BIOGEOCHEMISTRY OF WET ECOSYSTEMS: FROM ROOT ZONE TO LANDSCAPE

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ABSTRACT

The biogeochemistry of wetland ecosystems varies, causing them to act as sources, sinks, filters or transformers of nutrients and pollutants. Wetland plants play important roles in the cycling of elements in wet ecosystems. The structural and physiological adaptations that allow these plants to colonize wetland habitats as emergent or submerged species contribute to biogeochemical processes in wetland substrates. Rhizosphere (root zone) oxidation, iron and manganese oxide precipitation, acidification of the rhizosphere, root exudation, and microbial activity influence the mobility of elements in wetland substrates. Both emergent and submerged wetland plants can alter conditions in the rhizosphere that influence the mobility of elements. These plants are also capable of removing elements such as Cd, Cu, Fe, Mn, N, P and Zn from solution and accumulating them in their tissues.

Root zone studies were carried out in the greenhouse using the wetland plants *Typha angustifolia* (cattail) and *Rumex crispus* (curly dock) and in the field using *Triglochin maritima* (seaside arrowgrass) to determine differences in element concentrations in the root and bulk zone under different soil moisture conditions. Studies involving shallow lakes of Minnesota were carried out to determine relationships among (1) landscape variables (e.g. lake watershed size, percent agriculture, percent woodland), water and sediment characteristics (turbidity, chlorophyll-*a*, organic content, particle size), (3) element concentrations in waters and sediments, and (3) plant abundance and community composition.

The studies reported here showed that different factors influenced the distribution of multiple elements in the root zone of emergent wetland plants and in waters and sediments of shallow lakes. First, the root zone studies indicated that pH, redox and moisture content of wetland soils influenced the distribution of elements in the rhizosphere and subsequent uptake of these elements by wetland plants. Second, the shallow lake study showed that land cover uses (agriculture and woodland), lake watershed size, and sediment physical characteristics (organic content and particle size) influenced the distribution of elements in waters and sediments of shallow lakes. Concentrations of these elements, land cover uses, open water area, turbidity, chlorophyll-*a* concentrations and sediment physical characteristics influenced abundance and distribution of submerged and floating plants.

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CHAPTER 1. GENERAL INTRODUCTION

1.1. Background

The primary goal of my research was to examine the differences in biogeochemistry in 1) the root zone soil of wetland plants, and 2) shallow lake ecosystems. One aspect of this research examined biogeochemical differences in the root and bulk zone of emergent plants under different soil moisture conditions using greenhouse and field experiments. Interest in these differences arose from the suggestion of a link between rhizosphere biogeochemistry and metal tolerance in wetland plants (McCabe et al. 2001, Otte et al. 2004). The second aspect of the research examined biogeochemical differences in the sediment and water associated with submerged vegetation in shallow lakes of varying turbidities. This was to determine relationships between macrophyte communities and water and sediment chemistry. The first section of this review focuses on rhizosphere processes because of their major role in plant element uptake and distribution. Section two focuses on the biogeochemical effects of emergent plants. Adaptations to flooding, iron oxidation, influences on element concentrations in soil and sediment and element accumulation in plant tissues are reviewed in this section. Section three focuses on submerged plants and relationships with biogeochemistry. Plant influences on element concentrations in sediments and water, plant indicators of biogeochemical conditions and lake turbidity will be reviewed in this section. Finally, I have developed a conceptual model to summarize what is known about the biogeochemical effects of plants in wet ecosystems and to identify the gaps in our knowledge that my research will address.

1.2. Rhizosphere processes

1.2.1. The rhizosphere defined

The rhizosphere has been defined as the interfacial zone between the root and the soil (Alloway 1995), the section of the soil or sediment that is directly influenced by the presence of living roots (Jacob and Otte 2003), the volume of soil around the roots that has increased microbial activity (Kapulnik and Okon 2002), and the region of the soil which is modified by uptake and release of substances by living roots (Singleton and Sainsbury 1987). The rhizosphere environment differs in chemistry, biochemistry, and biology from the non-rhizosphere soil (Foster et al. 1983; Kapulnik and Okon 2002). These differences are a result of the physical and chemical changes caused by root growth and metabolism

(Badalucco and Kuikman 2001; Wang et al. 2002). These changes limit the distribution of organisms in the plant rhizosphere (Hawkes et al. 2007). Root modifications of the surrounding soil due to gaseous exchange and removal of nutrients and water create conditions in the rhizosphere that are very different in biota compared to bulk soil (Robinson et al. 2003).

1.2.2. Rhizosphere biogeochemistry

Plants from various ecological groups may experience different biogeochemical changes in their rhizosphere. Dryland plants (those that grow in non-flooded or unsaturated soil) may experience gradients in nutrient concentration, pH, redox potential, root exudates and microbial activity in the rhizosphere (Marschner 1995). Youssef and Chino (1989b), observing pH gradients at the soil-root interface of barley and soybean, concluded that the extent of this gradient depends upon plant species and the initial pH of the bulk soil. Wetland plants also experience these gradients, but to different degrees due to different influencing factors. Distinct differences in redox potential in the rhizosphere occur between plants grown in aerated or oxidized soils (dryland plants) and those grown in submerged, chemically reduced soils (wetland plants) (Marschner 1995). Radial oxygen loss is minimal in plants growing in aerated soils due to the absence of aerenchyma and lower oxygen diffusion rates within the plant and the lack of redox gradients in the soil (Mendelssohn 1993).

Changes in the rhizosphere soil pH can be due to carbon dioxide dissolution (Neumann and Römheld 2002), proton release, cation/anion uptake (Begg et al. 1994; Tinker and Nye 2000; Kirk 2004), release of root exudates (Brimecombe et al. 2001; Walker et al. 2003), and Fe oxidation (Begg et al. 1994; Tinker and Nye 2000; Neumann and Römheld 2002; Kirk 2004). The pH levels in the rhizosphere can also be influenced by soil and plant components such as soil buffering capability, soil moisture and aeration, acid production by microbes, carbon dioxide production by plant roots and soil microorganisms, plant genotype, absorption of soil nitrogen, plant nutrient status (Neumann and Römheld 2002), and release of organic acids and H^+ ions by plants (Marschner et al. 1986; Begg et al. 1994; Kirk and Bajita 1995; Hinsinger 2001; Kirk 2004).

Variations in pH greatly influence processes in the rhizosphere (Begg et al. 1994) such as the dissolution or precipitation of nutrients, in particular, changes in the solubility of metals such as Al, Zn, Fe, Mn, Cu, and Mo (Jungk 2002). Researchers observed pH gradients at the soil-root interface that

corresponded with increased solubility of Zn and Fe in the rhizosphere of barley and soybean (Youssef and Chino 1991) and increased bioavailability of Al and Fe in the rhizosphere of tea plants (Chen et al. 2006). Differences in pH between the rhizosphere and the bulk soils can result in adsorption, desorption, precipitation and volatilization of trace elements (Lombi et al. 2001), and dissolution and mobilization of minerals (Jones et al. 1996; Hinsinger 2001; Puschenreiter et al. 2005).

Trace element adsorption is also influenced by soil mineral composition and organic matter content (Lombi et al. 2001). Plants are the primary contributors of organic matter and humus to soils (Brooker et al. 2008) which increases soil nutrient status, improves soil texture, and forms complexes with metals, thus reducing their availability for plant uptake (Antonovics et al. 1971). Organic matter tends to accumulate in wetlands due to low oxygen availability resulting in decreased decomposition (Gambrell & Patrick 1978; Foster et al. 1983) and hence plays an important role in controlling metal mobility in wetland soils or sediments (Davies 1994; Doyle & Otte 1997).

Redox potential, pH, root exudation and root-microorganism interactions are key factors that play a role in the mobility and bioavailability of elements in the rhizosphere (Lombi et al. 2001). The redox potential of the rhizosphere can be altered by microbial activity, by the release of reducing agents (Lombi et al. 2001), or by radial oxygen loss by plants which will be discussed in more detail later. Root exudates are a complex mixture of compounds such as organic acids, sugars, amino acids, phenolics and various other metabolites which play a role in modifying the biochemical and physical properties of the rhizosphere (Walker et al. 2003). Root exudates also provide organic ligands for element complexation, facilitate microbial activity and nutrient uptake (Jungk 2002), and influence the speciation, solubility, and availability of elements in the soil (Marschner 1995; Kabata-Pendias and Pendias 2001; Wang et al. 2002; Graham and Stangoulis 2003; Reichman and Parker 2005).

1.3. Biogeochemical effects of emergent wetland plants

Plants are characterized commonly by their water requirements or adaptations. Wetland plants depend on an abundant supply of water and grow either partly or fully submerged in water (Raven et al. 1992). For the purposes of this review, we will refer to wetland plants as those species that grow in or on water or inhabit flooded or saturated soils, i.e., emergent, submerged, floating-leaved and free-floating plants (Cronk and Fennessy 2001). Zonation of wetland plant communities tends to follow a sequence

parallel to shores in wet ecosystems, with submerged species occurring in deeper water giving way to a zone of floating-leaved plants closer to the shore, followed by a zone of emergent plants on the margins in water of about 1 m to saturated soil on the shore (Figure 1.1) (Sculthorpe 1967). Emergent plants inhabit shallow waters, are rooted in the substrate with their basal portions typically below the water's surface, and their photosynthetic and reproductive parts above the surface (Sculthorpe 1967; Cronk and Fennessy 2001). The most common emergent species are from large families of monocotyledons such as Poaceae, Cyperaceae, Juncaceae and Typhaceae and they tend to dominate fresh and saltwater marshes (Cronk and Fennessy 2001).

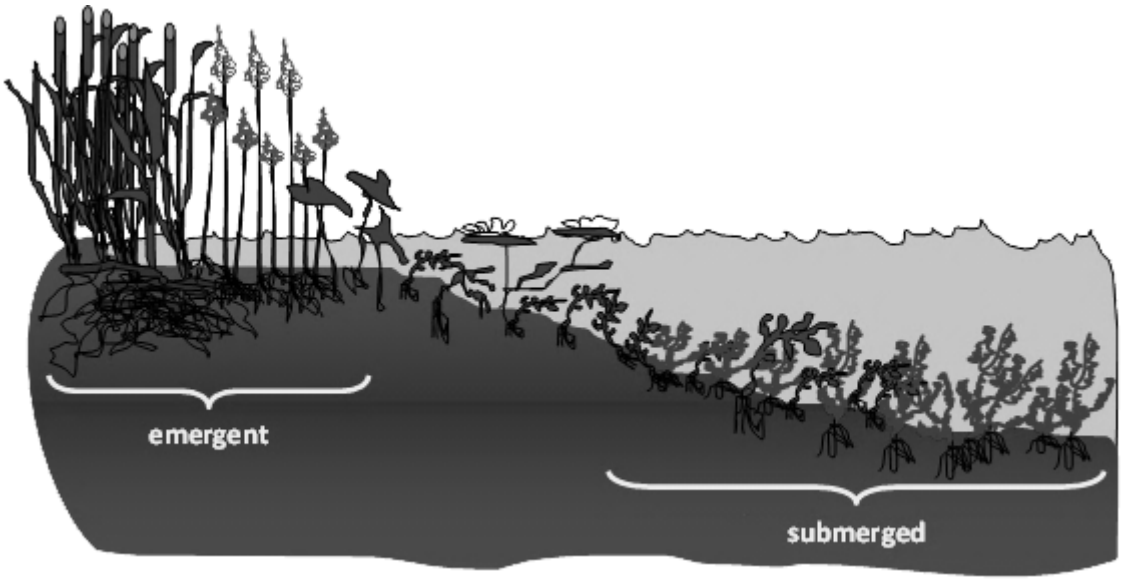

Figure 1.1. Characteristic distribution of emergent and submerged plants in a wet ecosystem (after Sculthorpe 1967).

1.3.1. Adaptations to soil flooding and waterlogging

Wetland plants have special structural adaptations which allow them to aerate their roots and rhizomes and thus alleviate oxygen shortages (Cronk and Fennessy 2001; Mitsch and Gosselink 2007). Some of these adaptations include aerenchyma formation, radial oxygen loss, adventitious roots, stem hypertrophy, stem buoyancy, fluted trunks, growth dormancy, shallow root systems, lenticels and pneumatophores (Cronk and Fennessy 2001; Mitsch and Gosselink 2007). The development of aerenchyma, which creates a gaseous pathway from the stomata in the leaves to the roots, is one of the most important adaptations in wetland plants that allow them to colonize flooded anoxic soils (Armstrong 1978; Bodelier 2003).

Wetland plants have the advantage of a constant water supply, and they have many physiological adaptations to deal with the consequences of living under these conditions (Kirk 2004). When soil is submerged, gaseous exchange between the air and soil is disrupted (Justin and Armstrong 1987) and the limited oxygen present is used up by soil microorganisms, creating a chemically reduced soil environment (Trolldenier 1988). The aerenchyma transports oxygen between the aerial parts and the respiring root tissues of the plant (Armstrong 1967; Armstrong 1978; Justin and Armstrong 1987; Trolldenier 1988; Jungk 2002; Kirk 2004). Some of the oxygen that is transported to the roots diffuses via radial oxygen loss into the adjacent soil, resulting in an oxidized rhizosphere and increased redox potential (Foster et al. 1983; Mendelssohn 1993; Davies 1994; Tinker and Nye 2000; Jacob and Otte 2003). The extent of the oxidation zone around the roots is influenced by the reducing capacity of the soil and the oxygen supply to the roots (Flessa and Fischer 1992). The rhizosphere becomes an interface between oxic and anoxic environments which is able to host diverse microbial populations (Neubauer et al. 2007). Rhizosphere oxidation is a physiological adaptation which prevents the deterioration of roots, maintains nutrient and water uptake in anaerobic soil conditions (Kirk 1994), and prevents the toxic buildup of Fe^{2+}, Mn^{2+}, H_2S and monocarboxylic acids (Neumann and Römheld 2002). Plants with greater rates of oxygen diffusion are able to tolerate stronger reducing conditions (Armstrong 1964). Other physiological adaptations in response to flooding include pressurized gas flow, decreased water uptake, altered nutrient absorption, sulfide avoidance and anaerobic respiration (Mitsch and Gosselink 2007).

1.3.2. Iron oxide formation

Rhizosphere oxidation in the root zone of wetland plants is apparent by the presence of reddish brown Fe^{3+} deposits on root surfaces (Armstrong 1967; Trolldenier 1988; Sadana and Claassen 1996; Kirk 2004) referred to as Fe plaque (Mendelssohn 1993; Otte et al. 1995). Ferrous iron (Fe^{2+}), the dominant form of iron present in reduced soils, can be oxidized in the rhizosphere to form ferric iron (Fe^{3+}) oxyhydroxides (Gambrell and Patrick 1978; De Laune et al. 1981; Mendelssohn 1993). A concentration gradient is established as the Fe^{2+} concentrations near the root decrease, thus causing more Fe^{2+} to diffuse towards the oxidized zone near the root (Otte et al. 1995; Tinker and Nye 2000; Kirk 2004). Oxygen diffusion via specialized air tissues into the roots and then into the surrounding soil is evident in the depletion of Fe^{2+} and the accumulation of Fe^{3+} in the root zone (Kirk and Bajita 1995).

The accumulation of Fe plaque in the vicinity of the roots is dependent on the oxidizing capacity of the roots, soil texture, organic matter content, pH and redox status, the form and concentration of the Fe present in the soil (Mendelssohn 1993), and the activity of iron oxidizing bacteria in the vicinity of the roots (Emerson et al. 1999; Weiss 2003). Soil pH influences Fe dissolution and the resolubilization of precipitated Fe in the rhizosphere (Mendelssohn 1993). Begg et al. (1994) reporteded increasing iron concentratons at the root surface and decreasing soil pH due to iron oxidation reactions in the oxidized rhizosphere of rice. The ratio of Fe^{2+} and Fe^{3+} concentration in soil influences the redox potential which is a measure of the intensity of oxidation and reduction reactions in the soil (Gambrell and Patrick 1978). Hence, the redox potential of the soil gives an indication of the extent of Fe^{2+} oxidation.

Rhizosphere oxidation and the subsequent formation of Fe plaque has been observed in many emergent species in laboratory and field settings. For example, this plaque has been noted on the roots of *Typha latifolia* L. (Taylor et al. 1984), *Oryza sativa* L. (Bacha and Hossner 1977; Trolldenier 1988; Sadana and Claassen 1996), *Sagittaria latifolia* (Chen and Barko 1988), *Aster tripolium* L. (Otte et al. 1989), *Rumex crispus, Rumex maritimus, Rumex thyrsiflorus* (Laan et al. 1989), *Halimione portulacoides*, *Spartina anglica* (Otte et al. 1995), *Spartina maritima* (Sundby et al. 1998), *Caltha palustris* (van der Welle et al. 2007), and *Juncus effusus* (Neubauer et al. 2007).

1.3.3. Influence on element concentrations in the soil or sediment

Several emergent and submerged plant species have been observed to influence element concentrations in the soil and sediment (Table 1.1), and have the potential to be used in the phytoremediation and phytostabilization of elements (Salt et al. 1995; Fritioff and Greger 2003; Weis and Weis 2004; Azaizeh et al. 2006). The presence of Fe plaque on and around plant roots affects rhizosphere metal concentrations (Otte et al. 1991; Otte et al. 1995; Doyle and Otte 1997). Oxygen release from plant roots and subsequent oxidation of sulfides in the rhizosphere (Choi et al. 2006) can result in the release and mobilization of metals from their associated sulfides (Roden and Wetzel 1996; Jacob and Otte 2004a), but these metals may then be immobilized due to precipitation in the oxidized rhizosphere and subsequent binding with Fe plaque (Barko et al. 1991; Jacob and Otte 2003). The hydrous oxides of Fe and Mn tend to have high adsorption affinities for trace cations (Davies 1994). Copper, Zn (Otte et al. 1989 1995) and As (Otte et al. 1995) have the potential to bind with Fe plaque. In

a study involving *Typha latifolia*, Ye et al. (1998) reported that Zn concentrations correlated positively with Fe concentrations on the root surface, which showed that Fe plaque can accumulate Zn. Blute et al. (2004) found proportions of As(III) and As(V) strongly adsorbed to Fe plaque on roots of *Typha latifolia*. The adsorption of As to Fe plaque on *Typha* roots resulted in decreased concentrations of both As(III) and As(V) in groundwater.

Rhizosphere oxidation and the presence of Fe plaque has been shown to influence metal concentrations in the rhizosphere. Kirk and Bajita (1995) observed zones of Zn accumulation and depletion associated with the accumulation of Fe^{3+} and soil acidification in the rhizosphere of lowland rice. Zn from highly insoluble fractions in the soil was released due to Fe oxidation and then this Zn was readsorbed by Fe plaque and organic matter. Otte et al. (1995) reported that the binding of As and Zn with Fe plaque resulted in a decreasing concentration gradient of metals towards plant roots. In studies comparing rhizosphere and bulk soil, the rhizosphere was reported to have higher concentrations of As, Fe and Zn (Otte et al. 1991; Otte et al. 1995; Doyle and Otte 1997; Wright and Otte 1999) and in some cases lower concentrations of Fe (Otte et al. 1995). Accumulation of Fe and As was probably due to oxidation processes in the rhizosphere (Otte et al. 1991). In studies comparing vegetated and non-vegetated flooded sediments, the vegetated sediments had higher concentrations of Zn (Jacob and Otte 2004a; Choi et al. 2006), As and Fe (Doyle and Otte 1997). The presence of living plant roots appears to enhance metal mobility by inducing the oxidation of the sediments and metal sulfides (Jacob and Otte 2004a).

1.3.4. Element accumulation by emergent plants

An oxidized rhizosphere and the presence of Fe plaque on plant roots has also been shown to influence metal uptake by plants (Mitsui 1965; Otte et al. 1989; Otte et al. 1991; Ye et al. 1997b; Ye et al. 1998). Armstrong (1978) and Gambrell and Patrick (1978) stated that the formation of Fe plaque on roots served as a sink for metals and was consequently a hindrance to nutrient uptake by wetland plants. However, a number of studies have reported that the precipitation of Fe in the rhizosphere of wetland plants does not necessarily reduce metal uptake (Otte et al. 1989; Ye et al. 1998). Iron plaque accumulation in the rhizosphere appears to be a secondary source of metals to wetland plants and not a physical barrier (Ye et al. 1997b). Wetland plants have the ability to remobilize metals that become

Table 1.1. Evidence of plant influence on element concentrations in soil (s), sediment (sd), porewater (pw) and/or water column (wc) (↓ indicates decreased element concentrations; ↑ indicates increased element concentrations).

Plants	Elements	Compartment	References
Emergent			
Aster tripolium	↑As, ↑Fe	s	Otte et al.1991
Atriplex portulacoides, Spartina townsendii; *Halimione portulacoides*, *Spartina anglica*; *Typha latifolia*	↑As, ↑Fe, ↑Zn	s	Doyle and Otte 1997; Otte et al.1995; Jacob and Otte 2004b
Menyanthes trifoliata	↓Fe, ↓Fe^{2+}, ↓P, ↓Mn	pw	Moore et al.1994
Oryza sativa	↑Fe, ↑Fe^{2+}; ↑Zn	s	Begg et al.1994; Kirk and Bajita 1995
Sagittaria latifolia	↓Fe, ↓N, ↓P	sd	Chen and Barko 1988
Scirpus subterminalis Torr.	↓Ca, ↓K, ↓Mg, ↓N, ↓Na	w	Mickle and Wetzel 1978
Typha angustifolia	↓P	w	Horrpila and Nurminen 2005
Typha latifolia	↑Fe, ↑Fe^{2+}, ↑Zn	pw	Wright and Otte 1999
Submerged			
Ceratophyllum demersum	↓Cu, ↓Pb, ↓Zn	w	Keskinan et al.2004
Elodea nuttallii, Myriophyllum spicatum, *Potamogeton pectinatus*, *Ranunculus penicillatus, Sparganium emersum*	↓C, ↓N, ↓P	sd	Clarke and Wharton 2001
Hydrilla verticillata	↓N, ↓P; ↓Fe, ↓N, ↓P	sd	Barko et al.1988; Chen and Barko 1988

Table 1.1 (continued)

Isoetes braunii, Myriophyllum tennellum	↑Fe, ↑P	sd	Jaynes and Carpenter 1986
Isoetes lacustris	↑Fe, ↑Mn	sd	Tessenow and Baynes 1975
Isoetes lacustris, Littorela uniflora	↓Fe, ↓Mn, ↓P	sd	Christensen et al.1998
Mentha aquatica, Myriophyllum aquaticum, Ludwigina palustris	↓Cu, ↓Fe, ↓Hg, ↓ Zn	w	Kamal et al.2004
Myriophyllum heterophyllum Michx.	↓Ca, ↑K, ↓N	w	Mickle and Wetzel 1978
Myriophyllum spicatum	↑C, ↓N, ↓P, ↑K; ↓Cu, ↓Pb, ↓Zn	pw; w	Carignan 1985; Keskinan et al.2003
Myriophyllum spicatum; Potamogeton pectinatus, *Vallisneria americana;*	↓N, ↓P	sd	Prentki 1979; Wigand et al.2001;
Ceratophyllum demersum, Ranunculus circinatus	↑N	sd	Nurminen and Horppila 2009
Myriophyllum spicatum; Egeria densa, Hydrilla verticillata, Myriophyllum spicatum; Ceratophyllum demersum, Potamogeton obtusifolius, Ranunculus circinatus;	↓P	sd	Peverly and Brittain 1978; Barko and Smart 1980; Horrpila and Nurminen 2005;
Myriophyllum sibiricum	↑P	pw	Cronin et al.2006

adsorbed onto Fe plaque and make them available for uptake by acidification of the rhizosphere (Kirk and Bajita 1995), releasing reducing agents, enzymes (Jungk 2002), chelates or protons (Otte et al. 2004) from their roots.

Several studies reported that metals are not permanently immobilized by Fe plaque, but may still be available for uptake by the plant. It was reported that *Typha latifolia* accumulated Pb and Cd in its root tissues while Zn was accumulated in the shoots indicating that the root surface and Fe plaque are not the main barriers to the transport of these metals (Ye et al. 1998). In a study comparing plant uptake and accumulation with and without Fe plaque present, Cu was found adsorbed to Fe plaque on the root surface, and plants with Fe plaque took up more Cu than those without Fe plaque (Ye et al. 1997b). This study also concluded that root tissue and not Fe plaque or the root surface was the main barrier to metals. A study by Otte et al. (1989) reported that Zn does not permanently adsorb to Fe plaque since root-induced acidification of the rhizosphere resulted in the remobilization of Zn. Iron plaque appeared to enhance Zn uptake since roots with Fe plaque took up more Zn than roots without and the amount taken up was dependent on the amount of plaque present (Otte et al. 1989). McCabe et al. (2001) and Otte et al. (2004) stated that wetland roots may be exposed to relatively higher levels of metals which in turn may have led to the development of metal tolerance in wetland plants because metals adsorb to Fe plaque and may still be available for uptake.

A number of studies have reported that wetland plants accumulate elements in their roots and/or shoots. *Phragmites communis* accumulated C, N and P in the shoots and Cd, Cr, Cu, Fe, Mn, Ni, Pb, S, and Zn in the roots (Baldatoni et al. 2004). Matthews et al. (2004) reported that *Glyceria fluitans* accumulated Zn more in the roots and dead leaves compared to the live leaves. Elevated concentrations of Fe, Cd, Co, Cr, Cu, Mn, Ni, Pb and Zn were detected in *Calamagrostis epigeios, Carex remota, Iris pseudoacorus, Juncus bulbosus, Juncus effusus, Phragmites australis, Phalaris arundinacea, Polygonum hydropiper, Schoenoplectus lacustris,* and *Typha angustifolia* (Samecka-Cymerman and Kempers 2001). Szymanowska et al. (1999) found elevated concentrations of Cd, Co, Cr, Hg, Pb and Zn in *Phragmites communis, Typha latifolia,* and *Schoenoplectus lacustris*. The elements in the plant tissues tend to reflect the element composition of the substrate or sediments (Guilizzoni 1991). As for macronutrients, significant decrease of N and P concentrations in the output from a constructed wetland receiving tile

drainage from agricultural fields indicates that the wetland was an efficient sink for nutrients (Hoagland et al. 2001). The most abundant species in this wetland were *Polygonum amphibium, Phalaris arundinea, Carex* spp., and *Polygonum punctatum*, which accumulated nutrients mainly in their belowground tissues.

1.4. Submerged plants: relationships with biogeochemistry

Submerged plants tend to inhabit shallow or deep open water depending on light availability (Sculthorpe 1967; Eggers and Reed 1997) and most are rooted in the substrate with their photosynthetic tissues below the water's surface (Sculthorpe 1967; Cook 1990; Cronk and Fennessey 2001). Compared with other types of wetland plants, submerged plants are exposed to lower oxygen and carbon dioxide concentrations within the water column due to the slow diffusion rate of gases in water (Sculthorpe 1967; Cronk and Fennessy 2001). Growth within the water column also exposes these plants to lower light conditions than emergent, free floating or floating-leaved species (Cronk and Fennessey 2001). Submerged species tend to form canopies concentrating their leaf biomass close to the water surface in order to maximize their light harvesting potential and to have greater access to carbon dioxide for photosynthesis (Cronk and Fennessey 2001). Submerged plant species have the ability to release oxygen from their roots and thus oxygenate their rhizosphere (Barko and Smart 1983). Rhizosphere oxidation in submerged plants was first reported by Wium-Andersen and Andersen (1972). Oxygen release from the roots has been observed in the submerged species *Isoetes lacustris, Litorella uniflora, Lobelia dortamana, Potamogeton crispus* L., *P. friesii* Ruprecht, *P. pectinatus*, *Sparganium simplex* Hudson, *Zostera marina* (Sand-Jensen et al. 1982), *Ranunculus circinatus* L., *Myriophyllum verticillatum L.*, (Flessa 1994), *Elodea canadensis* (Hupfer and Dollan 2003), and *Myriophyllum spicatum* (Laskov et al. 2006). Oxygen release from submerged plant roots may also influence metal nutrient retention, pH and oxidation-reduction status (Wium-Andersen and Andersen 1972; Tessenow and Baynes 1975; Jaynes and Carpenter 1986; Jackson et al. 1993), nitrification rates and sulfide oxidation in the sediments (Kemp and Murray 1986).

1.4.1. Influence on element concentrations in the sediment and water

Rooted submerged plants provide a link between the sediments and the overlying water, which has implications for nutrient cycling in freshwater ecosystems (Carpenter and Lodge 1986; Barko and James 1998). Submerged plants act as nutrient transporters or pumps as they move nutrients from the

sediment via uptake into their tissues and then to the water column via senescence of those tissues (Carignan and Kalff 1980; Jackson et al. 1991 1994; Cronk and Fennessy 2001). Rooted submerged species acquire most of their nutrients from the sediment and some from the water column (Sculthorpe 1967; Barko and Smart 1980; Barko et al. 1991; Clarke and Wharton 2001). The sediment is the primary source of N, P, Fe, Mn, and trace elements whereas the water column is the primary source of Ca, Mg, Na, K, sulfate, and chloride (Barko and Smart 1986; Barko and James 1998; Barko et al. 1991; Jackson and Kalff 1994).

Nutrient and trace element distribution in the sediment and water, and availability to plants, is influenced by organic matter, pH, nutrient concentrations, redox potential, calcium carbonate concentrations, light, microbial activity (Guilizzoni 1991), sediment oxidation (Tessenow and Baynes 1975), and the activity of benthic invertebrates (Barko et al. 1991). Rhizosphere oxidation and the subsequent formation of Fe plaque has been observed in a number of submerged species in laboratory and field settings and has important biogeochemical consequences in the cycling of nutrients (Tessenow and Baynes 1978; Jaynes and Carpenter 1986). Fe plaque has been observed on the roots of *Isoetes lacustris* (Tessenow and Baynes 1975, 1978), *Littorella uniflora* (Christensen et al. 1994), *Lobelia dortmana* (Christensen and Wigand 1998), *Vallisneria americana* Michx., *Heterathera dubia* (Jacq.) MacM (St-Cyr and Campbell 1996), *Hydrilla verticillata* (Wigand et al. 1997), *Elodea canadensis* (Hupfer and Dollan 2003), *Eriocaulon aquaticum* (Urban et al. 2006), *Potamogeton crispus* (Hupfer and Dollan 2003; Mi et al. 2008), and *Cymodocea serrulata* (Povidisa et al. 2009).

Emergent plants tend to have a greater ability to oxidize the rhizosphere compared to submerged plants (Barko and Smart 1983). This may be due to a lack of direct contact with the atmosphere and less aerenchyma tissue in submerged plants (Barko and Smart 1983; Cronk and Fennessy 2001). However, Christensen et al. (1998) indicated that submerged plants appear to produce more root plaque compared to emergent plants. They reported that *L. uniflora* and *I. lacustris* produced about 3500 µmol g root DW^{-1} Fe plaque whereas other researchers have reported emergent species produce about 1500 µmol g root DW^{-1} Fe plaque (Chen et al. 1980; McLoughlin et al. 1985; Macfie and Crowder 1987; St-Cyr and Crowder 1989). Oxygen release by the roots of submerged macrophytes and the subsequent Fe oxidation in the vicinity of plant roots has implications for the bioavailability of Fe, Mn (Tessenow and

Baynes 1975) and P (Jaynes and Carpenter 1986; Christensen and Wigand 1998; Hupfer and Dollan 2003) which are known to have an affinity for Fe plaque. Fe and Mn oxyhydroxides precipitate in the oxidized root zone of submerged plants (Tessenow and Baynes 1978; Christensen et al. 1998; Christensen and Wigand 1998) and may play a dominant role in the cycling of P as well as trace elements in aquatic ecosystems (St-Cyr and Campbell 1996).

Phosphorus concentrations in the sediments and overlying water are influenced by seasonal and daily fluctuations in pH and dissolved oxygen concentrations (Barko and James 1998) and by the binding capacity of sediments and minerals of Ca, Fe, and Al (Clarke and Wharton 2001). In general, isoetid species are known to influence the sediment redox potential and available sediment P by releasing oxygen produced during photosynthesis into the surrounding sediments via their extensive root systems (Wium-Andersen and Andersen 1972; Jackson and Kalff 1993; Christensen et al. 1998). The binding of P to Fe plaque plays an important role in the bioavailability of P in lakes during the growing season (Hupfer and Dolan 2003). These plaques may serve as a temporary sink for P because during senescence and subsequent decrease in radial oxygen loss, chemically reduced conditions will result in the dissolution of these plaques and the release of P into the water column (Hupfer and Dolan 2003).

The release of dissolved nutrients to the water column, deoxygenation and accumulation of sediments are important biogeochemical consequences associated with macrophyte decomposition in freshwater ecosystems (Carpenter and Lodge 1986). Phosphorus taken up by plants from the sediments can be released into the water column by excretory and decay processes (Barko and Smart 1980). Submerged plants mobilize sediment phosphorus via root uptake and senescence of plant tissues (Barko and James 1998). The mobilization of phosphorus and its subsequent release into the water column represents internal loading of P which contributes to increased phytoplankton productivity (Landers 1979; Barko and Smart 1980). Numerous studies have documented P uptake and release into the water column by senescing tissues of submerged plants (Welch et al. 1979; Barko and Smart 1980 1981; Carignan and Kalff 1980; Smith and Adams 1986). In addition to P, metals such as Cd and Zn can be released into the water column from senescing tissues (McIntosh et al. 1978; Jackson 1998).

Element concentrations in waters and sediments may be influenced by the presence of submerged plants. Studies that compared vegetated and non-vegetated sediments reported that

exchangeable N and extractable P concentrations were decreased and K concentrations were increased within the root zone of live submerged vegetation (Carignan and Kalff 1985; Barko et al. 1988). Wigand et al. (2001) reported that nutrient pools of porewater associated with vegetated sediments were significantly lower compared to non-vegetated sediments. Trisal and Kaul (1983) reported decreased concentrations of N, P, K, and C in sediment associated with *Ceratophyllum demersum, Myriophyllum spicatum,* and *Potamogeton lucens* during the growing season and increased concentrations during months of plant dormancy. In the presence of submerged vegetation, porewater had higher Ca, Mg, Si, Fe, Cu, and Zn and lower P concentrations whereas the overlying water had lower Ca, Mg, and Si and higher P and Cu concentrations (Mi et al. 2008). These differences in concentrations may be due to plant uptake, root oxygen release, increased pH levels, precipitation, and mineral deposition on plant leaves (Mi et al. 2008). Lower N and P concentrations in the sediments during the growing season are probably due to plant uptake, and higher concentrations detected during plant dormancy are probably due to senescence and decay (Landers 1979; Carignan and Kalff 1980; Carignan 1985). Senescing and decomposing submerged macrophyte stands release substantial amounts of nutrients from the littoral to the pelagic zone resulting in nutrient enrichment of lakes (Lie 1979; Carignan 1980; Landers 1982).

