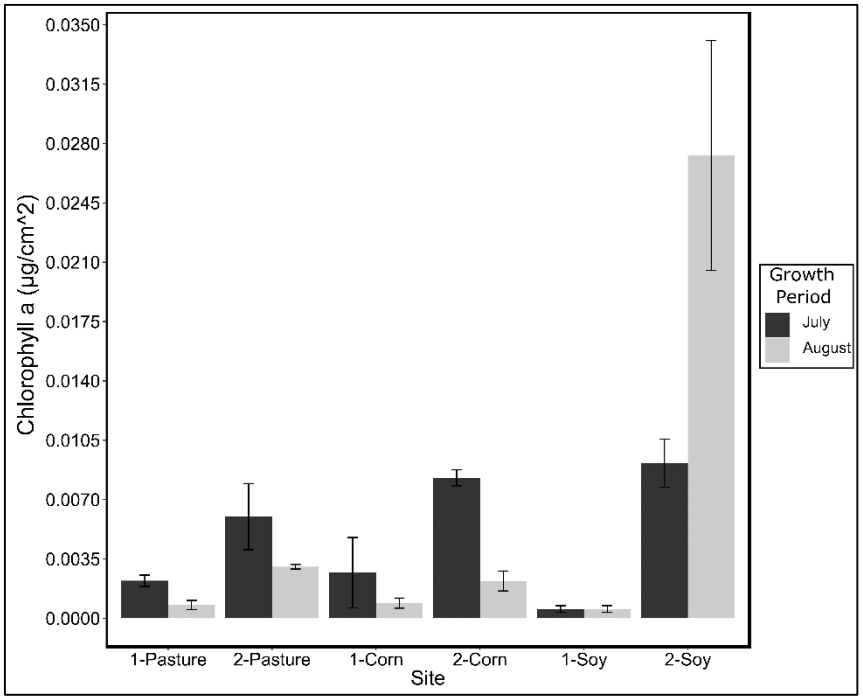

Figure 3.11: Variation in periphytic chlorophyll *a* content (±SE) in µg·cm^{-2} across the sampling period.

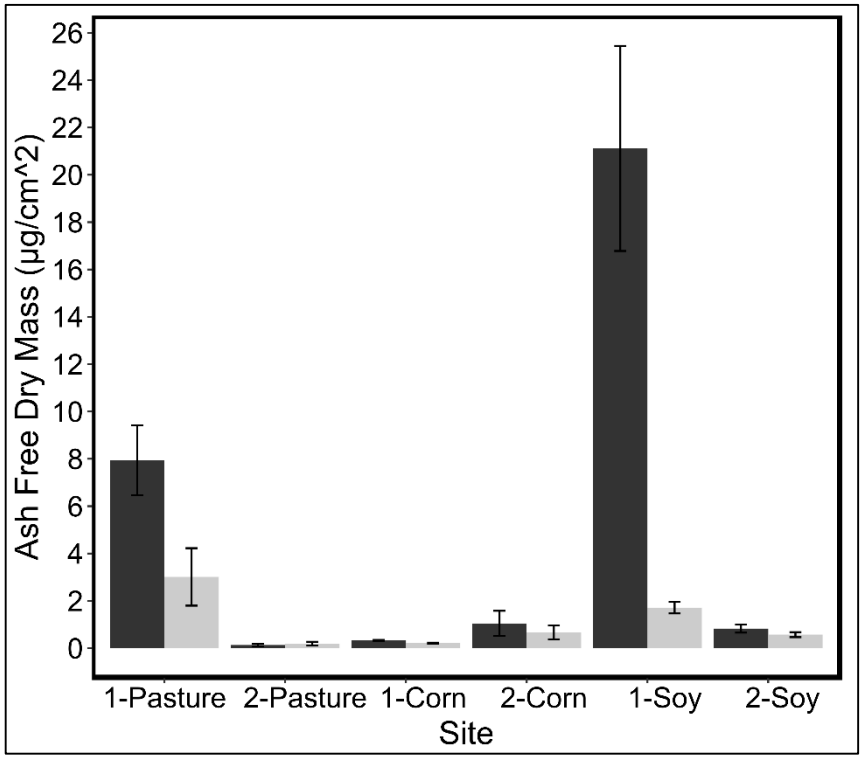

Figure 3.12: Variation in periphytic ash free dry mass content (±SE) in µg·cm^{-2} across the sampling period.

Table 3.2: Results of one-way ANOVAs for detectable site differences of water quality. Data from all sampling periods (n=3) were pooled in order for comparisons to be made. Statistical significance is set at α=0.05 and statistically significant differences in water quality parameters are shown in bold.

Parameter	Site	Mean	SD (±)	DF	F Value	*p*-value
Log DO	1-Pasture	9.42	0.46	5	4.259	**0.0185**
	2-Pasture	5.10	2.35			
	1-Corn	2.33	1.76			
	2-Corn	6.23	1.9			
	1-Soy	9.37	0.5			
	2-Soy	5.00	2.03			
Log pH	1-Pasture	8.03	0.16	5	7.435	**0.00218**
	2-Pasture	7.36	0.29			
	1-Corn	6.99	0.14			
	2-Corn	7.57	0.19			
	1-Soy	7.61	0.06			
	2-Soy	7.46	0.12			
Log TEMP	1-Pasture	18.49	1.34	5	2.215	0.12
	2-Pasture	16.12	0.56			
	1-Corn	15.62	1.27			
	2-Corn	16.73	0.46			
	1-Soy	15.48	0.16			
	2-Soy	17.13	1.71			
Log COND	1-Pasture	602	19.44	5	4.306	**0.0178**
	2-Pasture	997.33	342.92			
	1-Corn	407.33	10.21			
	2-Corn	606.67	120.93			
	1-Soy	800	31.54			
	2-Soy	876.67	239.32			
Log TURB	1-Pasture	2.13	0.4	5	2.579	0.083
	2-Pasture	7.76	2.61			
	1-Corn	5.01	1.05			
	2-Corn	56.02	60.57			
	1-Soy	9.68	8.03			
	2-Soy	7.92	3.95			
Log TP	1-Pasture	0.11	0.01	5	0.846	0.543
	2-Pasture	0.16	0.05			
	1-Corn	0.18	0.04			
	2-Corn	0.13	0.05			
	1-Soy	0.12	0.02			
	2-Soy	0.14	0.06			
Log TNN	1-Pasture	0.27	2.36	5	21.24	**<0.001**

Table 3.2 (cont.)

	2-Pasture	9.15	41.43			
	1-Corn	0.14	2.36			
	2-Corn	22.54	136.95			
	1-Soy	15.47	17			
	2-Soy	1.91	227.54			
Log TAN	1-Pasture	1.67	0.03	5	3.189	**0.0463**
	2-Pasture	90	3.33			
	1-Corn	8.33	0.04			
	2-Corn	203.33	6.78			
	1-Soy	16.67	6.24			
	2-Soy	173.33	2.26			
Log CHL	1-Pasture	0.00150	0.00101	5	2.764	0.124
	2-Pasture	0.00452	0.00209			
	1-Corn	0.00179	0.00127			
	2-Corn	0.00524	0.0043			
	1-Soy	0.00055	3.42×10^{-6}			
	2-Soy	0.01822	0.0128			
Log AFDM	1-Pasture	5.475	3.486	5	3.113	0.0996
	2-Pasture	0.1630	0.0437			
	1-Corn	0.2715	0.0823			
	2-Corn	0.8590	0.266			
	1-Soy	11.413	13.722			
	2-Soy	0.7005	0.187			

Table 3.3: Results of one-way ANOVAs for detectable sampling period differences of water quality. Data from all sampling sites (n=6) were pooled in order for comparisons to be made. Statistical significance is set at α=0.05 and statistically significant differences in water quality parameters are shown in bold.

Parameter	Sample Period	Mean	SD (±)	DF	F value	p-value
Log DO	July Deployment	7.035	2.46	2	0.746	0.491
	July Retrieval	6.76	2.77			
	August Retrieval	5.21	3.25			
Log pH	July Deployment	7.55	0.39	2	0.963	0.516
	July Retrieval	7.58	0.35			
	August Retrieval	7.36	0.32			
Log TEMP	July Deployment	17.18	1.30	2	0.724	0.501
	July Retrieval	16.8	1.65			
	August Retrieval	16.18	0.97			
Log COND	July Deployment	671.67	234.19	2	0.075	0.928
	July Retrieval	701	238.21			
	August Retrieval	743	311.35			
Log TURB	July Deployment	6.01	4.76	2	1.2	0.329
	July Retrieval	6.59	4.23			
	August Retrieval	31.09	49.82			
Log TP	July Deployment	0.14	0.07	2	0.722	0.502
	July Retrieval	0.13	0.05			
	August Retrieval	0.16	0.04			
Log TNN	July Deployment	8.98	8.85	2	0.137	0.873
	July Retrieval	9.33	10.38			
	August Retrieval	6.07	6.43			
Log TAN	July Deployment	66.67	113.43	2	1.35	0.289
	July Retrieval	47.92	84.03			
	August Retrieval	150.83	186.36			
Log CHL	July Retrieval	0.00482	0.00351	1	0.043	0.84
	August Retrieval	0.0058	0.0106			
Log AFDM	July Retrieval	5.23	8.33	1	1.088	0.321
	August Retrieval	1.06	1.10			

Table 3.4: Results of one-way ANOVAs for detectable crop type differences of water quality. Data for each agricultural type across the sampling period (n=6) were pooled in order for comparisons to be made. Statistical significance is set at α=0.05 and statistically significant differences in water quality parameters are shown in bold.

Parameter	Crop type	Mean	SD (±)	DF	F value	p-value
Log DO	Pasture	7.27	3.01	2	2.053	0.163
	Corn	4.28	2.93			
	Soy	7.19	2.89			
Log pH	Pasture	7.70	0.45	2	2.229	0.142
	Corn	7.28	0.37			
	Soy	7.53	0.13			
Log TEMP	Pasture	17.31	1.72	2	0.996	0.392
	Corn	16.18	1.21			
	Soy	16.31	1.61			
Log COND	Pasture	799.67	343.03	2	5.238	**0.0188**
	Corn	507.00	144.07			
	Soy	838.33	191.63			
Log TURB	Pasture	4.95	3.70	2	1.605	0.233
	Corn	30.52	54.61			
	Soy	8.80	7.00			
Log TP	Pasture	0.13	0.05	2	0.351	0.71
	Corn	0.15	0.06			
	Soy	0.13	0.05			
Log TNN	Pasture	4.71	5.51	2	0.216	0.808
	Corn	11.34	13.35			
	Soy	8.69	9.03			
Log TAN	Pasture	45.83	58.09	2	0.297	0.748
	Corn	105.83	150.55			
	Soy	95.00	196.47			
Log CHL	Pasture	0.00	0.00	2	0.86	0.455
	Corn	0.00	0.00			
	Soy	0.01	0.01			
Log AFDM	Pasture	2.82	3.67	2	0.885	0.446
	Corn	0.57	0.38			
	Soy	6.06	10.05			

3.3.2 Principal Components Analyses (PCA) on water quality

PCA was performed on *log* transformed, normalised (i.e. centre-standardized) nutrient data over the three sampling dates (Figure 3.13). Combined, the first and second principal components explained 57.49% of the overall variation in the water quality data set. The PCA biplot allowed for the visualization of the mostly unique driver of each site, even across the 3 sampling periods. The soy bean agricultural sites varied across an axis of high TNN (1-Soy) and inversely high TAN (2-Soy), whereas pasture sites varied across an axis of high COND, high pH and high TEMP (1-Pasture) and low COND, low TEMP, pH and AFDM (2-Pasture). Corn agricultural type was the most variable, with site 2-Corn expressing no clustering between the sampling periods and site 1-Corn strongly driven by low pH, TEMP, AFDM and neutral levels of most other nutrients. The variability between the sampling sites with related agricultural types is visually displayed in the lack of clustering between the two sites of each agricultural type.

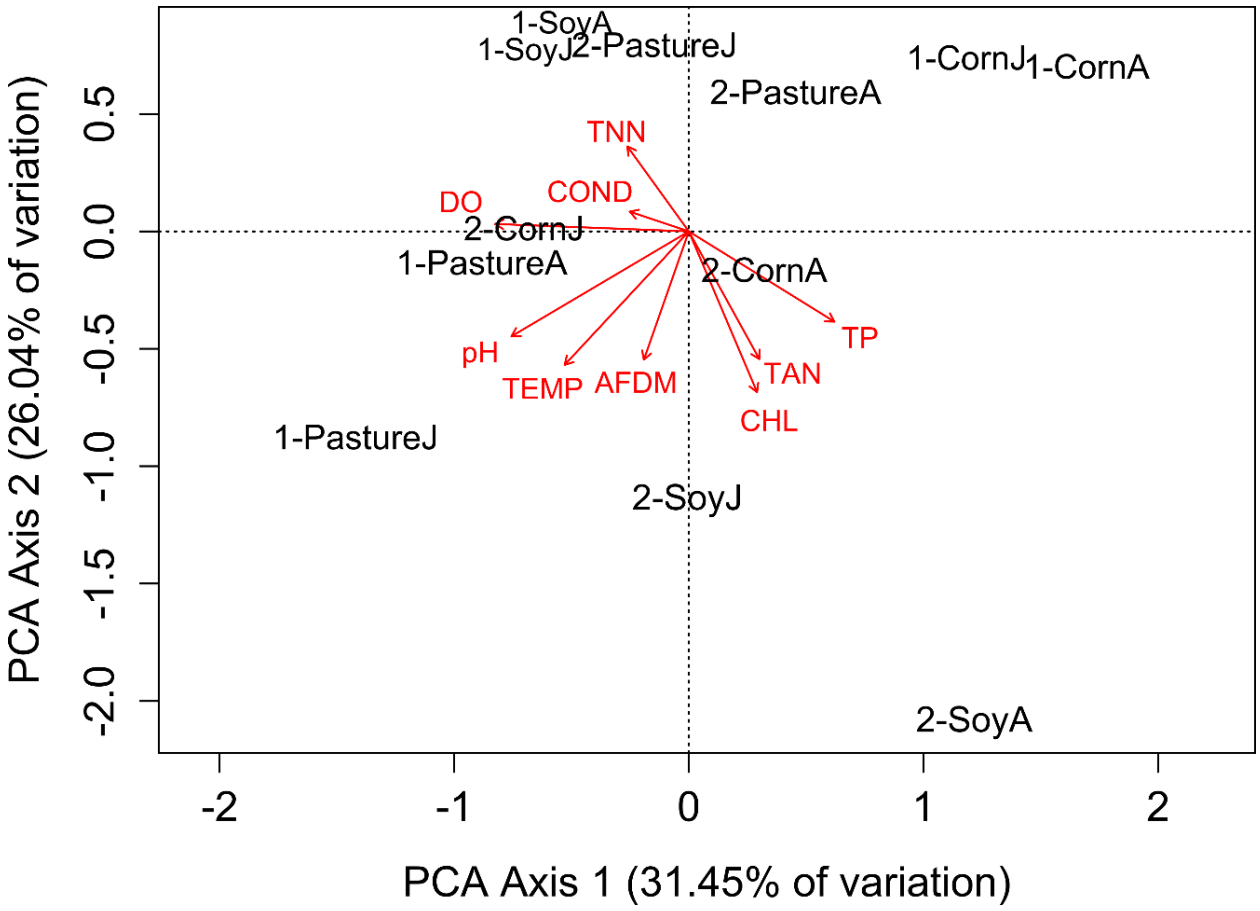

Figure 3.13: Principal component analysis on water quality during the July and August retrieval of algal assay experimental substrate.