Most biogeochemical studies involving submerged plants tend to focus on the cycling and uptake of P and N because these are the limiting nutrients in aquatic ecosystems (Prentki 1979; Barko and Smart 1980; Barko et al. 1991; Stephen et al. 1997; Hoagland et al. 2001; Horppila and Nurminen 2003; Hupfer and Dollan 2003; Rooney et al. 2003). Few studies have focused on the influences of submerged wetland plants on the biogeochemistry of multiple elements such as Al, Cd, Cr, Cu, Fe, Mn, Ni, and Zn (Jackson et al. 1994; St-Cyr et al. 1994; Mi et al. 2008). Some studies have shown that submerged plants can accumulate multiple elements such as Cu, Cd, Cs, Fe, Hg, Pb, and Zn from water (Kamal et al. 2004; Fritioff and Greger 2006; Pinder et al. 2006) and sediment (Barko and Smart 1980; Jackson et al. 1994) and have the potential for the phytoremediation of contaminated waters (Fritioff and Greger 2003).

1.4.2. Indicators of biogeochemical conditions

The most abundant macrophyte species in Minnesota include *Typha latifolia, Potamogeton pectinatus, Potamogeton richardsonii, Najas flexilis, Sagittaria latifolia, Scirpus acutus,* and *Ceratophyllum demersum* (Moyle 1945). The distribution and abundance of macrophytes are influenced

by local geology, hydrology, land use, and water and sediment chemistry (Thiébaut and Muller 1998; Koch 2001; Lougheed et al. 2001). Macrophytes have adaptations that are exclusive to conditions of their habitat (Cronk and Fennessy 2001). Moyle (1945) identified three groups of plants in Minnesota lakes, 1) macrophytes of soft-water lakes which occur most often in northeastern Minnesota, 2) macrophytes that inhabit the hard-water moraine lakes in central, northern and southern Minnesota and, 3) macrophytes of alkali or high sulfate lakes in the western and southwestern prairies of Minnesota. *Isoetes Braunii* Dur., *Nitella* spp., *and Lobelia dortmanna* are found in soft-water lakes, *Chara* spp., *Myriophyllum spicatum, and Najas flexilis* are found in hard-water lakes and *Ruppia occidentalis, Stuckenia pectinata* (formerly *Potamogeton pectinatus*), and *Zannichellia palustris* are found in alkali or sulfate-rich lakes (Moyle 1945; Mackie 2004).

Macrophytes have special adaptations or tolerances allowing them to attain optimum growth under particular chemical conditions (Moyle 1945). Some macrophytes are tolerant of nutrient-poor and/or clear conditions while some are tolerant of nutrient-rich and/or turbid conditions (Lougheed et al. 2001; Mackie 2004). Povidisa et al. (2009) reported that *Stuckenia pectinata*, *Ceratophyllum submersum,* and *Lemna* spp. were tolerant of nutrient enriched conditions while *Isoetes* spp. *Myriophyllum alterniflorum*, *Utricularia australis,* and *Nitella translucens* were tolerant of nutrient-poor systems and sensitive to eutrophication. Species reported to be tolerant of turbid conditions include *Potamogeotn pectinatus, Potamogeton crispus, Potamogeon foliosus, Potamogeton pusillus, Ceratophyllum demersum, Elodea candensis, Heteranthera dubia, Ranunculus longirostris, Butomus umbellatus,* and *Myriophyllum spicatum* (Stuckey 1989; van Dijk and van Vierssen 1991). Some macrophytes are tolerant of slightly brackish to saline conditions (Stewart and Kantrud 1972). Species such as *Potamogeton gramineus, P. pusillus, P. richardsonii,* and *Ceratophyllum demersum* were common in fresh and slightly brackish waters while *Chara* spp., *Stuckenia pectinata,* and *Ruppia maritima* were common in brackish and saline conditions (Stewart and Kantrud 1972). The distribution of macrophytes depends on water depth, water chemistry, sediment characteristics (organic versus inorganic), and competition among species for nutrients and space (Mackie 2004).

1.4.3. Role in lake turbidity

Submerged plants play an important role in the water quality of shallow lakes by stabilizing sediments, reducing the effects of benthivorous fish, wind and wave action (Borman et al. 1997; Eggers and Reed 1997; Scheffer 2004), and by inhibiting sediment erosion and suspension (Barko and James 1986). Minimized suspension of sediments affects the exchange of nutrients between the sediments and overlying water (Nurminen and Horppila 2009) and contributes to decreased water turbidity and internal loading of P to the water column (Horppila and Nurminen 2003). Benthivorous fish favor non-vegetated (uncovered) sediments in which they can easily forage and thus contribute to increased turbidity by disturbing these sediments (Faafeng and Mjelde 1998).

In shallow lakes, non-vegetated sediments are more vulnerable to disturbance and resuspension than plant-covered sediments (Faafeng and Mjelde 1998). Decreased sediment resuspension and internal P loading was observed within emergent and submerged macrophyte stands indicating that macrophytes in general contributed to decreased turbidity compared to non-vegetated areas (Horppila and Nurminen 2005). *Chara* beds play an important role in the cycling of nutrients in shallow lakes (Scheffer 2004). They serve as a sink for nutrients by trapping sediments and restricting the release of sediment bound nutrients (van den Berg et al. 1998; Kufel and Kufel 2002; Scheffer 2004). Significant sediment retention has also been observed in dense beds of *Callitriche cophocarpa* and *Elodea canadensis* (Sand-Jensen 1998). Submerged plants affect nutrient availability by influencing environmental conditions (pH and redox status), blocking nutrient access to phytoplankton by competing for nutrient uptake and restricting the release of nutrients from the sediments (Scheffer and Jeppesen 1998; Sondergaard and Moss 1998, Scheffer 2004). Submerged vegetation contributes to decreased P loading through the retention of P by calcite precipitates formed during the uptake of carbon from bicarbonate (Sondergaard and Moss 1998) and by Fe oxides formed as a result of root oxygen release and sediment oxygenation (Kufel and Kufel 2002). Faafeng and Mjelde (1998) found that shallow lakes with high densities of vegetation-covered sediments had higher water transparencies compared to lakes with lower vegetation cover. Water turbidity, concentration of suspended solids, and sediment resuspension rates were significantly lower within plant beds compared to open water (van den Berg et al. 1998; Horppila and Nurminen 2003).

Vegetation-dominated, clear-water shallow lakes typically favor high densities of zooplankton populations which use submerged vegetation as a refuge from predators (Sondergaard and Moss 1998; Scheffer 2004). Cronin et al. (2006) observed increased abundance of invertebrates in the presence of macrophytes probably due to the habitat and surfaces they provided. Higher grazing pressures are exerted by zooplankton (filter feeders) within compared to outside plant beds (Sondergaard and Moss 1998, van den Berg et al. 1998) thus controlling phytoplankton populations (Scheffer 2004). Loss of vegetation and large-bodied zooplankton results in increased phytoplankton biomass especially when nutrient levels are high (Scheffer 2004). Increased turbidity contributes to further loss of vegetation due to light limitations (Scheffer 2004). In the absence of vegetation, the turbid state is exacerbated due to increased phytoplankton biomass and sediment resuspension by wave action and benthivorous fish (Scheffer 2004). Nutrients released from the sediment and resuspended sediment particles contribute to increased turbidity (Scheffer 2004). Thus, the loss of aquatic vegetation causes a cascade of negative ecological effects in which the water quality and habitat for plants and animals are adversely affected.

Shallow lakes can switch from a clear, vegetation-dominated regime to a turbid phytoplankton-dominated regime and back again (Zimmer et al. 2009). These shifts can occur over a short period of time (within 1 year) or during longer transitions (several years) (Bayley et al. 2007). The mechanisms responsible for these shifts are not understood. The switch from a macrophyte-dominated to a turbid regime in shallow lakes may be due to increased and continuous nutrient loading (Scheffer and Jeppesen 1998). Scheffer and Jeppesen (1998) indicated that several mechanisms may be responsible for vegetation collapse and the shift from macrophyte-dominated to turbid regimes such as the introduction of carp, herbivory, herbicide applications, violent storms, and increased water levels. Blindow et al. (1998) indicated that fluctuations in both water levels and spring temperatures may contribute to mechanisms involved in the transition from a clear-water to turbid regime in shallow lakes. Hupfer and Dollan (2003) indicated that seasonal increased retention of P may support the switch to a vegetation-dominated, clear lake.

1.5. Conceptual model – a synthesis

Plants influence nutrient concentrations directly via uptake, or indirectly by altering the biogeochemistry of the sediment or water. Nutrient availability and the distribution of elements are

influenced by pH and redox gradients created at the root surface in the substrate due to the presence of plants or microbial activity (Figure 1.2). Root oxygen release, rhizosphere oxidation and subsequent Fe plaque formation have been observed in both emergent and submerged macrophyte species. Most studies have indicated that rhizosphere oxidation and Fe plaque play a key role in the cycling of nutrients in wet ecosystems. Microbial activity has been shown to play a role in the formation of Fe plaque and in the availability of metals in the rhizosphere of emergent plants. However, literature on the role of microbial activity in Fe plaque formation and element availability in submerged plants is lacking.

The role of the rhizosphere in the biogeochemistry of multiple elements is a crucial area of study that is important in understanding the processes controlling their total and bioavailable concentrations in wet ecosystems. Most rhizosphere studies have focused on dryland plants such as *Brassica napus* (rape) (Kuchenbuch & Jungk 1982), *Hordeum vulgare* L. var. Dorirumugi (barley) (Youssef and Chino 1989b; Youssef & Chino 1991; Højberg and Sørensen 1993), *Glycine max* L. var. Haweye (soybean) (Youssef & Chino 1991), *Brassica napus* L. cv. Sprinter (canola) (Wenzel et al. 2001), *Triticum aestivum* L. (wheat) (Wang et al. 2001; Wang et al. 2002), and *Lycopersicon esculentum* L. (tomato) (Cornu et al. 2007). The few rhizosphere studies involving wetland plants have focused on rhizosphere oxidation, Fe plaque or single element concentrations in the rhizosphere (Armstrong et al. 1992; Armstrong and Armstrong 2001; Begg et al. 1994, Kirk and Bajita 1995; Otte et al. 1995; Doyle and Otte 1997; Jacob and Otte 2004b), but none have investigated the distribution of multiple elements across the rhizosphere. The research presented here introduces a novel approach to explaining metal mobility in the rhizosphere of wetland plants, and may underpin the proposed theory of why many wetland plants display tolerance to high concentrations of metals.

Most biogeochemical studies involving submerged macrophytes have focused on their impact on nutrient cycles in particular N, P, and C (Barko and Smart 1980; Stephen et al. 1997; Hoagland et al. 2001; Horppila and Nurminen 2003; Rooney et al. 2003). Few studies have focused on the effects of submerged plants on concentrations of other elements in the sediment or water (Brix and Lyngby 1983; Jackson et al. 1994; St-Cyr et al. 1994, Mi et al. 2008). This study will determine the biogeochemical effects of emergent and submerged plants by examining pH, redox potential and the distribution of multiple elements in the root zone compared to bulk zone. This research is important because it will

increase our knowledge of the role of wetland vegetation in the biogeochemical cycling of elements in wet ecosystems. This research will also increase our knowledge of ecosystem dynamics and gather valuable information for the management of wetlands.

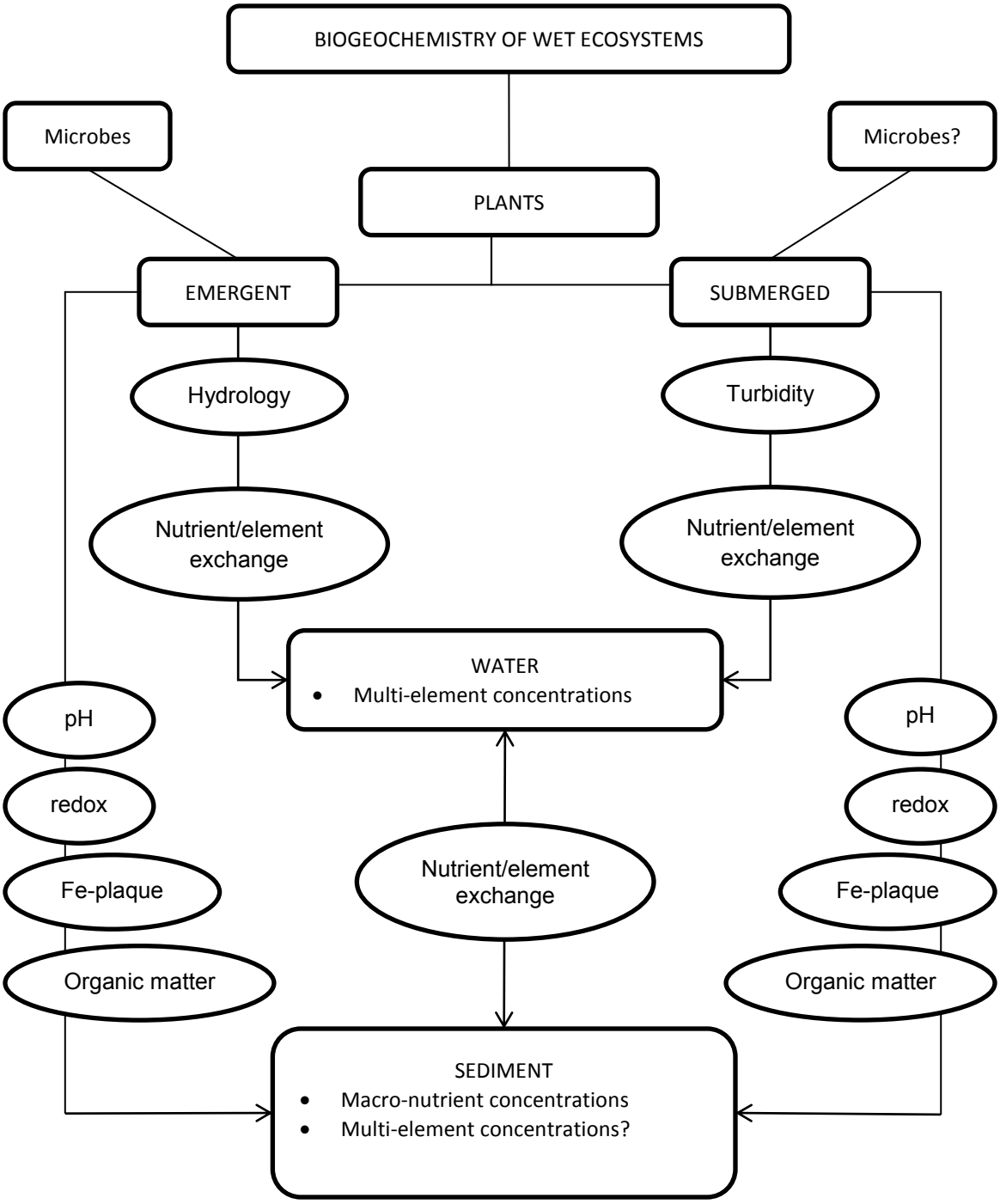

Figure 1.2. Conceptual diagram of the influences of submerged and emergent plants on the sediments and water as reported in the literature (rectangles indicate components in wet ecosystems, ovals indicate influencing factors, question mark indicates gaps or uncertainty in the literature).

1.6. Objectives of this research

The main objectives of my research were to examine the differences in element concentrations in 1) the root zone soil of wetland plants under different moisture conditions, and 2) waters and sediments of shallow lakes of varying plant abundance and turbidity. I also aimed to identify the important factors that influenced the distribution of elements in the root zone of wetland plants and in the waters and sediments of shallow lakes.

The following hypotheses were proposed for the root zone studies:

- Elements accumulate in the root zone more under flooded (reduced) compared to non-flooded (oxidized) conditions.
- This accumulation of elements in the root zone leads to greater exposure and uptake of these elements by wetland plants.

The following hypotheses were proposed for the shallow lakes study:

- High plant abundance in shallow lakes will coincide with low turbidities.
- Low plant abundance will be associated with high multi-element concentrations in the water and sediment.
- Lakes with high multi-element concentrations will be associated with agriculture-dominated watersheds.

Root zone studies were carried out in the greenhouse using the wetland plants *Typha angustifolia* and *Rumex crispus* to determine differences in element concentrations in the root and bulk zone of flooded and non-flooded soil. These species were chosen because of their ability to grow well in a wide range of moisture conditions from very wet to somewhat dry. A third rhizosphere experiment was carried out to determine if similar patterns of element accumulation in the root zone occurred under field conditions using *Triglochin maritima* from soils of varying moisture content. Most root zone studies carried out by other researchers used dryland plants such as *Brassica napus*, *Hordeum vulgare* L. var. Dorirumugi (Kuchenbuch and Jungk 1982; Youssef and Chino 1989b), *Glycine max* L. var. Haweye, *Hordeum vulgare* L. var. Dorirumugi (Youssef and Chino 1991), *Triticum aestivum* L. (Wang et al. 2001, 2002), and the wetland plant *Oryza sativa* L. (Begg et al. 1994; Kirk and Bajita 1995). Neither of these

studies compared element concentrations across the rhizosphere under flooded and non-flooded conditions and this is what my root zone studies intended to accomplish.

The shallow lake studies involved 44 shallow lakes in Minnesota from which I gathered data on the water and sediment chemistry, and the macrophyte abundance and community composition. This study was carried out to determine 1) whether or not different landscape variables (e.g. lake watershed area, open water area, percent agriculture, percent woodland) were associated with macrophyte abundance and community composition, and water and sediment chemistry of shallow lakes and; 2) if macrophyte community composition was related to the water and sediment chemistry of the shallow lakes. Previous studies have focused mostly on the cycling of phosphorus in these systems (Barko and Smart 1980; Stephen et al. 1997; Hoagland et al. 2001) while few studies have focused on other elements such as Al, Cd, Cr, Cu, Fe, Mn, Ni and Zn (Jackson et al. 1994; St-Cyr et al. 1994; Mi et al. 2008). My study examined the concentrations, in both water and sediments, of multiple elements such as As, Ba, Ca, Cs, Dy, Nd, Ni, Mg, Mn, Na, S, Sc, Sm, Sr, V, Y, Zn, and Zr and their relationships with macrophyte abundance and landscape variables.

1.7. Dissertation outline

The first chapter consists of a general introduction which reviews the relevant literature. The following four chapters include the methods and results of my greenhouse and field studies. The concluding chapter includes the general discussion and final conclusions associated with my findings.

Section 1 – root zone studies

- **Chapter 2 – Multi-element accumulation near *Rumex crispus* roots under wetland and dryland conditions (published in Environmental Pollution (2010) 158: 1834-1841).**
  This greenhouse study examined the differences in soil redox status, pH and multi-element concentrations of soil in the vicinity of plant roots (rhizosphere/root zone) to the soil furthest from the influence of plant roots (bulk zone) in both flooded and non-flooded soil under *Rumex crispus* plants. The possible mechanisms for these differences were discussed.
- **Chapter 3 – *Typha angustifolia* is exposed to multiple elements under wetland versus dryland conditions (published in Environmental and Experimental Botany (2011) 72: 232-241).**

This greenhouse study examined the differences in redox potential, pH and multi-element concentrations of soil from the root zone to the bulk zone below *Typha angustifolia* under flooded and non-flooded conditions. The possible mechanisms for these differences were discussed.

- **Chapter 4 – Multi-element accumulation in soils along a moisture gradient associated with the salt marsh plant *Triglochin maritima*.**

  This study was conducted in the field at Kellys Slough, North Dakota to determine differences in multi-element concentrations in root and bulk zone soils of varying moisture content below *Triglochin maritima*. The possible mechanisms for my findings were discussed.

Section 2 – shallow lake studies

- **Chapter 5 – Shallow lakes: variations in aquatic vegetation and biogeochemistry.**

  This study was carried out on 44 shallow lakes in Minnesota to determine 1) if the composition of macrophyte communities is related to lake turbidity and multi-element concentrations of the water and sediment and, 2) if predominant land use of the lake watershed was related to the macrophyte community composition, water and sediment chemistry.

SECTION 1 – ROOT ZONE STUDIES

CHAPTER 2. MULTI-ELEMENT ACCUMULATION NEAR RUMEX CRISPUS ROOTS UNDER WETLAND AND DRYLAND CONDITIONS[1]

2.1. Abstract

Rumex crispus was grown under wet and dry conditions in two-chamber columns such that the roots were confined to one chamber by a 21 µm nylon mesh, thus creating a soil-root interface ('rhizoplane'). Element concentrations at 3 mm intervals below the 'rhizoplane' were measured. The hypothesis was that metals accumulate near plant roots more under wetland than dryland conditions. Patterns in element distribution were different between the treatments. Under dryland conditions Al, Ba, Cu, Cr, Fe, K, La, Mg, Na, Sr, V, Y and Zn accumulated in soil closest to the roots, above the 'rhizoplane', only. Under wetland conditions Al, Fe, Cr, K, V and Zn accumulated above as well as 3 mm below the 'rhizoplane' whereas La, Sr and Y accumulated 3 mm below the 'rhizoplane' only. Plants on average produced 1.5 times more biomass and element uptake was 2.5 times greater under wetland compared to dryland conditions.

2.2. Introduction

In contrast to 'dryland' plants, many wetland plants display constitutive tolerance to elevated metal concentrations in the soil, meaning that they are tolerant to metals regardless of the metal concentrations at their location of origin (McNaughton et al. 1974; Ye et al. 1997a; McCabe et al. 2001; Matthews et al. 2004). Otte and co-workers (McCabe et al. 2001; Otte et al. 2004) suggested that the development of metal tolerance in wetland plants may be attributed to the biogeochemistry of wetland substrates. They proposed that the formation of Fe plaque deposits in the vicinity of wetland plant roots contributes to higher metal mobility and thus greater metal accumulation near plant roots. As a consequence wetland plants have been exposed to higher concentrations than dryland plants over the course of evolution, which favored selection for constitutive metal tolerance.

The bioavailability and mobility of chemical elements are influenced by changes in soil properties surrounding living plant roots including pH, organic content, cation exchange capacity, redox potential

[1] The material in this chapter was co-authored by La Toya Kissoon, Donna Jacob and Marinus Otte (published in Environmental Pollution 158: 1834-1841). La Toya Kissoon had the primary responsibility for the experimental design and collection and analysis of samples in the greenhouse experiments. La Toya Kissoon was the primary developer of the conclusions, drafted, and revised all versions of this chapter. Donna Jacob and Marinus Otte provided guidance, comments, and suggestions, and served as proofreaders.

(Eh), moisture status and temperature (Davies 1994; Alloway 1995; Jacob and Otte 2003). Plant roots influence the environment directly adjacent to them in order to obtain access to nutrients, in particular the essential macro- and micro-nutrients (Marschner et al. 1986; Mehra and Farago 1994; Jungk 2002; Neumann and Römheld 2002; Inderjit and Weston 2003). Wetland plants can modify redox conditions, pH and organic matter of the soil or sediment and thus affect the mobility (Wright and Otte 1999) and chemical speciation of metals in waterlogged environments (Jacob and Otte 2003). Knowledge of the biogeochemistry of metals and the processes affecting their mobility and trophic transfer is important, (1) because of their potential ecotoxicological effects, (2) because recent research has shown that less-studied elements such as the rare earth elements may be beneficial to plant growth (Chang 1991; Hong 2002), and (3) because the increasing demand for less-studied metals, such as the rare earth elements, for development of new technologies and their subsequent potential environmental impacts.

Hinsinger and Courchesne (2008) emphasize that rhizosphere studies play a key role in research on the biogeochemistry of elements. Most rhizosphere studies have used dryland plants such as *Brassica napus* (rape) (Kuchenbuch and Jungk 1982), *Hordeum vulgare* L. var. Dorirumugi (barley) (Youssef and Chino 1989b; Youssef and Chino 1991) and *Glycine max* (soybean) (Youssef and Chino 1991). The few studies using wetland plants include *Oryza sativa* L. (rice) (Begg et al. 1994; Kirk and Bajita 1995), *Halimione portulacoides* (sea purslane), *Spartina townsendii* (cord grass) (Doyle and Otte 1997), *Spartina anglica* (common cordgrass) (Otte et al. 1995) and *T. latifolia* (narrow leaf cattail) (Jacob and Otte 2004b). But none of these studies have compared element concentrations across the rhizosphere under flooded (wetland) and non-flooded (dryland) conditions. The aim of this study was to investigate the hypothesis that metals accumulate in the direction of plant roots in flooded soil more than in non-flooded soil and that this would lead to greater uptake of those metals in plants.

2.3. Materials and methods

2.3.1. Seed collection and soil preparation

Mature *Rumex crispus* fruits were collected in West Fargo, North Dakota (N 46° 52’ 30.7”, W 96° 58’ 08.7”) in October, 2006, and stored at 5 °C (Baskin and Baskin 1978) for 5 months. The seeds were washed in distilled water after sepal removal and germinated on moistened sterile sand for 2 weeks in an incubator (14 hour photoperiod, 25 °C). The seedlings were planted in 5 cm potting soil (Sun Gro

Sunshine LG3 germinating mix with vermiculite) and allowed to develop roots for about 6 weeks in a greenhouse (16 hour photoperiod, 3.03 log lumen m^{-2} (mean day time), 20-30 °C).

Local farmland soil was obtained from near Casselton in Cass County, North Dakota (N 46° 50' 51.4", W 97° 09' 20.0", 280 m). This soil was selected because it was more representative of natural conditions compared to substrates such as potting soil or sand. The soil was determined to be silty clay with 4.1% organic matter, bulk density of 1.04 g cm^{-3} and particle size 5.8% sand, 47.9% silt and 46.3% clay (North Dakota State University Soil Testing Lab). The soil was oven-dried (60 °C) to constant weight, crushed and passed through a 2 mm screen. The soil was amended with sterile sand (Quikrete Premium Play Sand) at a ratio of 3:1 soil to sand (by weight) to aid root penetration in the clay-rich soil.

2.3.2. Column apparatus assembly

The columns consisted of 2 sections of soil which were separated by 21 µm nylon mesh (Nylon 21/17, Miami Aqua-culture, and Inc.). The mesh restricted root growth to the upper section of the column while allowing diffusion of nutrients and water throughout the soil. The mesh was considered the rhizoplane because it separated the roots from the soil in the lower section of the column. This design enabled soil sampling in two different regions of the soil column; 1) above the rhizoplane (upper section of column) and 2) below the rhizoplane at distinct distances (lower section of column) (Figure 2.1).

The columns consisted of PVC pipe (9 cm diameter) cut into two sections measuring 10 cm (lower section) and 6 cm (upper section). The mesh was attached to one end of the 6 cm section. The two-chamber column was assembled by securing the 10 cm section to the 6 cm section with 5-cm wide waterproof Duct Tape (Nashua® Tape) with the nylon mesh in between. Both sections were filled with the prepared, homogenized soil/sand mixture, the lower section with 600 g to which 300 ml of distilled water was added evenly and the upper section with 300 g to which 150 ml of distilled water was also added evenly. To prevent soil loss from the column and still allow water movement, a 2 mm mesh was secured to the bottom end. The lower section of the column was inverted, filled with soil and secured with the 2 mm mesh before soil was added to the upper section to ensure contact between the bottom soil and the rhizoplane. The seedlings were removed from the potting soil, washed gently with distilled water and planted in the upper section of the column (1 seedling per column).

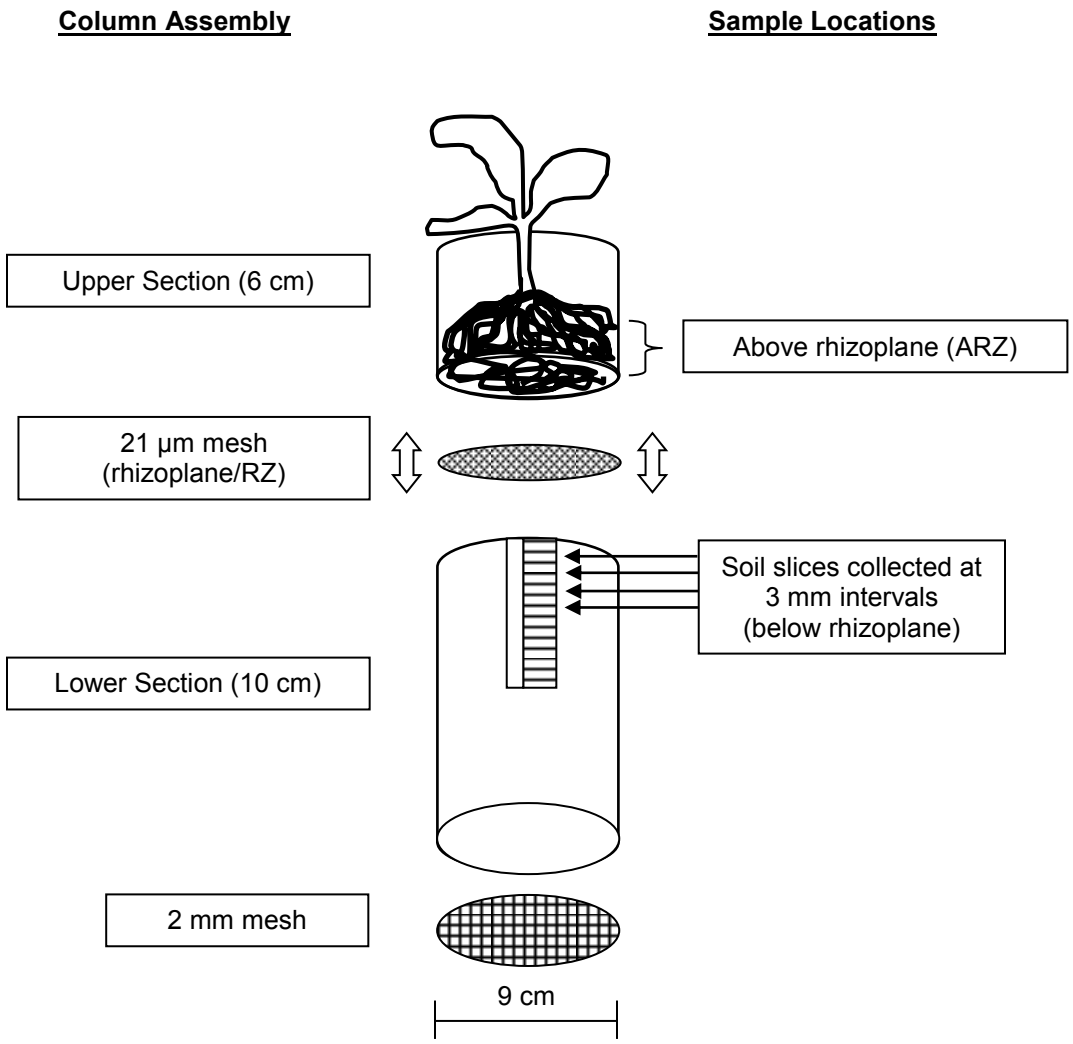

Figure 2.1. Column Apparatus for *Rumex crispus* experiment showing column components on the left and sampling locations on the right.

2.3.3. Soil flooding and monitoring moisture

This experiment was carried out in a greenhouse and the treatments arranged using complete randomized design. The columns were placed into 2 L containers. The plants were allowed to establish for two weeks prior to beginning the moisture treatments. The flooded treatment (n = 10) consisted of adding distilled water to the containers such that the surface of the soil was below 5 mm of water. The non-flooded treatment consisted of columns (n = 10) that received water as needed according to their wilting point weights (see below).

Both treatments were monitored daily to determine when water addition was necessary. Sterilized cotton wicks with one end in sealed bottles of distilled water and the other end inserted into the

soil above the rhizoplane were used continuously to maintain saturation of the flooded treatment. The same approach was used for the non-flooded treatment when water addition was necessary. The wicks were inserted in the soil in the upper section of the column and spread between the rhizoplane and the soil above the rhizoplane with any exposed portion of the wick wrapped securely with plastic. Water levels for the flooded treatment were restored when necessary to the marked lines of the initial water level (water was added to the larger container outside the column). The weights and plant height of the non-flooded treatments were monitored daily to determine if they were within 1 g of the wilting point weight. The wilting point had been determined previously by saturating the soil of four *R. crispus* plants growing in columns and then allowing the soil to dry. The weights of the columns containing plant and soil when the plant showed signs of wilting were determined. These weights were used to calculate an estimate of the weight of a column containing wilted plant and soil. *R. crispus* plants grown for 8 weeks were assessed for their height and weight which was used to obtain a linear equation with which to make adjustments when calculating the soil weight in the columns.

2.3.4. Soil sampling – pH and redox potential measurements

After 13 weeks, soil samples were collected from columns selected in random order. Each column was cut carefully to separate the upper and lower sections. The plant and soil in the upper section of the column were removed and soil was shaken from the roots. The soil remaining on the roots was collected and considered 'above rhizoplane' soil. The soil immediately below the nylon mesh (rhizoplane) was sampled using 60 ml syringes with the tips removed so they became small soil corers (2.5 cm diameter). The column was inverted and 3 syringes were inserted into the soil at the center, away from the column edges. The soil was extruded from each syringe in 3 mm intervals, sliced carefully and retained for analysis. Seven samples were collected from each syringe at 3, 6, 9, 12, 15, 18 and 21 mm intervals. Samples from the three syringes were pooled for each increment to obtain enough soil (at least 3 g) for analysis. Immediately upon obtaining a sample, pH and Eh were measured using a VWR Symphony SP90M5 Handheld Multimeter. Approximately 1 g of fresh soil sample was used to determine the soil pH in a 1:2 soil:water ratio (Gavlak et al. 2003). A soil paste (about 500 mg fresh soil sample and 3 ml water) was used to measure the Eh (Patrick et al. 1996).

2.3.5. Ferrous iron (Fe^{2+}) concentration and multi-element analysis of soil and plants

Fe^{2+} concentration was determined using a method modified from Roden and Wetzel (1996). Fe^{2+} standards were prepared from a stock solution containing 100 mg L^{-1} $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ in 1% (v/v) 6 M HCl. A fresh soil sample of known weight (about 0.5 g), was immediately transferred to 5 ml of 0.5 M HCl and extraction allowed overnight. The extraction was then filtered (0.45 µm pressure filter, Pall Corporation Supor®-450), diluted (flooded samples – 1:40 dilution, non-flooded samples – 1:10 dilution) and 0.25 ml of the diluted sample or of standard was added to 1.25 ml of FerroZine solution (1% wt/wt FerroZine in 50 mM HEPES buffer). After about 5 minutes, the absorbance was measured using a Helios Gamma UV-Vis Spectrophotometer at λ=562.

The remaining soil was oven-dried (60 ^{o}C) until constant weight, crushed using mortar and pestle and homogenized. The samples were then analyzed for multiple elements (37 elements) via Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) by a commercial laboratory (Activation Laboratories, Ltd, Analysis by Aqua Regia Extraction with ICP/OES finish). Method detection limits in mg kg^{-1} were as follows; Ag 0.2; Al 100; As 2; B 10; Ba 10; Be 0.5; Bi 2; Ca 100; Cd 0.5; Co 1; Cr 1; Cu 1; Fe 100; Ga 10; Hg 1; K 100; La 10; Mg 100; Mn 5; Mo 1; Na 10; Ni 6; P 10; Pb 7; S 100; Sb 2; Sc 1; Sr 1; Te 1; Ti 100; Tl 2; U 10; V 1; W 10; Y 1; Zn 2 and Zr 1 (Accredited Laboratory; ISO/IEC 17025:2005).

The plants were washed gently in distilled water, separated into aboveground and belowground material, oven-dried (60 ºC) until constant weight, crushed and homogenized. A known amount of this plant material (approximately 250 mg) was digested in 5 ml HNO_3 and 5 ml distilled water in a MARS Xpress Microwave Digester (1600W, 100% Power, ramped to 200 ºC). The digested samples were cooled and analyzed in our laboratory for multiple elements with a Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP). A continuing control verification (CCV) was done after every 10 samples to check that variability was within 10% for Al and Ca while all other elements were monitored. These samples were also diluted (1:100 in 5% HNO_3) and analyzed for the same 32 elements using the ICP-OES. Method detection limits in mg/kg for these elements were as follows: Ag 0.03; Al 22; As 1; B 0.5; Ba 0.006; Be 0.005; Ca 0; Cd 0.13; Ce

0.3; Co 0.14; Cr 0.1; Cu 0.1; Fe 0.1; K 0; Li 0.06; Mg 0.9; Mn 0.02; Mo 0.3; Na; 0.2; Ni 0.4; P 2; Pb 0.5; S 1.7; Sb 0.2; Si 0.07; Sn 1.4; Sr 0.06; Ti 0.02; Tl 1.2; V 0.4; Zn 0.2; Zr 0.002.

2.3.6. Statistical analysis

Data for concentrations were $\log_{10}$ transformed before statistical analysis to obtain normal distribution and homogeneity of variance. Significance of differences (probability) was determined by a General Linear Model (two-way ANOVA, $p<0.05$) and Multiple Comparison tests by the Tukey Method ($p<0.01$) using Minitab statistical software (Minitab® 15 ©2006 Minitab Inc.). Data for soil pH, Eh and element concentrations were analyzed for the following significant differences: 1) between moisture treatments regardless of sampling interval, 2) between sampling intervals regardless of moisture treatment , 3) between moisture treatments for the equivalent sampling intervals, 4) between sampling intervals of the flooded treatment, and 5) between sampling intervals of the non-flooded treatment. To test for relationships between element concentrations, pH and Eh, Pearson correlations and *P* values were calculated using Minitab. Here we consider only correlations with $r \geq 0.707$, that is, those correlations explaining 50% or more of the variation (McClave and Sincich, 2006).

2.4. Results

2.4.1 Soil

The results for pH, Eh and element concentrations in the soil focus on four sampling intervals; above the rhizoplane (ARZ), the 3, 6 and 9-21 mm intervals. Within each moisture treatment, concentrations at the sampling intervals 9, 12, 15, 18 and 21 mm were not significantly different from each other for any of the elements and so were pooled for statistical comparisons.

2.4.1.1. Soil pH

There were significant differences in soil pH between moisture treatments and between sampling intervals (Table 2.1). There were also significant interactions for pH between the moisture treatments and the sampling intervals, indicating that patterns in pH across intervals were not the same in flooded compared to non-flooded treatments. The soil pH was significantly higher in the non-flooded treatment compared to the flooded treatment for all sampling intervals (Figure 2.2). For each moisture treatment separately, the non-flooded soil showed no significant variations in pH above or below the rhizoplane but

the flooded soil showed significantly lower pH above the rhizoplane compared to sampling intervals below.

Table 2.1. Significance of differences (probability) in soil pH, Eh and element concentrations between the moisture treatments (flooded and non-flooded) and between sampling intervals (above rhizoplane (ARZ), 3, 6 and 9-21 mm below rhizoplane) as determined by Two-Way ANOVA (NS indicates non-significance; $p<0.05$, $n = 10$).

	Source of Variation		
	A. Moisture Treatments	B. Sampling Intervals	Interaction (A x B)
		p-value	
pH	0.000	0.000	0.001
Eh	0.000	0.000	0.000
Al	0.01	0.000	0.000
Ba	0.044	0.000	0.021
Cr	0.001	0.000	0.000
Cu	0.014	0.000	0.003
Fe	0.004	0.000	0.000
Fe^{2+}	0.000	0.000	0.000
K	0.002	0.000	0.000
La	0.005	0.000	0.020
Mg	NS	0.000	0.001
Na	0.000	0.000	0.000
Sr	0.000	0.000	0.000
V	0.003	0.000	0.000
Y	0.001	0.000	0.007
Zn	0.012	0.000	0.001

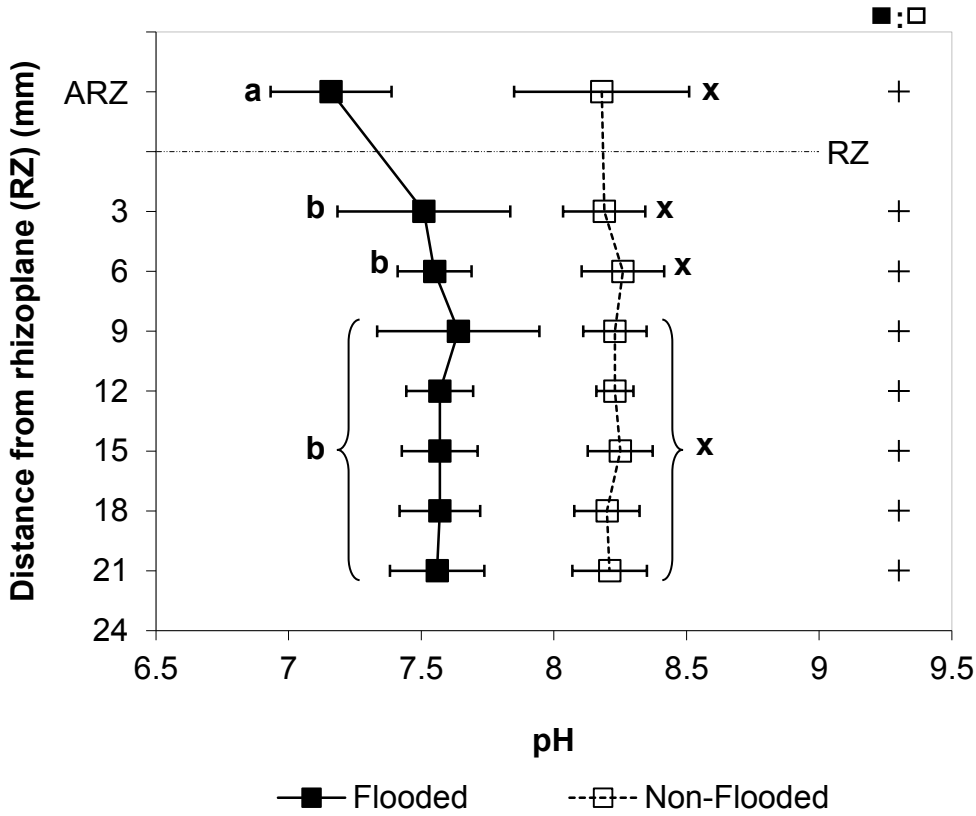

Figure 2.2. Mean soil pH in the moisture treatments (flooded; non-flooded) for the different sampling intervals (above rhizoplane (ARZ) and below rhizoplane) under Rumex crispus (RZ = rhizoplane, n = 10, except for 9-21 mm interval; n = 50). Different letters within each moisture treatment indicates significant variation between sampling intervals (ARZ, 3 , 6 and 9-21 mm) at $p<0.01$. The results of the comparison between moisture treatments for each interval appears to the right of the graph (the small black and white shapes at the top right of the graph indicate this comparison (e.g. ■:□ means filled squares compared to open squares); + indicates significant differences and – indicates no significant differences between moisture treatments, $p<0.01$).

2.4.1.2. Redox potential and Fe oxidation

Significant differences for Eh and Fe^{2+} occurred between moisture treatments and between sampling intervals (Table 2.1). There were also significant interactions for Eh and Fe^{2+} between the moisture treatments and the sampling intervals. Eh was significantly higher in the non-flooded treatment compared to the flooded treatment for all sampling intervals except for the soil above the rhizoplane (Figure 2.3). In the non-flooded treatment, the Eh above the rhizoplane was significantly lower than soil 9-21 mm below the rhizoplane. The flooded treatment showed significantly higher Eh above the rhizoplane than the sampling intervals below the rhizoplane. For these intervals below the rhizoplane, the soil Eh showed no significant differences.

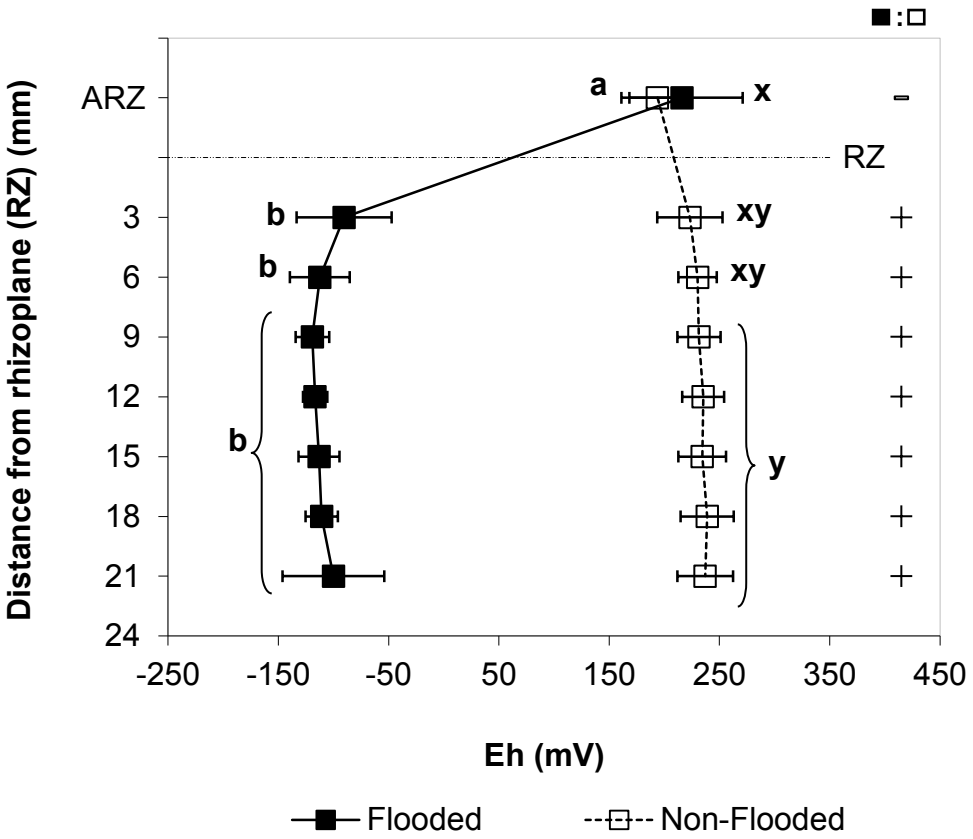

Figure 2.3. Mean Eh in the moisture treatments and statistical differences as in Figure 2.2.

Concentrations of Fe^{2+} were significantly higher in the flooded treatment compared to the non-flooded treatment for all the sampling intervals (Figure 2.4). The non-flooded treatment showed no significant differences in the Fe^{2+} concentrations between sampling intervals. However, in the flooded treatment the Fe^{2+} concentrations were significantly lower above the rhizoplane than the sampling intervals below the rhizoplane which did not vary significantly from each other. In the non-flooded treatment, no precipitation of Fe-oxyhydroxide (reddish-brown precipitate) was observed anywhere in the soil column. However, in the flooded treatment a reddish-brown precipitate was clearly visible on the plant roots, rhizoplane and soil above the rhizoplane.

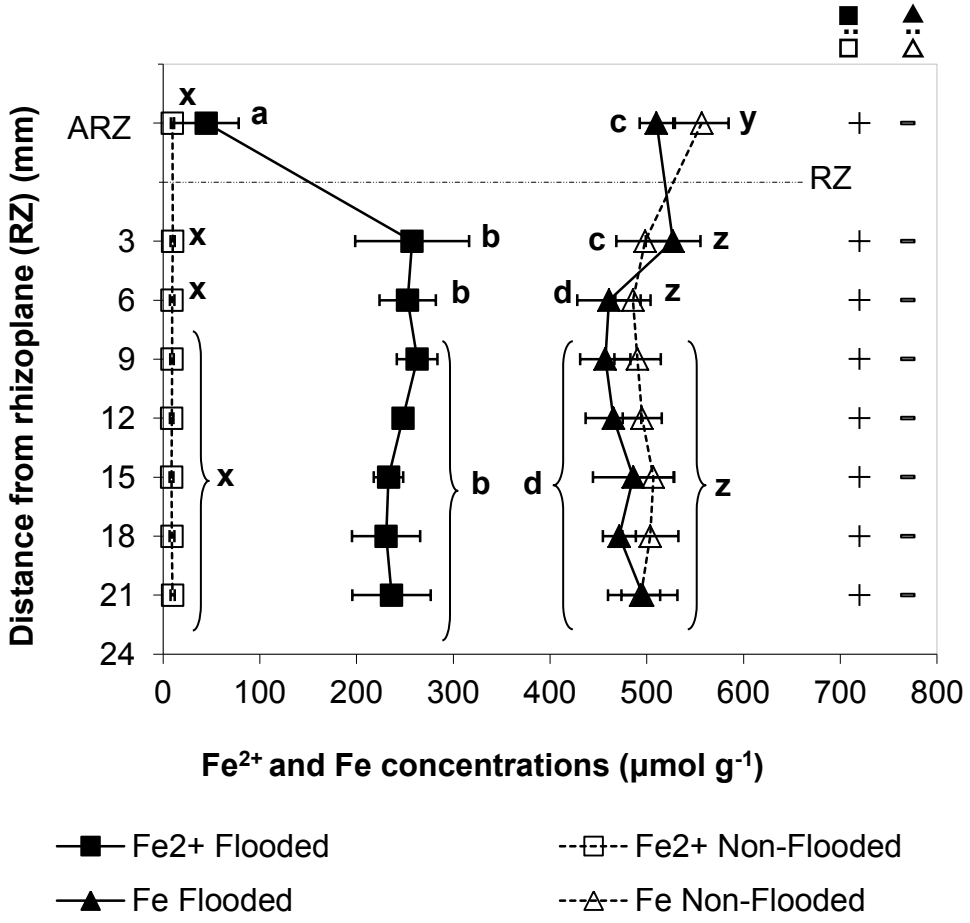

Figure 2.4. Mean Fe and Fe^{2+} concentrations (µmol g^{-1} dry soil) in the moisture treatments and statistical differences as in Figure 2.2.

2.4.1.3. Multiple element analysis

Element concentrations were at or below detection limits for Ag, As, B, Be, Cd, Mo, Bi, Ga, Hg, Pb, Sb, S, Sc, Te, Ti, Tl, U, W and Zr. A few other elements were easily detectable but showed no significant variation – for these the mean element concentrations ± standard deviation in µmol g^{-1} dry soil, averaged for all samples were calculated, as follows: Ca (465±38), Co (0.21±0.02), Mn (13.1±2.8), Ni (0.6±0.1), and P (14.1±1.5). These elements will not be discussed further.

Significant variation was observed for Al, Ba, Cr, Cu, Fe, K, La, Mg, Na, Sr, V, Y and Zn concentrations between sampling intervals and (except for Mg) between moisture treatments (Table 2.1). In addition, significant interactions were found between the moisture treatments and sampling intervals. The non-flooded compared to the flooded treatment had significantly higher Al, Cr, Cu, K, Sr, V and Y concentrations above the rhizoplane and significantly higher Al, Cr, Fe, K, Sr, V and Zn concentrations at

the 9-21 mm interval (Table 2.2). Na concentrations were significantly higher in the flooded compared to the non-flooded treatment at the 3, 6 and 9-21 mm intervals.

In the non-flooded treatment, Al, Cr, Fe, K, La, Mg, Na, Sr, and V concentrations were significantly higher above the rhizoplane than below the rhizoplane (Table 2.2, Figure 2.4 and Figure 2.5). Concentrations of Ba, Cu, Y and Zn were significantly higher above the rhizoplane compared to the 6 and 9-21 mm interval. Na concentrations at the 9-21 mm interval were significantly lower than above the rhizoplane but higher than the 3 mm interval.

The flooded treatment showed significantly higher concentrations of Al, Fe, K, and V above the rhizoplane compared to the 6 mm and 9-21 mm intervals (Table 2.2, Figure 2.4). Above the rhizoplane Cr concentrations were significantly higher than the 6 mm interval whereas Zn concentrations were significantly higher than the 9-21 mm interval (Figure 2.5). Concentrations of Al, Fe, Cr, K, La, Sr, V and Zn were significantly higher at the 3 mm interval than at the 6 mm interval. Ba, Mg and Na showed no significant variation between intervals of the flooded treatment.

Correlation analysis of the data was carried out to ascertain possible underlying patterns regardless of the treatments. Some elements that varied significantly between the sampling intervals for the moisture treatments (Al, Cr, Fe, K, La, Sr, V, Y and Zn) correlated significantly with each other (Table 2.3). Ba correlated significantly with Al, K and V. Fe^{2+} correlated significantly with Eh and pH. Covariate analysis showed Fe to be a significant covariate with other elements (Table 2.4).

2.4.2. Plants

In both treatments, *R. crispus* formed a dense mat of roots on the surface of the mesh, the rhizoplane. Root growth in the flooded treatment was also densely distributed throughout the soil, but in the non-flooded treatment the roots were not as widespread. Plant biomass (mean ± standard deviation) was significantly higher ($p<0.001$) for the flooded treatment (289±39 mg live aboveground biomass, 1569±236 mg belowground biomass) compared to the non-flooded treatment (99±33 mg live aboveground biomass, 1067±155 mg belowground biomass). Differences in dead aboveground biomass (120±33 mg) were not significant between treatments. For the element analysis of the 32 elements in the plant material, not all yielded results suitable for statistical analysis. Element concentrations in some plant material were at or below the detection limits (Ag, As, Be, Cd, Ce, Co, Mo, Pb, Sb, Sn, Tl, V, Zr) or

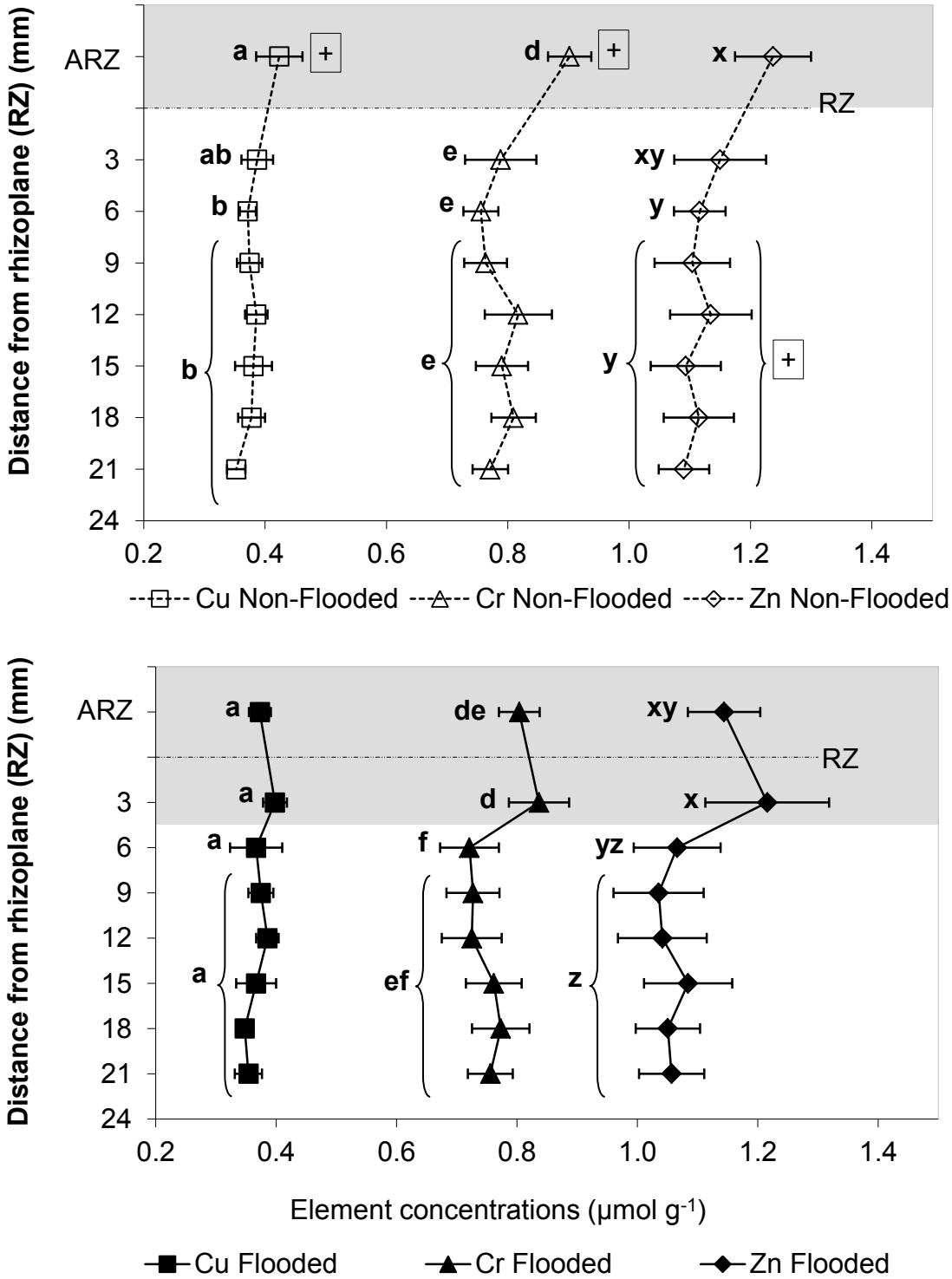

Figure 2.5. Mean Cu, Cr and Zn concentrations (µmol g^{-1} dry soil) in the moisture treatments and statistical differences as in Figure 2.2. Where there is a box containing a plus sign (+) to the right of a point marking an interval in the non-flooded treatment indicates significant differences (p<0.01) between that interval and the corresponding interval in the flooded treatment (no boxes indicate no significant differences). The shaded regions of the graph represent zones of element accumulation.

Table 2.2. Mean element concentrations (μmol g^{-1} dry soil) in the moisture treatments for the different sampling intervals (mean ± standard deviation, n = 10, except for * *n* = 50, different letters within each moisture treatment indicates significant variation between sampling intervals at *p*<0.01, significant differences between moisture treatments for equivalent intervals is marked by † before the significantly higher value for a particular interval).

Element	Element concentrations															
	Flooded								Non-Flooded							
	ARZ		3 mm		6 mm		*9-21 mm		ARZ		3 mm		6 mm		*9-21mm	
Al	922±36	a	954±76	a	797±61	b	812±51	b	†1033±65	x	867±53	y	841±31	y	†858±50	y
Ba	1.28±0.08	a	1.33±0.10	a	1.20±0.16	a	1.18±0.11	a	1.47±0.16	x	1.27±0.20	xy	1.21±0.08	y	1.25±0.13	y
K	104±5	a	111±8	a	92±9	b	95±7	b	†119±7.9	x	102±7.1	y	98.2±5.0	y	†101±6.4	y
La	0.17±0.02	ab	0.17±0.01	a	0.15±0.01	b	0.16±0.01	b	0.19±0.01	x	0.17±0.01	y	0.16±0.01	y	0.16±0.01	y
Mg	543±19	a	568±30	a	538±39	a	537±34	a	587±40	x	537±18	y	517±23	y	538±25	y
Na	27.0±3.4	a	†25.2±3.4	a	†23.5±2.3	a	†24.7±2.6	a	25.7±1.4	x	16.5±1.8	y	19.1±2.3	yz	21.1±2.1	z
Sr	0.50±0.02	ab	0.53±0.03	a	0.48±0.05	b	0.49±0.0	b	†0.56±0.05	x	0.51±0.02	y	0.50±0.02	y	†0.51±0.02	y
V	1.93±0.06	a	1.99±0.12	a	1.70±0.15	b	1.75±0.12	b	†2.14±0.10	x	1.85±0.11	y	1.79±0.07	y	†1.85±0.09	y
Y	0.14±0.01	ab	0.15±0.01	a	0.14±0.01	ab	0.13±0.01	b	†0.16±0.01	x	0.15±0.01	xy	0.14±0.01	y	0.14±0.01	y

Table 2.3. Pearson correlations for pH, Eh and element concentrations in soil below *Rumex crispus*. Correlations with r≥0.707 (that explain 50% or more of variation) are shown (p<0.001).

	Al	Cr	Fe	Fe^{2+}	K	La	Sr	V	Y
pH				-0.726					
Eh				-0.964					
Ba	0.715				0.722			0.772	
Cr	0.900								
Fe	0.883	0.828							
K	0.966	0.907	0.900						
La	0.766	0.705	0.738		0.762				
Sr	0.861	0.841	0.812		0.886	0.727			
V	0.935	0.915	0.907		0.952	0.745	0.840		
Y	0.832	0.783	0.795		0.830	0.786	0.796	0.794	
Zn	0.901	0.837	0.846		0.888	0.734	0.788	0.859	0.864

Table 2.4. Analysis of covariance for element concentrations with moisture treatments, sampling intervals and interaction between the two as fixed variables and Fe as a covariate (NS indicates non-significance; p<0.05).

	Source of Variation			
Element	Fe (covariate)	A. Moisture Treatments	B. Sampling Intervals	Interaction (A x B)
		p-value		
Al	0.000	NS	0.000	0.007
Ba	0.000	NS	NS	NS
Cr	0.000	NS	0.002	0.046
Cu	0.000	NS	NS	0.035
K	0.000	NS	0.004	0.050
La	0.000	NS	NS	NS
Mg	0.000	NS	NS	0.014
Na	0.000	0.000	0.000	0.001
Sr	0.000	0.031	NS	0.008
V	0.000	NS	0.000	0.018
Y	0.000	NS	0.000	NS
Zn	0.000	NS	0.000	NS

Table 2.5. Mean element concentration of shoot and root (μmol g^{-1}) and mean element content of whole plant (μmol $plant^{-1}$) for the different moisture treatments (mean ± standard deviation, n = 10, different letters within each plant compartment indicates significant variation between moisture treatments at $p<0.05$).

	Shoot				Root				Whole Plant			
	Flooded		Non-Flooded		Flooded		Non-Flooded		Flooded		Non-Flooded	
Al	12±8	a	30±14	b	95±52	d	54±18	d	157±92	x	63±25	y
B	4.0±0.6	a	5.8±1.6	a	1.3±0.2	d	1.6±1.1	d	3.2±0.5	x	2.3±1.0	y
Ba	0.42±0.05	a	0.54±0.14	a	0.4±0.1	d	0.3±0.1	d	0.7±0.2	x	0.3±0.1	y
Ca	715±79	a	530±126	b	219±28	d	220±84	d	551±92	x	283±78	y
Cu	0.2±0.02	a	0.2±0.04	a	0.2±0.03	d	0.1±0.03	e	0.35±0.06	x	0.15±0.02	y
Fe	4±2	a	10±4	b	53±20	d	28±35	e	85±36	x	29±29	y
K	533±71	a	416±75	b	336±55	d	236±25	e	679±124	x	292±42	y
Li	0.7±0.3	a	1.0±0.4	a	0.4±0.2	d	0.4±0.3	d	0.9±0.3	x	0.5±0.3	y
Mg	432±39	a	463±102	a	125±20	d	164±81	d	323±66	x	219±78	y
Mn	5.6±1.4	a	0.6±0.1	b	6.2±3.3	d	0.8±1.1	e	11±5.8	x	0.8±0.9	y
Na	113±72	a	233±173	a	58±7.6	d	45±43	d	121±21	x	71±44	y
Ni	0.07±0.03	a	0.08±0.07	a	0.13±0.09	d	0.08±0.05	d	0.2±0.1	x	0.09±0.06	y
P	98±16	a	64±22	b	67±6.6	d	72±15	d	133±20	x	83±20	y
S	92±33	a	114±57	a	46±4.3	d	45±10	d	98±14	x	60±14	y
Si	59±9	a	84±18	b	94±15	d	80±18	d	167±38	x	95±30	y
Sr	0.7±0.1	a	0.6±0.1	a	0.40±0.03	d	0.37±0.05	d	0.82±0.09	x	0.46±0.06	y
Ti	0.2±0.1	a	0.4±0.1	a	1.2±0.6	d	1.0±1.2	d	2.0±1.1	x	1.1±0.97	y

showed no significant variation. For the latter, the mean element concentrations ± standard deviation in μmol g^{-1} aboveground or belowground tissue, averaged for all samples were as follows; Cr (0.03±0.01 μmol g^{-1} aboveground, 0.08±0.07 μmol g^{-1} belowground), Zn (0.5±0.2 μmol g^{-1} aboveground, 0.4±0.2 μmol g^{-1} belowground). The flooded treatment had higher concentrations of Ca, K, Mn and P per gram of aboveground tissue and higher concentrations of Cu, Fe, K and Mn per gram of belowground tissue compared to the non-flooded treatment (Table 2.5). The non-flooded treatment had higher concentrations of Al, Fe and Si concentrations per gram of aboveground tissue compared to the flooded treatment. The plants of the flooded treatment showed significantly higher element content of Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, Ni, P, S, Si, Sr and Ti compared to plants of the non-flooded treatment.

2.5. Discussion

Despite significant differences in leaf and root biomass, both flooded and non-flooded plants formed a dense mat of roots covering a similar surface area at the rhizoplane. Surface area plays an important role in the transport of materials and plants usually increase root surface area to volume ratios to facilitate efficient nutrient uptake (Jungk 2002). However, by forcing the plant roots to grow along the mesh in this experiment, it was ensured that the effective surface area was similar in both the flooded and non-flooded treatments.

Some studies have shown that living plant roots have the ability to influence the soil chemistry (Youssef and Chino 1989b; Kirk and Bajita 1995; Wright and Otte 1999; Neumann and Römheld 2002; Jacob and Otte 2003; Hinsinger and Courchesne 2008). This study assumes that observed changes in the soil chemistry are plant-induced in addition to the activity of microbes and inorganic processes in the soil (Neumann and Römheld 2002). Soil pH in the vicinity of roots can be influenced by nutrient availability, uptake ratio of anions and cations (Tinker and Nye 2000; Gerendas and Ratcliffe 2002; Neumann and Römheld 2002), iron oxidation (Begg et al. 1994; Kirk and Bajita 1995; Tinker and Nye 2000), soil moisture and aeration, CO_2 production by roots (Neumann and Römheld 2002) and by root exudation (Begg et al. 1994; Neumann and Römheld 2002). The difference in pH between moisture treatments may be due to differences in CO_2 dissolution in response to flooding (Ponnaperuma 1972). In turn, changes in soil pH influence the mobility, solubility and availability of micronutrients (Youssef and Chino 1991; Mendelssohn 1993; Kirk and Bajita 1995; Luo et al. 2000; Jacob and Otte 2003).

Acidification associated with low pH enhances the plant's ability to accumulate metals near the roots (Kirk and Bajita 1995; van der Welle et al. 2007). In the flooded treatment in the study reported here the change in pH near the roots was observed in the same zone of change in element concentrations (between the soil above the rhizoplane and the 6 mm interval). However, soil pH did not significantly correlate with element concentrations observed in the soil, probably because the range of pH overall was narrow. Youssef and Chino (1989a) observed small changes in soils of similar pH (8.4) which was attributed to a high buffering capacity (Tao et al. 2004). Our observations are consistent with typical pH ranges (7.4-8.4) reported by the USDA for the soil used in this study (Fargo-Hegne) (Soil Survey Staff 2008).

The Eh of the non-flooded treatment indicated that the soil was oxidized throughout the soil column whereas in the flooded treatment the soil was reduced except for above the rhizoplane, which was as oxidized as the non-flooded treatment. In anaerobic, chemically reduced environments, Eh tends to increase towards plant roots due to radial oxygen loss (ROL) and oxidation of ferrous iron (Flessa and Fischer 1992; Davies 1994). This in turn can lead to an influx of metals that have an affinity for Fe plaque (Otte et al. 1995) in the direction of plant roots and subsequent accumulation in the rhizosphere (Wright and Otte 1999; Jacob and Otte, 2003).

With the exception of Ba, Fe was a significant covariate with the elements that showed variation in the soil columns (i.e. Al, Cr, K, La, Sr, V, Y and Zn). This suggests an important role of Fe in the underlying mechanisms of mobility of elements in the soil - Fe colloidal oxides are known to act as carriers of other metals (Shuman 2005). Movement of iron as Fe(II) from the reduced soil layer to the oxidized soil above the rhizoplane most likely followed a concentration gradient caused by changes in Eh (De Laune et al. 1981; Neumann and Römheld 2002; van der Welle et al. 2007), while other elements behaved similarly because they are redox sensitive (Kirk 2004) and/or they have a high affinity to co-precipitate or form complexes with secondary minerals of Fe (Mathys 1980; Otte et al. 1991; Kabata-Pendias and Pendias 2001; Kirk 2004). In contrast, Ba has chemical properties similar to Ca and Sr (Suarez 1996) and is usually associated with K in geochemical processes (Kabata-Pendias and Pendias 2001). It typically has no strong geochemical relationship with Fe and so does not follow a similar pattern. Al appears to be a dominant element in this clay-rich soil and it too correlated with other

elements that showed variation in the soil columns (Ba, Cr, Fe, K, La, Sr, V, Y and Zn). This suggests that these elements may also be associated with the colloidal surfaces of clay minerals in this soil or hydrous oxides of Al (McBride 1994; Kabata-Pendias and Pendias 2001; Shuman 2005).