3.3.3 Summary of fatty acid composition and fatty acid profiles of first- and second-order agriculturally fed streams

In order to better characterize periphyton and understand how agriculture type is having an effect on periphyton community structure, analysis of FAs was conducted, which included the identification of proportions of 14 different FAs, with varying chain length (Table 3.6). FA profiles were created using boxplots expressing the distribution of FA proportions throughout the algal assay experiments at each site and the mean value of the two experiments (Figures 3.14-3.19). There was a large amount of diversity in each FA profile, as well as the identification of all 14 FAs present in at least one of the samples. In comparison, relative abundance of each FA profile from the total at each site can be compared between the two algal growth assays (Figure 3.20 and 3.21). There was a large increase in the proportion of linoleic acid (C18:2ω6) during the August 1-Pasture assay, though this was unique to this time period and site. To better understand the distribution of grouped FA classification (e.g. MUFA, PUFA, SAFA) relative abundance was determined for each sites, grouping each FA into these classifications for each growth assay (Figure 3.22 and 3.23). Due to the large increase in the proportion of C18:2ω6 at site 1-Pasture during August, there was also an increase in the proportion of PUFAs.

When comparing sites, there were no statistically significant differences between the relative abundances of any of the individual FAs (Table 3.6). There was also no statistically significant differences in the proportions of grouped FAs for their classification when comparing pooled site differences (Table 3.7). Individual proportions of FAs were also not statistically significantly different when comparing between month (Table 3.8) and between crop type

(Table 3.10) differences. Grouped FA classification showed similar trends, except with a statistically significant decrease in the proportion of MUFAs from the July to August algal assay (Table 3.9). Finally, there were no statistically significant differences in grouped FA classification related to crop type (Table 3.11).

Table 3.5: Fatty acids identified by their fatty acid methyl ester products. Fatty acid classifications: saturated fatty acid (SAFA), monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids (PUFA).

Common Name	Fatty Acid Equivalent	Lipid Number	Molecular Formula	Fatty Acid Classification
Caprylic acid methyl ester	Caprilic acid	C8:0	$C_9H_{18}O_2$	SAFA
Capric acid methyl ester	Capric acid	C10:0	$C_{11}H_{22}O_2$	SAFA
Lauric acid methyl ester	Lauric acid	C12:0	$C_{13}H_{26}O_2$	SAFA
Myristic acid methyl ester	Myristic acid	C14:0	$C_{15}H_{30}O_2$	SAFA
Palmitic acid methyl ester	Palmitic acid	C16:0	$C_{17}H_{34}O_2$	SAFA
Palmitoleic acid methyl ester	Palitoleic acid	C16:1	$C_{17}H_{34}O_2$	MUFA
Stearic acid methyl ester	Stearic acid	C18:0	$C_{19}H_{38}O_2$	SAFA
Oleic acid methyl ester	Oleic acid	C18:1ω9	$C_{19}H_{36}O_2$	MUFA
Linoleic acid methyl ester	Linoleic acid	C18:2ω6	$C_{19}H_{34}O_2$	PUFA
Arachidic acid methyl ester	Arachidic acid	C20:0	$C_{21}H_{42}O_2$	SAFA
Linolenic acid methyl ester	α-Linolenic acid	C18:3ω3	$C_{19}H_{34}O_2$	PUFA
Behenic acid methyl ester	Behenic acid	C21:0	$C_{23}H_{46}O_2$	SAFA
Euricic acid methyl ester	Euricic acid	C22:1ω9	$C_{23}H_{44}O_2$	MUFA
Lignoceric acid methyl ester	Lignoceric acid	C23:0	$C_{25}H_{50}O_2$	SAFA

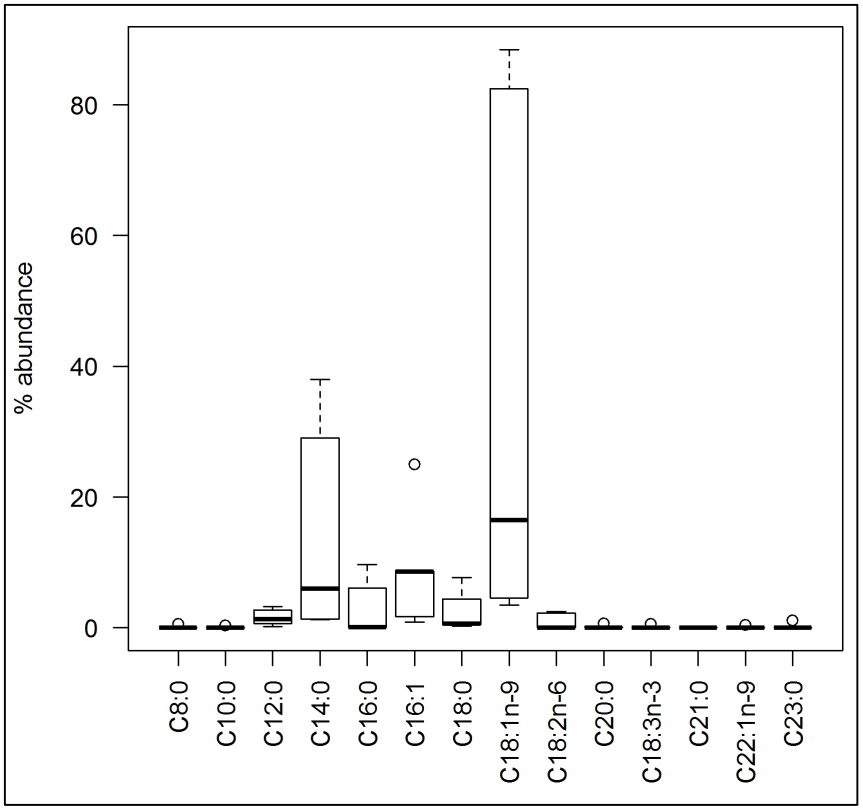

Figure 3.14: Boxplot of fatty acid biomarkers used to characterize the overall FA profile between the July and August algal growth assays at site 1-Pasture. The *thick middle line* represents the median, the *box* is the first and third quartiles, *points* represent outliers and the *whiskers* represent the 5th and 95th percentiles.

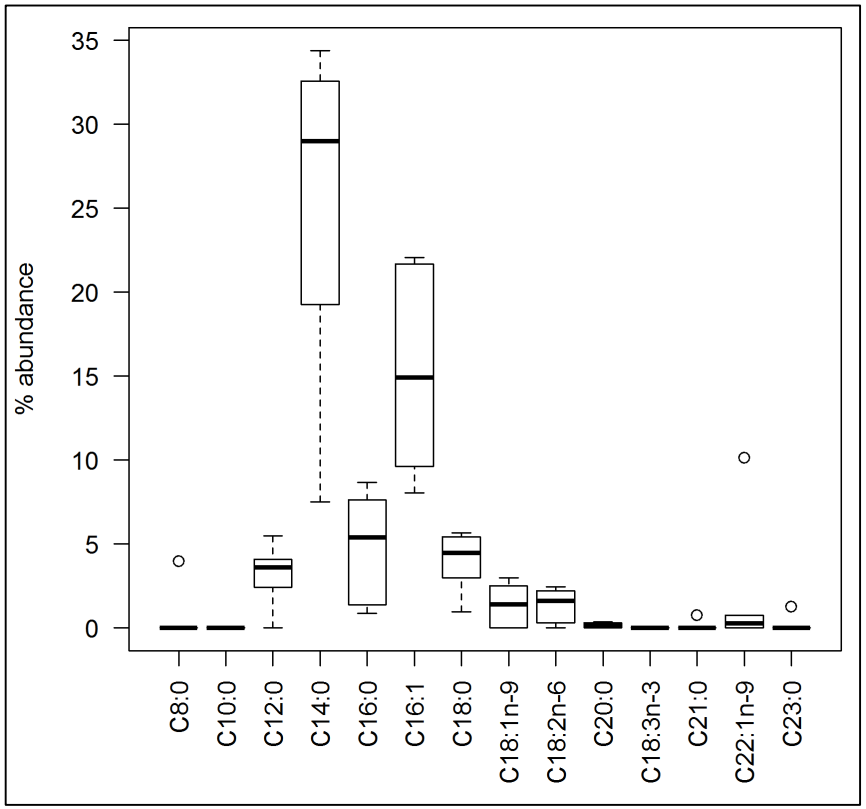

Figure 3.15: Boxplot of fatty acid biomarkers used to characterize the overall FA profile between the July and August algal growth assays at site 2-Pasture. The *thick middle line* represents the median, the *box* is the first and third quartiles, *points* represent outliers and the *whiskers* represent the 5th and 95th percentiles.

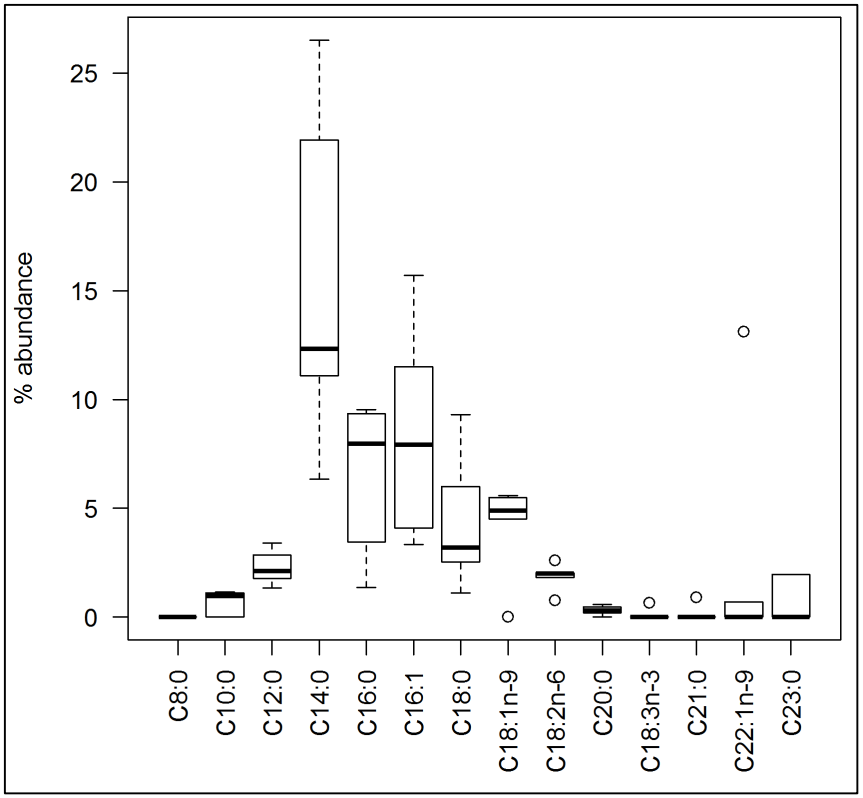

Figure 3.16: Boxplot of fatty acid biomarkers used to characterize the overall FA profile between the July and August algal growth assays at site 1-Corn. The *thick middle line* represents the median, the *box* is the first and third quartiles, *points* represent outliers and the *whiskers* represent the 5th and 95th percentiles.

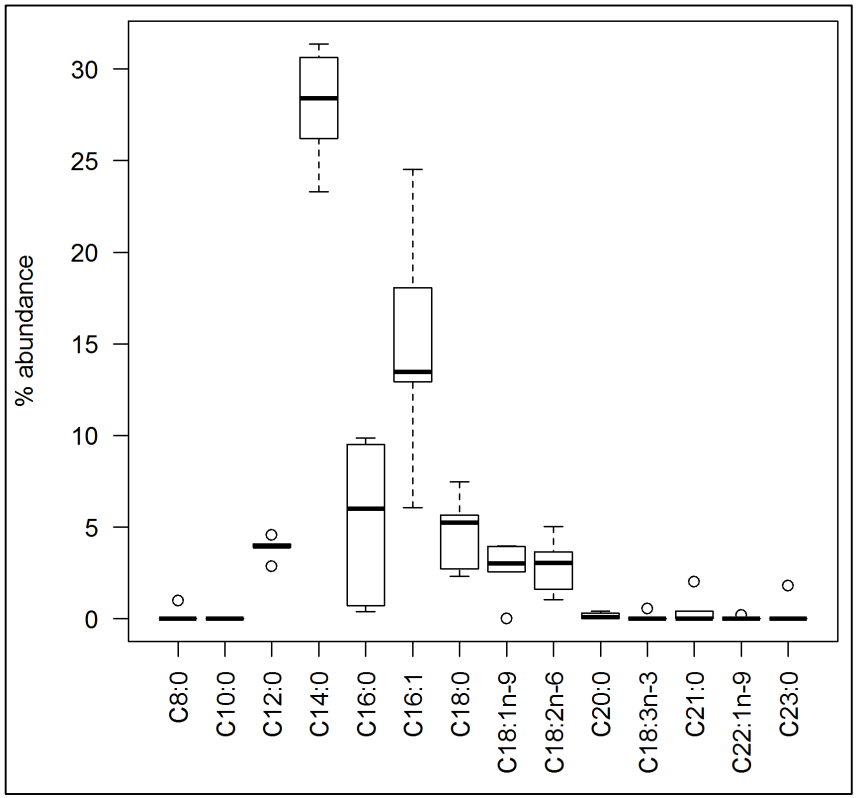

Figure 3.17: Boxplot of fatty acid biomarkers used to characterize the overall FA profile between the July and August algal growth assays at site 2-Corn. The *thick middle line* represents the median, the *box* is the first and third quartiles, *points* represent outliers and the *whiskers* represent the 5th and 95th percentiles.