Patterns in Eh coincided with those of Fe^{2+} concentrations in both treatments. The low and non-variable Fe^{2+} concentrations detected in the non-flooded treatment indicated that this soil was homogeneously oxidized. In the flooded treatment the presence of high soluble iron (Fe^{2+}) concentrations below the rhizoplane is indicative of reducing conditions (Justin and Armstrong 1987). The Fe plaque is visible evidence of oxidation in the vicinity of plant roots (Armstrong 1967). In the flooded treatment, low Fe^{2+} concentrations above the rhizoplane and high Fe^{2+} concentrations below the rhizoplane showed that the soil above the rhizoplane was oxidized compared to the soil below, implying an oxidized-reduced boundary layer. This boundary layer of soil in the flooded treatment facilitates conditions for the oxidation of Fe^{2+} to Fe^{3+} (Mendelssohn 1993), resulting in decreased Fe^{2+} concentrations (Otte et al. 1995) and Fe oxide precipitation near plant roots (Mendelssohn 1993; Sadana and Claassen 1996).

Element concentrations in the non-flooded treatment may be expected to be similar throughout the soil column (Youssef and Chino 1989b; Lorenz et al. 1997; McGrath et al. 1997; Luo et al. 2000;), but this treatment showed element (Al, Ba, Cr, Cu, Fe, K, La, Mg, Na, Sr, Y, V and Zn) accumulation in the soil near the roots, above the rhizoplane. This may have been due to the release of chelators, protons or other exudates from roots and microbial activity (Marschner et al. 1986; Youssef and Chino 1989b; Zhang et al. 1991; Parker et al. 2005). Another possible explanation may be transport of solutes to the roots via mass flow exceeding uptake by plants, resulting in accumulation of elements near the roots (Hinsinger and Courchesne 2008). The flooded treatment showed a different pattern of accumulation compared to the non-flooded treatment. Higher element accumulations above the rhizoplane as well as at the 3 mm interval below the rhizoplane compared to sampling intervals below were observed in the flooded treatment. Higher metal concentrations in the rhizosphere of wetland plants were also observed by Otte et al. (1991), Begg et al. (1994), Kirk and Bajita (1995), Otte et al. (1995), Doyle and Otte (1997) and Wright and Otte (1999).

A possible explanation for why the concentrations in the zone above the rhizoplane were lower in the flooded treatment compared to the non-flooded treatment is as follows. Fe plaque serves as a sink

for metals accumulating around wetland plant roots (Howeler 1973; Armstrong 1978; Gambrell and Patrick 1978; Taylor and Crowder 1983). Some studies have shown that Fe plaque does not necessarily reduce metal uptake and may indeed enhance it (Otte et al. 1989; Ye et al. 1997b; Ye et al. 1998; Ye et al. 2001; Liu et al. 2008). Zn and As become adsorbed to Fe plaque, but through root exudation can still be made available for uptake by plants (Otte et al. 1989; Otte et al. 1991; Zhang et al. 1998; McCabe et al. 2001). This may explain the lower concentrations of elements above the rhizoplane in the flooded compared to the non-flooded treatment. Adsorption to Fe oxides at the rhizoplane as well as plant uptake may have lowered the concentrations in the soil immediately surrounding the roots.

Concentrations of elements in plants are not good measures of element uptake, because differences in biomass affect concentrations due to dilution effects. Small plants usually have higher concentrations than large plants under otherwise comparable conditions (Ernst 1995). Element uptake in plants can therefore only be accurately measured as the total amount of element per plant, particularly when significant differences in biomass occur between the groups being compared. In this experiment, plant growth was significantly affected by the treatments. Element uptake expressed as amount per plant was higher under flooded conditions compared to non-flooded conditions.

It would be ideal if we were able to calculate a mass balance to show that the amounts of element taken up in the plants explained the lower concentrations in the 'above rhizoplane' compartment of the flooded treatment compared to the non-flooded treatment, or to quantify the zones of accumulation in both treatments, but this is not possible with the experimental set-up used here. Soil analyzed as the 'above rhizoplane' soil was not a representative sample of that entire compartment, because it was taken from soil adhering to the roots. Soil in that same compartment a few millimeters away from the roots was not analyzed. It is therefore not possible to calculate the amount of element present in that compartment.

2.6. Conclusions

What this research has shown is (1) that patterns in element distribution in the soil as affected by the roots vary significantly between wetland and dryland conditions, (2) that the zones of accumulation differ in size between wetland and dryland conditions, and (3) that when the same plant species is grown under wetland and dryland conditions, the plants grown under wetland conditions take up more element per plant than those grown under dryland conditions.

CHAPTER 3. MULTIPLE ELEMENTS IN *TYPHA ANGUSTIFOLIA* RHIZOSPHERE AND PLANTS: WETLAND VERSUS DRYLAND[2]

3.1. Abstract

In a recent study, researchers found that multiple elements accumulated near the roots of *Rumex crispus* more under wetland conditions and element uptake was significantly greater in the plants grown under wetland compared to dryland conditions. The study reported here also found that elements accumulated in the root zone (up to 3 mm beyond the rhizoplane) of *Typha angustifolia* grown under wetland conditions. In comparison to the bulk zone, Be, Cu, Fe, Li, Sr and Zn accumulated more in the root zone of the flooded treatment whereas Ni and Sr accumulated more in the root zone of the non-flooded treatment. On average, *T. angustifolia* produced 4 times more biomass and element uptake was 2 to 27 times greater under wetland compared to dryland conditions.

3.2. Introduction

The mobility and bioavailability of elements in the rhizosphere is influenced by changes in the chemical and physical properties of the soil. Researchers found that *Rumex crispus* had greater element accumulations in the rhizosphere and took up more elements when grown under wetland compared to dryland conditions (Kissoon et al., 2010). Redox potential and pH gradients were observed in their wetland treatment and probably played a role in the mobility and uptake of elements in the rhizosphere. Hinsinger and Courchesne (2008) reported that biogeochemical gradients such as element concentrations, organic ligand concentrations, pH, pCO_2, pO_2 and redox potential in proximity of plant roots determine the mobility and bioavailability of elements at the soil-root interface. Chemical gradients exist from the root surface to the bulk soil and are influenced by physical, chemical and biological processes associated with living plant roots (Hinsinger and Courchesne, 2008). Determination of element concentration gradients at the soil-root interface is important in the assessment of their bioavailability

[2] The material in this chapter was co-authored by La Toya Kissoon, Donna Jacob and Marinus Otte (published in Environmental and Experimental Botany 72: 232-241). La Toya Kissoon had the primary responsibility for the experimental design and collection and analysis of samples in the greenhouse experiments. La Toya Kissoon was the primary developer of the conclusions, drafted, and revised all versions of this chapter. Donna Jacob and Marinus Otte provided guidance, comments, and suggestions, and served as proofreaders.

which in turn helps estimate their uptake and potential adverse effects on the food chain (Hinsinger and Courchesne, 2008).

Rhizosphere oxidation and subsequent Fe plaque formation has been shown to influence metal concentrations in the rhizosphere of wetland plants. Kirk and Bajita (1995) observed zones of Zn accumulation and depletion associated with the accumulation of Fe^{3+} and soil acidification in the rhizosphere of lowland rice. Zn from highly insoluble fractions in the soil was released due to Fe oxidation and then this Zn was readsorbed by Fe plaque and organic matter. Otte et al. (1995) found that the binding of As and Zn with Fe plaque resulted in a decreasing concentration gradient of metals towards plant roots. In studies comparing rhizosphere and bulk soil, the rhizosphere was found to have higher concentrations of As, Fe and Zn (Otte et al., 1991; Otte et al., 1995; Doyle and Otte, 1997; Wright and Otte, 1999) and in some cases lower concentrations of Fe (Otte et al., 1995). Accumulation of Fe and As was probably due to oxidation processes in the rhizosphere (Otte et al., 1991). In studies comparing vegetated and non-vegetated flooded sediments, the vegetated sediments had higher concentrations of Zn (Jacob and Otte, 2004a; Choi et al., 2006), As and Fe (Doyle and Otte, 1997). The presence of living plant roots appears to enhance metal mobility by inducing the oxidation of the sediments and metal sulfides (Jacob and Otte, 2004a).

An oxidized rhizosphere and the presence of Fe plaque on plant roots has also been shown to influence metal uptake by plants (Mitsui, 1965; Otte et al., 1989; Otte et al., 1991; Ye et al., 1997b; Ye et al., 1998). Armstrong (1978) and Gambrell and Patrick (1978) suggested that the formation of Fe plaque on roots served as a sink for metals and was consequently a hindrance to nutrient uptake by wetland plants. Iron plaque accumulation in the rhizosphere appears to be a secondary source of metals to wetland plants and not a physical barrier. This occurs because plants have the ability to remobilize metals that become adsorbed onto Fe plaque and make them available for uptake by acidification of the rhizosphere (Kirk and Bajita, 1995), releasing reducing agents, enzymes (Jungk, 2002) chelates or protons (Otte et al., 2004) from their roots. Several studies have suggested that metals are not permanently immobilized by Fe plaque but may still be available for uptake by the plant (Otte et al., 1989; Ye et al., 1997b; Ye et al., 1998). These studies observed that plants with Fe plaque present took up more metals than plants without Fe plaque. Because metals adsorb to Fe plaque and may still be

available for uptake, McCabe et al. (2001) and Otte et al. (2004) suggested that wetland roots may be exposed to relatively higher levels of metals which in turn may have led to the development of metal tolerance in wetland plants.

Most rhizosphere studies have focused on dryland plants. These include, for example, *Brassica napus* (rape) (Kuchenbuch and Jungk 1982), *Hordeum vulgare* L. var. Dorirumugi (barley) (Youssef and Chino, 1989b; Youssef and Chino, 1991; Højberg and Sørensen, 1993), *Glycine max* L. var. Haweye (soybean) (Youssef and Chino 1991), *Brassica napus* L. cv. Sprinter (canola) (Wenzel et al., 2001), *Triticum aestivum* L. (wheat) (Wang et al., 2001; Wang et al., 2002), and *Lycopersicon esculentum* L. (tomato) (Cornu et al., 2007). Most rhizosphere studies involving wetland plants have focused on rhizosphere oxidation, Fe plaque or single element concentrations in the rhizosphere (Armstrong et al., 1992; Begg et al., 1994; Kirk and Bajita, 1995; Otte et al., 1995; Doyle and Otte, 1997; Armstrong and Armstrong, 2001; Jacob and Otte, 2004b) and one study has investigated the distribution of multiple elements across the rhizosphere (Kissoon et al., 2010). Hinsinger and Courchesne (2008) stated that studies involving rhizosphere biogeochemistry are important for understanding the mechanisms controlling the mobility and bioavailability of trace elements.

In a previous study using *Rumex crispus* we found that elements accumulated in the vicinity of the roots more under wetland than dryland conditions and there was greater uptake of these elements. In the present study we aimed to determine if element accumulation in the rhizosphere and plant will also occur in *Typha angustifolia* grown under wetland and dryland conditions. We hypothesized that *Typha angustifolia* would have 1) greater accumulation of multiple elements in the rhizosphere and 2) greater uptake of these elements under wetland compared to dryland conditions.

3.3. Materials and methods

3.3.1. Plant collection and soil preparation

Typha angustifolia was collected from the edge of a ditch in West Fargo, North Dakota (N 46º 53' 06.9" W 96º 58' 06.5") in October 2006. Average rhizome lengths have been found to range from 14 – 40 cm (White and Ganf, 1998; Sharma et al., 2008). Plants were collected from a depth of approximately 20-30 cm in order to obtain adequate rhizome material. The plants were taken to the North Dakota State University greenhouse facility and washed gently to remove excess soil. The plants were then planted in

buckets containing moist potting soil (Sun Gro Sunshine LG3 germinating mix with vermiculite) to establish stock plants (16 hour photoperiod, 2.97 log lumen m^{-2} (mean daytime), 26±9 ºC). Cuttings of young *T. angustifolia* of approximately the same size in terms of aboveground and belowground biomass were taken from the stock plant collection to be used in this study (stock plant collection established over 41 week period).

Soil for this experiment was obtained from farmland near Casselton in Cass County, North Dakota (N 46º 50' 51.4" W 97º 09' 20.0" elevation 280 m) because it was more representative of natural conditions compared to substrates such as potting soil or sand. The soil was determined a silty clay Fargo-Hegne soil (Soil Survey Staff, 2008) with 4.1% organic matter, bulk density of 1.04 g cm^{-3} and particle size of 5.8% sand, 47.9% silt and 46.3% clay (North Dakota State University Soil Testing Laboratory). The soil was air dried at room temperature (about 25 ºC) until constant dry weight, crushed and passed through a 2 mm screen. Because of the high clay content and to aid root penetration, the soil was amended with sterile sand (Quikrete Premium Play Sand) at a ratio of 3:1 soil to sand by weight.

3.3.2. Column apparatus assembly

The columns used in this experiment contained two sections of soil which were separated by a 21 µm nylon mesh (Nylon 21/17, Miami Aqua-culture, Inc.), and designed based on a similar experiment carried out previously using *Rumex crispus* (Kissoon et al., 2010). The mesh restricted root growth to the upper chamber of the column and so was considered the rhizoplane. The diffusion of nutrients and water was possible across the nylon mesh barrier due to its porous nature. This column design facilitated soil sampling in the different compartments in the soil column 1) above the rhizoplane (upper section of the column) and 2) at distinct distances below the rhizoplane (lower section of the column) (Figure 3.1).

The columns consisted of PVC pipe (15 cm diameter) cut into two sections both measuring 20 cm. The mesh was attached to one end of a 20 cm section. The two-chamber column was assembled using caulk and 5-cm wide waterproof Duct Tape (Nashua® Tape) to secure the two 20 cm sections together with the nylon mesh in between. The lower section of the column was filled with 3.77 kg of prepared soil to which 1.34 L of distilled water was added evenly and the upper section of the column with 3.1 kg to which 500 ml of distilled water was also added evenly. To prevent soil loss from the column and still allow water flow, a 2 mm fiberglass mesh was secured to the bottom end. To ensure contact between

soil in the lower section of the column and the rhizoplane, the lower section of the column was inverted, filled with soil and secured with the 2 mm mesh before soil was added to the upper section. The roots of single *T. angustifolia* cuttings were washed gently with distilled water and planted in the top section of each column. A total of 10 columns were prepared for the experiment. The *Typha angustifolia* cuttings were distributed between the two treatments so that there was not a bias in the initial plant biomass. Plant biomass (mean ± standard deviation) was not significantly different for the treatments (p>0.05, 2.66±3.36 g non-flooded treatment, 2.70±2.87 g flooded treatment).

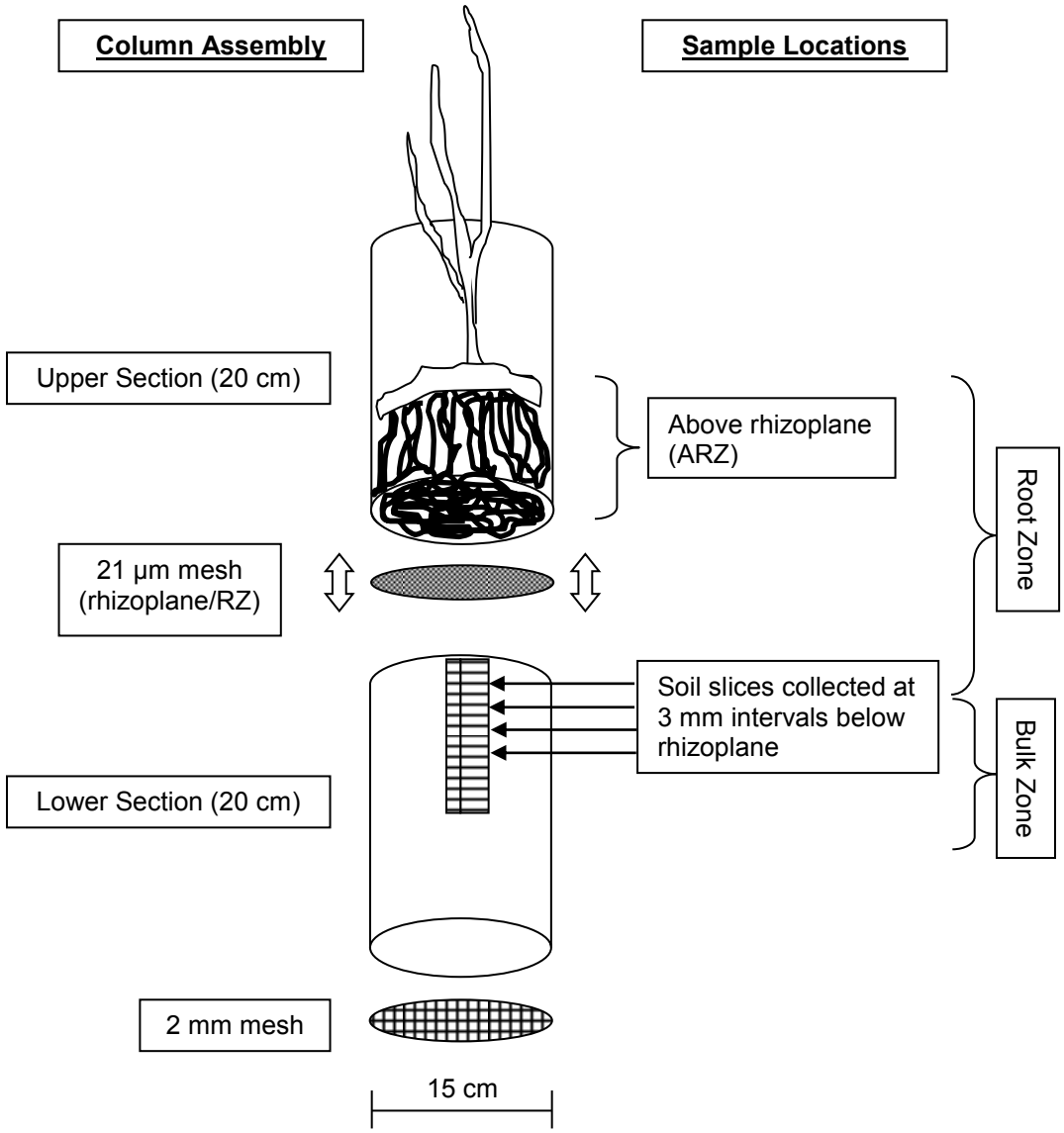

Figure 3.1. Column Apparatus for *Typha angustifolia* experiment showing column components on the left and sampling components on the right.

3.3.3. Soil flooding and monitoring moisture

This experiment was carried out in a greenhouse using *Typha angustifolia* grown in two moisture treatments (non-flooded and flooded soil) in two-chamber columns that were arranged using a complete randomized design. The columns were placed in 94 L cylindrical containers containing 500 g (2 cm) sterile sand to capture drainage from columns. The containers were wrapped in black plastic to exclude light and reduce algae growth. The plants were allowed to establish for 9 weeks before the start of the experimental treatments. During this period they were watered twice a week by adding 500 ml of distilled water to the bottom of the container. After this 9 week period, 5 columns of plants, the flooded treatment, were flooded by adding equal volumes of distilled water to each container such that the surface of the soil was below 3 cm of water. The water levels were marked on the inside of the container with water proof tape for future reference, monitored daily and restored when necessary to the initial water level line.

The remaining 5 columns, the non-flooded treatment, received water as needed according to their wilting point weights. The weights and plant height of the non-flooded treatments were monitored daily to determine if they were below 1 kg of the wilting point weight. When water addition was necessary the amount of water needed to bring the weight within 1kg of the wilting point was calculated and this water was added to the bottom of the outer container. Wilting point was determined previously by saturating the soil of five *T. angustifolia* plants growing in columns and then allowing the soil to dry. When the plants showed signs of wilting, the weights of the columns containing plant and soil were determined. These weights were used to calculate an estimate of the weight of a column containing wilted plant and soil. Some plants similar in size to the plants used in the wilting point experiment were taken from the stock collection and assessed for height and weight. Plant height was plotted against weight to obtain a linear equation with which to make adjustments when calculating the soil weight in the columns.

3.3.4. Soil sampling – pH and redox potential measurements

After 42 weeks, soil samples were collected from the columns selected in random order. Each column was cut carefully with a stainless steel hack saw to separate the upper and lower section. The plant and soil in the upper section were removed from the column and soil shaken from the roots. The soil still attached to the roots was collected and considered “above rhizoplane” soil. The lower section of the column was inverted and soil was carefully removed from the column leaving the five centimeters of

soil that was located immediately below the nylon mesh (rhizoplane). Following methods used by Kissoon et al. (2010), this soil was sampled using 60 ml syringes with the tips removed (2.5 cm diameter). Six syringes were inserted into the soil towards the center and away from the edges of the column. The soil collected was extruded from each syringe in 3 mm increments, sliced carefully and retained for analysis. Seven samples were collected from each syringe at 3, 6, 9, 12, 15, 18 and 21 mm increments. From each increment, samples from each syringe were pooled to obtain enough soil (at least three grams) for analysis.

Immediately upon obtaining a fresh soil sample, the pH was measured using a Corning pH Meter 430 and the redox potential (Eh) was measured using a VWR Symphony SP90M5 Handheld Multimeter. The redox electrode was checked with two calibration solutions (pH 4 and pH 7 buffer solution quinhydrone mixture). Eh measurements were taken by placing the redox electrode in a soil paste (Patrick et al., 1996) made with approximately 500 mg of soil sample and 3 ml distilled water. The pH electrode was calibrated using buffer solutions pH 4 and 10. The soil pH was measured using a 1:2 soil:water ratio recommended for soils within the pH range of 4-9 (Gavlak et al., 2003). Prior to this experiment, equivalent soils were dried and the water content was calculated to estimate how much water would be required for each sample to achieve the same moisture proportions for the different treatments. The average water content of the two moisture treatments were: Non-Flooded - 23%; Flooded - 39%. Based on this, approximately 1 g of fresh soil sample was placed in a clean test tube with 1.8 ml distilled water if a non-flooded sample or with 1.6 ml distilled water if a flooded sample. The soil and water mixtures were shaken vigorously and left to stand for about 10 minutes. The mixture was swirled carefully and the electrodes were placed in the slurry and a reading was taken after the pH stabilized.

3.3.5. Ferrous iron (Fe^{2+}) concentration and multi-element analysis of soil and plants

The ferrous iron concentration was determined using a modified method from Roden and Wetzel (1996). Ferrous iron standards were prepared from a stock solution containing 100 mg L^{-1} $FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$ and 1% (v/v) 6 M HCl. Fresh soil samples of a known weight (approximately 0.5 g), were immediately transferred to test tubes containing 5 ml of 0.5 M HCl to minimize oxidation and fix the Fe^{2+} in the sample. The mixture was shaken vigorously and extraction was allowed for approximately 24 hours. For each of the flooded samples, 0.25 ml of extract was collected and placed in a test tube

containing 9.75 ml distilled water (1:40 dilution). For each of the non-flooded samples, 0.5 ml of extract was collected and placed in a test tube containing 4.5 ml distilled water (1:10 dilution). After each diluted extract solution was shaken vigorously, 0.25 ml of sample or standard was added to 1.25 ml of FerroZine solution. The color of the resulting solutions was allowed to develop for five minutes and the absorbance was measured using a Helios Gamma UV-Vis Spectrophotometer at a wavelength of 562. A standard curve was constructed and used to calculate the ferrous iron concentrations in each sample.

The remaining soil was weighed, oven-dried at 60 ºC until constant weight, crushed using mortar and pestle and homogenized. A known amount of this soil (approximately 500 mg) was digested in 10 ml of HNO_3 in a MARS Xpress Microwave Digester (16 total vessels (XPRESS 55 ml PFA Venting Vessels), 1600W, 100% Power, ramped to 185 ºC over 10 minutes and held at this temperature for 5 minutes). The digested samples were cooled, filtered using Whatman® 1 filter paper and then analyzed for multiple elements with Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP). This analysis used a four-point calibration using individual or a combination of standards in a five percent HNO_3 matrix. A continuing control verification (CCV) was done after every 10 samples to check that variability was within 10% for Al and Ca while all other elements were monitored. These samples were also diluted (1:100 in 5% HNO_3) and analyzed for the same 32 elements using the ICP-OES. Method detection limits in mg/kg for these elements were as follows: Ag 0.08; Al 4; As 0.2; B 0.2; Ba 0.002; Be 0.002; Ca 0; Cd 0.07; Ce 0.3; Co 0.1; Cr 0.08; Cu 0.03; Fe 0.002; Hg 0.08; K 4.9; Li 0.04; Mg 0; Mn 0.01; Mo 1.9; Na 0; Ni 0.2; P 0.4; Pb 1; Sb 1; Si 0.2; Sn 0.3; Sr 0.005; Ti 0.02; Tl 1; V 0.4; Zn 0.03; and Zr 0.04.

The plants were washed gently in distilled water, separated into roots and shoots, oven-dried (60 ºC) until constant weight, crushed and homogenized. Following methods used by Kissoon et al. (2010), known amounts of plant material (approximately 250 mg) were digested in 5 ml HNO_3 and 5 ml water using a MARS Xpress Microwave Digester and then analyzed for 32 elements using a Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP). Method detection limits in mg/kg for these elements were as follows: Ag 0.03; Al 22; As 1; B 0.5; Ba 0.006; Be 0.005; Ca 0; Cd 0.13; Ce 0.3; Co 0.14; Cr 0.1; Cu 0.1; Fe 0.1; K 0; Li 0.06; Mg

0.9; Mn 0.02; Mo 0.3; Na; 0.2; Ni 0.4; P 2; Pb 0.5; S 1.7; Sb 0.2; Si 0.07; Sn 1.4; Sr 0.06; Ti 0.02; Tl 1.2; V 0.4; Zn 0.2; Zr 0.002.

3.3.6. Statistical analysis

Statistical analysis of the data was carried out using Minitab statistical software (Minitab® 15 ©2006 Minitab Inc.). Prior to statistical analysis, the concentration data were $\log_{10}$ transformed in order to obtain normality and homogeneity of variance. Significance of differences (probability) was determined using a General Linear Model (two-way ANOVA, $p<0.05$) and multiple comparison tests using the Tukey Method ($p<0.05$). The data for soil pH, redox potential (Eh) and element concentrations were analyzed for significant differences 1) between moisture treatments regardless of sampling interval, 2) between sampling intervals regardless of moisture treatment, 3) between sampling intervals of the flooded treatment, 4) between sampling intervals of the non-flooded treatment and 5) between moisture treatments for each equivalent sampling interval. To test for relationships between element concentrations, pH and Eh, Pearson correlations and *P* values were calculated using Minitab. Here we consider only correlations with $r \geq 0.475$, that is, those correlations explaining 22% or more of the variation ($p<0.001$) (McClave and Sincich, 2006). Covariate analysis was carried out using soil Fe concentrations as a covariate ($p<0.05$).

3.4. Results

3.4.1. Soil

The results reported here for soil pH, redox potentials and iron concentrations focus on three sampling intervals; above the rhizoplane (ARZ), 3 mm and the 6-12 mm intervals below the rhizoplane. Within each moisture treatment, the sampling intervals 6, 9, 12, 15, 18 and 21 mm were not significantly different from each other and so the sampling intervals 6-12 mm were pooled for statistical comparisons.

3.4.1.1. Soil pH

Analysis of variance indicated significant variations in soil pH between moisture treatments but not between sampling intervals. There were also significant interactions for soil pH between the moisture treatments and between the sampling intervals indicating that patterns in pH across intervals were not the same in the flooded compared to non-flooded treatments. The soil pH was significantly lower in the

flooded treatment compared to the non-flooded treatment for the sampling intervals above and below the rhizoplane (Figure 3.2).

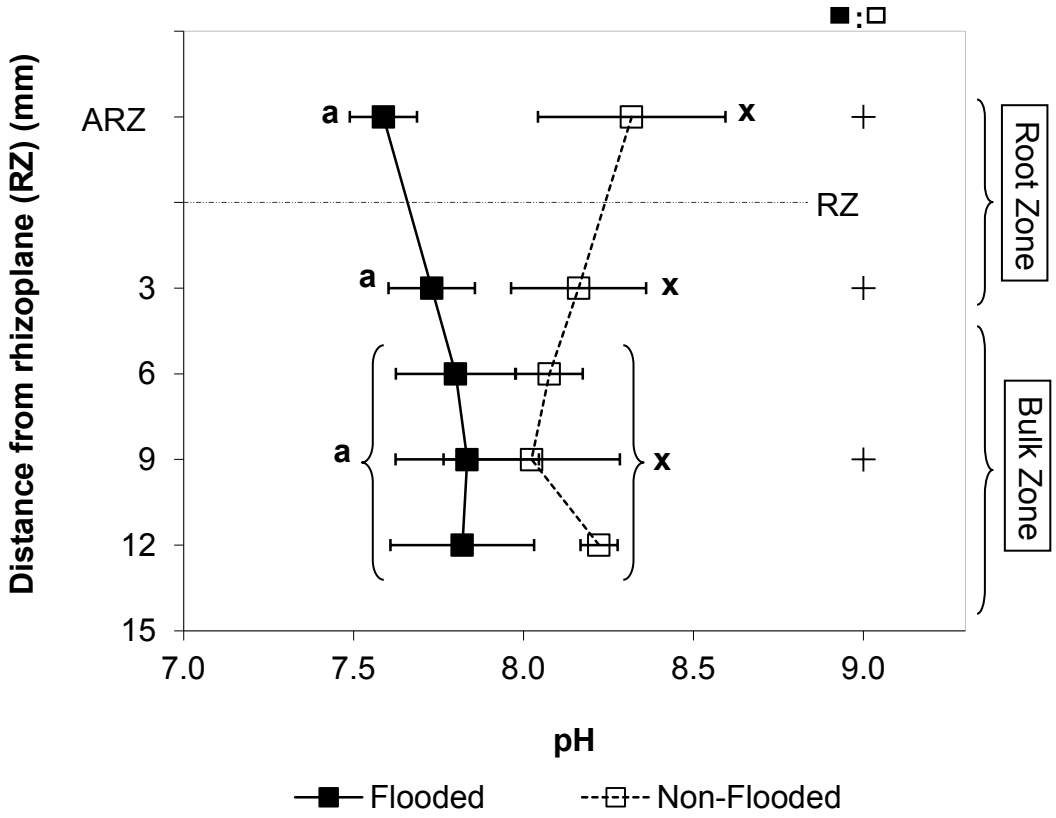

Figure 3.2. Mean soil pH in the moisture treatments (flooded; non-flooded) for the different sampling intervals (above rhizoplane (ARZ) and below rhizoplane) under *Typha angustifolia* (RZ = rhizoplane, *n* = 5, except for 6-12 mm interval; *n* = 15). Different letters within each moisture treatment indicates significant variation between sampling intervals (ARZ, 3, 6-12 mm) at $p<0.01$. The results of the comparison between moisture treatments for each interval appear to the right of the graph (the small black and white shapes at the top right of the graph indicate this comparison (e.g. ■:□ means filled squares compared to open squares); + indicates significant differences and – indicates no significant differences between moisture treatments, $P < 0.01$).

3.4.1.2. Redox Potential and Fe Oxidation

Significant variations for redox potential and Fe^{2+} concentrations occurred between moisture treatments and between sampling intervals. There were also significant interactions for redox potential and Fe^{2+} concentrations between the moisture treatments and the sampling intervals. The redox potential of the non-flooded treatment was significantly higher than the flooded treatment for all the sampling intervals (Figure 3.3). The non-flooded treatment showed no significant variation between the sampling intervals. In the flooded treatment, the redox potential in the soil above the rhizoplane was significantly

higher than all other sampling intervals. For these other intervals, the mean redox potential values ranged from –75 to –79 mV and showed no significant differences.

The flooded treatment showed significantly higher Fe^{2+} concentrations compared to the non-flooded treatment for all the sampling intervals (Figure 3.4). The non-flooded treatment showed no significant variation in Fe^{2+} concentrations. In the flooded treatment, the Fe^{2+} concentrations were significantly lower above the rhizoplane compared to the sampling intervals below the rhizoplane which were not significantly different from each other. The non-flooded treatment showed no precipitation of Fe-oxyhydroxide (reddish-brown discoloration) anywhere in the soil column. However, in the flooded treatment the reddish-brown discoloration of the Fe-oxyhydroxide precipitation was clearly visible on the plant roots, on the rhizoplane and on the soil surrounding the roots.

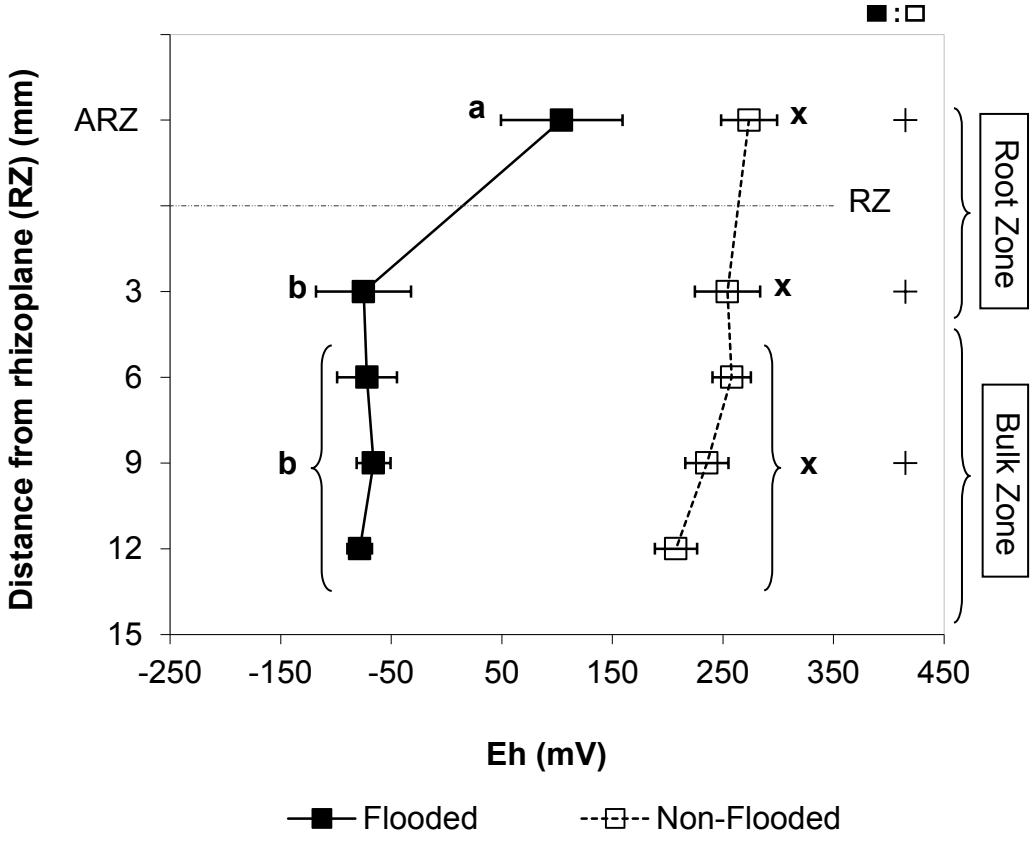

Figure 3.3. Mean Eh in the moisture treatments and statistical differences as in Figure 3.2.

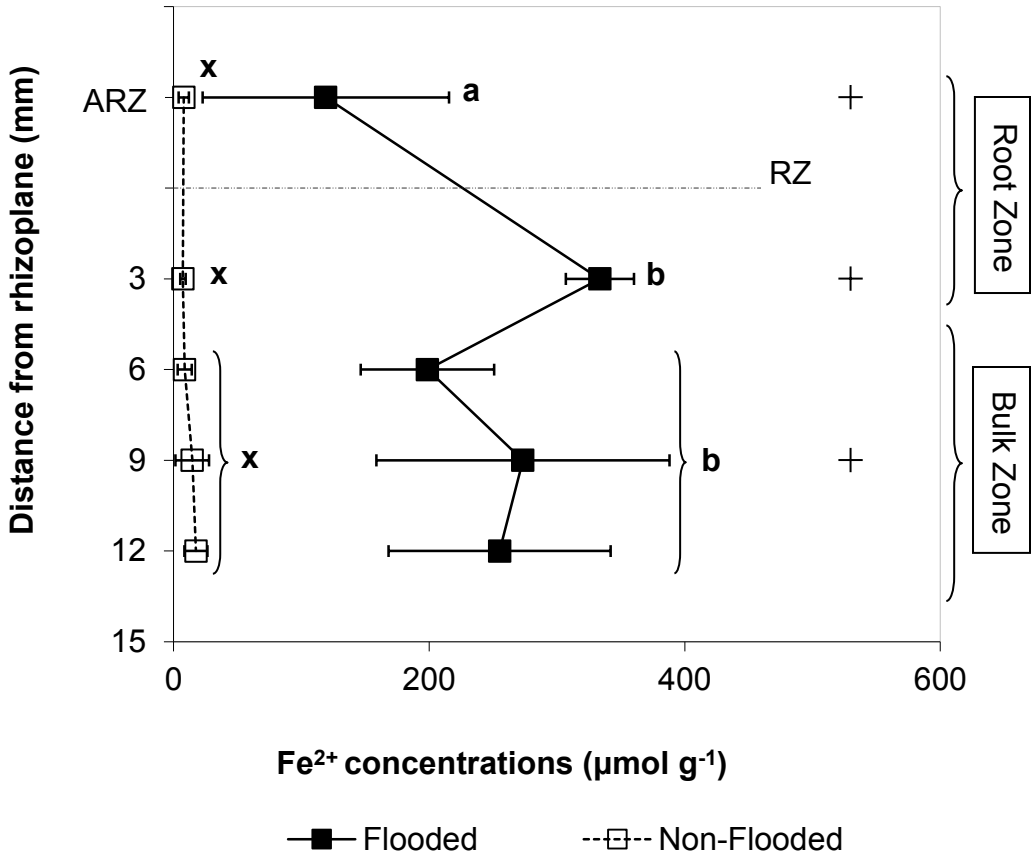

Figure 3.4. Mean Fe^{2+} concentrations (µmol g^{-1} dry soil) in the moisture treatments and statistical differences as in Figure 3.2.

3.4.1.3. Multiple element analysis

For the element analysis, 32 elements were measured, but not all yielded results suitable for statistical analysis. For instance, element concentrations in some samples were at or below detection limits for Ag, As, Cd, Hg, Mo, Pb, Sb, Si, Sn and Tl, or showed lack of variation. For the latter, the mean concentrations ± standard deviation in µmol g^{-1} dry soil, averaged for all samples were as follows; Al (829±126), B (1.40±0.18), Ba (1.06±0.18), Ca (359±55), Ce (0.23±0.02), Co (0.11±0.01), Cr (0.41±0.04), K (141±14), Mg (295±17), Mn (7.58±1.35), P (9.10±0.57), Ti (1.08±0.29), V (0.70±0.09) and Zr (0.18±0.02). These elements will not be discussed further. Preliminary statistical analysis showed the element concentrations in the soil above the rhizoplane and 3 mm below the rhizoplane were not statistically different and so these two intervals were pooled for statistical comparisons and considered the root zone because of its proximity to the plant roots. The sampling intervals 6, 9, 12, 15, 18 and 21

mm showed no significant variations and so only the sampling intervals 6, 9 and 12 were used for statistical comparisons with the root zone. This area of the soil that is 6-12 mm below the rhizoplane was considered the bulk zone.

Analysis of variance indicated significant differences for Fe, Na and Zn between moisture treatments and for Be, Cu, Fe, Li, Ni, Sr and Zn between sampling intervals. There was also significant interactions between moisture treatments and between sampling intervals for Zn. Sodium concentrations did not vary between sampling intervals for either moisture treatment but were significantly higher in the bulk zone of the non-flooded treatment compared to the flooded treatment (Fig. 3.5e). Iron concentrations were also significantly higher in the bulk zone of the non-flooded treatment compared to the flooded treatment (Fig. 3.5c). In the non-flooded treatment, Ni and Sr concentrations were significantly higher in the root zone compared to the bulk zone (Fig. 3.5f and 3.5g). The flooded treatment showed significantly higher concentrations in the root zone compared to the bulk zone for Be, Cu, Fe, Li, Sr and Zn (Fig. 3.5).

Correlation analysis of the data was carried out to determine possible underlying patterns regardless of the treatments. Some elements correlated significantly with each other (Be, Cu, Fe, Li, Ni, Sr and Zn) (Table 3.1). Eh correlated with pH, Fe, Fe^{2+} and Na and pH correlated with Fe^{2+} and Na. Fe, via covariate analysis, was shown to influence Be, Cu, Li, Ni and Zn concentrations and may account for some of their variation (Table 3.2). In addition, the factors ‘moisture treatments’ and ‘sampling intervals’ were significant for Be, Cu, Ni, Sr and Zn and ‘moisture treatments’ were significant for Be, Cu, Li, Na, Ni, Sr and Zn.

3.4.2. Plants

In both the non-flooded and flooded treatments, *T. angustifolia* formed a dense mat of roots at the surface of the mesh (rhizoplane). In the flooded treatment, root growth was densely distributed throughout the soil compared to the non-flooded treatment where root growth occurred mainly at the surface of the mesh. Plant biomass (mean ± standard deviation) was significantly higher for the flooded treatment (6.7±1.5 g aboveground biomass, 24±18 g belowground biomass) compared to the non-flooded treatment (2.3±2.2 g aboveground biomass, 5.2±4.8 g belowground biomass).

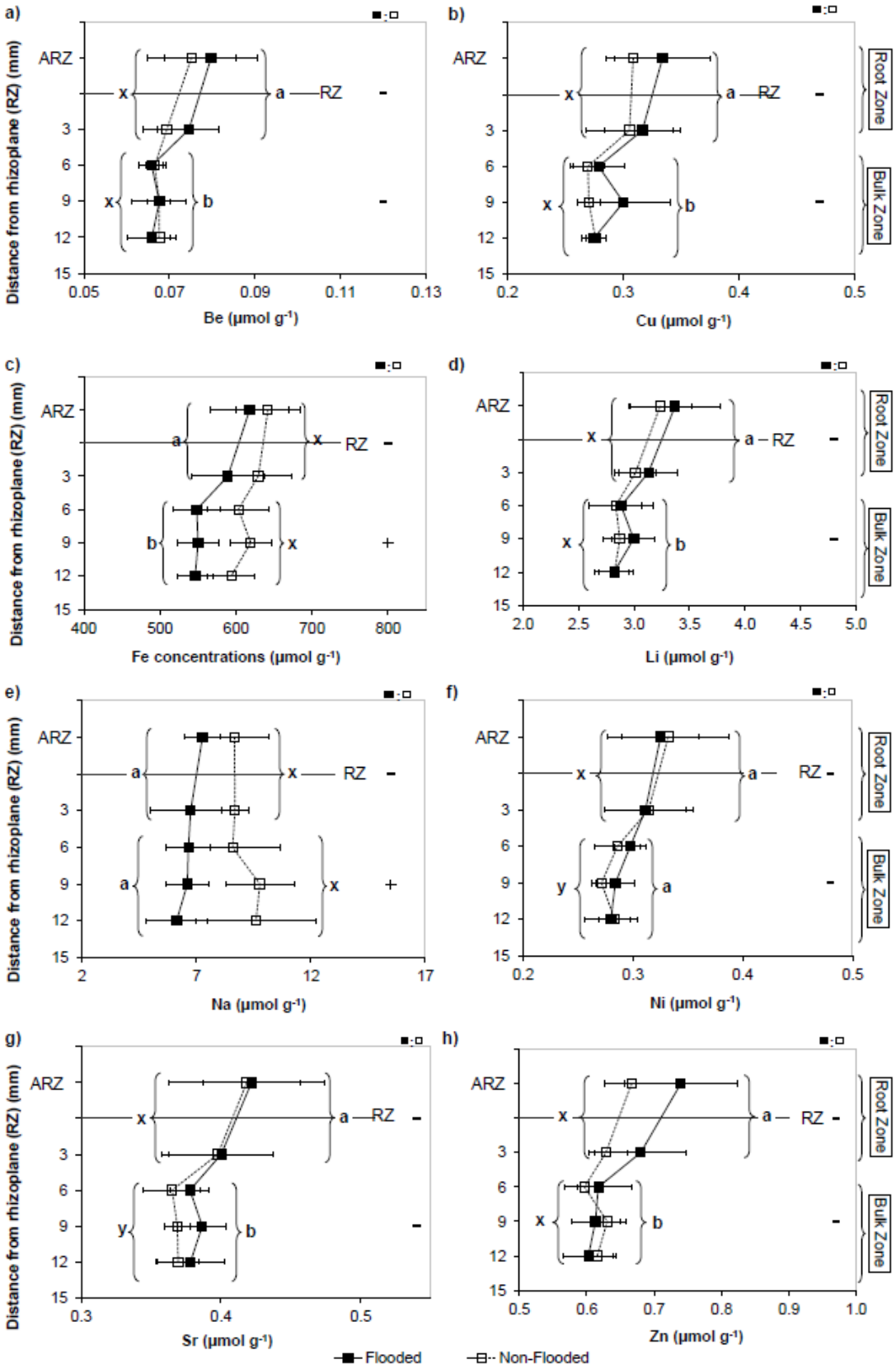

Figure 3.5. Mean element concentrations (µmol g^{-1} dry soil) in the moisture treatments for the different sampling intervals (root zone; n = 10, bulk zone; *n* = 15) and statistical differences as in Figure 3.2.

Table 3.1. Pearson correlations for pH, Eh and element concentrations in soil below *Typha angustifolia*. Correlations with r≥0.475 (that explain 22% or more of variation) are shown (*p*<0.001).

	pH	Eh	Be	Cu	Fe	Fe^{2+}	Li	Na	Ni	Sr
Eh	0.596									
Cu			0.730							
Fe		0.570	0.481	0.528						
Fe^{2+}	-0.633	-0.947			-0.496			-0.578		
Li			0.809	0.837	0.650					
Na	0.475	0.641								
Ni			0.801	0.715	0.510		0.742			
Sr			0.828	0.618			0.721		0.750	
Zn			0.922	0.793	0.487		0.819		0.762	0.743

Table 3.2. Analysis of covariance for element concentrations with moisture treatments, sampling intervals and interaction between the two as fixed variables and Fe as a covariate (NS indicates non-significance; *p*<0.05).

Element	Source of Variation			
	Fe (Covariate)	A. Moisture Treatments	B. Sampling Intervals	Interaction (A x B)
	p-value			
Be	0.001	0.006	0.019	NS
Cu	0.000	0.000	0.013	NS
Li	0.000	0.000	NS	NS
Na	NS	0.000	NS	NS
Ni	0.000	0.035	0.024	NS
Sr	NS	0.037	0.000	NS
Zn	0.000	0.000	0.015	NS

Table 3.3. Mean element concentration of shoot and root (μmol g^{-1}) and mean element content of whole plant (μmol $plant^{-1}$) for the different moisture treatments (mean ± standard deviation, n = 5, different letters within each plant compartment indicates significant variation between moisture treatments at p<0.05).

Element	Shoot				Root				Whole Plant			
	Flooded		Non-Flooded		Flooded		Non-Flooded		Flooded		Non-Flooded	
Al	20±21	a	27±33	b	99±38	d	58±43	d	1610±763	x	131±137	y
B	1.7±0.8	a	1.9±0.7	a	1.1±0.4	d	0.9±0.3	d	32±8.2	x	8.1±7.3	y
Ba	0.1±0.04	a	0.1±0.07	a	0.6±0.2	d	0.2±0.1	d	14±13	x	0.8±0.8	y
Be	0.0001±0	a	0.003±0.004	a	0.02±0.01	d	0.009±0.007	e	0.5±0.3	x	0.05±0.04	y
Ca	220±39	a	325±117	b	565±280	d	210±117	d	14400±10900	x	1530±1340	y
Cu	0.2±0.05	a	0.2±0.07	a	0.3±0.07	d	0.3±0.1	e	7.0±3.8	x	1.4±1.2	y
Fe	4.3±5.6	a	7.4±9.2	b	99±40	d	20±10	e	2260±1810	x	83±86	y
K	467±127	a	616±106	b	338±81	d	251±141	e	10500±5510	x	3000±2820	y
Li	0.3±0.2	a	0.4±0.1	a	0.6±0.2	d	0.3±0.2	d	15±7.9	x	2.0±1.9	y
Mg	63±27	a	95±41	a	239±96	d	111±27	d	6030±4910	x	687±601	y
Mn	18±8.5	a	2.7±2.4	b	18±7.4	d	2.3±3.1	e	530±456	x	8.8±4.1	y
Na	73±22	a	119±59	a	173±52	d	75±30	d	4210±2440	x	604±532	y
P	53±18	a	54±32	b	49±15	d	100±72	d	1390±670	x	356±292	y
S	46±13	a	108±45	a	30±7.3	d	55±11	d	1000±730	x	470±410	y
Si	21±26	a	36±39	b	67±25	d	64±23	d	1480±766	x	339±320	y
Sr	0.3±0.05	a	0.5±0.2	a	0.4±0.2	d	0.3±0.2	d	12±7.9	x	2.2±1.9	y
Ti	0.1±0.2	a	0.2±0.3	a	1.1±0.5	d	0.4±0.3	d	22±9.3	x	2.2±3.0	y
Zn	0.3±0.3	a	0.3±0.2	a	0.4±0.2	d	0.3±0.2	d	12±8.7	x	1.0±0.8	y

For the element analysis of the plant material 32 elements were measured, but not all yielded results suitable for statistical analysis. Element concentrations were at or below the detection limits for Ag, As, Cd, Ce, Co, Mo, Ni, Pb, Sb, Sn, Tl, V and Zr. The non-flooded treatment had higher concentrations of S per gram of aboveground tissue and per gram of belowground tissue (Table 3.3). The flooded treatment had higher concentrations of Mn per gram of aboveground tissue and higher concentrations of Ba, Ca, Cr, Fe, Mg, Mn, Na and Ti per gram of belowground tissue. The plants of the flooded treatment had significantly higher element content of Al, B, Ba, Be, Ca, Cu, Cr, Fe, K, Li, Mg, Mn, Na, P, Si, Sr, Ti and Zn compared to plants of the non-flooded treatment. The plant S content did not vary between treatments.

3.5. Discussion

Changes in the rhizosphere soil pH influence the mobility and bioavailability of metals (Davies, 1994; Wright and Otte, 1999; Jacob and Otte, 2003). The pH in the rhizosphere is influenced by Fe oxidation (Begg et al., 1994; Tinker and Nye, 2000; Neumann and Römheld, 2002; Kirk, 2004) cation and anion uptake (Begg et al., 1994; Tinker and Nye, 2000; Kirk, 2004), soil buffering capability, soil moisture and aeration, acid production by microbes, CO_2 production by plant roots and soil microorganisms, release of root exudates, plant genotype, absorption of soil nitrogen and plant nutrient status (Neumann and Römheld, 2002). In general the pH in the rhizosphere differs from that in the bulk soil by about 2-3 units (Neumann and Römheld, 2002), hence the conditions in the rhizosphere are quite different from the soil some distance away from the roots (Kapulnik and Okon, 2002). In the study reported here, there was no pH gradient present in the flooded or non-flooded soil columns. The lack of a pH gradient in the flooded columns contrasts with findings by Kissoon et al. (2010) for flooded columns containing *Rumex crispus*. This is probably due to interspecies differences and the soil buffering capacity. The lower pH in the flooded compared to the non-flooded treatment could be a result of CO_2 dissolution causing slightly acidic conditions upon flooding (Ponnamperuma, 1972). Soil pH correlated positively with Eh and Na and correlated negatively with Fe^{2+} concentrations. The relationship between soil pH and Eh suggests that the redox status as a result of flooding or not flooding the soil columns influenced the soil pH. Alkaline soil conditions are usually associated with elevated concentrations of Na (McBride, 1994). This may explain why Na was higher in the non-flooded treatment which had a higher and more alkaline soil pH

than the flooded treatment. The relationship between soil pH and Fe^{2+} concentrations indicate that Fe oxidation and subsequent Fe^{2+} depletion probably played a role in the soil pH.

High and non-variable Eh values and low and non-variable Fe^{2+} concentrations in the non-flooded treatment indicate that this soil was oxidized throughout the column compared to the flooded treatment which had lower Eh values and higher Fe^{2+} concentrations. In the flooded treatment, gaseous exchange between the air and soil was disrupted when the soil was submerged (Justin and Armstrong, 1987) and the limited oxygen present was used up by soil microorganisms creating a chemically reduced soil environment (Trolldeneir, 1988; Sadana and Claassen, 1996). Higher Eh and lower Fe^{2+} concentrations in the soil above the rhizoplane of the flooded treatment suggests that this soil was oxidized compared to the soil below the rhizoplane. This oxidized zone in the vicinity of the roots is evidence of radial oxygen loss, resulting in an oxidized rhizosphere and increased redox potential (Foster et al., 1983; Mendelssohn, 1993; Davies, 1994; Tinker and Nye, 2000; Jacob and Otte, 2003). Under flooded conditions, the rhizosphere becomes an interface between oxidized and reduced environments which host diverse microbial populations (Neubauer et al., 2007), play a role in metal mobility (Jackson, 1998) and facilitate Fe^{2+} oxidation and subsequent Fe plaque formation (Mendelssohn, 1993; Hupfer and Dolan, 2003).

Studies have shown that element concentrations tend to be similar throughout soils under non-flooded conditions (Youssef and Chino, 1989b; Lorenz et al., 1997; McGrath et al., 1997; Luo et al., 2000). Element concentrations in the non-flooded treatment were similar throughout the soil column except for Ni and Sr concentrations which were higher in the root zone than in the bulk zone. In general, the lack of variation in element concentrations may be due to the lack of soil pH and Eh gradients in the non-flooded soil columns (Kissoon et al., 2010). Release of root exudates influences element mobility and availability and also influences the physical, chemical and biological properties of the rhizosphere (Deiana et al., 2001). The higher concentrations of Ni and Sr in the root compared to the bulk zone may be due to root exudation and subsequent mobilization of elements for plant uptake (Marschner et al., 1986; Lombi et al., 2001; Jungk, 2002; Parker et al., 2005). In the flooded treatment, higher concentrations of Be, Cu, Fe, Li, Sr and Zn in the root zone compared to the bulk zone may be due to the presence of an Eh gradient, Fe plaque and/or root exudation. Studies have reported greater As, Fe and

Zn concentrations in the rhizosphere compared to the bulk soil under wetland conditions (Otte et al., 1991; Otte et al., 1995; Doyle and Otte, 1997; Wright and Otte, 1999). Otte et al., (1991) attributed metal accumulations near the roots to rhizosphere oxidation processes.

With the exception of Na and Sr, Fe was a significant covariate with elements that showed variation in the soil columns (Be, Cu, Li, Ni and Zn). This indicates that Fe plays an important role in the mobility of these elements in the soil. When Fe^{2+} is oxidized in the rhizosphere, a concentration gradient forms as Fe^{2+} diffuses towards the roots (Sheppard and Evenden, 1991). Some of these elements may follow the movement pattern of Fe along a concentration gradient as Fe(II) diffuses from the reduced to oxidized soil layers, some may be redox sensitive (McBride, 1994; Hinsinger, 2001), co-precipitate with or have an affinity for the Fe oxides in the rhizosphere (McBride, 1994; Kirk, 2004). Zinc and copper are associated with insoluble sulfides which result in low mobility under reducing conditions and have high affinity for colloidal oxides of Al, Mn and Fe in soils (McBride, 1994). Both of these elements have a high affinity for these oxides, which may explain the increased concentrations in the oxidized root zone. The mobility of Ni is also restricted under reducing conditions due to incorporation with sulfides and also readily co-precipitates with Mn and Fe oxides (McBride, 1994). Iron oxides provide sorption sites for cations (McBride, 1994), act as carriers of metals (Shuman, 2005) and are one the most important oxides in soil that influence trace element behavior (Kabata-Pendias and Pendias, 2001). Transport of trace elements through the soil is influenced by the free ion concentrations, redox status, complexation with ligands, sorption with oxides, organic matter and/or clay minerals and precipitation or co-precipitation with hydroxides, carbonates, sulfides or phosphates (Kirk, 2004). The concentrations of trace elements such as Fe, Cr, Cu, V and Mn at the soil-root interface depend on their oxidation state which in turn, influences their speciation, mobility and availability for uptake (Deiana et al., 2001).

Plant biomass was significantly different between the flooded and non-flooded treatments, however, isolation of the roots to the upper section of the column forced them to grow along the surface of the mesh where they formed a dense mat of roots. This ensured that the effective surface area at the rhizoplane was similar for both moisture treatments. Root surface area (Mengel and Kirkby, 1978; Jungk, 2002), substrate element concentrations and element bioavailability are important for element uptake by plants (Jackson et al., 1991). Studies have reported that Fe plaque appears to have no impact on

element uptake (Liu et al., 2008; Jiang et al., 2009) and in some instances appears to enhance uptake (Otte et al., 1991; Zhang et al., 1998).

A number of studies have shown that wetland plants accumulate high concentrations of multiple elements in their tissues (Szymanowska et al., 1999; Samecka-Cymerman and Kempers, 2001; Matthews et al., 2004). The elevated metal concentrations in these plants tend to reflect high available concentrations in the sediments. In the study reported here, element uptake was significantly greater in plants grown under flooded compared to non-flooded conditions for Al, B, Ba, Ca, Cu, Fe, K, Li, Mg, Mn, Na, S, Si, Sr, Ti and Zn. Of these elements concentrations of Cu, Fe, K, Li, Sr and Zn were higher in the root zone compared to the bulk zone under flooded conditions. The accumulation of these elements in the root zone of the plants in the flooded treatment may be due to rhizosphere oxidation processes and may have resulted in greater exposure of the plants to higher bioavailable concentrations of these elements. This greater exposure to elements may be an explanation for why the plants under flooded conditions took up more elements than the plants grown under non-flooded conditions. Rhizosphere oxidation promotes element uptake (Mitsui, 1965), influences the sequestering of nutrients and creates concentration gradients which stimulates the movement of nutrients in the direction of the roots (Moore et al., 1994).

The results of this study coincide with findings of a similar study using *Rumex crispus*. Observations of Eh, Fe^{2+} concentrations and Fe plaque were consistent with the findings of Kissoon et al. (2010). They found that *Rumex crispus* accumulated multiple elements (Al, Cr, Fe, K, La, Sr, V, Y and Zn) near the roots and took up significantly more elements under flooded compared to non-flooded conditions. Researchers have suggested that the biogeochemical conditions of the wetland rhizosphere may contribute to increased metal exposure which may have led to the development of metal tolerance in wetland plants (McCabe et al., 2001; Otte et al., 2004). Kissoon et al. (2010) proposed that element accumulation near the roots and subsequent element uptake indicate that wetland plants may indeed be exposed to more metals that are available for uptake. The study reported here provides evidence to further support this theory.

3.6. Conclusions

This study has shown that when plants of the same species are grown under wetland and dryland conditions, 1) multiple elements accumulate more in the root zone compared to the bulk zone under wetland conditions and 2) there is greater element uptake by the plants grown under wetland compared to dryland conditions.

CHAPTER 4. MULTI-ELEMENT ACCUMULATION IN SOILS ALONG A MOISTURE GRADIENT ASSOCIATED WITH THE SALT MARSH PLANT *TRIGLOCHIN MARITIMA*

4.1. Abstract

This study examined multiple element concentrations in soils of high and low moisture content associated with *Triglochin maritima* in the Kellys Slough salt marsh to determine if elements accumulate in the root zone and in the plant tissues of the high moisture content soils. This study found that several elements accumulated in soils with higher moisture content and that plants growing in these soils tend to take up more of these elements in their tissues. The results indicated that moisture content played a role in element distribution in wetland soils. Other factors, such as pH, redox, Fe^{2+} and sulfide concentrations, LOI and particle size may also play a role in element distribution in wetland soils.

4.2. Introduction

The purpose of the research presented here was to establish if patterns of accumulation of metals and other elements in the rhizosphere of wetland plants, as observed in greenhouse experiments (Kissoon et al. 2010, 2011) could be confirmed in the field. Such information is important for our understanding of spatial and temporal variation of elements, some of which are among the most studied pollutants, in the environment, and therefore important in monitoring and assessment approaches. The concentrations of multiple elements were examined including metals such as As, Ba, Co, Cu, Fe, Mg, Mn, Ni, Sr, Ti, V and Zn.

Element distribution in the rhizosphere is influenced by biogeochemical gradients in element concentration, pH, redox potential and organic compounds (Hinsinger and Courchesne 2008). Studies have shown that pH and redox potential influence metal mobility and accumulation (De Laune et al. 1981; van der Welle et al. 2007). Knowledge of the movement and distribution of trace elements in soils and plants is critical for agriculture and human nutrition (Morrissey and Guerinot 2009). Studies have reported differences in biogeochemical processes between the root zone and the non-vegetated or bulk zones in soil (Barber 1962; Eberhard et al. 1994; Assadian and Fenn 2001; Hinsinger and Courchesne 2008). The presence of an oxidation-reduction gradient between the root and bulk zone in wetland soils influences element mobility (Doyle and Otte 1997; Kirk and Bajita 1995; Frommer et al. 2011) and leads to element accumulation of Fe, Mn, As and Zn in the rhizosphere (Otte et al. 1991, 1995; Kirk and Bajita 1995; Doyle

and Otte 1997; Frommer et al. 2011) and many other elements, and to greater uptake of those elements in plants (Kissoon et al. 2010, 2011).

The pH of the rhizosphere is influenced by the exchange of CO_2 between roots and soil (Begg et al. 1994), root exudation (Hinsinger 2001), the anion-cation uptake ratio (Gerendas and Radcliffe 2002), soil moisture and aeration, soil buffering capacity, plant nutritional status, assimilation of soil nitrogen and microbial activity (Neumann and Römheld 2002). Root exudation influences the availability of trace elements in soil by affecting acidification, precipitation, and redox reactions in the rhizosphere (Tao, et al. 2004). Plant-induced changes in pH may cause the dissolution or precipitation of nutrients, thus influencing their mobility in the rhizosphere (Begg et al. 1994; Jungk 2002). Localized reoxidation of waterlogged soils is made possible by the presence of well-ventilated roots through which oxygen diffuses, leading to the formation of an oxidized rhizosphere (Justin and Armstrong 1987). The oxidizing activity of some wetland plant roots is evident by the deposition of reddish brown Fe oxyhydroxide precipitates on and around the roots (Armstrong 1967; Flessa and Fischer 1992). Iron oxidation reactions in flooded soils produce iron oxide precipitates and H^+ ions, resulting in increased acidity (Begg et al. 1994).

My previous studies found that elements accumulated in the root zone of *Rumex crispus* (Kissoon et al. 2010) and *Typha angustifolia* (Kissoon et al. 2011) more under flooded (wetland) compared to non-flooded (dryland) conditions in controlled laboratory experiments. The question then arises how such differences change along natural moisture gradients. Few plant species grow naturally across a wide range of soil moisture conditions. The plants used for those studies, *Typha angustifolia* and *Rumex crispus*, are both able to grow under widely varying moisture conditions, for example, wet during spring and early summer, then drying later in the year. I am not aware of any situation where these plants truly grow along a spatial gradient from wet to dry, completing their life cycles along that gradient. However, as observed in another study arising from my research group (Sunwar 2011), *Triglochin maritima* does. This plant was chosen for this study because it was found growing along a moisture gradient at Kellys Slough, North Dakota. The purpose of my study was therefore to determine the distribution of multiple elements in the root zone of *T. maritima* under different moisture conditions in the Kellys Slough. I hypothesized that (1) soils with higher moisture content would have significant accumulation of multiple

elements in the root zone, because such conditions can lead to steep biogeochemical gradients in the rhizosphere of plants, and (2) that plants on these soils would show greater uptake of these elements.

4.3. Materials and methods

4.3.1. Sample collection

The study site was located in Kellys Slough Wildlife Refuge in Grand Forks County, North Dakota (47°59'52.4" N, 97°14'28.17" W) (Figure 4.1). The soil was characterized as Lallie silty clay loam (Soil Survey Staff 2001). In September 2009, samples, consisting of approximately 8 L of soil with intact *Triglochin maritima* plants were collected using a spade. Five samples were collected from each of the three sample locations of varying elevation at different distances from a small creek; 1) less than 0.5 m from the creek (lowest elevation), 2) approximately 6 m from the creek, and 3) approximately 10 m from the creek (highest elevation) which was approximately 30 cm higher than the sampling locations at less than 0.5 m from the creek (Figure 4.2). *T. maritima* plants were the dominant plant species in the area sampled and their growth was uniform and continuous within about 10 m from the creek. The plant and soil samples were transported to the laboratory in plastic bags and stored in a refrigerator overnight at 4 °C until analysis.

A portion of soil was collected from each sample location, avoiding the surface and outermost soil layers, for determination of the moisture content. This was estimated by first determining the fresh weight and then the dry weight after drying at 60 °C. Soil was then collected from the root zone and bulk zone within each sample avoiding the oxidized surface layer and outermost soil layers. Soil from two different compartments was collected by hand: bulk zone soil (away from the root surface) and root zone soil (scraped from the root surface).

4.3.2. Soil sample analyses

Immediately upon collecting fresh soil samples from the bulk and root zones, the pH was measured in each using a Corning 430 pH Meter, and the redox potential (Eh) was also measured using a VWR Symphony SP90M5 Handheld Multimeter. The pH electrode was calibrated using buffer solutions pH 4, 7 and 10. The soil pH was measured by placing the electrode in a paste made with 6-12 drops of distilled water and approximately 1 g of fresh soil sample (United States Salinity Laboratory Staff 1954). The redox electrode was verified with two calibration solutions (pH 4 and pH 7 buffer solution

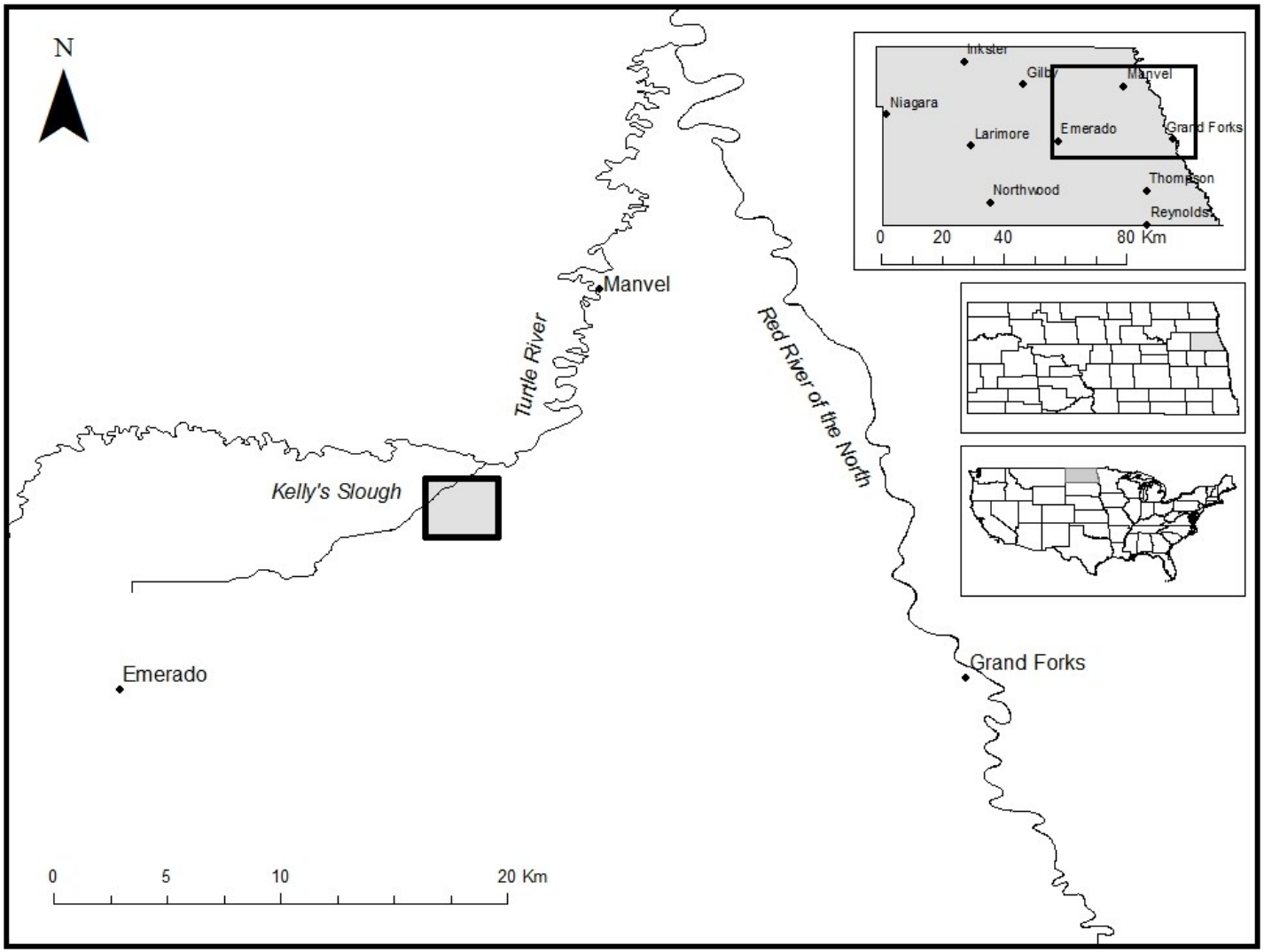

Figure 4.1. Map of general study area in the Kellys Slough, North Dakota.