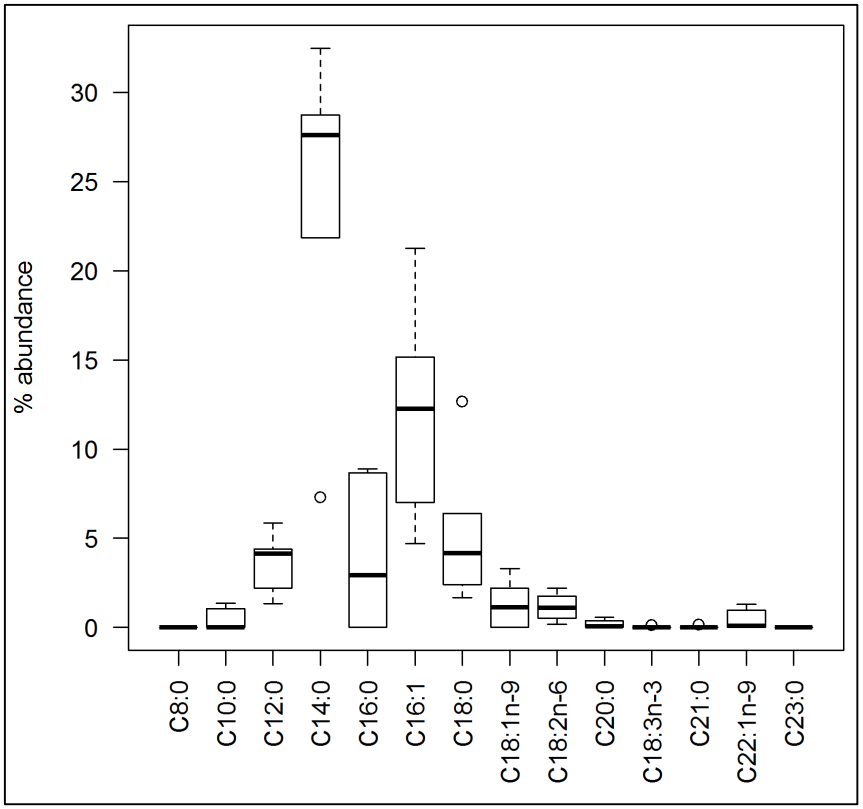

Figure 3.18: Boxplot of fatty acid biomarkers used to characterize the overall FA profile between the July and August algal growth assays at site 1-Soy. The *thick middle line* represents the median, the *box* is the first and third quartiles, *points* represent outliers and the *whiskers* represent the 5th and 95th percentiles.

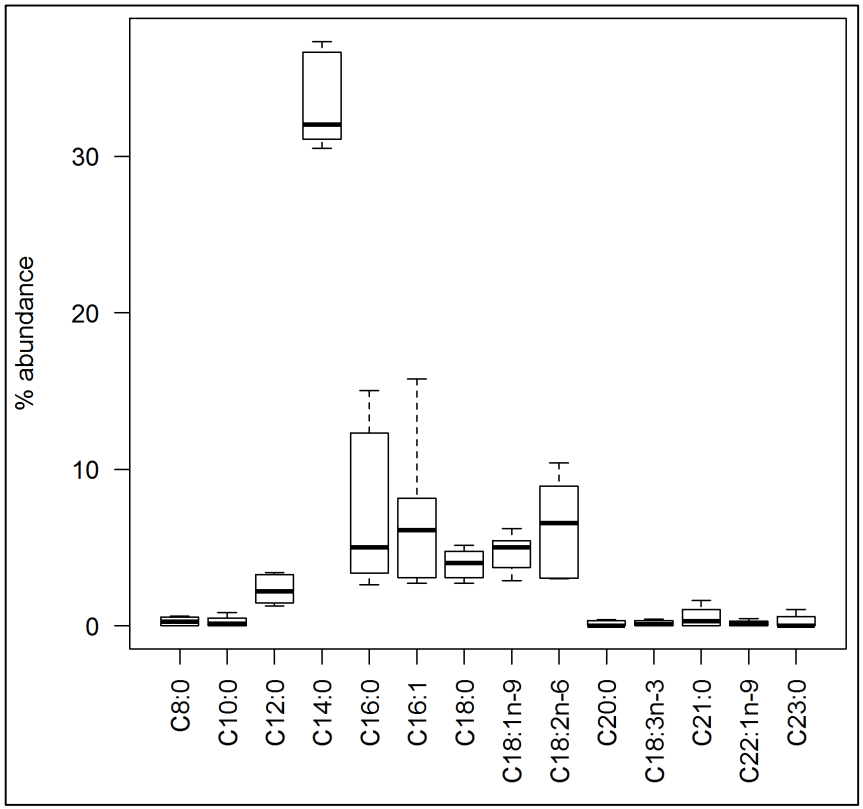

Figure 3.19: Boxplot of fatty acid biomarkers used to characterize the overall FA profile between the July and August algal growth assays at site 2-Soy. The *thick middle line* represents the median, the *box* is the first and third quartiles, *points* represent outliers and the *whiskers* represent the 5th and 95th percentiles.

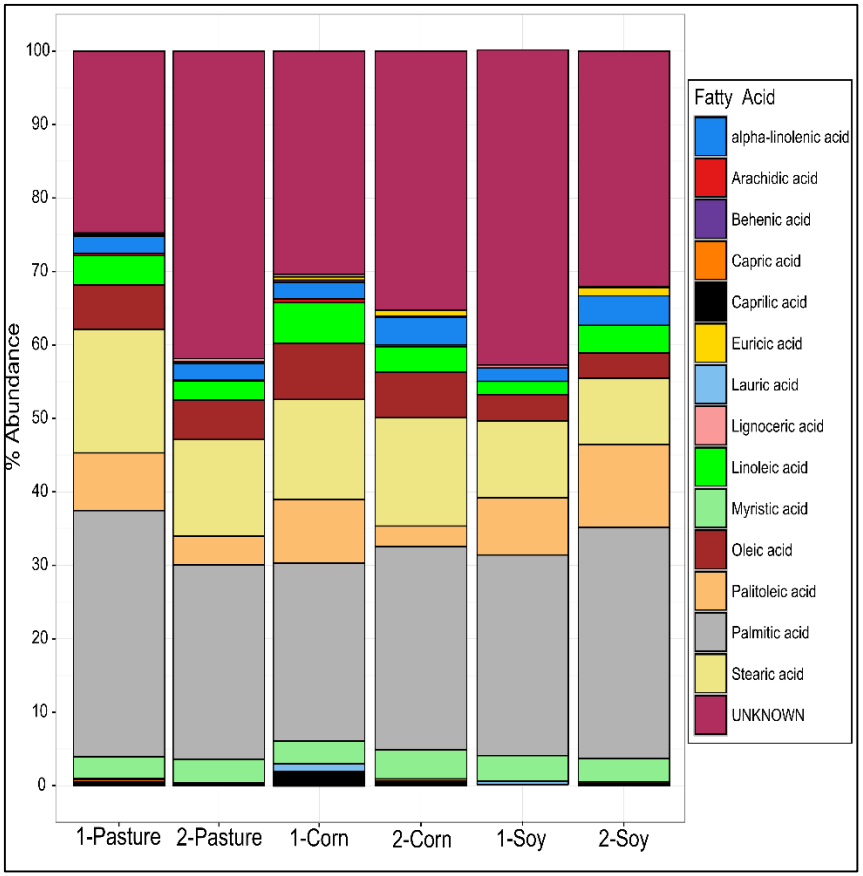

Figure 3.20: Relative abundance of periphytic fatty acids identified from the July algal growth assay (n=3).

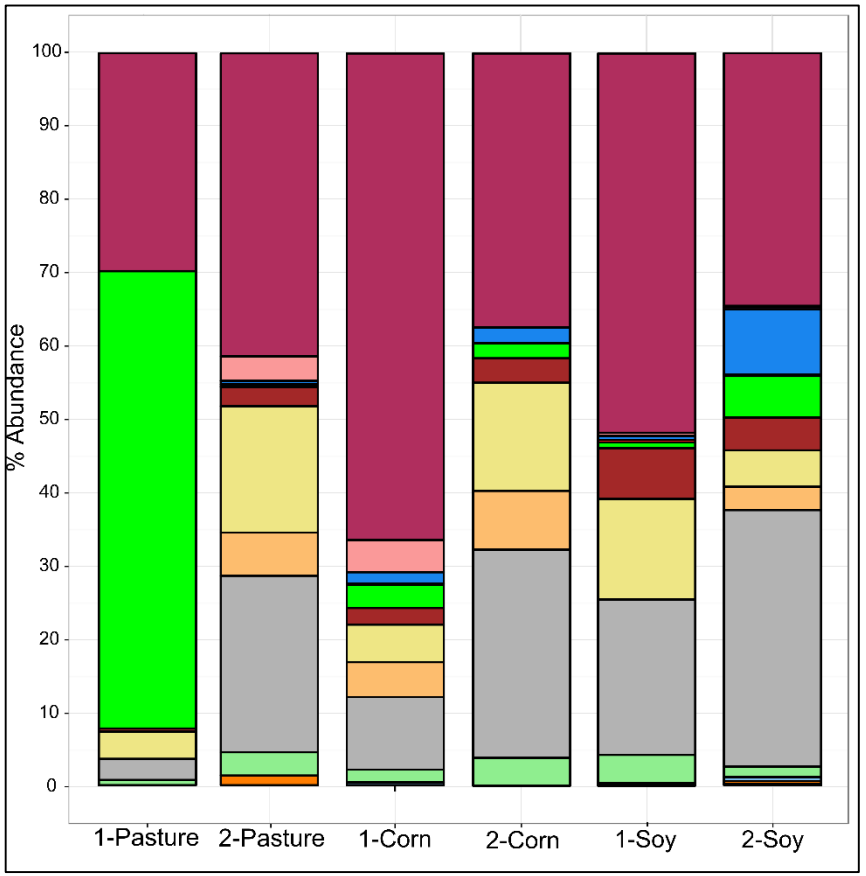

Figure 3.21: Relative abundance of periphytic fatty acids identified from the August algal growth assay (n=3).

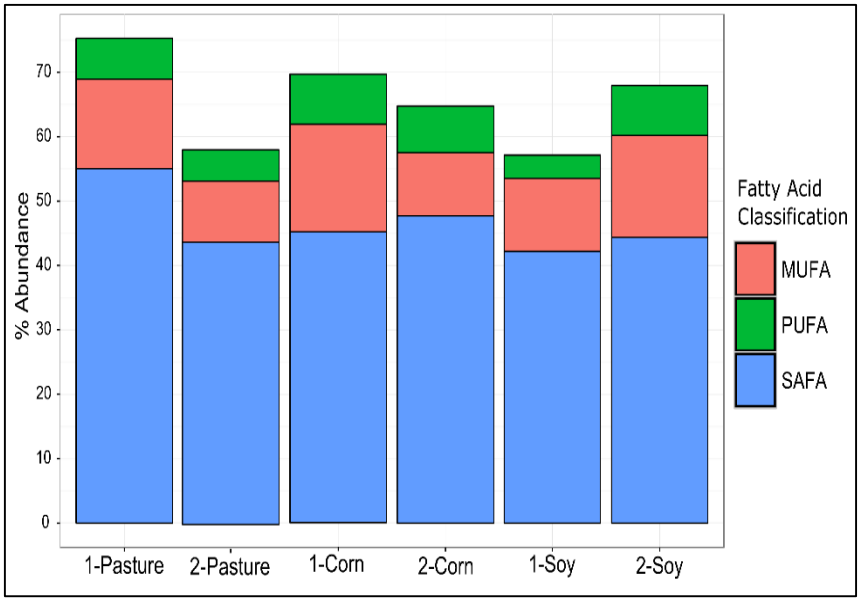

Figure 3.22: Relative abundance of grouped periphytic fatty acids identified from the July algal growth assay (n=3). Fatty acids are classified into monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and saturate fatty acids (SAFA).

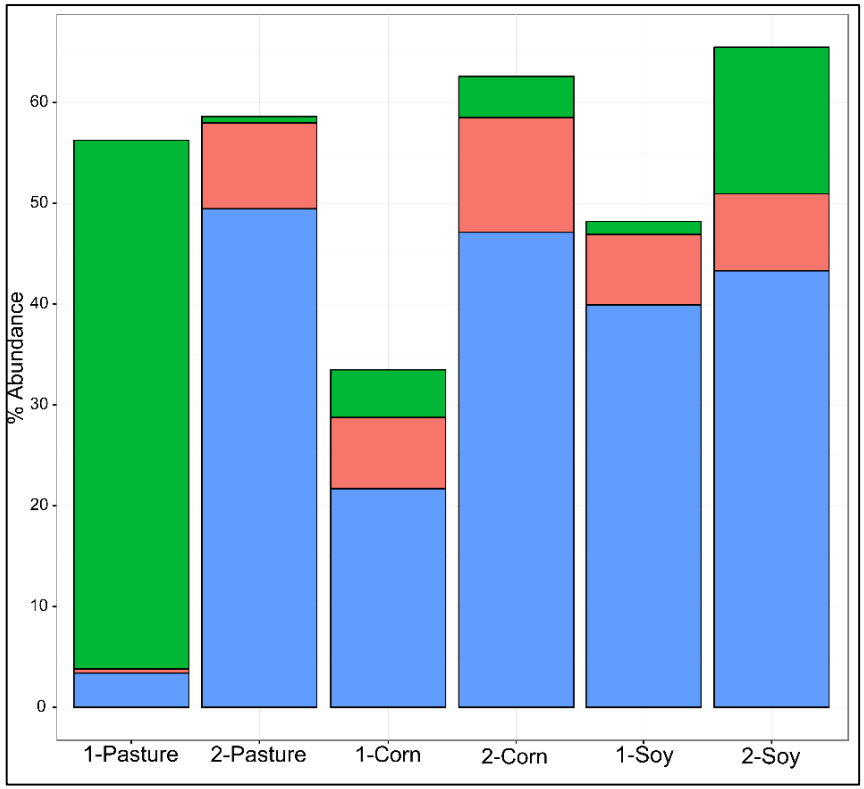

Figure 3.23: Relative abundance of grouped periphytic fatty acids identified from the August algal growth assay (n=3). Fatty acids are classified into monounsaturated fatty acids (MUFA), polyunsaturated fatty acids (PUFA) and saturate fatty acids (SAFA).

Table 3.6: Results from one-way ANOVAs for detectable differences in proportions of fatty acids across sites. Data from both algal growth assay experiments (n=2) were pooled in order for comparisons to be made. Statistically significant (p<0.05) relationships are shown in bold.

Fatty Acid Classification	Site	Mean	SD (±)	DF	F value	p-value
C8:0	1-Pasture	0.28	0.28	5	0.596	0.706
	2-Pasture	0.21	0.21			
	1-Corn	0.98	0.98			
	2-Corn	0.30	0.30			
	1-Soy	0	0			
	2-Soy	0.27	0.07			
C10:0	1-Pasture	0.14	0.14	5	1.526	0.309
	2-Pasture	0.66	0.66			
	1-Corn	0	0			
	2-Corn	0.16	0.16			
	1-Soy	0	0			
	2-Soy	0.28	0.07			
C12:0	1-Pasture	0.08	0.08	5	0.81	0.582
	2-Pasture	0.4	0.05			
	1-Corn	0.72	0.33			
	2-Corn	0	0			
	1-Soy	0.27	0.27			
	2-Soy	0	0			
C14:0	1-Pasture	1.84	1.13	5	1.619	0.286
	2-Pasture	3.67	0.19			
	1-Corn	2.44	0.69			
	2-Corn	3.2	0.01			
	1-Soy	2.3	0.87			
	2-Soy	3.89	0.06			
C16:0	1-Pasture	18.17	15.33	5	1.898	0.229
	2-Pasture	25.28	1.17			
	1-Corn	17.07	7.15			
	2-Corn	28.04	0.38			
	1-Soy	24.26	3.05			
	2-Soy	33.27	1.8			
C16:1	1-Pasture	3.94	3.91	5	2.706	0.129
	2-Pasture	4.89	1			
	1-Corn	6.72	1.94			
	2-Corn	5.42	2.61			
	1-Soy	3.91	3.87			
	2-Soy	7.23	4.06			

Table 3.6 (cont.)

C18:0	1-Pasture	10.27	6.54	5	0.853	0.56
	2-Pasture	15.21	2.06			
	1-Corn	9.36	4.24			
	2-Corn	14.75	0.01			
	1-Soy	12.11	1.62			
	2-Soy	7	2.01			
C18:1ω9	1-Pasture	3.23	2.8	5	0.667	0.663
	2-Pasture	3.99	1.37			
	1-Corn	4.96	2.68			
	2-Corn	4.78	1.43			
	1-Soy	5.25	1.66			
	2-Soy	3.96	0.51			
C18:2ω6	1-Pasture	33.22	29.24	5	0.969	0.504
	2-Pasture	1.38	1.22			
	1-Corn	4.33	1.2			
	2-Corn	2.75	0.74			
	1-Soy	1.29	0.51			
	2-Soy	4.71	0.98			
C20:0	1-Pasture	0.16	0.14	5	0.16	0.969
	2-Pasture	0.16	0.04			
	1-Corn	0.34	0.18			
	2-Corn	0.15	0.09			
	1-Soy	0.18	0.18			
	2-Soy	0.12	0.12			
C18:3ω3	1-Pasture	1.18	1.18	5	1.455	0.328
	2-Pasture	1.37	0.9			
	1-Corn	1.91	0.31			
	2-Corn	2.91	0.83			
	1-Soy	1.15	0.66			
	2-Soy	6.43	2.42			
C21:0	1-Pasture	0.13	0.13	5	0.625	0.689
	2-Pasture	0	0			
	1-Corn	0.16	0.16			
	2-Corn	0.09	0.09			
	1-Soy	0.02	0.02			
	2-Soy	0.16	0.16			
C22:1ω9	1-Pasture	0	0	5	0.518	0.756
	2-Pasture	0.12	0.12			
	1-Corn	0.22	0.22			

Table 3.6 (cont.)

	2-Corn	0.4	0.4			
	1-Soy	0.03	0.03			
	2-Soy	0.54	0.54			
C23:0	1-Pasture	0.1	0.09	5	1.469	0.324
	2-Pasture	1.9	1.47			
	1-Corn	2.36	2.01			
	2-Corn	0.03	0.03			
	1-Soy	0.41	0.02			
	2-Soy	0.16	0.01			

Table 3.7: Results from one-way ANOVAs for detectable differences in proportions of grouped fatty acid classification across sites. Data from both algal growth assay experiments (n=2) were pooled in order for comparisons to be made. Statistically significant ($p<0.05$) relationships are shown in bold.

FA Classification	Site	Mean	SD (±)	DF	F value	p-value
MUFA	1-pasture	7.14	6.74	5	0.220	0.941
	2-soy	9.18	2.18			
	3-corn	11.90	4.84			
	4-pasture	9.01	0.49			
	5-soy	11.72	4.09			
	6-corn	10.59	0.78			
PUFA	1-pasture	29.40	23.06	5	1.125	0.437
	2-soy	2.43	1.17			
	3-corn	6.24	1.51			
	4-pasture	2.75	2.12			
	5-soy	11.14	3.40			
	6-corn	5.66	1.57			
SAFA	1-pasture	29.22	25.81	5	0.404	0.831
	2-soy	41.05	1.12			
	3-corn	33.43	11.73			
	4-pasture	46.63	2.83			
	5-soy	43.83	0.52			
	6-corn	47.42	0.29			

Table 3.8: Results from one-way ANOVAs for detectable differences in proportions of individual fatty acids between algal growth assay experiments. Data from all sites (n=6) were pooled in order for comparisons to be made. Statistically significant (p<0.05) relationships are shown in bold.

Fatty Acid Classification	Month	Mean	SD (±)	DF	F value	p-value
C8:0	July	0.55	0.62	1	3.513	0.0904
	August	0.03	0.07			
C10:0	July	0.12	0.14	1	0.3696	0.557
	August	0.28	0.48			
C12:0	July	0.24	0.38	1	0.178	0.682
	August	0.21	0.22			
C14:0	July	2.95	0.32	1	1.584	0.237
	August	2.46	1.22			
C16:0	July	24.78	3.12	1	0.008	0.929
	August	20.26	10.90			
C16:1	July	6.04	2.88	1	2.207	0.168
	August	3.66	2.94			
C18:0	July	11.65	2.59	1	0.984	0.345
	August	9.93	5.44			
C18:1ω9	July	4.67	1.48	1	0.322	0.583
	August	3.34	2.00			
C18:2ω6	July	11.94	1.16	1	1.057	0.328
	August	12.37	22.47			
C20:0	July	0.17	0.18	1	0.09	0.77
	August	0.17	0.11			
C18:3ω3	July	2.34	0.83	1	0.285	0.605
	August	2.25	3.04			
C21:0	July	0.11	0.13	1	0.105	0.752
	August	0.06	0.12			
C22:1ω9	July	0.37	0.40	1	4.867	0.0519
	August	0.01	0.02			
C23:0	July	0.22	0.16	1	2.338	0.157
	August	1.39	1.78			

Table 3.9: Results from one-way ANOVAs for detectable differences in proportions of grouped fatty acid classification between algal growth assay experiments. Data from all sites (n=6) were pooled in order for comparisons to be made. Statistically significant (p<0.05) relationships are shown in bold.

FA Classification	Month	Mean	SD (±)	DF	F value	p-value
MUFA	July	12.85	2.82	1	9.095	**0.013**
	August	6.99	3.30			
PUFA	July	6.25	1.55	1	0.669	0.432
	August	12.95	18.24			
SAFA	July	46.37	4.21	1	2.592	0.139
	August	34.15	16.43			

Table 3.10: Results from one-way ANOVAs for detectable differences of proportions of fatty acids related to crop type. Data for agricultural type across the sampling period (n=6) were pooled in order for comparisons to be made. Statistically significant (p<0.05) relationships are shown in bold.

Fatty Acid Classification	Site	Mean	SD (±)	DF	F value	p-value
C8:0	Pasture	0.20	0.46	2	0.394	0.685
	Corn	0.48	0.86			
	Soy	0.17	0.42			
C10:0	Pasture	0.38	1.14	2	0.615	0.562
	Corn	0.08	0.28			
	Soy	0.11	0.26			
C12:0	Pasture	0.03	0.09	2	1.221	0.339
	Corn	0.27	0.49			
	Soy	0.23	0.56			
C14:0	Pasture	2.28	1.83	2	0.584	0.578
	Corn	2.90	1.36			
	Soy	3.33	0.75			
C16:0	Pasture	18.94	14.75	2	1.283	0.323
	Corn	20.54	10.45			
	Soy	29.39	4.13			
C16:1	Pasture	3.76	3.80	2	1.157	0.357
	Corn	5.35	4.01			
	Soy	9.53	3.53			
C18:0	Pasture	11.34	8.49	2	0.249	0.785
	Corn	10.92	6.95			
	Soy	9.75	5.32			
C18:1ω9	Pasture	3.11	2.60	2	0.611	0.564
	Corn	4.23	2.71			
	Soy	3.52	0.89			
C18:2ω6	Pasture	16.97	32.33	2	0.88	0.448
	Corn	3.08	2.08			
	Soy	2.77	1.59			
C20:0	Pasture	0.14	0.20	2	0.03	0.97
	Corn	0.20	0.21			
	Soy	0.00	0.00			
C18:3ω3	Pasture	1.08	1.13	2	1.088	0.377
	Corn	2.22	1.39			
	Soy	2.91	1.62			
C21:0	Pasture	0.04	0.15	2	0.543	0.599
	Corn	0.10	0.24			

Table 3.10 (cont.)

	Soy	0.00	0.00			
C22:1ω9	Pasture	0.06	0.21	2	0.443	0.656
	Corn	0.28	0.61			
	Soy	0.54	0.67			
C23:0	Pasture	0.99	2.89	2	0.04	0.961
	Corn	1.17	3.77			
	Soy	0.29	0.53			

Table 3.11: Results from one-way ANOVAs for detectable differences of proportions of fatty acid classification related to crop type. Data for agricultural type across the sampling period (n=6) were pooled in order for comparisons to be made. Statistically significant ($p<0.05$) relationships are shown in bold.

Fatty Acid Classification	Crop Type	Mean	SD (±)	DF	F value	p-value
MUFA	Pasture	8.08	1.53	2	0.505	0.620
	Corn	11.25	1.55			
	Soy	10.45	1.78			
PUFA	Pasture	16.07	8.13	2	0.600	0.569
	Corn	5.95	0.77			
	Soy	6.79	1.14			
SAFA	Pasture	37.93	1.64	2	0.086	0.918
	Corn	40.42	0.94			
	Soy	42.44	0.58			

3.3.4 PCA, cluster analyses and RDA of fatty acid proportions and fatty acid classification

In order to better compare and understand how agriculture type was affecting the FA distribution, PCA was run on log transformed, normalised data for each of the algal assay experiments, represented by July and August (Figure 3.24 and 3.25). The first two principal component axes combined were able to explain 76.46% and 82.50% of the total variation in the FAs present for each assay, respectively. Similarly to water quality, there was no clustering of the sites in the PCA biplot that reflected the same agricultural type. PCA was also completed for grouped FA classifications, with the explanatory variables (FA classification) represented by the arrows from the centre of the biplot (Figure 3.26). Similarly to the individual FA analysis, there was no clustering of similar agricultural type, although there was 94.08% of the total variation explained by the first two PCA axes.

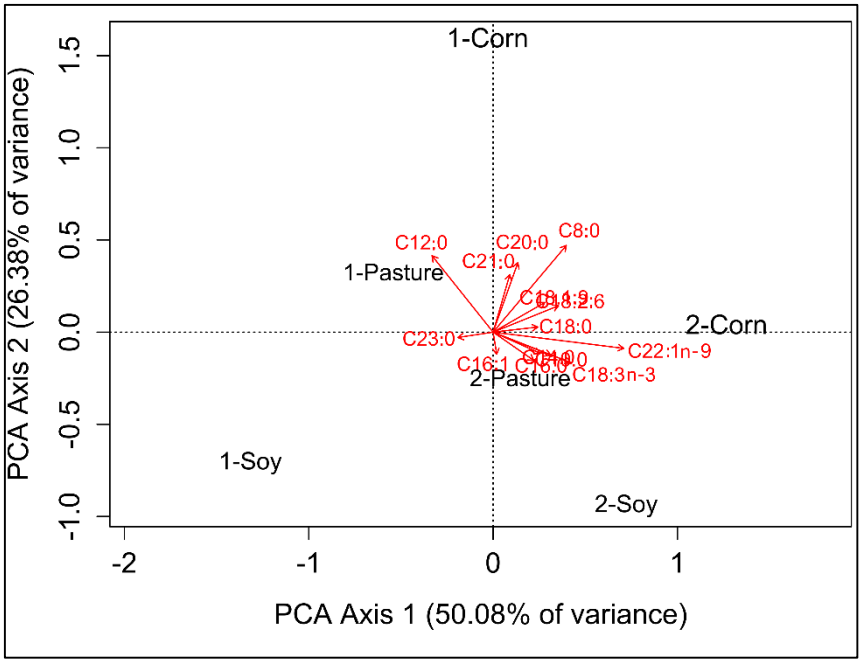

Figure 3.24: Principal component analysis of identified fatty acids extracted from algal growth assay in July.