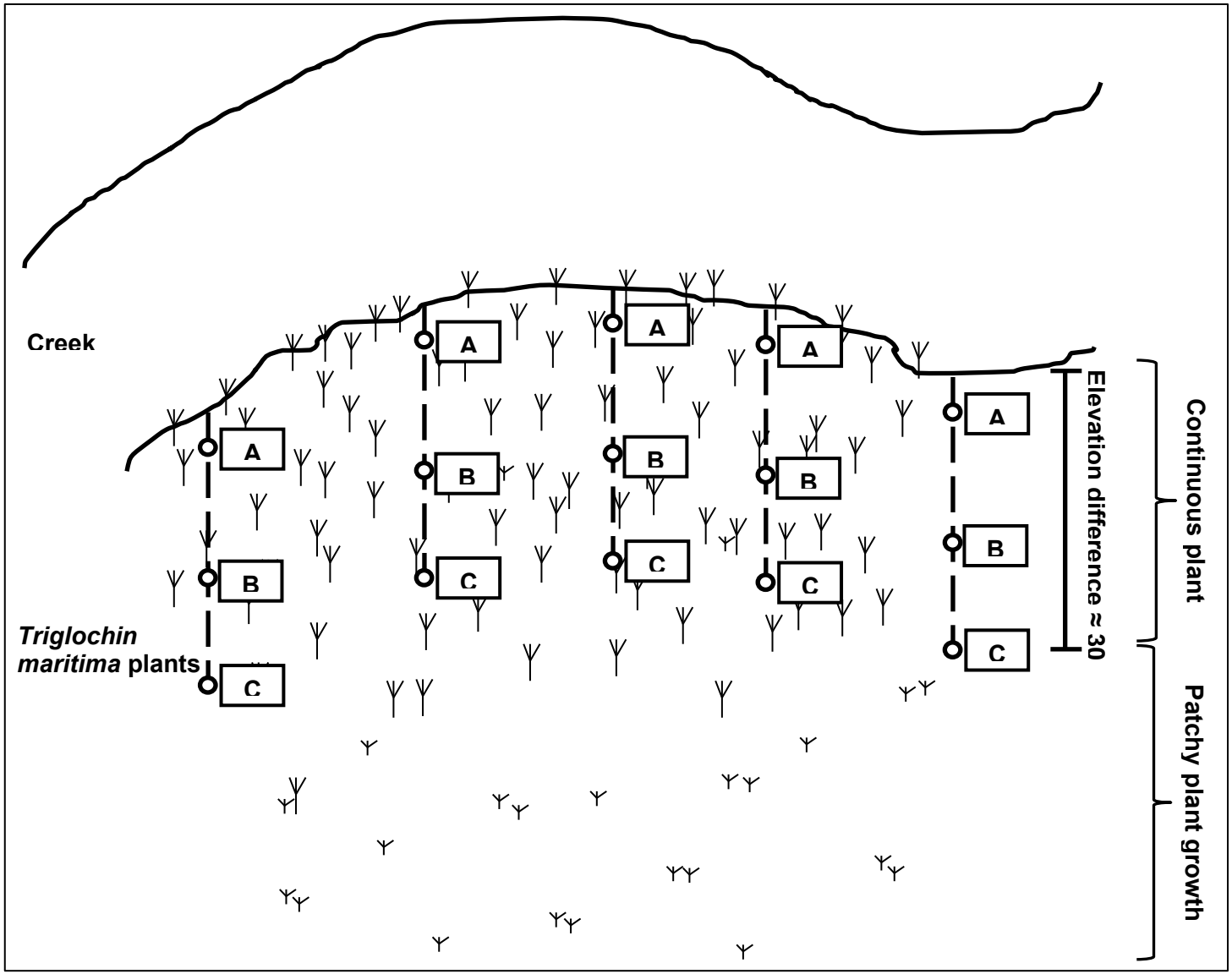

Figure 4.2. Diagram of sampling area (sampling distances from creek: A=0.5 m, B=6 m, C=10 m; approximate distance between samples of similar locations=2-7.5 m).

quinhydrone mixture). Eh measurements were taken by placing the redox electrode in a soil paste (Patrick et al. 1996) made with approximately 500 mg of fresh soil sample and 3 ml distilled water.

Ferrous iron concentrations were determined using a modified method by Roden and Wetzel (1996). Ferrous iron standards were prepared from a stock solution containing 100 mg L^{-1} ferrous ammonium sulfate hexahydrate and 1% (v/v) 6 M HCl. Fresh soil samples of a known weight (approximately 0.5 g), were immediately transferred to test tubes containing 5 ml of 0.5 M HCl to minimize oxidation and fix Fe^{2+} in the sample. The mixture was shaken vigorously and extraction was allowed for approximately 24 hours. For each of the samples, 0.25 ml of extract was collected and placed in a test tube containing 4.75 ml distilled water (1:20 dilution). After each diluted extract solution was shaken vigorously, 0.25 ml of sample or standard was added to 1.25 ml of FerroZine solution (1% wt/wt FerroZine in 50 mM HEPES buffer, pH adjusted to 7 with 1M NaOH). The color of the resulting solutions was allowed to develop for five minutes and the absorbance was measured using a Spectronic Helios Gamma UV-Vis Spectrophotometer at a wavelength of 562. A standard curve was constructed and used to calculate the ferrous iron concentrations in each sample.

The remaining soil samples were dried at 60 °C until constant weight, crushed using mortar and pestle and homogenized. These dried samples were reserved for loss-on-ignition (LOI, a measure of organic matter content), particle size and multi-element analysis. LOI of bulk and root zone samples was determined by drying the samples in an oven at 105 °C for two hours, weighing, then ashing in a furnace at 360 °C for two hours. After ashing, the remaining material was cooled, weighed and then passed through a 63-µm sieve under running water to determine an estimate of the particle size. The amount of sample that passed through the sieve was considered the fraction of soil particles smaller than 63 µm (f<63 µm).

A known amount of soil sample (approximately 500 mg dry material) was digested in 10 ml of HNO_3 in a MARS Xpress Microwave Digester (16 total vessels (XPRESS 55 ml PFA Venting Vessels), 1600W, 100% Power, ramped to 185 °C for 10 minutes and held at this temperature for 5 minutes). The digested samples were cooled, filtered with 3 – 1ml aliquots of water using Whatman® 1 filter paper and then analyzed for multiple elements with Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES) with Crossflow nebulizer, Side-On-Plasma (SOP). A four-point calibration using

individual or a combination of standards in a five percent HNO_3 matrix was used for this analysis. A continuing control verification (CCV) was done at the beginning and after every 20 samples of the analysis to check that variability was within 10% for Al and Ca while all other elements were monitored. Method detection limits in μmol g^{-1} for these elements were as follows: Ag 0.003; Al 0.05; As 0.05; B 0.06; Ba 0.003; Be 0.003; Ca 0.03; Cd 0.001; Ce 0.009; Co 0.006; Cr 0.006; Cu 0.02; Fe 0.02; Hg 0.01; K 0.6; Li 0.1; Mg 0.2; Mn 0.003; Mo 0.03; Na 0.3; Ni 0.02; P 0.3; Pb 0.01; S 0.3; Sb 0.04; Si 0.05; Sn 0.02; Sr 0.01; Ti 0.002; Tl 0.01; V 0.006; Zn 0.02; and Zr 0.002.

4.3.3. Plant sample analysis

The plant samples, separated into roots and shoots, were washed gently in distilled water, dried at 60 °C until constant weight, crushed using mortar and pestle and homogenized. A known amount of plant sample (approximately 250 mg) was pre-digested in 5 ml HNO_3 for 2 hours in a fume hood. Following pre-digestion, 5 ml distilled water was added to each sample and they were digested in a MARS Xpress Microwave Digester (16 total vessels (XPRESS 55 ml PFA Venting Vessels), 1600W, 100% Power, ramped to 185 °C over 10 minutes and held at this temperature for 5 minutes). After complete digestion, samples were cooled, transferred to clean vials with 3 ml aliquots of distilled water and then analyzed for multiple elements with ICP-OES following the same method as described above for the soil digests. Method detection limits in μmol g^{-1} for these elements were as follows: Ag 0.003; Al 0.2; As 0.06; B 0.05; Ba 0.008; Be 0; Ca 0.1; Cd 0.001; Ce 0.004; Co 0.005; Cr 0.004; Cu 0.02; Fe 0.06; Hg 0.01; K 0.6; Li 0.01; Mg 0.2; Mn 0.003; Mo 0.07; Na 0.6; Ni 0.01; P 0.2; Pb 0.008; S 0.4; Sb 0.04; Se 0.08; Si 0.06; Sn 0.01; Sr 0.007; Ti 0.002; Tl 0.009; V 0.004; Zn 0.004; and Zr 0.002. Element uptake by plants from each soil moisture group was determined by first calculating the total plant biomass per square meter (g m^{-2}) and then multiplying that value by the element concentration of each element (μmol g^{-1}). Element upatake is reported as the amount of element per plant per square meter (mmol $plant^{-1}$ m^{-2}).

4.3.4. Statistical analysis

The concentration data were log_{10} transformed before statistical analysis to obtain homogeneity of variance. Significance of differences (probability) was determined by two-way ANOVA ($p<0.05$) using Minitab Statistical software (Minitab®15 ©Minitab Inc.). To test for relationships between element concentrations, pH, Eh, LOI and moisture content, Pearson correlations and *p*-values were also

calculated using Minitab®15. I report correlations where r≥0.707 (p<0.001), that is, explaining 50% or more of the variation (McClave and Sincich 2006).

4.4. Results

4.4.1. Soil

4.4.1.1. Moisture content

Moisture content was significantly different between sample locations (p<0.001). Soil from less than 0.5 m from the creek had significantly higher moisture content than locations at 6 and 10 m from the creek (Figure 4.3). However, moisture content of the sampling locations at 6 and 10 m from the creek were not significantly different from each other and so only the locations at less than 0.5 m and 10 m were used for further statistical comparisons. I will refer to these sample locations as different moisture groups, samples taken at less than 0.5 m from the creek will be referred to as the high moisture content group and samples taken at 10 m will be referred to as the low moisture content group.

4.4.1.2. Soil pH, redox potential and Fe^{2+} concentrations

Soil pH was not significantly different between the bulk and root soil zones, but was significantly higher in the low moisture group compared to the high moisture group (Table 4.1, Figure 4.4). Redox potential was significantly higher in the low moisture content soils compared to the high content soils for both the root and bulk zones, but that difference was much more pronounced in the bulk soil, which explains the significant interaction between the two factors. Ferrous iron concentrations were much higher in the high moisture soil group compared to the low moisture groups, but similar between the root and bulk soil zones (Figure 4.4). Significant interaction indicated that the difference between high and low moisture soils in Fe^{2+} concentrations was greater in the bulk zone compared to the root zone soils. Fe plaque was visible adjacent to roots in the high moisture content soils.

4.4.1.3. Loss-on-ignition and particles smaller than 63 µm

LOI was similar between moisture groups and soil zones (Table 4.1), but the significant interaction between the factors indicates lower LOI in low moisture soils compared to high moisture soils only in the bulk zone soils (Figure 4.4). The fraction of particles smaller than 63 µm (mean ± the standard deviation) was similar between moisture groups and soil zones and was very high averaging 99±0.2% dry soil.

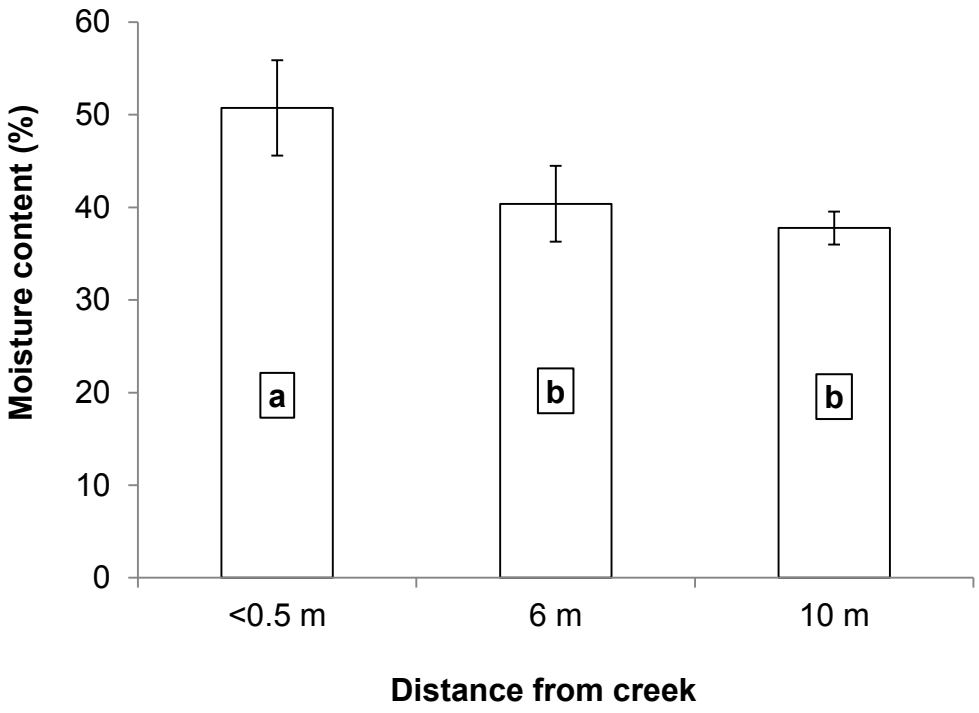

Figure 4.3. Mean soil moisture content and standard deviation (error bars) for the sample locations at different distances from a creek under *Triglochin maritima* (different letters indicate significant differences between sample locations) (n=5).

4.4.1.4. Multi-element concentrations

Not all of the 34 element concentrations measured yielded results suitable for statistical analysis. Element concentrations were at or below the detection limits for Ag, As, Cd, Hg, Mo, Pb, Sb, Se, Si, Sn and Tl. A few elements were detectable but showed no significant variation and the mean concentrations ± the standard deviation in µmol g^{-1} dry soil averaged for all samples were calculated as follows: B: 5.6±1.2, Ba: 0.6±0.1, Fe: 255±36, P: 23±4, S: 109±42 and Ti: 0.8±0.4.

The concentrations of Be, Cu, Li, Ni, Zn and Zr varied significantly between both the moisture groups and soil zones, while concentrations of Ca, Ce, Co, Cr, Mg, Mn, Na, Sr and V varied between the moisture groups only, and Al and K between the soil zones only (Table 4.1). There were no significant differences in element concentrations between the root zone and bulk zone within either moisture group. On the other hand, comparison of root zone soils (Table 4.2) showed that the high moisture content soil had significantly higher concentrations of Ce, Fe^{2+} and Zn compared to the low moisture content soil, but the reverse was true for Ca, Na and Sr. Comparison of the bulk zone soils showed that the high moisture

content soil had significantly higher concentrations of Be, Ce, Fe^{2+}, Ni, Zn and Zr and the low moisture content soil had significantly higher Ca, Mg, Mn, Na and Sr.

Correlation analysis was carried out to identify possible underlying patterns regardless of the moisture groups or soil zones. Several elements showed highly significant correlations with each other (Al, Be, Cr, Cu, Ni, V, Zn and Zr; Ca, Fe^{2+}, Mn and Sr; Ce, Na, Zn and Zr) (Table 4.3). Other correlations included Eh with Ca, Fe^{2+}, Mn, Ni, Sr and Zn; water content with Ca, Fe^{2+}, Li, Mg and Sr; Ca with Ce, Mg and Na; Ce with Cr, Ni and Sr; Co with Al, Ni, Zn, and Zr; K with Al, Cu and Li; and Li with K, Mg and Mn.

4.4.2. Plants

Plant biomass per square meter (mean ± standard deviation) was similar for the different moisture groups (low moisture: 127±129 g aboveground biomass m^{-2}, 244±66 belowground biomass m^{-2}; high moisture: 65±42 g aboveground biomass m^{-2}, 155±80 belowground biomass m^{-2}). Element concentrations in some plant material were at or below the detection limit (Ag, As, Cd, Ce, Co, Cr, Hg, Mo, Ni, Pb, Sb, Se, Sn, Tl and Zr). These elements will not be discussed further. Some elements were detectable but showed no significant variation and the mean element concentrations ± the standard deviation in μmol g^{-1} dry weight averaged for all samples were as follows: Al (7±6 aboveground, 41±47 belowground tissue), Ba (0.02±0.01 aboveground, 0.1±0.05 belowground tissue), Ca (210±72 aboveground, 125±39 belowground tissue), Cu (0.2±0.2 aboveground, 0.3±0.2 belowground tissue), K (759±181 aboveground, 429±203 belowground tissue), Li (3±2 aboveground, 1±0.3 belowground tissue), Mn (1±0.5 aboveground, 3±1 belowground tissue), Na (1898±1041 aboveground, 580±413 belowground tissue), P (129±36 aboveground, 99±20 belowground tissue), Si (6±5 aboveground, 64±37 belowground tissue), Sr (3±1 aboveground, 3±0.4 belowground tissue), Ti (0.06±0.04 aboveground, 0.3±0.4 belowground tissue), V (0.01±0.01 aboveground, 0.1±0.1 belowground tissue) and Zn (0.5±0.2 aboveground, 0.5±0.2 belowground tissue).

Plants from the low moisture content soil had significantly higher concentrations of B, Mg and S in their aboveground tissues and higher concentrations of B, Fe and Mg in their belowground tissues compared to plants from the high moisture content soils (Table 4.4). Whole plants from the high moisture content soils had significantly higher element contents (mmol $plant^{-1}$ m^{-2}) of Fe and Mn compared to plants from the low moisture content soils.

Table 4.1. Significance of differences (probability) in element concentrations, soil Eh, pH and LOI between moisture groups (high and low moisture content) and between soil zones (root and bulk zone) as determined by Two-Way ANOVA (ns indicates non-significance, i.e. $p>0.05$, $n=5$)

	Source of Variation		
	A. Moisture Groups	B. Soil Zones	Interaction (A x B)
Variable	*p*-value		
Eh	0.000	0.004	0.005
pH	0.005	ns	ns
LOI (% dry soil)	ns	ns	0.000
Al	ns	0.049	ns
Be	0.001	0.023	ns
Ca	0.000	ns	ns
Ce	0.000	ns	ns
Co	0.007	ns	ns
Cr	0.004	ns	ns
Cu	0.019	0.017	ns
Fe^{2+}	0.000	ns	0.027
K	ns	0.024	ns
Li	0.005	0.017	ns
Mg	0.002	ns	ns
Mn	0.000	ns	ns
Na	0.000	ns	ns
Ni	0.000	0.047	ns
Sr	0.000	ns	ns
V	0.025	ns	ns
Zn	0.000	0.025	ns
Zr	0.002	0.036	ns

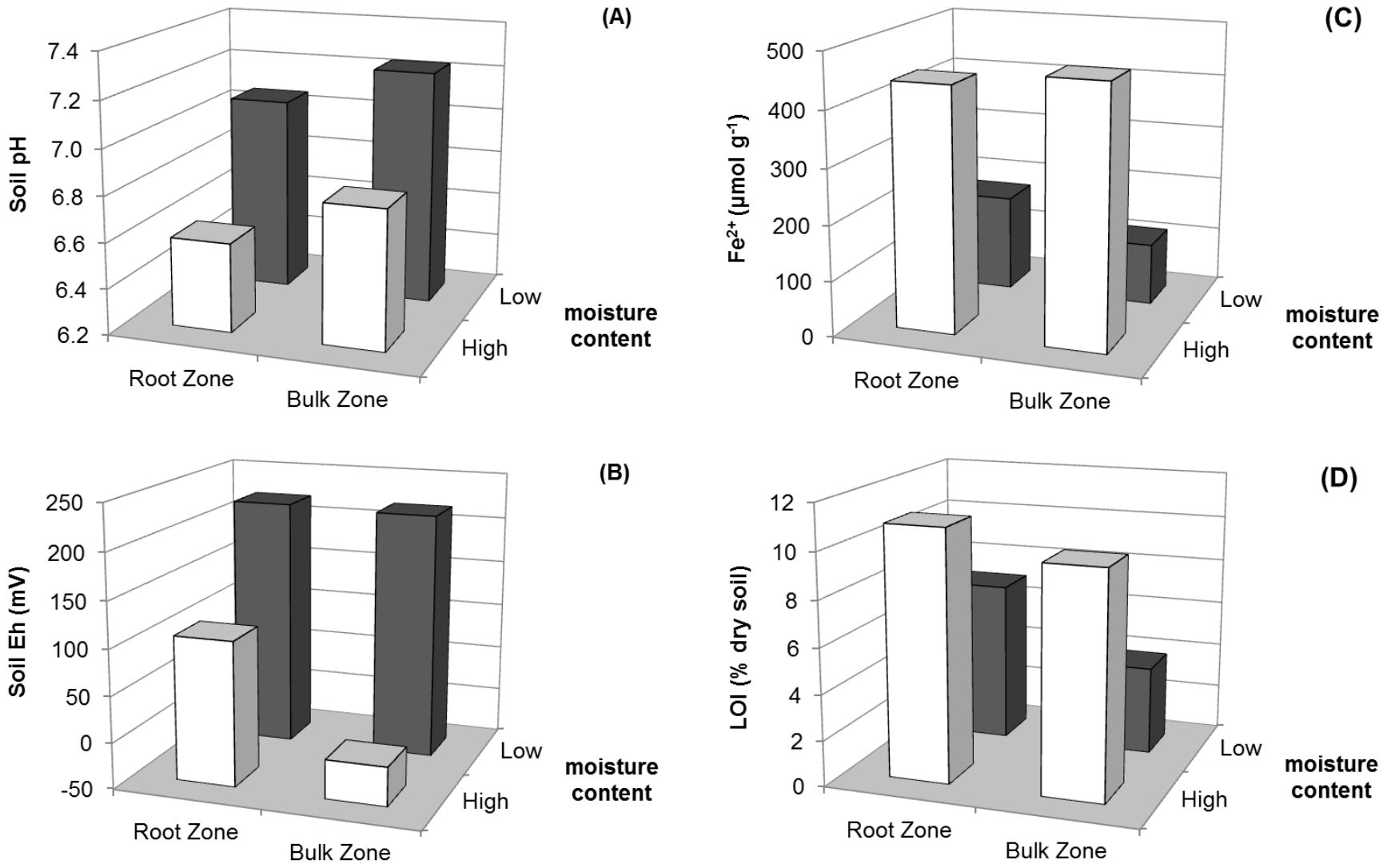

Figure 4.4. (A) Mean soil pH, (B) Eh (redox potential), (C) Fe^{2+} concentrations and (D) loss on ignition (LOI, % dry soil) of the root and bulk zone soils under *Triglochin maritima* for the different moisture content groups.

Table 4.2. Mean element concentrations (μmol g^{-1} dry soil) in the root and bulk zone soils under *Triglochin maritima* for the different moisture groups (mean ± standard deviation, *n*=5, significant differences between moisture groups within each soil zone is marked by * after the higher value for a particular soil zone).

Element	Root Zone		Bulk Zone	
	High H_2O content	Low H_2O content	High H_2O content	Low H_2O content
Al	500±70	443±88	625±122	511±107
Be	0.06±0.009	0.04±0.005	0.07±0.01*	0.05±0.008
Ca	368±215	1220±278**	460±227	1560±493**
Ce	0.26±0.05*	0.19±0.01	0.27±0.04*	0.20±0.03
Co	0.09±0.02	0.07±0.01	0.11±0.01	0.09±0.01
Cr	0.35±0.05	0.28±0.05	0.41±0.08	0.31±0.05
Cu	0.24±0.05	0.20±0.04	0.30±0.05	0.24±0.05
Fe^{2+}	465±63***	112±23	441±148***	175±35
K	110±14	112±20	136±20	132±28
Li	4.3±0.5	5.7±1.5	5.4±1.0	7.8±2.1
Mg	291±59	404±75	343±57	515±121*
Mn	6.0±2.0	10±4.6	5.5±2.2	13±3.0**
Na	249±122	452±134*	224±51	389±41*
Ni	0.27±0.04	0.21±0.04	0.32±0.02*	0.23±0.04
Sr	3.1±1.2	8.7±1.8***	3.8±1.5	11±3.0***
V	0.63±0.08	0.53±0.12	0.8±0.2	0.60±0.11
Zn	0.77±0.09*	0.61±0.06	0.89±0.07**	0.66±0.09
Zr	0.23±0.04	0.18±0.03	0.28±0.04*	0.21±0.03

Table 4.3. Pearson correlations for Eh, moisture content and element concentrations in soil (pooled for all samples) below *Triglochin maritima*. Correlations with r≥0.707 (that explain 50% or more of variation) only are shown (p<0.0001).

	Eh	Water Content	Al	Be	Ca	Ce	Co	Cr	Cu	Fe^{2+}	Li	Mg	Ni	Sr	V	Zn
Be	--	--	0.880	--	--	--	--	--	--	--	--	--	--	--	--	--
Ca	0.716	-0.735	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Ce	--	--	0.750	0.838	-0.811	--	--	--	--	--	--	--	--	--	--	--
Co	--	--	0.713	--	--	--	--	--	--	--	--	--	--	--	--	--
Cr	--	--	0.945	0.968	--	0.852	--	--	--	--	--	--	--	--	--	--
Cu	--	--	0.751	0.906	--	--	--	0.809	--	--	--	--	--	--	--	--
Fe^{2+}	-0.841	0.834	--	--	-0.803	--	--	--	--	--	--	--	--	--	--	--
K	--	--	0.846	--	--	--	--	--	0.774	--	0.712	--	--	--	--	--
Li	--	-0.744	--	--	--	--	--	--	--	--	--	--	--	--	--	--
Mg	--	-0.777	--	--	0.814	--	--	--	--	--	0.918	--	--	--	--	--
Mn	0.736	--	--	--	0.881	--	--	--	--	-0.773	0.751	0.83	--	0.885	--	--
Na	--	--	--	--	0.708	-0.803	--	--	--	--	--	--	--	0.714	--	-0.756
Ni	-0.723	--	0.804	0.906		0.782	0.878	0.864	0.867	--	--	--	--	--	--	--
Sr	0.756	-0.728	--	--	0.982	-0.824	--	--	--	-0.828	--	0.762	--	--	--	--
V	--	--	0.898	0.898	--	--	--	0.935	0.725	--	--	--	0.796	--	--	--
Zn	-0.743	--	0.834	0.936	--	0.864	0.797	0.903	0.845	--	--	--	0.927	--	0.783	--
Zr	--	--	0.933	0.974	--	0.866	0.722	0.988	0.829	--	--	--	0.895	--	0.928	0.919

Table 4.4. Mean element concentrations in plant parts (μmol g^{-1}) and mean element content of whole plant (mmol $plant^{-1}$ m^{-2}) for the different moisture groups (mean ± standard deviation, *n*=5, significant differences between moisture groups (high moisture content or low moisture content) for each plant part concentration or whole plant content is marked by * after the higher value).

Element	Aboveground tissue		Belowground tissue		Whole plant	
	(μmol g^{-1})		(μmol g^{-1})		(mmol $plant^{-1}$ m^{-2})	
	High	Low	High	Low	High	Low
Al	8.34±8.84	5.25±1.19	30.6±11.2	51.8±67.4	8.98±3.27	13.1±20.5
B	4.32±0.6	6.54±1.25**	11.3±3.74	17.3±3.2*	3.25±1.1	3.3±1.81
Ba	0.02±0.01	0.03±0.01	0.13±0.04	0.12±0.06	0.04±0.01	0.02±0.023
Ca	200±98.7	221±39.3	122±20.2	129±54.7	64.3±50.2	39.1±20.7
Cu	0.14±0.13	0.25±0.2	0.34±0.29	0.24±0.09	0.08±0.031	0.05±0.036
Fe	4.46±1.76	3.64±1.09	162±54	68.4±20.8**	40±16.7**	12.3±10.1
K	738±192	780±188	474±230	384±187	193±58.5	112±29.4
Li	1.95±1.29	3.49±1.78	0.97±0.25	1.21±0.31	0.62±0.59	0.5±0.24
Mg	183±45.1	263±62.3*	116±7.79	174±33.2**	56.3±38.5	48.4±18.7
Mn	1.42±0.55	0.97±0.22	4.03±0.66	2.95±0.95	1.19±0.37*	0.59±0.44
Na	1490±959	2300±1060	496±356	663±489	398±373	288±119
P	139±45.4	118±23.1	94.3±27.5	104±8.57	37.1±14.8	22.6±5.63
S	174±28.9	233±44.3*	113±20.7	118±23.3	52.3±32.6	35.9±13.2
Si	6.86±6.71	5.8±2.24	66.2±24.8	61.7±48.9	17.1±4.45	13.4±16.7
Sr	2.56±1.17	3.84±0.55	2.71±0.36	2.37±0.37	1.09±0.63	0.66±0.23
Ti	0.07±0.052	0.05±0.012	0.2±0.05	0.41±0.59	0.06±0.02	0.11±0.18
V	0.01±0.01	0.01±0	0.1±0.02	0.17±0.1	0.02±0.01	0.03±0.04
Zn	0.41±0.18	0.51±0.3	0.44±0.12	0.54±0.23	0.14±0.03	0.12±0.08

4.5. Discussion

Studies have found that several elements accumulated in wet and dry soils associated with different wetland plants (Kissoon et al. 2010, 2011). Doyle and Otte (1997), found that As, Fe, and Zn accumulated in salt marsh soils associated with *Atriplex portulacoides* and *Spartina townsendii.* Similarly, under wet conditions, Be, Ce, Fe^{2+}, Ni, Zn, and Zr accumulated in the soils associated with *T. maritima*, while under dry conditions Ca, Mg, Mn, Na, and Sr accumulated in the soils associated with *T. maritima*. Plants grown under wet conditions took up more Fe and Mn than plants grown under dry conditions. B, Ba, Ca, Cu, K, Li, Mg, Na, P, S, Si, Sr, and Zn displayed a similar trend of uptake but the differences were not significantly different. Similarly, studies have found that other wetland plants take up significantly more Al, B, Ba, Ca, Cu, Fe, K, Li Mg, Mn, Na, Ni, P, S, Si, Sr, Ti, and Zn when grown under wetland compared to dryland conditions (Kissoon et al. 2010, 2011).

The patterns of element accumulation in the root zone and bulk zone of *T. maritima* are different from my greenhouse studies (Kissoon et al. 2010, 2011). These differences could be attributed to several factors including differences in the experimental setup and sampling technique. First, and probably most important, the plant species used in this study, *T. maritima*, is very different from those used in the greenhouse experiments, i.e. *Rumex crispus* and *Typha angustifolia*. Second, the plants in the greenhouse studies were grown from seed for several months while plants in the field study most likely would have been several years old with developed root systems. Another reason for differences is that the root zone in this study was not as clearly defined and localized as in my greenhouse experiments (Kissoon et al. 2010, 2011). In the green house experiments, the root zone included soil attached to roots and within 3 mm of the root surface while the root zone described in the present study was soil scraped from the root surface. Furthermore, in the greenhouse experiments, gradients in element concentrations towards the roots were limited to the total amount available in the soil columns. In contrast, element accumulation in the root zone in the field and subsequent uptake by plants can be compensated for by migration of elements from farther away from the plant. Variations in element concentrations in the field were due to an equilibrium that had several years to form while in the greenhouse studies variations in element concentrations formed over several months only.

Soil properties such as moisture content, pH and redox conditions are altered by living plant roots and in turn can influence the bioavailability and mobility of chemical elements in soil (Cataldo and Wildung 1978; Alloway 1995; Deiana et al. 2001; Hinsinger and Courchesne 2008). Element mobility can also be influenced by the soil solution composition, type and density of charge in soil colloids, the reactive surface area (Cataldo and Wildung 1978) and formation of complexes in the rhizosphere (Deiana et al. 2001). The availability of elements for plant uptake is also dependent on soil factors such as organic content, Ca content, cation exchange capacity and temperature (De Laune et al. 1981; Davies 1994). A combination of differences in moisture content, pH, Eh and LOI may explain the different patterns of element accumulation that were observed in this study.