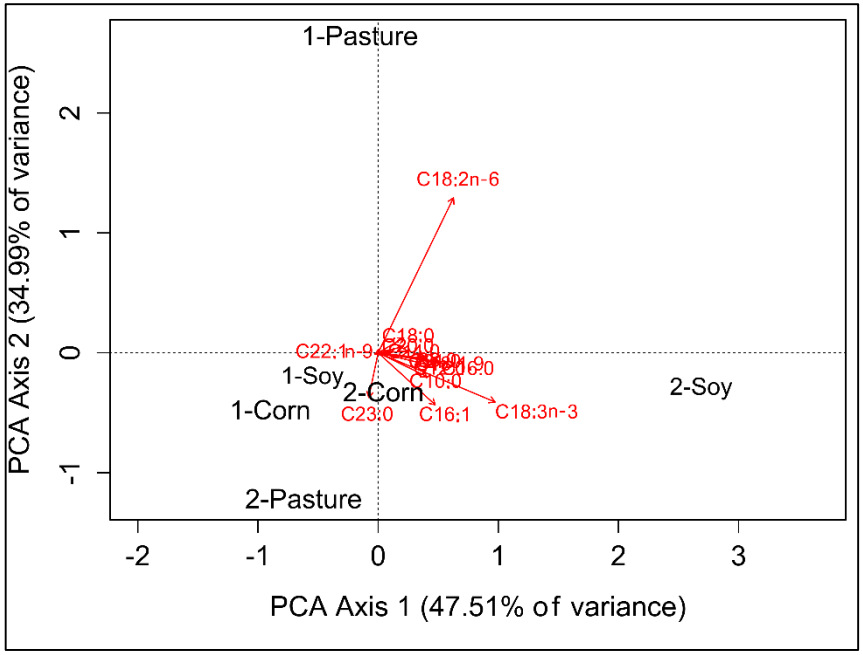

Figure 3.25: Principle component analysis of identified fatty acids extracted from algal growth assay in August.

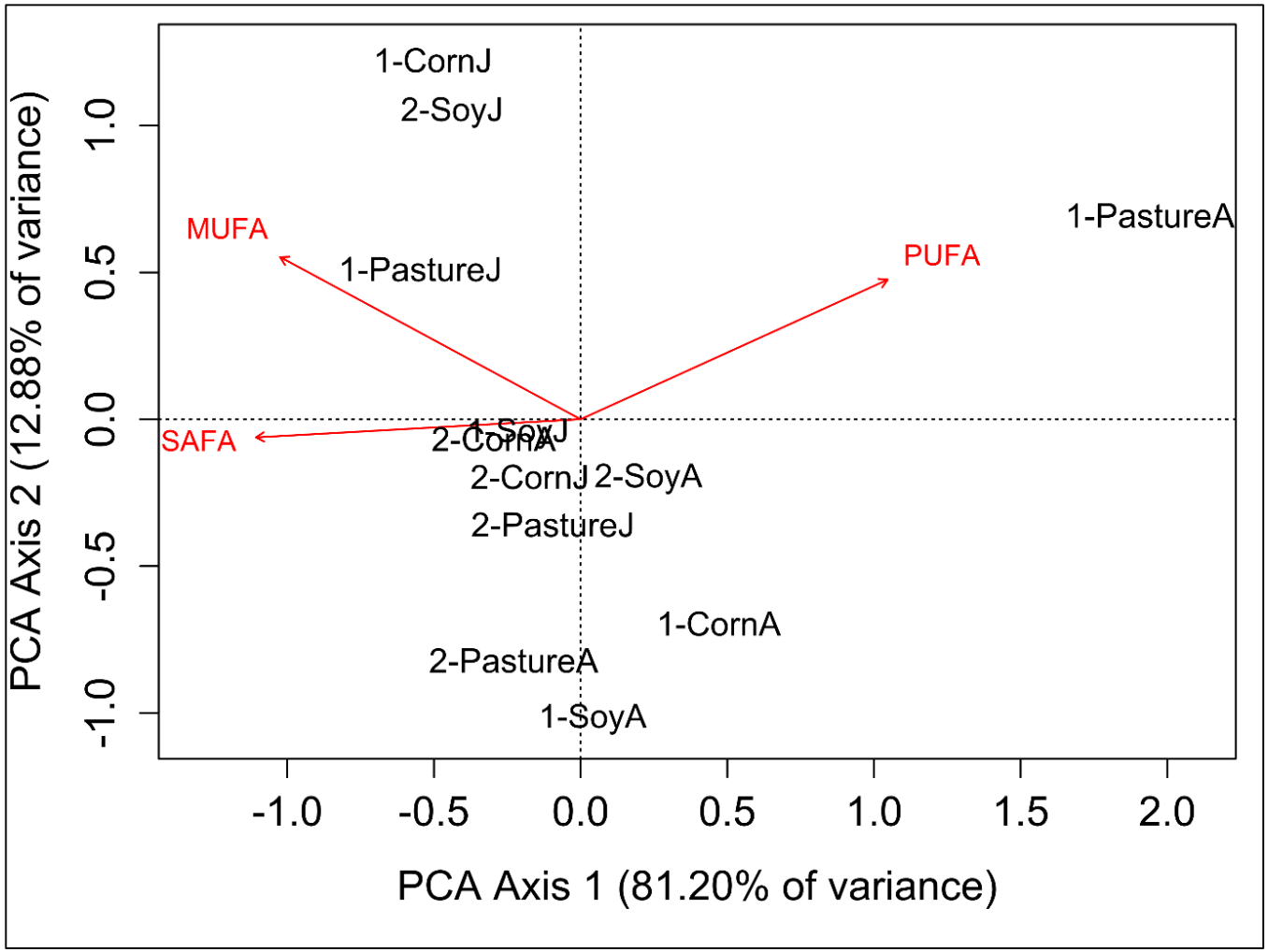

Figure 3.26: Principal components analysis of grouped fatty acid classifications extracted from July and August algal growth assays. Experimental month is denoted as (J) for July and (A) for August.

Further confirmation that agriculture type did not have an effect on FA profile was found through Bray-Curtis Dissimilarity cluster analyses were completed for each individual algal growth assay (Figure 3.27 and 3.28). This cluster analysis grouped closely related FA profiles closest together. Neither of the two algal assay growth periods had clustering at the least dissimilar level, having at minimum ~40% dissimilar profiles, further highlighting the variability between systems. Though agriculture type did not seem to affect the water quality or FA profile of the different sites, RDA was run in order to determine how water quality was controlling the variability in the FA profiles (Figure 3.29), as well as the grouped FA classification (Figure 3.30). Individual FAs showed very limited correlation with the water quality parameters contained within this study, as seen by the large amount of clustering around the centre of the biplot, even though 64.14% of the variation was explained by RDA axis 1 and 2. FA classification was also limited in its ability to be explained by water quality variation, with only 52.32 % of the total variation explained by RDA axis 1 and 2. There was a strong relationship between high PUFA proportion and high TEMP and pH, and low TNN and COND, whereas MUFA and SAFA were strongly related to high TP.

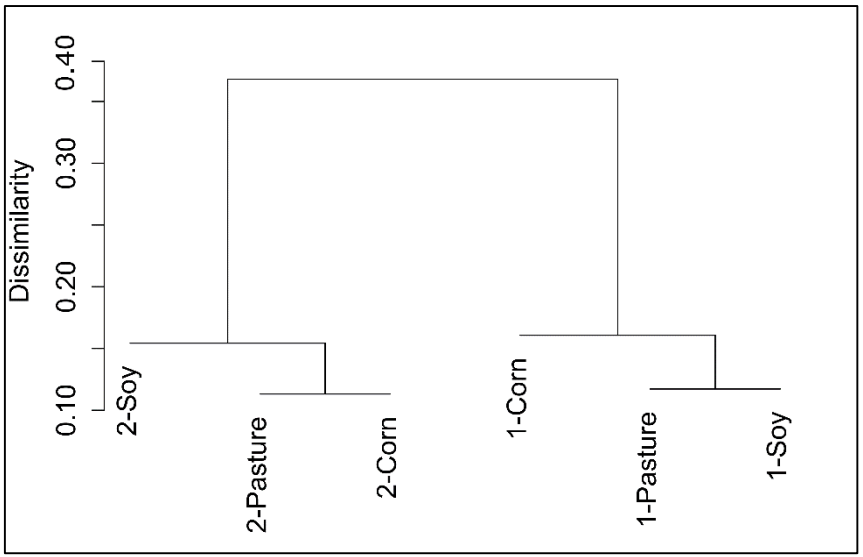

Figure 3.27: Cluster analysis using Bray Curtis dissimilarity to assess difference between the fatty acid profiles from algal growth assays in July.

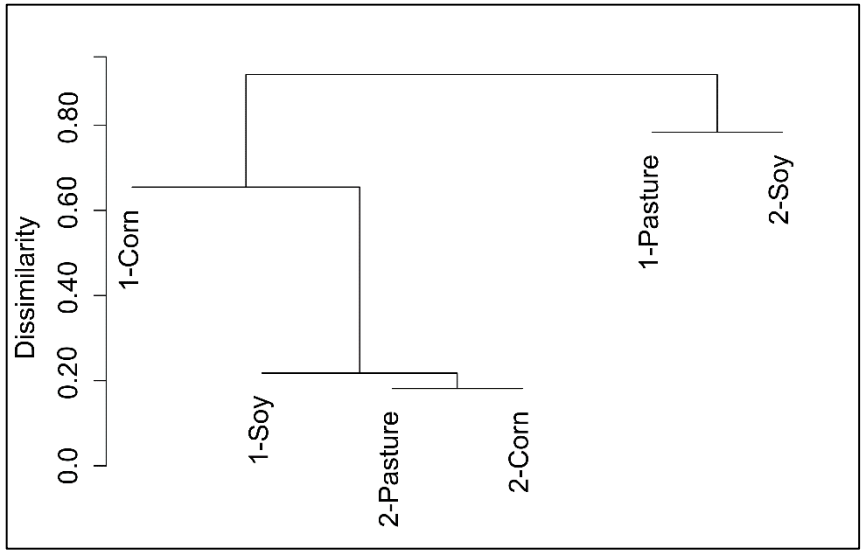

Figure 3.28: Cluster analysis using Bray Curtis dissimilarity to assess difference between the fatty acid profiles from algal growth assays in August.

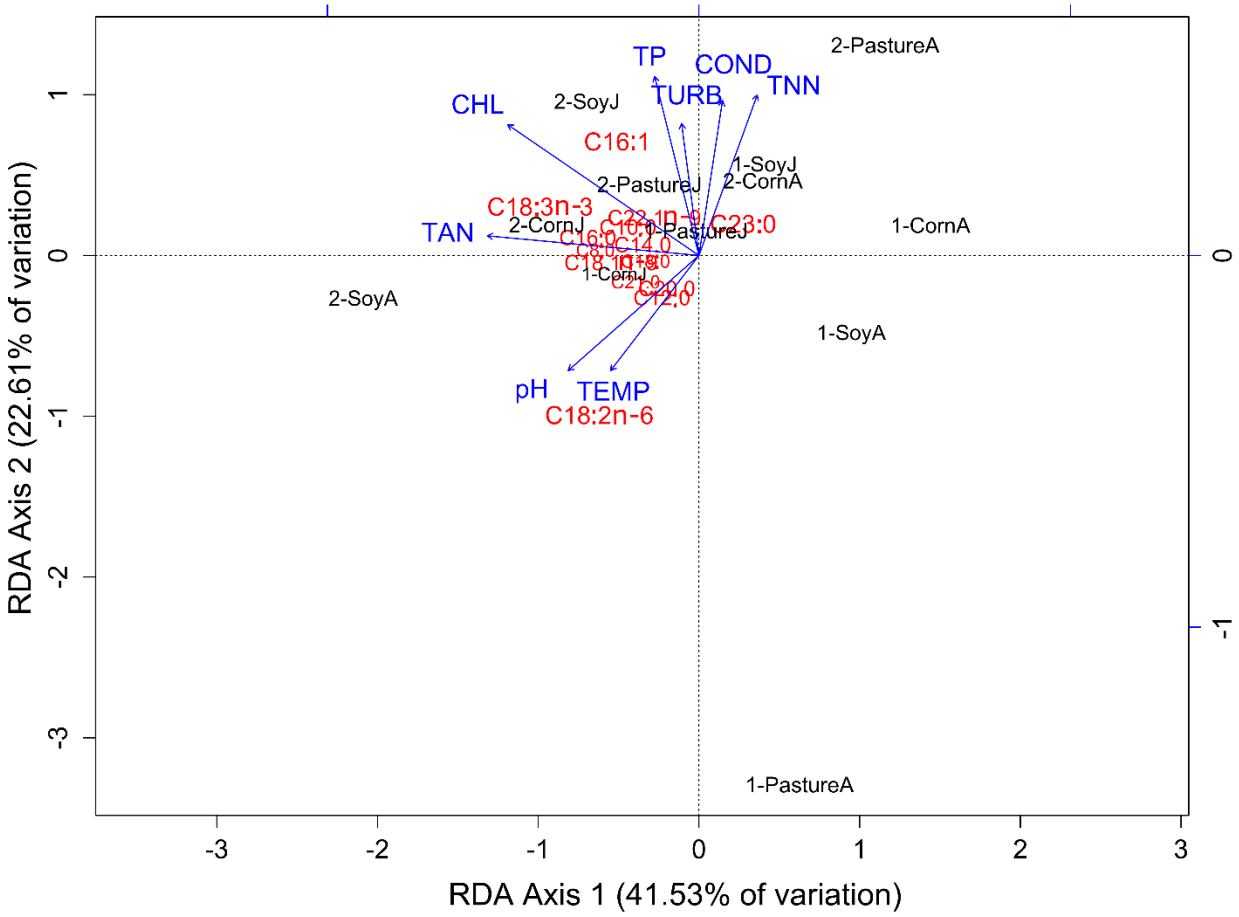

Figure 3.29: Resulting biplot using redundancy analysis to determine relationships between water quality and individual fatty acids extracted from the July (denoted as J) and August (denoted as A) algal growth assays.