My greenhouse studies showed differences in the patterns of element accumulation in rhizosphere of wetland plants under different moisture conditions. For *Rumex crispus*, rhizosphere accumulations of Al, Cr, Fe, K, La, Sr, V, Y and Zn occurred under wetland conditions and Al, Ba, Cu, Cr, Fe, K, La, Mg, Na, Sr, V, Y and Zn occurred under dryland conditions (Kissoon et al. 2010). For *Typha angustifolia*, rhizosphere accumulations of Be, Cu, Fe, Li, Sr and Zn occurred under wetland conditions and Ni and Sr occurred under dryland conditions (Kissoon et al. 2011). In a study of dryland plants, Assadian and Fenn (2001) found that Cu, Pb, Mn and Zn concentrations and organic carbon were significantly greater at the soil-root interface compared to bulk soil. Several studies have reported patterns of element accumulation or mobility in the rhizosphere of wetland plants for As, Fe and Zn (Otte et al. 1991; Kirk and Bajita 1995; Otte et al. 1995; Doyle and Otte 1997; Wright and Otte 1999; Jacob and Otte 2004b). In a tailings pond, Jacob and Otte (2004a) found that flooded zones showed higher metal mobility than dryer zones. The study reported here found no differences between the root and bulk zone of either soil of different moisture content, but within the zones, differences in element concentrations were found depending on the mositure content. This seems to be a contradiction to my earlier findings *with R. crispus* and *T. angustifolia*, but it is not. What was defined as root zones here is not the same as what was called the rhizosphere in my other studies. In the greenhouse experiments (Kissoon et al. 2010, 2011), the soils were either fully saturated or dry (just above the wilting point of the plants), while the rhizosphere was a clearly defined layer, no more than a few millimeters across, immediately adjacent to the roots. The more pronounced differences between the ‘bulk soil’ and the ‘rhizopshere soil’ in the

greenhouse experiments ocurred in the continously saturated treatments. From this it seems that water content of the soil is the most important factor determining element concentrations in wetland soils. The effects of rhizosphere oxidation of the roots then are particularly prominent in soils that are water-saturated most of the time. Such conditions exist, for example, in coastal salt marshes, which is where Doyle and Otte (1997) found strong As, Fe and Zn accumulation in the rhizosphere of salt marsh plants. The site in this study, Kellys Slough marsh, an inland salt marsh, goes through prolonged periods of drought and may over the year be much drier than the studies by Doyle and Otte (1997) and the greenhouse studies by Kissoon et al. (2010, 2011)

Soil pH and redox status of the soil in the present study were different from those reported in the greenhouse studies (Kissoon et al. 2010, 2011) and may explain the different patterns of element accumulation. The moisture content influences the pH and redox status of soil (Ponnaperuma 1972; Nye 1981; Sajwan and Lindsay 1985; Mendelssohn 1993) and hence influence the element concentrations of the soil. In both the low and high moisture soil groups the pH ranges were lower than what we observed previously for flooded soil (6.93-8.20) and for non-flooded soil (7.52-8.73) (Kissoon et al. 2010, 2011). However, both the greenhouse and field studies observed differences in pH between the two moisture treatments or groups which may be due to acidification of the saturated or high moisture soil caused by accumulation and subsequent dissolution of CO_2 (Ponnamperuma 1972). The soil in the present study was not as reduced as soils in the earlier studies with *Typha angustifolia* and *Rumex crispus*, which reached redox potentials as low as -113 mV and -140.5 mV (Kissoon et al. 2010, 2011). The Kellys Slough soils experience prolonged dry periods and is not continuously saturated like the wetland treatment in the greenhouse studies. Hence, the Kellys Slough soils would not be able to reach the reduced conditions observed in the greenhouse studies. Similarly to the greenhouse studies, the high moisture content soil had lower redox potential and hence was more reduced compared to the low moisture content soils. The redox status of soil influences the availability of redox sensitive elements (Davies 1994; Hinsinger 2001; Kirk 2004; Kidd et al. 2009). The difference in redox potential between the high moisture and low moisture content soils may account for why these soils had higher or lower concentrations of specific elements. Some of the elements that occurred at higher or lower concentrations in the high moisture content soils also correlated with redox potential (Ca, Fe^{2+}, Mn, Ni, Sr,

Zn), which implies that redox status played a role in their distribution. In waterlogged soils, the redox potential of the rhizosphere increases as a result of oxygen leaking from the plant roots via radial oxygen loss (Davies 1994). In the high moisture content soils, the root zone had a higher redox potential than the bulk zone, which is evidence of rhizosphere oxidation. Rhizosphere oxidation can lead to the precipitation of Fe and Mn and subsequent co-precipitation of other elements at the soil-root interface (Hinsinger 2001). The presence of a redox gradient in the high moisture content soils did not correspond with an element concentration gradient as seen in previous studies (Voegelin et al. 2007; Kissoon et al. 2010, 2011). This may be due to the extent and impact of rhizosphere oxidation being less in the field studies due to the moisture content of the high moisture soils not being saturated continuously as in the greenhouse studies.

The high Fe^{2+} concentrations of waterlogged soils are indicative of low redox potential and reduced conditions (Justin and Armstrong 1987), which is the case for the high moisture content soils in the present study. Ferrous iron concentrations were higher in both the high and low moisture content soils compared to soils in the previous studies where the average Fe^{2+} concentrations detected were 115±121 and 130±130 μmol g^{-1} (Kissoon et al. 2010, 2011). This may due to years of Fe^{2+} accumulation in the Kellys Slough soil. In wetland soils with high Fe^{2+} concentrations, sulfur can bind with iron to form insoluble ferrous sulfides, which can then reduce the mobility and availability of trace elements (Mitsch and Gosselink 2007). Sulfur concentrations in the Kellys Slough are high compared to sulfur concentrations in the greenhouse studies (Kissoon et al. 2010, 2011) and in freshwater wetlands (Jones et al. 1982) but lower than that of coastal salt marshes where there is constant sulfate input by seawater (Ferdelman et al. 1991). Under reduced conditions, metals form insoluble metal sulfide precipitates thus making them unavailable for uptake (Gambrell 1994; Kirk 2004; Choi 2006). In flooded soils Fe^{2+}, Mn^{2+}, Hg^{2+} and Cu^{2+} form relatively stable insoluble sulfides (Kabata-Pendias and Pendias 2001). Metals such as Cd, Co, Ni, Sn, Ti and Zn may coprecipitate with iron sulfides thus reducing their mobility (Jenne 1977; Choi 2006). Metal sulfide precipitation may account for the lack of element concentration gradients between the root and bulk zone soils and may explain differences in element accumulation with the greenhouse studies.

Previous studies found that Fe and LOI (Otte et al. 1991; Otte et al. 1995; Doyle and Otte 1997; Jacob and Otte 2003; Kissoon et al. 2010, 2011) play an important role in the distribution patterns of elements in wetland soils. Total Fe concentrations did not vary significantly in this study but we found that Fe^{2+} concentrations correlated with a few elements. LOI (indicative of organic content) was higher in both the high and low moisture content soils compared to soils in the previous studies where LOI was less than 4% (Kissoon et al. 2010, 2011). High organic matter in soil can form complexes with metals making them unavailable for uptake and hence decreasing their concentrations in soil (Antonovics et al. 1971; Davies 1994). Doyle and Otte (1997) indicated that high LOI may interfere with oxidation patterns of Fe and the binding of metals with Fe oxides in wetland soils. I did not find significant correlations for LOI or Fe with any of the elements that showed variation in the study. This may be due to interference by the high organic content detected in the Kellys Slough soil.

Although particle size did not vary in this study, it may still influence the distribution of elements in the soil. Aluminum is an important component of silicate and clay minerals (Bertsch and Bloom 1996) and appears to be dominant in the clay-rich soil of the Kellys Slough. Transport of trace elements in soils is impacted by their affinity to adsorb to clay minerals in the soil (Kabata-Pendias and Pendias 2001; Kirk 2004). Aluminum correlated with several elements that showed significant variation and was a significant covariate with some elements including Be, Ce, Cr, K, Li, Ni, Zn and Zr. Aluminum may play an important role in the underlying patterns of distribution of these elements.

The greenhouse studies (Kissoon et al. 2010, 2011) and the study reported here had several similarities and differences. The greenhouse and field studies observed element accumulations in the soils of higher moisture content and greater uptake of these elements by wetland plants. However, patterns of element accumulation in the root zone and bulk zone were different. This could be due to a number of factors, which we have previously discussed. These studies have shown that water content of the wetland soils played a key role in the distribution of elements in these soils. Other factors may include differences in experimental growth conditions (e.g. greenhouse column experiments versus field grown plants), soil physiochemical conditions, trace metal availability and differences between plant species (e.g. root morphology, nature of root exudates, nutrient acquisition strategies) (Kidd, et al. 2009).

4.6. Conclusions

Results of this study indicated that moisture content played a role in element distribution in wetland soils. Other factors, such as pH, redox, Fe^{2+} and sulfide concentrations, LOI, particle size and wetland plant species may also play a role in element distribution in wetland soils. This study found that several elements accumulated in soils with higher moisture content and that plants growing in these soils tend to take up more of these elements in their tissues.

SECTION 2 – SHALLOW LAKES STUDY

CHAPTER 5. SHALLOW LAKES: VARIATIONS IN AQUATIC VEGETATION AND BIOGEOCHEMISTRY

5.1. Abstract

This study examined macrophyte abunadance and community composition, and multi-element concentrations of waters and sediments of 44 shallow lakes of varying turbidity and macrophyte cover in Minnesota. The results identified significant associations between several environmental variables and, 1) macrophyte cover, 2) macrophyte biomass, 3) macrophyte community composition, 4) water element concentrations, and 5) sediment element concentrations. Land cover uses (percent woodland and percent grassland), chlorophyll-*a*, and open water area were identified as significant sources of variance for macrophyte cover while sediment physical characteristics (loss-on-ignition and particles smaller than 63μm), land cover uses (percent cropland and percent woodland), and turbidity were identified as significant sources of variance of macrophyte biomass. Percent woodland and percent agriculture were significant sources of variance of element concentrations in the water while percent woodland, lake watershed area and sediment physical characteristics were significant sources of variance of elements in the sediments. Concentrations of Ca, Mg, Na, S and Sr in water and Al, Li, Nd, Pr, Sc and U in sediments were identified as significant predictors of macrophyte community composition. High macrophyte abundant lakes had higher Mn and lower Si concentrations in their waters and higher B, Ba, Ca, Mo, P, S, Sr, U and Zr in their sediments compared to low macrophyte abundant lakes.

5.2. Introduction

Plant community composition and distribution varies with climate, hydrology, substrate type and nutrient availability (Cronk and Fennessy 2001). Each macrophyte species has adaptations or tolerances allowing them to attain optimum growth under particular chemical conditions (Moyle 1945). Povidisa et al. (2009) found that natural (habitat and biotic variables) and anthropogenic effects influenced submerged community composition while land use variables (urban, forest, agriculture) influenced emerged and floating macrophytes. Different macrophyte species are tolerant of nutrient-poor and/or clear conditions while some are tolerant of nutrient-rich and/or turbid conditions (Lougheed et al. 2001; Mackie 2004). Povidisa et al. (2009) reported that *Potamogeton pectinatus*, *Ceratophyllum submersum* and *Lemna* spp. were tolerant of eutrophic systems while *Isoetes* spp. *Myriophyllum alterniflorum*, *Utricularia australis* and

Nitella translucens were tolerant of nutrient-poor systems and sensitive to eutrophication. Macrophytes also have adaptations that are exclusive to conditions of their habitat (Cronk and Fennessy 2001). Moyle (1945) identified three groups of plants in Minnesota lakes, 1) macrophytes of soft-water lakes which occur most often in northeastern Minnesota, 2) macrophytes that inhabit the hard-water moraine lakes in central, northern and southern Minnesota and, 3) macrophytes of alkali or high sulfate lakes in the western and southwestern prairies of Minnesota.

Shallow lakes are known to exhibit regime characteristics (Scheffer 2004) and Zimmer et al. (2009) reported that shallow lakes in Minnesota also conform to these dynamics. Turbid regimes are usually dominated by phytoplankton with little to no submerged vegetation while clear regimes are dominated by abundant submerged vegetation (Scheffer 2004). Studies have shown that shallow lakes can shift between turbid and clear regimes from year to year or after several years (Blindow et al. 1998; Bayley et al. 2007). Sediments in shallow lakes that have abundant plant cover are less vulnerable to disturbance and resuspension by wind or wave action and foraging activities of benthivorous fish (Faafeng and Mjelde 1998; Horppila and Nurminen 2003; Scheffer 2004). Submerged plants decrease sediment resuspension and subsequently reduce phosphorus loading to the water column and turbidity (Horppila and Nurminen 2003). Several studies have reported shifts in lake regimes or lake clarity in response to changes in water levels, plant biomass and spring temperatures (Blindow et al. 1998), total phosphorus concentrations (Bayley et al. 2007) and fish biomass (Zimmer et al. 2009). Scheffer and Jeppesen (1998) indicated that the switch from a macrophyte-dominated to a turbid regime in shallow lakes might be due to increased and continuous nutrient loading.

Land use is a major factor influencing the loading of nutrients into lakes and is dependent on the lake watershed transport capacity (Fraterrigo and Downing 2008). Plant community composition in lakes can be impacted by geology, land cover, water and sediment chemistry (Moyle 1945; Stewart and Kantrud 1972; Barko and Smart 1986; Barko et al. 1991; Koch 2001; Lougheed et al. 2001; Hansel-Welch et al. 2003; Del Pozo et al. 2011). Water chemistry (Nilsson and Håkanson 1992; Whigham and Jordan 2003; Fraterrigo and Downing 2008) and macrophyte community composition of lakes are influenced by the surrounding land use (Stewart and Kantrud 1972; Lougheed et al. 2001; Del Pozo et al. 2011). Lakes within developed or agricultural watersheds are subject to increased nutrient inputs due to runoff from

fertilizer use or animal waste (Atkinson et al. 2011). Wetlands impacted by agricultural activities tend to have higher nutrient concentrations, higher turbidities and lower species richness compared to wetlands not influenced by agriculture (Lougheed et al. 2001; Atkinson et al. 2011; Rowan et al. 2012). Unlike agricultural or developed land, forested land prevents sedimentation and the release of nutrients via runoff into surface waters due to the presence of constant vegetation cover (Lougheed et al. 2001).

The purpose of this study was to determine 1) if the composition of macrophyte communities in shallow lakes is related to lake turbidity and multi-element concentrations in the lake water and sediment and, 2) if predominant land use of the watershed was related to macrophyte community composition, water and sediment chemistry of shallow lakes. I hypothesized that 1) high plant abundance will coincide with low turbidities, 2) low plant abundance will coincide with high element concentrations in the associated water and sediment, and 3) lakes with high element concentrations will be associated with agriculture-dominated watersheds. Based on previous studies involving either emergent or submerged vegetation, we expected that elements such as Cu, Fe, Mn, P, Pb and Zn concentrations would be elevated in the water and sediments when there was low plant abundance (Chen and Barko 1988; Goulet and Pick 2001; Keskinan et al. 2004).

5.3. Materials and methods

5.3.1. Description of study sites

This study involved 38 shallow lakes in Minnesota which were sampled during August 9-19, 2010 and August 8-17, 2011. These included six shallow lakes in Grant County, six in Itasca State Park (Hubbard and Clearwater Counties), six on the Red Lake Indian Reservation (Clearwater and Beltrami Counties) that were sampled in 2010, and 20 in the Windom area that were sampled in 2011 (Jackson and Cottonwood Counties). In addition to these 38 lakes, six lakes located within a bog area on the Red Lake Indian Reservation (Clearwater County) were sampled July 19 and 21, 2010. Due to the isolation and the difficulty of transporting sampling equipment within the Red Lake bog area, cover data could not be collected, but some water and sediment samples were collected from these six lakes (RL07, RL08, RL09, RL10, RL11, RL12). For comparison purposes, I will refer to the study sites as occurring within different regions of Minnesota, referring to these regions as the Grant County (GC), Itasca (IT), Red Lake (RL), and Windom (W) regions (Figure 5.1). Shallow lakes in this study occurred within watersheds

ranging from 8-23953 ha, were from 1.8-59 ha in basin area and averaged 1.4±0.8 m deep (Table 5.1). Shallow lakes in the Itasca and Red Lake regions are located within areas where the dominant land cover (>40%) is forest (Minnesota Geospatial Information Office Staff 1999). Shallow lakes in Grant County and the Windom region occur in areas where dominant land cover is cultivated land (>79%). Lakes in the Red Lake and Itasca regions were located in areas dominated by woodland while those in Grant County and Windom were located in areas dominated by agriculture (Table 5.2).

Study lakes in the Itasca region occur within the Itasca moraine, Red Lake within Erskine moraine and Peat deposits, Grant County within Big Stone moraine, and Windom within Altamont moraine (Ojakangas and Matsch 1982; Lusardi 1997). Parent materials include glacial till or outwash except for the lakes located within the bogs of the Red Lake region, which are associated with organic material over till or glacio-lacustrine deposits (Soil Survey Staff 2012). In the Itasca region soils were well drained and were formed in loamy glacial till on moraines, very permeable soils formed on glacial outwash, and well drained soils formed on loamy sediments overlying sandy and gravelly sediments on glacial outwash (Soil Survey Staff 2012). Soils in the Red Lake region include fine loamy soils, sapric soils, very poorly drained organic soils overlying loamy glacial deposits on moraines, and very poorly drained soils that formed in woody materials over loamy calcareous glacial till or loamy lacustrine sediments (Soil Survey Staff 2012). Soils in the Grant County region include excessively drained soils that formed over coarse glacial outwash, well drained soils that formed in loamy glacial drift, well drained soils formed in calcareous glacial till, well drained soils that formed in glacial outwash deposits of loamy mantle over sandy deposits, and well drained soils that formed in loamy till (Soil Survey Staff 2012). Soils in the Windom region include well drained soils formed over glacial till, very poorly drained soils that formed in loamy till, very poorly drained soils that formed over calcareous loamy till, well drained soils that formed in calcareous loamy till on glacial moraines, and excessively drained soils formed in sandy outwash sediments (Soil Survey Staff 2012).

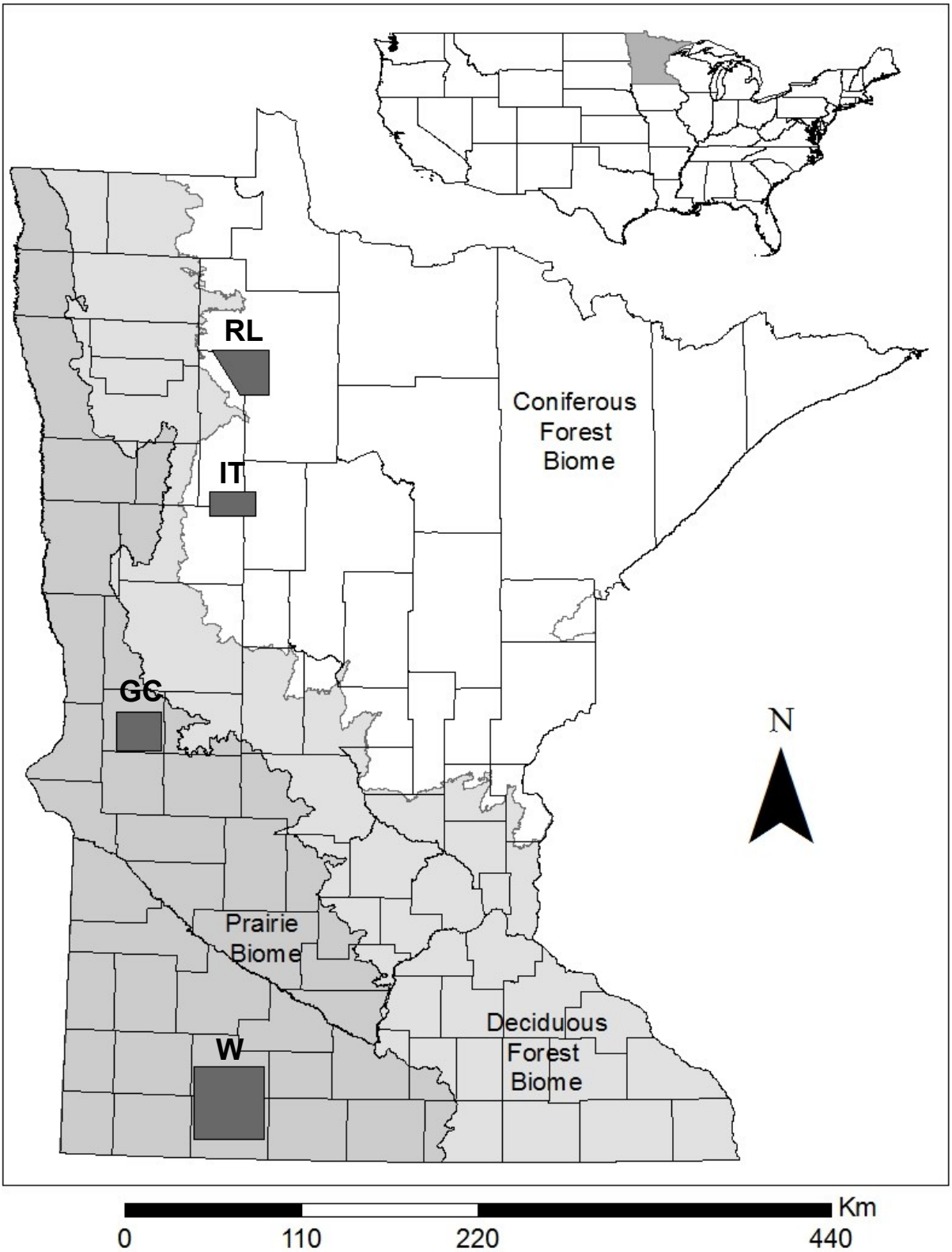

Figure 5.1. Map of Minnesota showing the locations of the study areas (regions: RL=Red Lake; IT=Itasca; GC=Grant County; W=Windom) containing the 44 shallow lakes sampled in 2010 and 2011.

Table 5.1. Size of study basin, watershed and average depth of 44 shallow lakes in Minnesota (study basin area includes the open water and emergent vegetation fringe determined to be part of the basin, lake watershed area entire includes land area draining to the outlet of the study lake).

Lake	Region	Lake watershed area	Study basin area	Open water area	Average depth
		ha	ha	ha	m
GC01	Grant County	12	4.3	4.1	1.61
GC02	Grant County	262	15	14	1.54
GC03	Grant County	265	32	31	1.69
GC04	Grant County	1384	22	22	1.42
GC05	Grant County	340	58	40	1.74
GC06	Grant County	537	7.4	4.7	0.88
IT01	Itasca	8.0	1.8	1.3	1.58
IT02	Itasca	13	4.0	3.7	1.98
IT03	Itasca	144	2.2	2.0	2.08
IT04	Itasca	35	8.2	6.4	3.85
IT05	Itasca	243	11	9.7	2.92
IT06	Itasca	128	4.7	3.9	3.05
RL01	Red Lake	14	4.5	3.1	1.19
RL02	Red Lake	15	7.1	5.1	1.31
RL03	Red Lake	46	6.5	2.9	1.01
RL04	Red Lake	100	9.1	4.1	0.76
RL05	Red Lake	50	5.4	3.3	0.82
RL06	Red Lake	97	12	2.7	1.25
RL07	Red Lake	23953	48	39	0.73
RL08	Red Lake	23953	3.3	3.0	1.01
RL09	Red Lake	23953	6.2	5.6	0.82

Table 5.1 (continued)

RL10	Red Lake	23953	8.0	4.5	0.73
RL11	Red Lake	23953	25	22	0.98
RL12	Red Lake	23953	7.6	6.4	0.70
W01	Windom	258	40	25	1.38
W03	Windom	466	41	40	1.37
W04	Windom	201	42	42	2.03
W05	Windom	762	27	25	0.51
W06	Windom	51	6.1	3.9	0.78
W07	Windom	845	38	18	0.52
W08	Windom	58	6.5	6.2	1.92
W09	Windom	222	26	26	2.39
W10	Windom	235	13	3.3	0.77
W11	Windom	196	12	11	0.98
W12	Windom	100	40	39	2.73
W13	Windom	101	12	2.9	0.62
W14	Windom	1061	32	8.1	0.84
W15	Windom	167	59	56	1.73
W16	Windom	412	37	35	1.44
W17	Windom	19	3.5	2.8	0.65
W18	Windom	21	3.8	2.7	0.64
W19	Windom	49	14	14	1.45
W20	Windom	640	32	30	0.81
W21	Windom	54	3.6	2.1	0.79

Table 5.2. Land cover proportions for the watersheds of 44 shallow lakes in Minnesota (total agriculture is a combination of the percent cropland for corn and soybeans and hay and grains).

Lake	Region	Grassland	Shrubland	Woodland	Corn and Soybeans	Hay and Grains	Total Agriculture
GC01	Grant County	0.95	0	0	0	0	0
GC02	Grant County	0.34	0.02	0.02	0.40	0.08	0.48
GC03	Grant County	0.15	0.01	0.04	0.34	0.29	0.63
GC04	Grant County	0.16	0.02	0.03	0.40	0.22	0.62
GC05	Grant County	0.40	0.09	0.01	0.38	0.00	0.38
GC06	Grant County	0.21	0.01	0.03	0.46	0.14	0.60
IT01	Itasca	0.21	0	0.71	0	0	0
IT02	Itasca	0.00	0.05	0.92	0	0	0
IT03	Itasca	0.01	0.02	0.79	0	0	0
IT04	Itasca	0.00	0	0.85	0	0	0
IT05	Itasca	0.04	0.001	0.80	0	0	0
IT06	Itasca	0.09	0.15	0.69	0	0	0
RL01	Red Lake	0.01	0	0.86	0	0	0
RL02	Red Lake	0	0	0.99	0	0	0
RL03	Red Lake	0	0	0.98	0	0	0
RL04	Red Lake	0.10	0.02	0.77	0	0	0
RL05	Red Lake	0.16	0.01	0.74	0	0	0
RL06	Red Lake	0.03	0	0.93	0	0	0
RL07	Red Lake	0.0001	0.001	0.39	0	0	0
RL08	Red Lake	0.0001	0.001	0.39	0	0	0
RL09	Red Lake	0.0001	0.001	0.39	0	0	0
RL10	Red Lake	0.0001	0.001	0.39	0	0	0

Table 5.2 (continued)

RL11	Red Lake	0.0001	0.001	0.39	0	0	0
RL12	Red Lake	0.0001	0.001	0.39	0	0	0
W01	Windom	0.08	0	0.05	0.58	0.20	0.78
W03	Windom	0.06	0	0.02	0.03	0.83	0.86
W04	Windom	0.04	0	0.04	0.22	0.59	0.81
W05	Windom	0.16	0.002	0.06	0.38	0.28	0.66
W06	Windom	0.21	0	0.001	0.22	0.47	0.69
W07	Windom	0.05	0.02	0.04	0.66	0.14	0.80
W08	Windom	0.04	0.0002	0.03	0.60	0.23	0.83
W09	Windom	0.11	0.007	0.03	0.14	0.68	0.82
W10	Windom	0.06	0	0	0.32	0.59	0.91
W11	Windom	0.23	0.04	0.04	0.04	0.34	0.38
W12	Windom	0.04	0	0.10	0.35	0.47	0.82
W13	Windom	0.21	0.03	0	0.18	0.57	0.75
W14	Windom	0.05	0	0	0.31	0.59	0.90
W15	Windom	0.08	0	0.01	0.70	0.16	0.86
W16	Windom	0.06	0	0.002	0.69	0.22	0.90
W17	Windom	0.40	0	0.02	0.57	0	0.57
W18	Windom	0.92	0	0	0	0	0
W19	Windom	0.05	0	0	0.58	0.16	0.74
W20	Windom	0.16	0.001	0.004	0.45	0.19	0.64
W21	Windom	0.25	0	0.02	0.49	0.10	0.59

5.3.2. Vegetation assessment

In each shallow lake, the macrophyte species were identified and their percent cover estimated using an acrylic glass bottom cylinder (scope-like device) at 10 stations within the perimeter of each lake, approximately equidistant from each other and at least 4 m from shore (Figure 5.2). Plant species were identified according to Fasset (1957) and Borman et al. (1997).

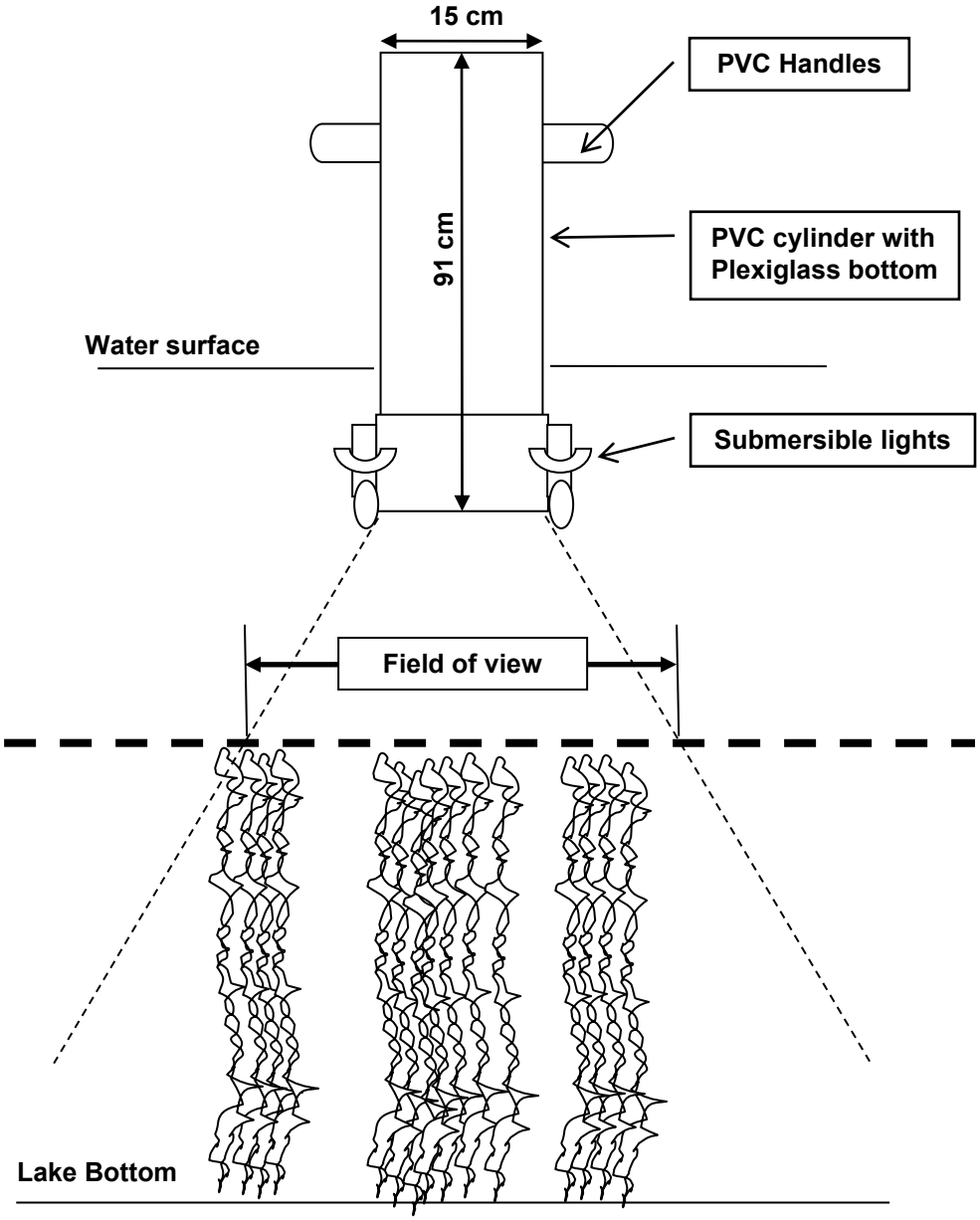

Figure 5.2. Acrylic glass bottom cylinder used for viewing submerged vegetation in shallow lakes.

Macrophyte biomass was determined by Minnesota Department of Natural Resources personnel on different days at 15 stations along 3 transects running the width of each lake. To collect macrophytes for mass estimates, a plant rake was cast at each station and dragged along the lake bottom for about 3 m. The macrophytes collected were weighed as grams per rake sample (hereafter g sample^{-1}) and averaged for each lake.

5.3.3. Water collection and analysis

Water samples were collected at approximately the same locations where the macrophyte cover was determined. Water samples were collected directly above the vegetation beds by placing the sample bottle approximately 25 cm below the water's surface, filling the bottle completely and replacing the cap before bringing the bottle back to the surface. Five water samples equidistant from each other were collected at each of the six Red Lake sites located within the bog area. A portion of each water sample was used to measure the turbidity using a HACH® portable turbidimeter (Model 2100P) and pH using a VWR Symphony SP90M5 Handheld Multimeter. The remaining water samples (approximately 50 ml) were filtered (0.45-µm pressure filter, Pall Corporation Supor® -450) and acidified with 0.1 ml (2 drops) of concentrated nitric acid. The samples were stored in a refrigerator at 4°C and then analyzed for 32 elements with a Spectro Genesis Inductively Coupled Plasma Optical Emission Spectrometer (ICP-OES). The method detection limits in µmol g^{-1} for these elements were as follows for the 2010 samples: Ag 0.05; Al 0.3; As 0.1; B 0.1; Ba 0.02; Be 0.002; Ca 0.1; Cd 0.01; Ce 0.1; Co 0.03; Cr 0.03; Cu 0.05; Fe 0.2; K 2; Li 0.03; Mg 0.8; Mn 0.02; Mo 0.04; Na 0.3; Ni 0.1; P 0.4; Pb 0.1; S 0.2; Sb 0.2; Se 0.2; Si 0.1; Sn 0.1; Sr 0.001; Ti 0.03; Tl 0.2; V 0.1; Zn 0.01. The method detection limits in µmol g^{-1} for these elements were as follows for the 2011 samples: Ag 0.01; Al 0.3; As 0.2; B 0.03; Ba 0.03; Be 0.001; Ca 0.04; Cd 0.01; Ce 0.1; Co 0.02; Cr 0.01; Cu 0.01; Fe 0.1; K 0.8; Li 0.004; Mg 0.2; Mn 0.01; Mo 0.03; Na 0.02; Ni 0.04; P 0.3; Pb 0.1; S 0.4; Sb 0.1; Se 0.3; Si 0.03; Sn 0.1; Sr 0.0004; Ti 0.01; Tl 0.2; V 0.02; Zn 0.01. In 2010, 5-10 water samples from each lake were used for element analysis while in 2011, 10 water samples from each lake were used for element analysis. Chlorophyll-*a* and total phosphorus concentrations for each shallow lake were determined by the Minnesota DNR in July of the same year.

5.3.4. Sediment collection and analysis

Sediment samples were collected with a sediment corer at approximately the same locations where the plants were surveyed and the water samples collected (Figure 5.3). For the six Red Lake sites located within the bog area, five sediment samples were collected equidistant from each other at approximately the same location where water samples were collected.