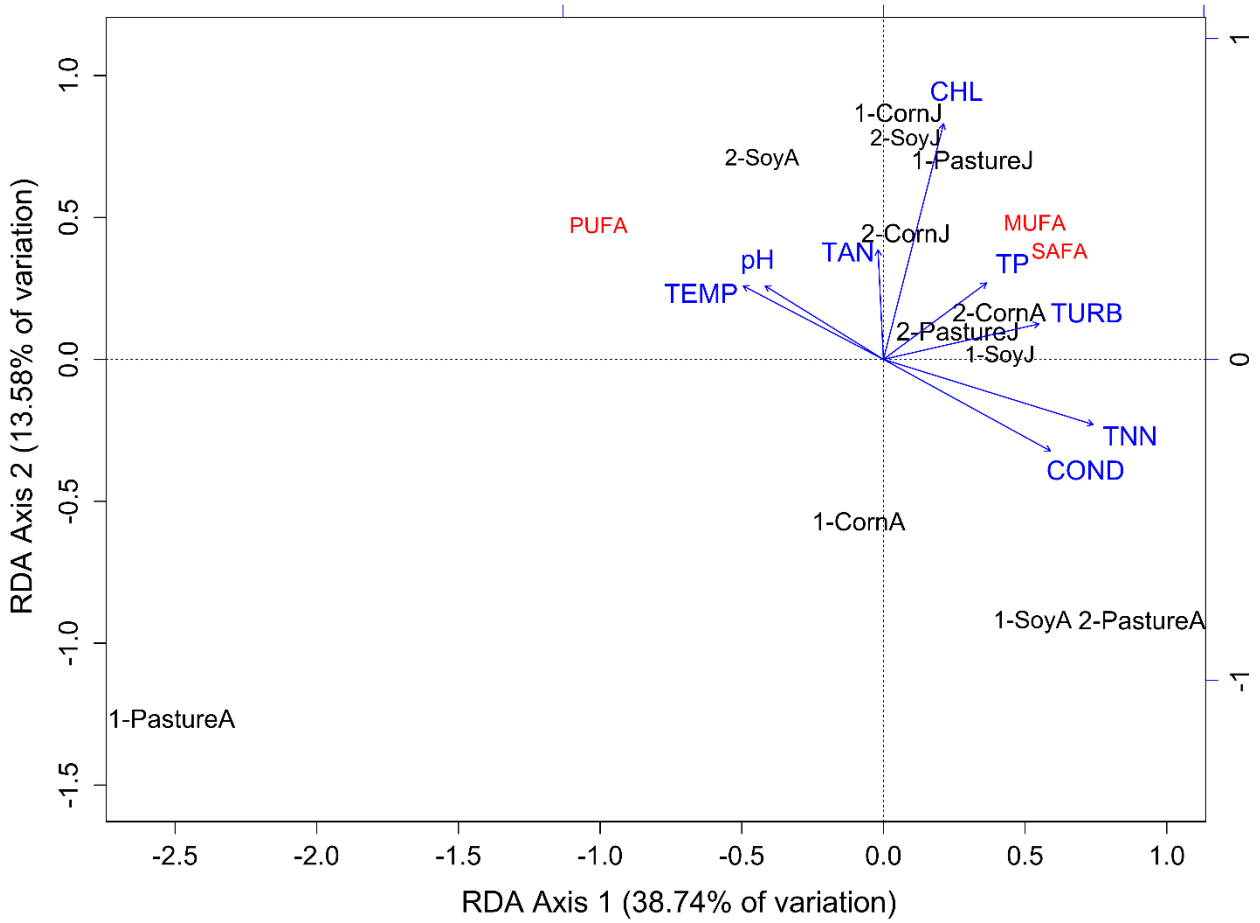

Figure 3.30: Resulting biplot using redundancy analysis to determine relationships between water quality and grouped classifications of fatty acids extracted from the July (denoted as J) and August (denoted as A) algal growth assays.

3.3.5 Pearson correlation analyses and multiple regression on fatty acid composition and water quality

Linear relationships between water quality parameters and biological data sets was determined using Pearson correlation analyses (Table 3.11). Significant correlations of water quality variables were identified, with moderate statistically significant correlations found between DO and pH, pH and TEMP, pH and TP (negative), TURB and both TAN/TNN. There were no statistically significant relationships between water quality and either CHL or AFDM respectively, as well as no statistically significant relationship between CHL and AFDM. When assessing the linear relationships of water quality on FA proportions, C18:0 was the only FA that had a statistically significant relationship, which was positively correlated with DO and pH, though multiple linear regression found that a combination of the two variables was not significant in determining C18:0 proportions. There were significant relationships between five of the individual FAs and CHL content, C10:0, C16:0, C18:1ω9, C18:3ω3 and C21:0, all with r^2 values above 0.60 ($p<0.05$). When grouped for FA classification, there was no statistically significant correlations with water quality or biological data. In order to better understand the multiple relationships between FAs and CHL, multiple stepwise regression was used to determine the variation in CHL that can be attributed to each FA (Table 3.12). Positive relationships of C10:0, C16:0, C18:3ω3 and C21:0 were determined in combination to be significant predictors of CHL from algal growth assays.

Table 3.12: Pearson correlation coefficient values for the pooled water quality and fatty acid proportion data sets over both the algal growth assay experiments (n=2). Statistically significant (p<0.05) relationships are shown in bold

Parameter	DO	COND	pH	Temp	TURB	TAN	TP	TNN	CHL	AFDM
DO	--									
COND	0.40	--								
pH	**0.77**	0.17	--							
Temp	0.49	-0.07	**0.78**	--						
TURB	0.09	0.22	-0.22	-0.33	--					
TAN	-0.12	0.11	-0.31	-0.39	**0.72**	--				
TP	-0.46	0.19	**-0.62**	-0.25	0.39	0.39	--			
TNN	0.40	0.42	0.20	-0.12	**0.60**	0.45	-0.14	--		
CHL	-0.23	0.16	0.09	0.11	0.20	0.38	0.42	-0.19	--	
AFDM	0.22	0.40	0.14	0.15	-0.20	-0.37	0.13	-0.47	0.24	--
C8:0	-0.20	-0.15	0.18	0.10	-0.16	-0.17	-0.08	-0.32	0.57	0.32
C10:0	-0.16	0.55	0.15	0.21	0	0.01	0.40	-0.04	**0.74**	0.37
C12:0	-0.37	-0.30	-0.16	-0.16	-0.05	0.04	0.18	-0.32	0.39	-0.26
C14:0	0.46	0.40	0.51	0.09	0.40	0.28	-0.18	0.39	0.51	0.21
C16:0	0.31	0.38	0.46	0.22	0.31	0.24	0.08	0.19	**0.74**	0.28
C16:1	-0.23	0.06	-0.13	0.02	0.05	-0.01	0.48	-0.06	0.41	0.26
C18:0	**0.61**	0.39	**0.67**	0.33	0.16	0.07	-0.34	0.31	0.31	0.20
C18:1ω9	0.16	0.07	0.41	0.14	0.24	0.13	-0.15	0.08	**0.64**	0.13
C18:2ω6	0.08	-0.4	0.36	0.33	-0.21	-0.14	-0.23	-0.49	0.36	0.31
C20:0	-0.23	-0.34	0.24	0.26	-0.16	0.03	-0.23	-0.21	0.41	-0.31
C18:3ω3	-0.18	0.06	0.02	-0.03	0.36	0.19	0.31	0.06	**0.68**	0.16
C21:0	-0.28	-0.17	0.20	0.21	-0.02	-0.06	0.06	-0.23	**0.67**	-0.08
C22:1ω9	-0.01	0.14	0	-0.36	0.14	-0.04	-0.31	0.07	0.12	0.38
C23:0	-0.48	0.05	-0.54	-0.39	-0.13	0.26	0.37	-0.16	0.09	-0.19
SAFA	-0.08	0.26	-0.12	-0.16	0.39	0.12	0.27	0.35	0.22	-0.06
MUFA	-0.17	0.14	-0.24	-0.24	0.32	0	0.29	0.21	0.14	0.02
PUFA	-0.03	-0.45	0.28	0.35	-0.3	-0.24	-0.14	-0.57	0.32	0.35

Table 3.13: Results of multiple stepwise regression modelling changes in CHL related to proportion of individual FA. Only significant (p<0.05) partial r2 values for predictors and significant overall regression models included. Parameters showing strong autocorrelation were removed from analysis.

Parameter	**Predictor** (Partial r^2)	**Sign of Coefficient**	**Regression r^2** (*p*-value)
Log CHL	C10:0 (0.2413)	+	0.8627
	C16:0 (0.1968)	+	(<0.001)
	C18:3ω3 (0.2526)	+	
	C21:0 (0.1721)	+	

3.4 Discussion

This chapter highlights the water quality and periphyton variability that can occur in small streams over the course of two growth periods, as well as the variability that can occur among similar land-use types. Although there was a limited number of replicate sites representing each land-use type, it is still clear that regardless of land-use type, each site was unique with respect to water quality and fatty acid profiles. Even with this apparent variability across sites, there were interesting patterns and relationships between water quality condition, algal biomass and FA composition.

Small lotic systems are highly influenced by flow characteristics, which due to the discrete sampling period of this study, may not have been properly characterized. Streams have the ability to become increasingly "flashy" due to small wetted width and the tilled nature of the agricultural landscape, leading to lower rates of water infiltration and higher overland run off rates (Allan and Castillo 2007). Though not evident during the study period, this may have played a role in the significant difference determined for DO concentrations, as DO is maintained in small streams through diffusion from the atmosphere, as well as release from photosynthesis (Allan and Castillo 2007). Decreased flow during the summer dry season, coupled with increased microbial decomposition processes in accumulated organic matter could be linked to areas of low DO (Allan and Castillo 2007). Linked with DO was pH, which was positively correlated with DO. Decreases in pH could be linked with increased microbial decomposition of organic matter, which would relate to the strong relationship between DO and pH (Qiu *et al*. 2013), while increases in pH are usually linked to a greater abundance of sedimentary rock in the stream substrate (Allan and Castillo 2007).

TNN concentrations exhibited a large distribution, which may be linked to the agricultural application of fertilizers and individual farming practices implemented at each site. . Corn fertility management (i.e. fertilization) is strongly connected with nitrogen levels, which can be applied in the form of urea-ammonium nitrate solutions or anhydrous ammonia, which may link the increased TNN at site 2-Corn with corn agriculture type (Ontario Ministry of Agriculture 2009). In congruence with this fertilization information, site 2-Corn also shows continuously high concentrations of TAN, which may be also related to its crop type of corn, but individualized farming practices differentiates it from site 1-Corn.

TAN concentrations have been found to be dependent on loads from surface waters rather than groundwater, linking TAN levels to agricultural applications (Sheibley *et al*. 2014). This is hypothesized to be the case in the study area, although it is difficult to gain access to fertilizer application information directly due to lack of limitations on fertilizer applications, only guidelines from the Ontario Ministry of Agriculture, Food and Rural affairs (OMAFRA) (Woyzbun 2011). With only approximately half of added agricultural fertilizers captured by crops, this can lead to the introduction of large concentrations to even small tributaries (Tilman *et al*. 2001). TAN concentrations at select sites approached the PWQO of 20 μg/L of un-ionized ammonia (a fraction of the total ammonia (Emerson *et al*. 1975)), which could be linked to agricultural practices or the timings of nutrient additions to agricultural land. The drastic increase of TAN concentrations at site 2-Soy from July to August could be linked to the addition of urea or calcium ammonium nitrate over the crop fields at 50kg/ha, which is the recommended fertilizer application if nodulation of soy bean plants does not occur (Ontario Ministry of Agriculture 2009).

All of the sites, indiscriminate of agriculture type, exhibited similar TP concentrations, which is ubiquitous with the use of P fertilizers for row crops (Howarth *et al*. 2002) and the potential for the contamination of surface water from manure at pasture sites (Harmel *et al*. 2006). Since no limitations on P addition for farming practices are present in Ontario (Ontario Ministry of Agriculture 2009), sources can either be from legacy P from previous applications of fertilizers (Sharpley *et al*. 2014) or from current fertilizer applications, but current run-off is known to play a larger role (Allan and Castillo 2007). TP concentrations, as well as TNN and TAN may also be contributing to the increased COND at site 2-Pasture. In similar agricultural streams, COND was linked with TP, TN, NH_4^+, suspended solids and animal density, (Lavoie *et al*. 2004), but also stream substrate and geology. High proportions of sedimentary rock as bedrock of streams, which can release significant amounts of Ca^{2+} ions, can contribute to increased COND (Liu *et al*. 2000).

Algal biomass in the forms of CHL and AFDM showed little relation to the water quality parameters measured in this study. Even though important nutrients such as TP and multiple forms of nitrogen have all been linked to increases in algal biomass in similar systems (Munn *et al*. 1989, Lavoie *et al*. 2004, Taylor *et al*. 2004), there were no such relationships. This either means that the algal community is not limited by nitrogen or phosphorus concentrations in these systems, or there were alternative parameters (e.g. herbicides) that were impacting algal growth. Inorganic nitrogen concentrations were only measured in this study, but TN:TP ratios could be limiting algal growth. As TN:TP ratios fluctuate between nitrogen limited (<18:1) and phosphorus limited (>65:1), co-limitation of algae by both nutrients can occur (Dzialowski *et al*.

2005). This form of co-limitation is the most common reaction when additional nutrients occur from point or non-point sources (Maberly *et al*. 2002).

The algal biomass metrics that were used in this study, CHL, representation of viable autotrophic algae and CHL containing detritus, and AFDM, which represents all organisms contained within the sample, including autotrophic and heterotrophic fungi, bacteria and protozoa (Biggs and Close 1989) showed little correlation with water quality. Though artificial substrate is conducive to growing benthic algae similar to those communities that are present on natural substrate (Lavoie *et al*. 2004), there is no exclusion of other benthic organisms that may be influencing the community structure. The variability related to both CHL and AFDM could be linked to variations in the microbial community structure that is attached to the artificial substrate or variations in the sediment, but are beyond the scope of this study.

The relationships between CHL content and individual FAs is of interest due to the fact that there are multiple significant relationships across the sampling period. The five different FAs that are linked with CHL (C12:0, C16:0, C18:1ω9, C18:3ω3 and C21:0) are from all three classifications of FAs (MUFA, PUFA and SAFA) which leads to the understanding that they are most likely not linked directly to any one specific group of algae or species in particular and are a universal constituent of many algal and bacterial species. The SAFAs are of limited importance due to the ability of many macroinvertebrate grazers to synthesize these from other constituents, but can also use them to synthesize MUFAs in some cases (Brett and Müller-Navarra 1997). Alternatively, the MUFA and PUFAs that were related to the increased CHL are of importance and have been shown to found in higher proportions when light availability increases and are also slightly influenced by nutrients, but not as significantly (Cashman *et al*.

2013), reinforcing the idea that photosynthetic pigments are the most important influencer of EFA content.

FAME profiles in this study were used to characterize the periphyton communities at each individual site and more importantly, characterize the effect that agriculture type may be having on periphyton FAs. FAME profiles were unique with regards to each site during both growth assay experiments, similar to water quality profiles, and there was no apparent effect from land-use type. As discussed previously, C18:3ω3, α-linolenic acid (ALA), was significantly higher at site 2-Soy, which may be directly related to the unique water quality and periphyton community that is present here. Increased levels of ALA have been associated with low CO_2 levels in some green algae (Thompson 1996), as well as with increased light availability (Cashman *et al*. 2013), which may be related to unique site characteristics, rather than water quality or agricultural type.

When comparing sites for SAFA, MUFA and PUFA proportions, there are fewer differences in the sites, with no significant differences and a larger amount of clustering in the PCA biplot around the origin. Agriculture type and nutrients do not appear to be affecting this ratio, which bodes well for periphyton biomass as a food source in these streams (Brett and Müller-Navarra 1997). It has been previously shown that changes in community dynamics can have a larger variation in FA content than nutrients (Galloway and Winder 2015), but ultimately nutrient availability will determine the community structure, so they are not exclusive mutually exclusive. SAFAs make-up the largest proportion of any of the three groups of FAs, but this may be due to the identification standard and its mix of FAs, which contains a higher proportion of SAFAs then other FAs. Though this is the case, the high proportion of SAFAs is an important

finding because both PUFAs and MUFAs are more important in aquatic primary production, as there are many PUFAs that are essential for macroinvertebrate growth and reproduction (Torres-Ruiz *et al*. 2007), the prime grazers of periphyton biomass. This may relate how agricultural land can decrease macroinvertebrate grazer taxon richness, potentially due to limitations in essential fatty acid needs from periphyton (Liess *et al*. 2012). Ultimately, this reduction in essential fatty acids (EFA) could cause bottom up control of these systems over time, with alterations to food webs at both low and high trophic levels (Larson *et al*. 2013).

Chapter 4: General Conclusion

The previous chapters have shown that the agricultural domination of the NVW, both at small scales and large ones, are impacting water quality in the NR and its tributaries. By analysing the land-use, water quality and algal biomass across the NVW, it is evident that negative impacts to water chemistry (nitrogen and phosphorus), water clarity (turbidity and total suspended solids) and biological parameters (algal abundance and FA content) are impacted and need to be further studied.

Chapter 2 highlights the need for further study in the Nottawasaga River in order to better understand not only what is driving the phytoplankton community, but also what is driving the nutrient enrichment that is consistently occurring throughout its waters. Due to the preliminary nature of this study, there are specific aspects that should be taken into account when studying this area further. Firstly, a better identification of the sources of nutrients, most importantly nitrogen and phosphorus needs to be determined in order to understand whether it is legacy nutrients causing these increased concentrations or if there are current strong influences. If present agricultural practices and anthropogenic inputs are in fact linked to increased nutrient loads, implementation and enforcement of best management practices by the Nottawasaga Conservation Authority and OMAFRA are essential in order to reduce nutrient enrichment, due to the limitations that were seen with using land-use alone as a driver of water quality degradation. Secondly, the high concentrations of suspended sediment enrichment that is consistently occurring linked with Innisfil Creek needs to be better understood. Limited information was gained from this study from direct influencers of Innisfil Creek due to study

design and the limitations of sampling site placement to the main branch of the NR. In order to better understand the hydrodynamic processes occurring within Innisfil Creek, directed studies should be undertaken to determine the source of nutrient impacts that are consistently linked with its outflow. Finally, information needs to be gathered in order to understand if the phytoplankton community is in fact light limited and if remediation of the suspended sediments from Innisfil Creek may cause further issues related to increased algal biomass. Lack of light penetration may be the leading cause of issues once the high nutrient concentrations disperse in Nottawasaga Bay, leading to fish and bird deaths in the area related to anoxic conditions (Rutledge *et al*. 2015). Light penetration and light availability was not within the scope of this study, as nutrients are in most systems more important than light availability, but due to the turbid nature of this system, it may be affecting the algal community.

Chapter 3 highlights the need for better understanding of agriculturally linked nutrients and their effect on primary producers, not only in their community structure, but ultimately how they can then control higher trophic levels that are of significance downstream of these areas. Water quality variability can be linked with land-use over large scales, but alternative variability in geography, geology, flow regime and many other environmental factors may play a larger role in streams of this size. Agricultural land has the effect of increasing nutrients such as N and P to aquatic systems and that can directly increase algal biomass, but we are starting to find out that there is more important factors that may be affected by these changes in nutrient dynamics. Though water quality and algal biomass can have direct downstream effects, this may be amplified when changes in FA structure are disturbed from their natural ratios. Due to the heavy agricultural land-use contained within the NVW, it is important to study how nutrient

additions and land-use changes will affect not only the directly adjacent streams and rivers, but also the main branch of the Nottawasaga River. FAs are known to be influenced in many different ways, which is unique to each system that is being studied, making this type of analysis difficult to relate to other information, highlighting the need for increased research in this area. Aquatic macroinvertebrates and fish species are some of the better protected organisms when it comes to conservation and preventing loss of species diversity, but it may be more important to understand how they will ultimately be affected by primary production and their food quality at the headwaters of streams.

One of the largest limitations of this study was the availability of study sites that fit the criteria needed in order to support the research questions. A larger representation of the NVW and of each crop type would allow for more consistent data, as well as the addition of continuous monitoring for water quality during algal growth assays. Information directly linked to farming practices, such as timing and amounts of fertilizer/herbicide additions, tilling practices, watering addition and soil characteristics would allow for a better understanding of relationships and explanation of the variability of these systems. Farming practices were not within the scope of this study, as it was only to determine if the crop type alone was having a significant effect on the algal community and water quality. Another one of the limitations of this study was the lack of periphyton community structure information from species counts, which would have allowed for a greater deal of information to be gained coupled with the analysis of fatty acid profiles within each algal growth assay. This information was not able to be included in this study due to time constraints. Accurate enumeration of algal communities, both for abundance and correct identification, is extremely time consuming and was not able to

be included. As exhibited by the differences in CHL and AFDM from the algal growth assays, there may have been large quantities of non-algal microbial growth, which may have required specialized culturing techniques in order to properly identify, which would also not be within the scope of this study, further adding to the time constraints.

In conclusion, it is important to continue to monitor the Nottawasaga River and its tributaries to assess how agricultural land-use and the expanding urban development in the watershed will alter water quality in the future. The Nottawasaga Valley Conservation Authority would benefit from further partnerships in order to conduct directed studies of water quality impairment in this system. In order to protect and conserve such a large system, a great deal of information is needed to make the best choices not only for the health of the river's ecosystem, but also for all stakeholders in the area. With important ecological areas such as the Minesing Wetlands contained within the NVW, as well as important economical areas such as Wasaga Beach at the outflow of the NR, all will benefit from proper management of this area.

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Appendix A

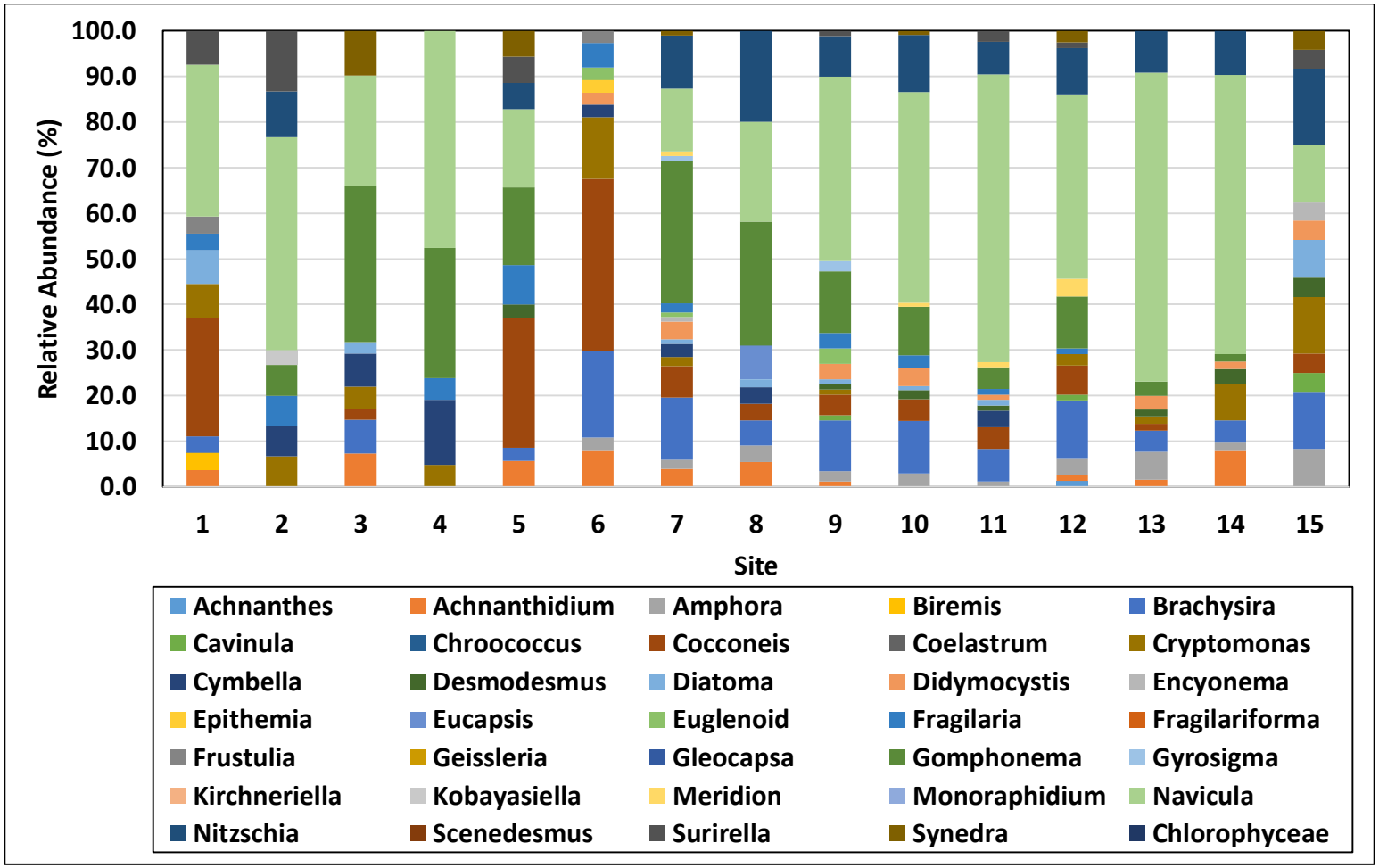

Figure A1: Variation of phytoplankton relative abundance at the studied sites of the Nottawasaga River during June.

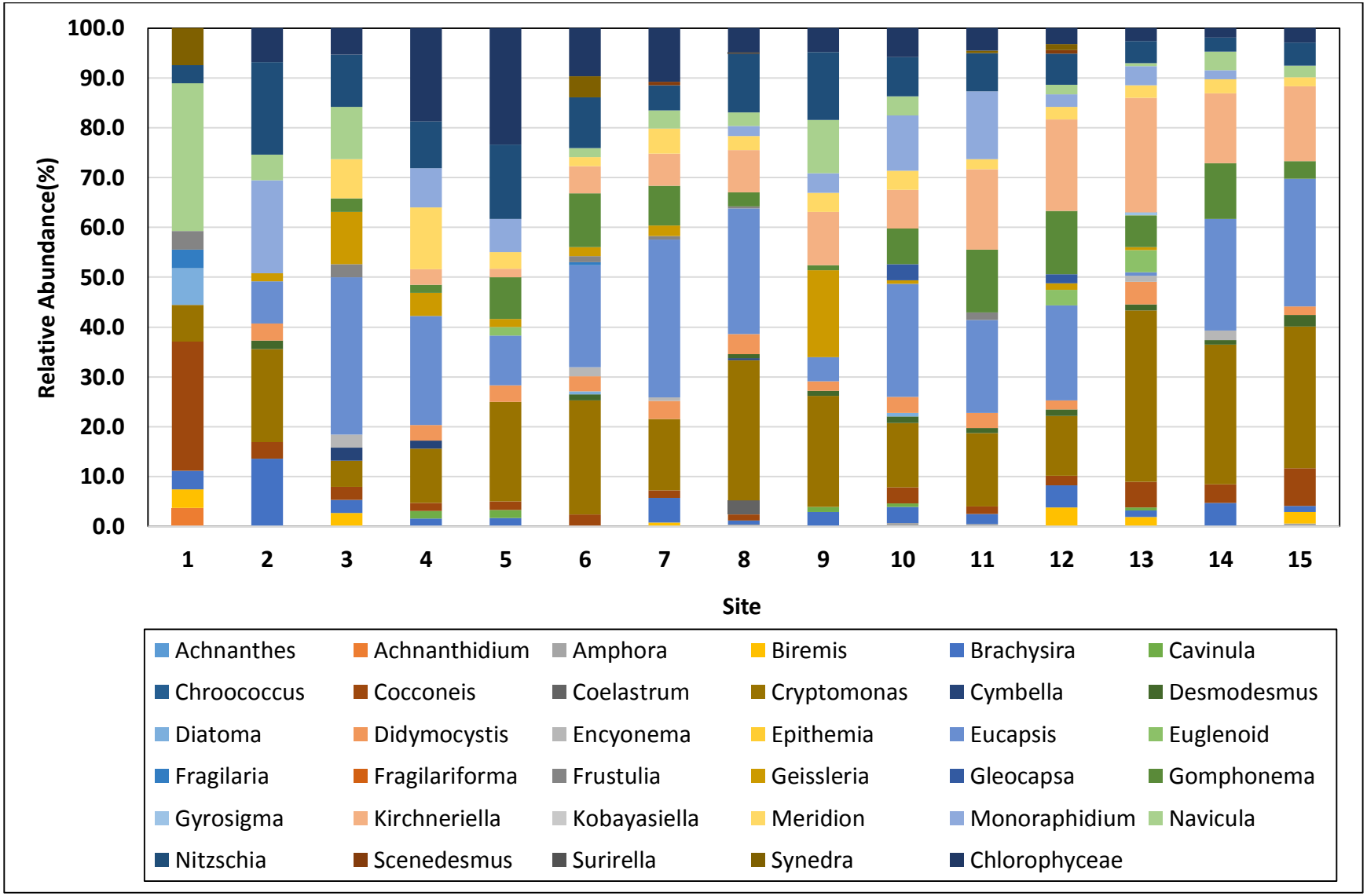

Figure A2: Variation of phytoplankton relative abundance at the studied sites of the Nottawasaga River during July.

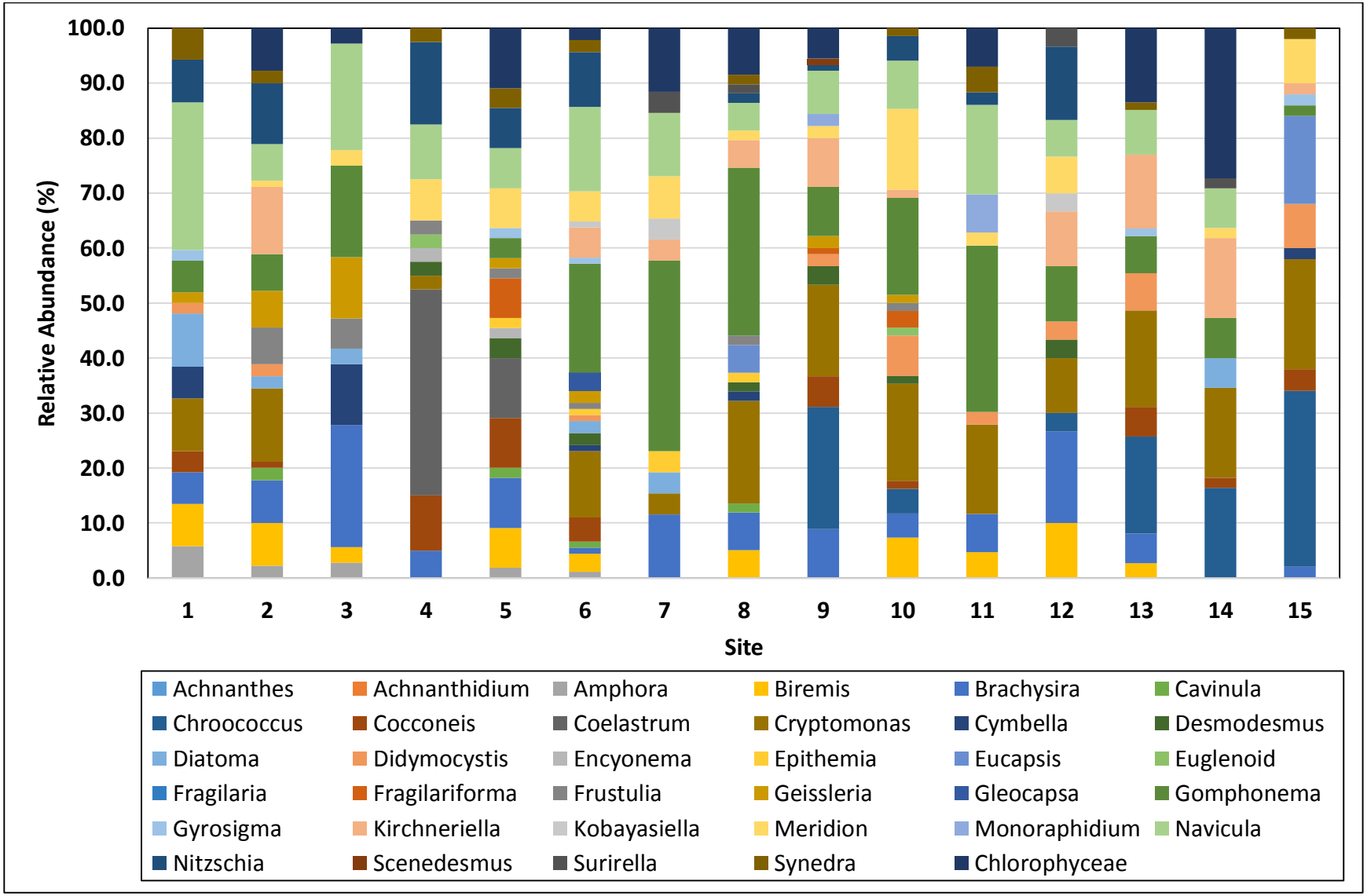

Figure A3: Variation of phytoplankton relative abundance at the studied sites of the Nottawasaga River during August.

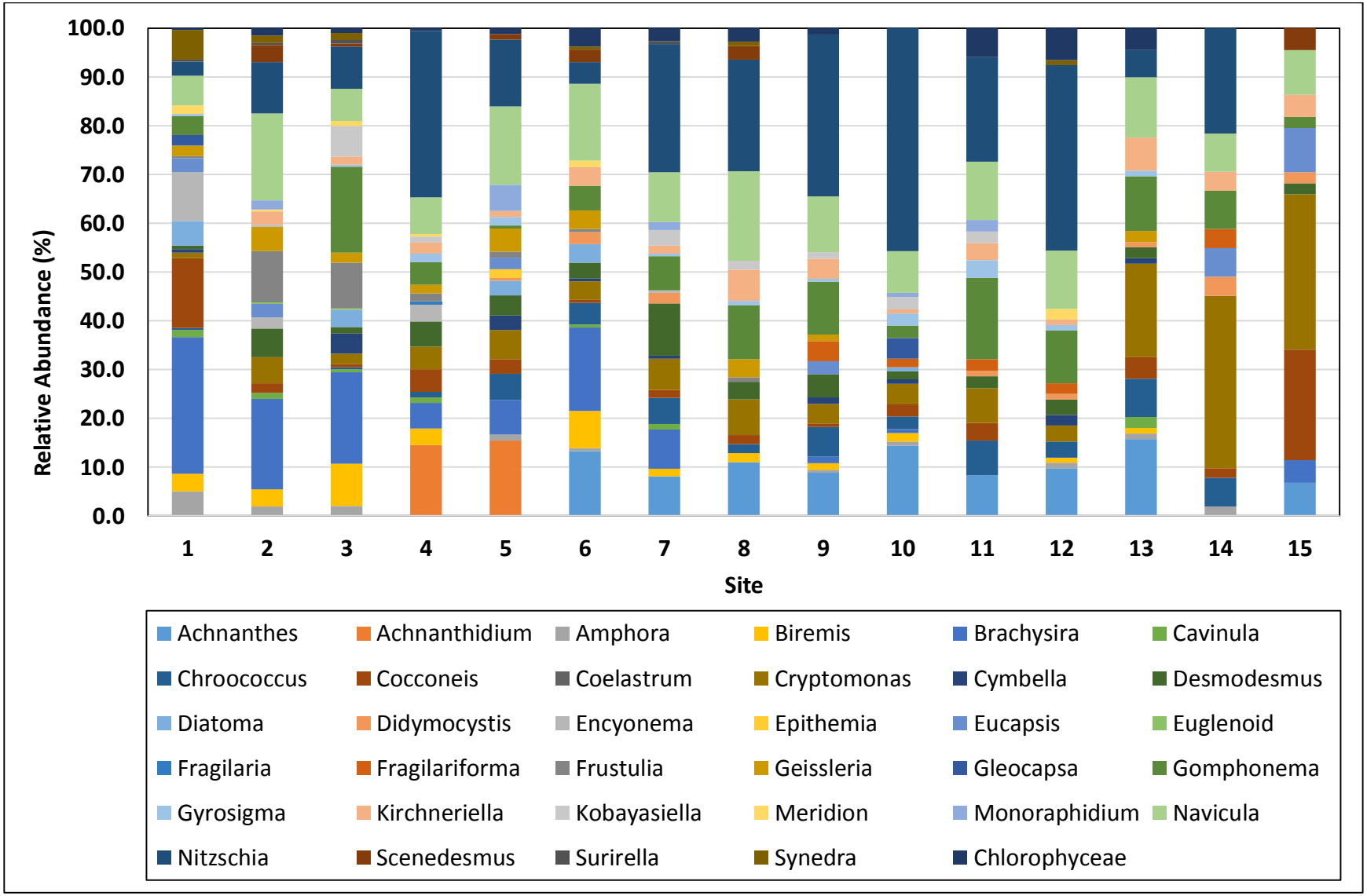

Figure A4: Variation of phytoplankton relative abundance at the studied sites of the Nottawasaga River during September.

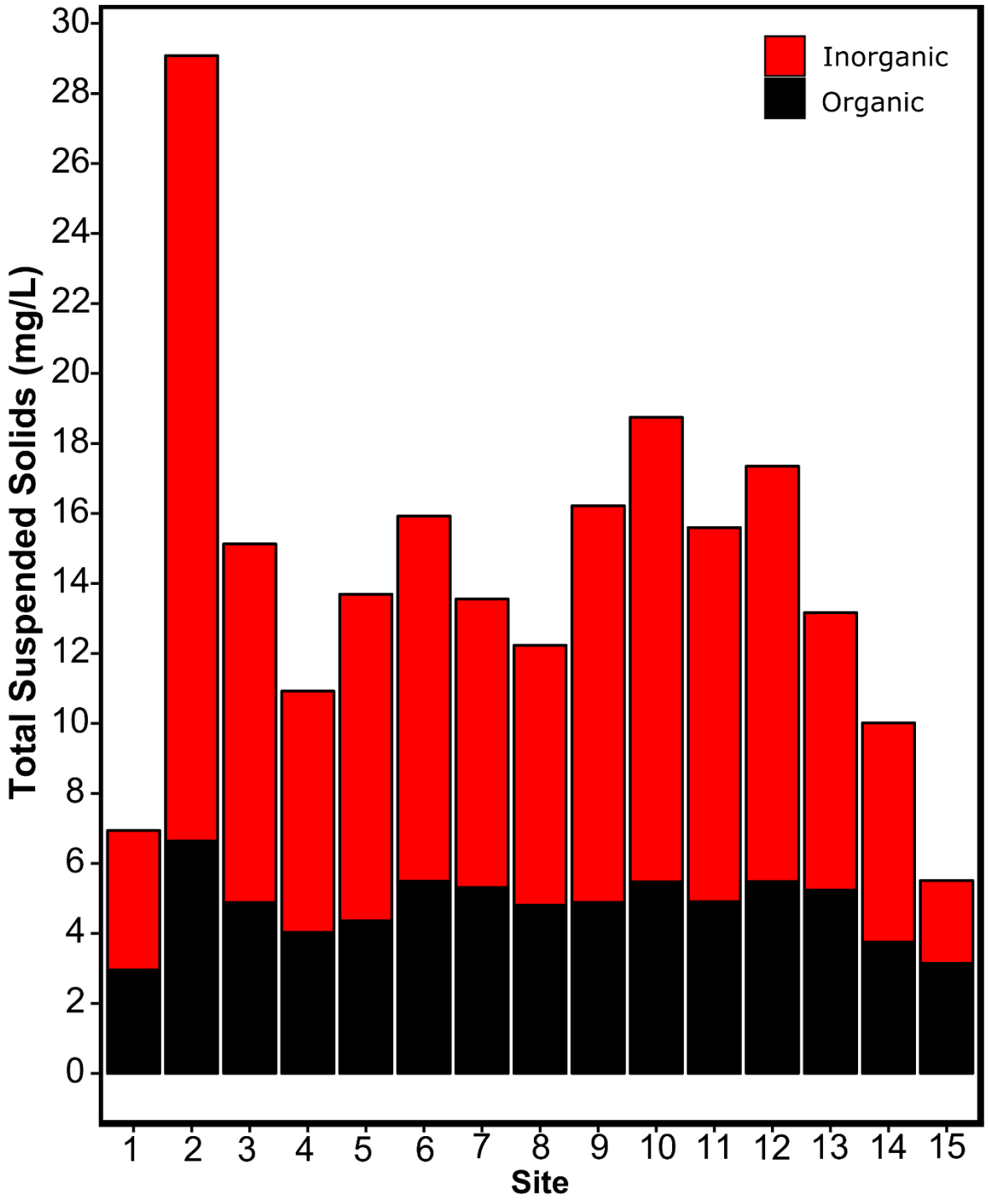

Figure A5: Relative mean monthly contributions of organic (black) and inorganic (red) constituents of TSS at each sapling site.