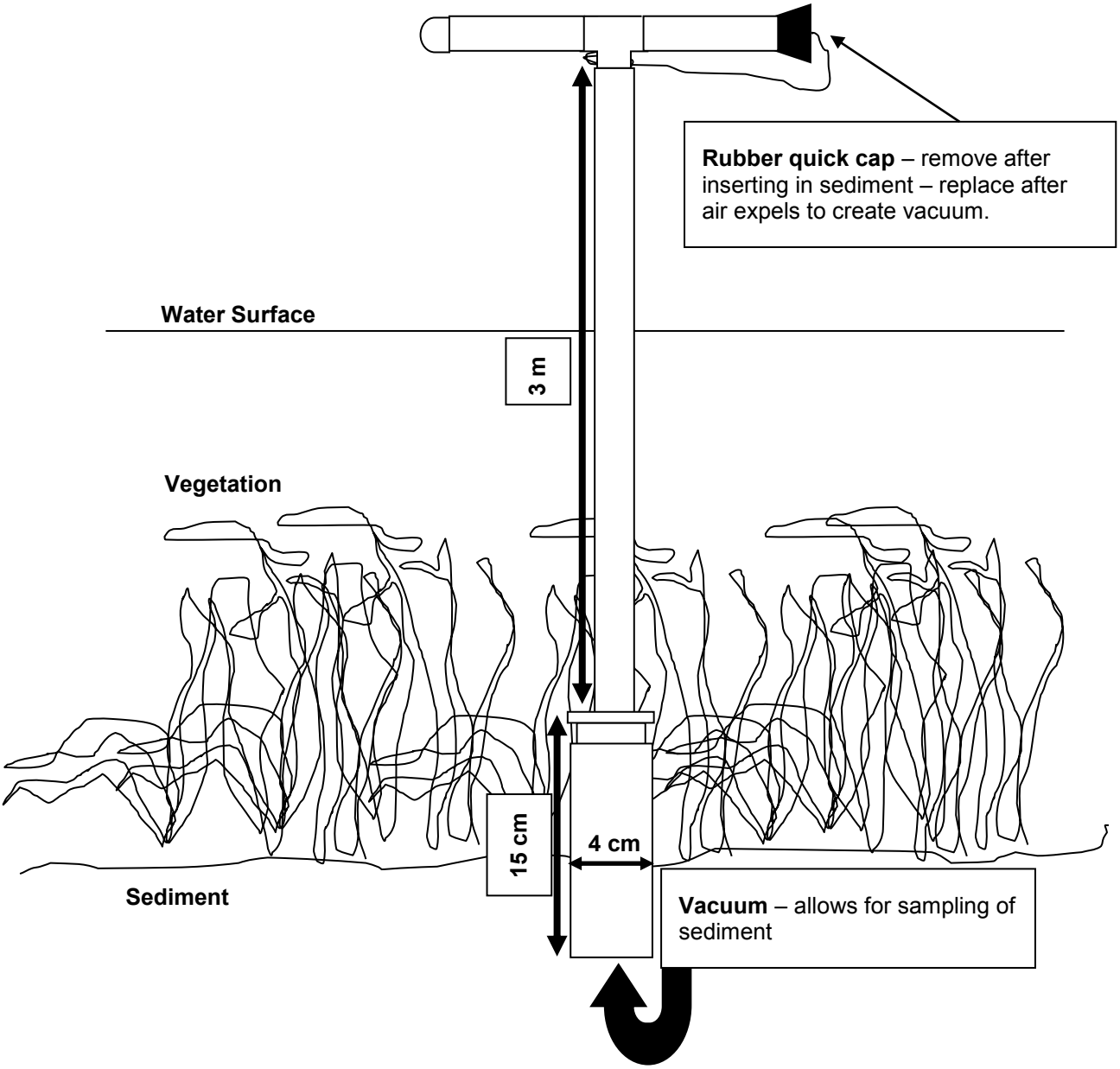

Figure 5.3. Sediment corer for sampling sediments in shallow lakes (adapted from Madsen et al. 2007).

Samples were transported in a cooler with ice to the lab where they were placed in paper bags and oven-dried until constant weight at 60°C, crushed, and homogenized. These dried samples were reserved for loss-on-ignition (LOI, a measure of organic matter content), particle size and multi-element analysis. LOI of sediment samples was determined by again drying the samples in an oven at 105 °C for two hours, weighing, and then ashing in a furnace at 360 °C for two hours. After ashing, the remaining sample material was cooled, weighed and then passed through a 63-µm sieve under running water to determine an estimate of particle size. The amount of sample that passed through the sieve was considered the fraction of sediment smaller than 63 µm (*f*<63 µm).

Another portion of the dried sediment samples were analyzed for 65 elements via Inductively Coupled Plasma Mass Spectrometry (ICP-MS) by a commercial laboratory (Activation Laboratories, Ltd, Analysis by Aqua Regia Digestion). Method detection limits for the 2010 samples in nmol g^{-1} for these elements: Ag 0.02, As 1.3, Au 0.003, B 93, Ba 3.6, Be 11, Bi 0.09, Cd 0.09, Ce 0.07, Co 1.7, Cr 9.6, Cs 0.2, Cu 0.2, Dy 0.006, Er 0.6, Eu 0.7, Ga 0.3, Gd 0.6, Ge 1.4, Hf 0.6, Ho 0.6, In 0.2, La 3.6, Li 14, Lu 0.6, Mn 18, Mo 0.1, Nb 1.1, Nd 0.14, Ni 1.7, Pb 0.05, Pr 0.7, Rb 1.2, Re 0.005, Sb 0.2, Sc 2, Se 1.3, Sm 0.7, Sn 0.4, Sr 5.7, Ta 0.3, Tb 0.6, Te 0.2, th 0.4, Tl 0.1, Tm 0.6, U 0.4, V 20, W 0.5, Y 0.1, Yb 0.6, Zn 1.5, Zr 1.1 and in µmol g^{-1} for these elements: Al 4, Ca 3, K 3, Mg 4, Na 0.4, P 0.3, S 0.3 and Ti 2 (Accredited Laboratory; ISO/IEC 17025:2005). Method detection limits for the 2011 samples in nmol $g^{-}1$ are the same except for Au 0.03. In 2010 and 2011, 5-10 sediment samples were used for element analysis.

5.3.5. Statistical analysis

Concentration data were log transformed prior to statistical analysis to help obtain homogeneity of variance and macrophyte data were relativized by maxima to reduce the influence of highly abundant species (McCune and Grace 2002). A General Linear Model with a nested design and multiple comparison tests by the Tukey method was used to determine significant differences among regions and among lakes within regions (p<0.01) using Minitab® statistical software (Minitab® 15 ©2006 Minitab Inc.). K-means cluster analysis (Lattin et al. 2003) was carried out in Minitab to classify lakes into two groups based on macrophyte cover and biomass, with the two groups consisting of lakes with low macrophyte abundance and high macrophyte abundance. A General Linear Model was then used to test for significant differences in element concentrations between the low macrophyte and high macrophyte lakes

(One-Way ANOVA, $p<0.05$). To test for relationships between element concentrations in the water and sediment, Pearson correlations and their associated *p*-values were calculated in Minitab. Here we consider only correlations that explained 25% or more of the variation ($r \geq 0.50$, $p<0.0001$) (McClave and Sincich 2006).

Water-column (hereafter water) and sediment element concentration matrices were analyzed using principal components analysis with a correlation cross-products matrix and Euclidean distances to determine trends in the element concentrations among the lakes (PCA in PC-ORD v. 6.0, McCune and Mefford 2010). Indicator Species Analyses were carried out in PC-ORD using the Dufrêne and Legendre (1997) method to determine significant indicator species for regions and macrophyte abundance groups.

Relationships between environmental variables (land cover proportions, lake watershed area, basin area, open water area, turbidity, chlorophyll-*a*, LOI, *f*<63 µm) and element concentrations of the water and sediment were assessed using partial Redundancy Analysis (pRDA) in CANOCO (©2005 CANOCO Version 4.5) where influence of region was assessed as a covariable in these models. Strength of relationships between the environmental variables and macrophyte cover were assessed using pRDA and between the environmental variables and plant biomass using Partial Canonical Correspondence Analysis (pCCA). Relationships between macrophyte community composition and element concentrations in the water and sediments were determined using RDA. Preliminary Detrended Correspondence Analysis (DCA) indicated that linear gradient analysis (RDA) was appropriate for analysis of the water, sediment, macrophyte cover and community composition data since the gradient lengths were less than two standard deviations and pCCA was deemed appropriate for analysis of the macrophyte biomass data since the gradient lengths were greater than two standard deviations (van Wijngaarden et al. 1995). Prior to analysis the rare macrophyte species (species that occurred in less than three lakes) were deleted from the macrophyte matrices to reduce dataset sparsity (McCune and Grace 2002; Peck 2010). Forward selection with Monte Carlo permutation tests (499 permutations) were used to determine the significant environmental variables which were included in final models ($p<0.05$). Methods of Borcard et al. (1992) were then used to partition the variance attributed to both environmental variables and covariables.

5.4. Results

5.4.1. Aquatic vegetation

Several macrophyte species only occurred in certain regions and so differences in cover and biomass could not be assessed for individual species. I included filamentous algae and *Chara* spp. in the analysis of all the macrophyte data. Total macrophyte cover and biomass varied significantly among regions and also among lakes within regions. Lakes in the Red Lake region had greater total macrophyte cover compared to lakes in Itasca, Grant County and Windom ($p<0.0001$) (Table 5.3). On the other hand, lakes in Grant County had significantly greater total macrophyte biomass than lakes in Itasca, Red Lake and Windom. Lakes in Itasca had significantly lower total macrophyte biomass than lakes in Red Lake and Windom ($p<0.0001$).

Species richness varied among the lakes with the most macrophyte species (12 species) detected in GC01 and the least species (one species) in IT03, W08, W19 and W20 (Table 5.4). No macrophyte species were detected in GC02, IT02, W03, W06 and W09. Results of the indicator species analysis showed that seven of the 27 macrophyte species detected by the rake and viewer method combined were identified as indicator species. These included filamentous algae, *Potamogeton richardsonii* and *Stuckenia pectinata* identified as significant indicators for Grant County, *Sagittaria cristata* and *Sparganium americanum* for Itasca, and *Potamogeton natans* and *Utricularia vulgaris* for the Red Lake region (Table 5.5).

Table 5.3. Average ± standard deviation of total macrophyte cover and biomass for the different regions sampled (different letters indicate significant differences among the regions for cover or biomass).

Regions	Total macrophyte cover	Total macrophyte biomass
	%	g rake sample^{-1}
Grant County	55±40 b	693±560 x
Itasca	40±49 b	64±89 y
Red Lake	83±40 a	359±410 z
Windom	47±44 b	378±407 z

Table 5.4. Macrophyte species and filamentous algae detected by the viewer or rake method in each shallow lake (each number identifies a lake in each region; GC = Grant County, IT = Itasca, RL = Red Lake, W = Windom).

Species	GC	IT	RL	W
Bidens beckii		6		
Brasenia schreberi		1	2, 7, 8, 12	
Ceratophyllum demersum	1, 3, 6	1	4	1, 5, 7, 10, 13 , 14, 15, 17, 18, 21
Chara spp.	1, 3, 5, 6	1, 5	2, 3, 4, 6, 7, 8, 9, 11	1, 5, 10, 13, 14, 17, 18
Drepanocladus spp.				13
Elodea canadensis				11, 17
Filamentous algae	1, 3, 4, 5, 6			4, 7, 10, 13, 14, 15
Heteranthera dubia		6		
Lemna minor				5, 13, 14
Lemna trisulca	1, 6		1	10, 13, 18, 21
Myriophyllum sibiricum	1, 3, 6		3, 4, 6, 8, 9, 10, 11	13, 17
Najas flexilis	1, 6	5	2, 4, 5, 6, 7, 8, 9, 10, 11, 12	5, 15, 17, 21
Nitella spp.		6		
Nuphar spp.		6	1	
Nymphaea spp.			1	
Potamogeton amplifolius		1	2, 5, 7, 12	
Potamogeton gramineus		6	2	
Potamogeton natans		1	1, 3, 4, 5, 9, 10	
Potamogeton praelongis				1
Potamogeton pusillus	1	1	2	1, 5, 7, 13, 14, 17, 18
Potamogeton richardsonii	1, 3, 6			1, 4, 12, 15, 16, 19
Potamogeton zosteriformis	1	4, 5	3, 4, 9, 11	1, 11, 18, 21
Ruppia occidentalis	3			
Sagittaria cristata	1, 6	3, 4, 5, 6	2	
Sparganium americanum		1, 4		14
Stuckenia Pectinata	1, 3, 4, 5, 6	1, 5, 6	4, 9	1, 4, 5, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 20, 21
Utricularia vulgaris	1, 5	1, 6	1, 2, 3, 6, 7, 8, 9, 10, 11, 12	10

Table 5.5. Indicator values for each macrophyte species and filamentous algae in the different regions as determined by indicator species analysis (*p*-values indicate the probability of the listed indicator values or higher given the species distributions, * denotes the significant species indicators, $p<0.05$).

Species	Region with maximum observations	Indicator Value	*p-value*
Bidens beckii	Itasca	20.0	0.2541
Brasenia schreberi	Red Lake	20.8	0.2545
Ceratophyllum demersum	Grant County	23.2	0.2917
Chara spp.	Grant County	28.1	0.3069
Drepanocladus spp.	Windom	5.9	1.0000
Elodea canadensis	Windom	11.8	0.6923
Filamentous algae*	Grant County	70.8	0.0002*
Heteranthera dubia	Windom	20.0	0.2541
Lemna minor	Windom	17.6	0.1794
Lemna trisulca	Grant County	22.3	0.1396
Myriophyllum sibiricum	Red Lake	32.1	0.0788
Najas flexilis	Red Lake	33.6	0.0632
Nitella spp.	Itasca	20.0	0.2541
Nuphar spp.	Itasca	14.1	0.5037
Nymphaea spp.	Red Lake	8.3	0.5569
Potamogeton amplifolius	Red Lake	20.8	0.2555
Potamogeton gramineus	Itasca	14.1	0.5065
*Potamogeton natans**	Red Lake	35.7	0.0494*
Potamogeton praelongis	Windom	5.9	1.0000
Potamogeton pusillus	Windom	18.9	0.3019
*Potamogeton richardsonii**	Grant County	37.8	0.0182*
Potamogeton zosteriformis	Red Lake	17.9	0.6601
Ruppia occidentalis	Grant County	20.0	0.2555
*Sagittaria cristata**	Itasca	49.9	0.0054*
*Sparganium americanum**	Itasca	34.9	0.0416*
*Stuckenia pectinata**	Grant County	35.8	0.0470*
*Utricularia vulgaris**	Red Lake	41.0	0.0220*

K-means cluster analysis classified 38 shallow lakes in this study into two groups characterized by differences in macrophyte cover and biomass. These two groups consisted of 18 lakes with low or no macrophyte occurrence which we considered the low macrophyte lakes, and 20 lakes with high macrophyte occurrence which we considered the high macrophyte lakes (Figure 5.4). Six lakes were not included in the cluster analysis because they did not have cover data available. Three of these lakes were assigned to the low macrophyte group if their biomass was less than 150 g sample^{-1} and the remaining three lakes to the high macrophyte group. Lakes classified as low macrophyte averaged 9% macrophyte cover and 46 g sample^{-1} of macrophytes, while lakes classified as high macrophyte averaged 93% macrophyte cover and 621 g sample^{-1} of macrophytes. Indicator species analysis showed that *Ceratophyllum demersum*, *Chara* spp., *Myriophyllum sibiricum* and *Potamogeton pusillus* were significant indicators of high macrophyte abundance lakes (Table 5.6).

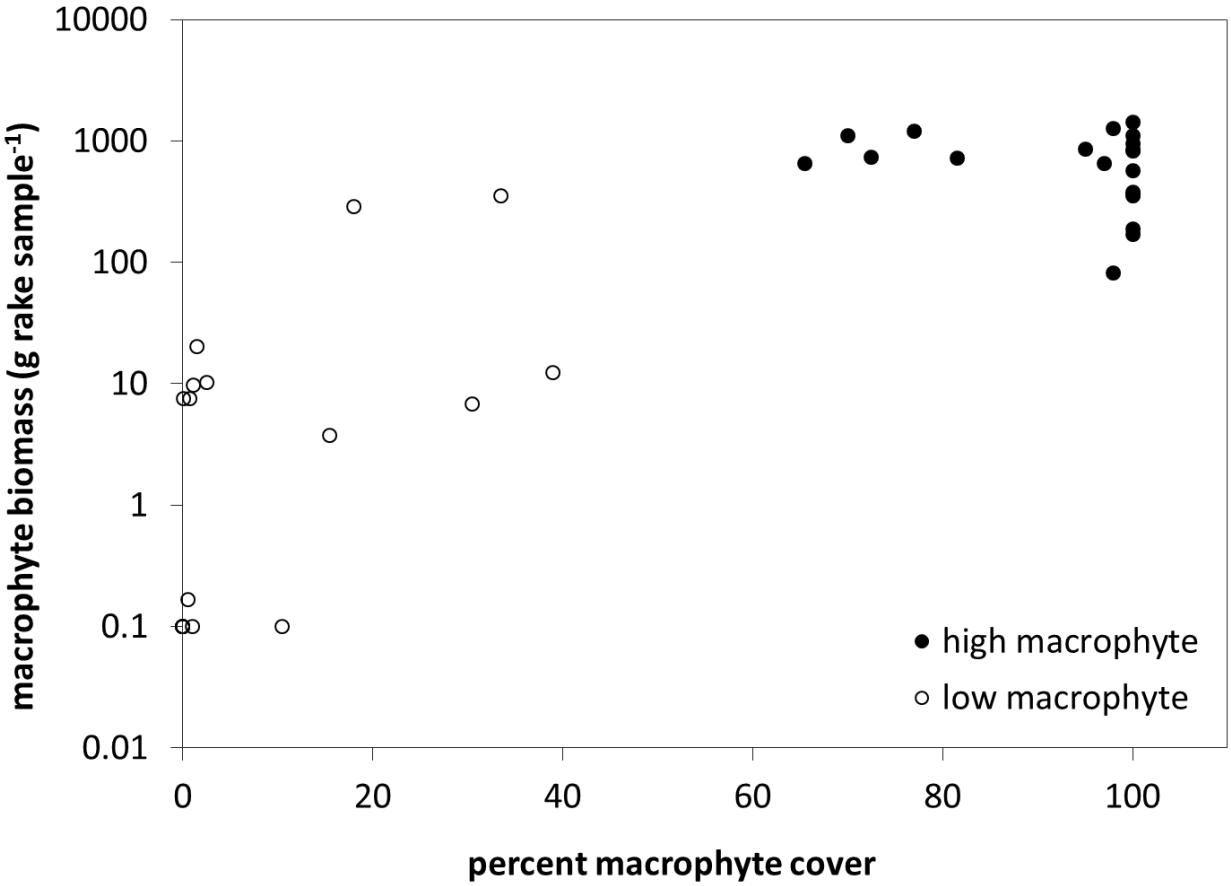

Figure 5.4. Average macrophyte and algae percent cover and biomass (g rake sample^{-1}) for 38 shallow lakes classified as high macrophyte and low macrophyte abundance lakes by *k*-means cluster analysis.

Table 5.6. Indicator values for each macrophyte species and filamentous algae in high and low macrophyte abundance lakes as determined by indicator species analysis (*p*-values indicate the probability of the listed indicator values or higher given the species distributions, * denotes the significant species indicators, $p<0.05$).

Species	Lake with maximum observations	Indicator value	*p-value*
Bidens beckii	low	6.2	0.4057
Brasenia schreberi	high	6.7	1.0000
*Ceratophyllum demersum**	high	50.5	0.0080
*Chara spp.**	high	71.8	0.0002
Drepanocladus spp.	high	4.3	1.0000
Elodea canadensis	low	3.7	1.0000
Filamentous algae	high	26.5	0.2913
Heteranthera dubia	low	6.2	0.4057
Lemna minor	high	13	0.2482
Lemna trisulca	high	21	0.2046
*Myriophyllum sibiricum**	high	37.9	0.0414
Najas flexilis	high	40.2	0.1030
Nitella spp.	low	6.2	0.4057
Nuphar spp.	low	12.5	0.1696
Nymphaea spp.	low	6.2	0.4213
Potamogeton amplifolius	high	6.7	1.0000
Potamogeton gramineus	low	3.7	1.0000
Potamogeton natans	high	13.8	0.6863
Potamogeton praelongis	low	6.2	0.4169
*Potamogeton pusillus**	high	33.7	0.0256
Potamogeton richardsonii	low	20.1	0.4319
Potamogeton zosteriformis	high	27.6	0.3281
Ruppia occidentalis	high	4.3	1.0000
Sagittaria cristata	low	9.7	1.0000
Sparganium americanum	high	5.1	1.0000
Stuckenia pectinata	high	45.5	0.2935
Utricularia vulgaris	high	25.3	0.5051

Results of a pRDA indicated that percent woodland, chlorophyll-*a*, percent grassland and open water area were significant sources of variance for macrophyte cover, which together explained 23.2% of the variation (Figure 5.5, Table 5.7). Region accounted for 2.7%, covariance for 0.7%, and 74.8% of the variance was unexplained.

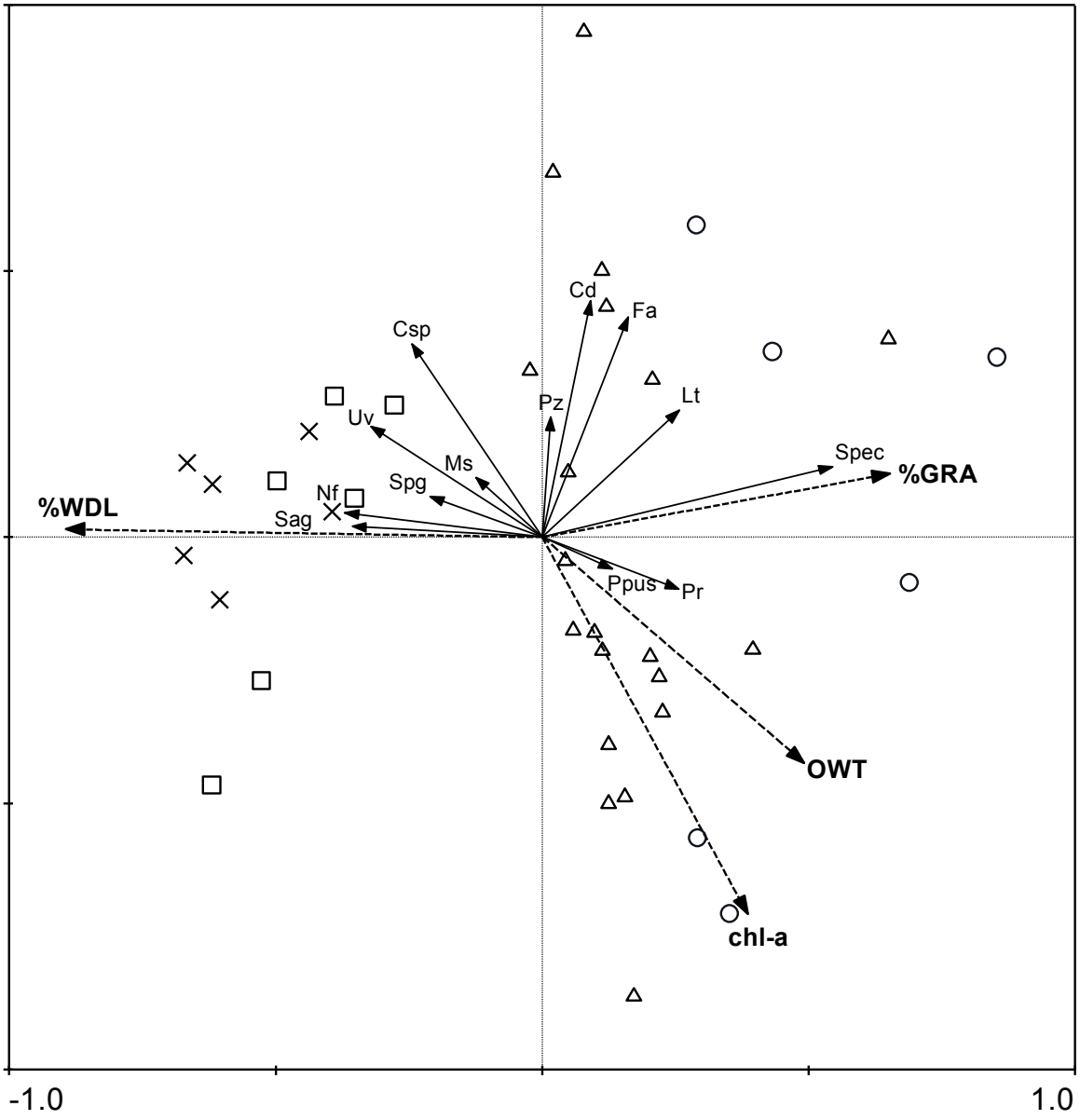

Figure 5.5. Ordination plot of the partial RDA of macrophyte cover constrained by environmental variables while controlling for region (Regions: Grant County (o), Itasca (□), Red Lake (X), Windom (Δ); Environmental Variables (in bold): chlorophyll-*a* concentrations (chl-*a*), open water area (OWT), percent grassland (%GRA), percent woodland (%WDL); common macrophyte or algae species (occurred in >7% of the shallow lakes sampled): *Ceratophyllum demersum* (Cd), *Chara* spp. (Csp), filamentous algae (Fa), *Lemna trisulca* (Lt), *Myriophyllum sibiricum* (Ms), *Najas flexilis* (Nf), *Potamogeton pusillus* (Ppus), *Potamogeton richardsonii* (Pr), *Potamogeton zosteriformis* (Pz), *Sagittaria cristata* (Sag), *Sparganium americanum* (Spg), *Stuckenia pectinata* (Spec), *Utricularia vulgaris* (Uv)).

The results of the pRDA showed a pattern similar to the results of the indicator species analysis where certain species were associated with different regions. *Chara* spp.*, Myriophyllum sibiricum, Utricularia vulgaris, Sparganium americanum, Najas flexilis* and *Sagitaria cristata* cover were positively associated with lakes in watersheds dominated by woodland. These included lakes in the Itasca and Red Lake regions. *Potamogeton zosteriformis, Ceratophyllum demersum,* filamentous algae, *Lemna trisulca* and *Stuckenia pectinata* cover were positively associated with lakes in watersheds dominated by grassland while *Potamogeton richardsonii* and *Potamogeton pusillus* were positively associated with lakes with large open water areas and high chlorophyll-*a* concentrations. These included lakes in the Grant County and Windom regions.

Results of a pCCA showed that *f*<63 µm, LOI, percent cropland (hay and grains), percent woodland, and turbidity were significant sources of variance of macrophyte biomass, and collectively explained 35.6% of the biomass variation (Figure 5.6, Table 5.7). Region accounted for 6.8%, covariance for 0.9*%,* and 58.5% of the variance was unexplained*. Potamogeton richardsonii* and *Stuckenia pectinata* were positively associated with turbidity while *Ceratophyllum demersum, Myriophyllum sibiricum, Lemna minor* and filamentous algae were positively associated with *f*<63 and percent cropland (hay and grains). *Potamogeton natans, Potamogeton amplifolius* and *Brasenia schreberi* were positively associated with lakes in watersheds dominated by woodland and high LOI.

5.4.2. Water chemistry

Lake turbidity, Ca, Mg, Mn, Na, S, Si and Sr concentrations varied among regions and also among lakes within regions (Table 5.8). Lakes in Windom had significantly higher turbidity than lakes in Grant County, Itasca and Red Lake. However, lakes in Itasca and Red Lake had similar turbidities. A comparison of lakes grouped according to macrophyte abundance showed that high macrophyte lakes tend to have significantly lower turbidities than low macrophyte lakes. Lakes also had significantly different pH among regions and among lakes within regions, and pH decreased in the order of W>GC>IT>RL. Lakes with low and high macrophyte abundance did not vary significantly in pH. Lakes in Grant County and Windom had higher Ca and Sr concentrations compared to lakes in Itasca and Red Lake. Lakes in Itasca had significantly lower concentrations of Ca compared to Red Lake. Concentrations of Mg, Na, S and Si varied significantly in the order of GC>W>RL>IT. Lakes in Grant

County, Itasca and Red Lake had similar concentrations of Mn and were all significantly higher in Mn compared to lakes in Windom. Lakes with high macrophyte abundance had higher Mn and lower Si concentrations compared to lakes with low macrophyte abundance.

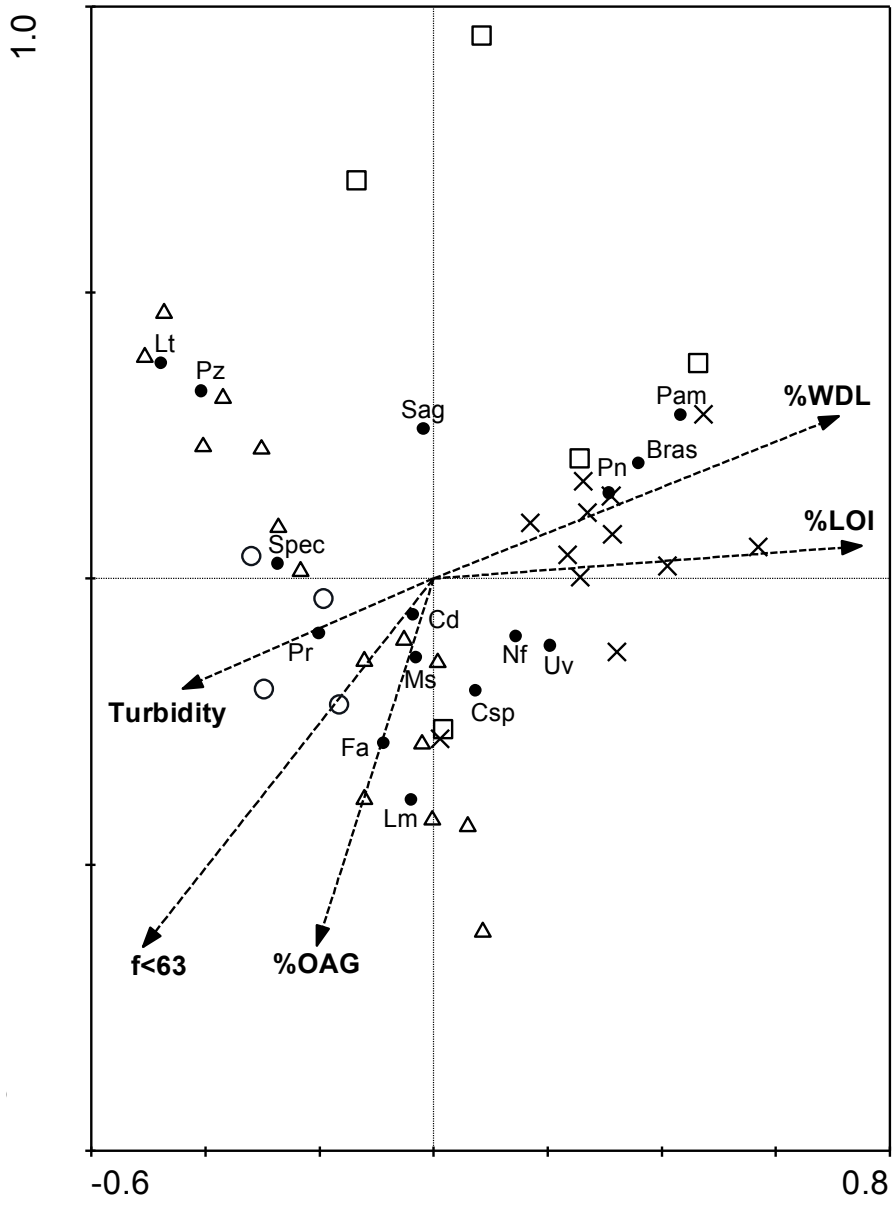

Figure 5.6. Ordination plot of the partial CCA of macrophyte biomass constrained by environmental variables while controlling for region (Regions: Grant County (o), Itasca (□), Red Lake (X), Windom (Δ); Environmental Variables (in bold): *f*<63 μm; %LOI, turbidity, percent cropland for hay and grains (%OAG), percent woodland (%WDL); common macrophyte or algae species (●) (occurred in >7% of the shallow lakes sampled): *Brasenia schreberi* (Bras), *Ceratophyllum demersum* (Cd), *Chara* spp. (Csp), filamentous algae (Fa), *Lemna trisulca* (Lt), *Lemna minor* (Lm), *Myriophyllum sibiricum* (Ms), *Najas flexilis* (Nf), *Potamogeton amplifolius* (Pam), *Potamogeton natans* (Pn), *Potamogeton richardsonii* (Pr), *Potamogeton zosteriformis* (Pz), *Sagittaria cristata* (Sag), *Stuckenia pectinata* (Spec), *Utricularia vulgaris* (Uv)).

Table 5.7. Results of partial redundancy or canonical correspondence analysis* models of macrophyte cover, macrophyte biomass, water and sediment chemistry using environmental variables and covariables (Variance proportions calculated according to Borcard et al. (1992); $p<0.05$).

Species matrix	Variance Class	Variables	%Variance explained	*p*-value
Macrophyte cover	Environmental	%Woodland	8.3	0.002
		Chlorophyll-*a*	14.8	0.002
		Grassland	19.9	0.006
		Open water area	23.7	0.008
	Covariable	Region	2.7	0.002
	Covariance		-0.7	
	Total		25.2	
Macrophyte biomass*	Environmental	LOI	13.4	0.002
		f<63μm	9.2	0.002
		%Hay and grains	7.1	0.004
		%Woodland	4.4	0.01
		Turbidity	1.5	0.006
	Covariable	Region	6.8	0.002
	Covariance		-0.9	
	Total		41.5	
Water chemistry	Environmental	%Agriculture	58.8	0.002
		%Woodland	2.5	0.002
	Covariable	Region	9.1	0.002
	Covariance		-3.0	
	Total		67.4	
Sediment chemistry	Environmental	%Woodland	43.2	0.002
		Lake watershed area	13.1	0.002
		f<63μm	3.2	0.006
		LOI	2.1	0.018
	Covariable	Region	3.0	0.002
	Covariance		3.7	
	Total		68.2	

Principal components analysis showed a grouping pattern distinguishing between lakes of the different regions based on water element concentrations (Figure 5.7). Variance explained by PCA ordination axis 1, the only significant axis (p<0.01) was 70%. Axis 1 was generally related to higher Ca, Mg, Na, S, Si, Sr, turbidity and chlorophyll-*a* while Axis 2 was related to higher Mn concentrations. Most of the lakes in the Windom region clustered together and were related to high Ca, Si, chlorophyll-*a* and turbidity and low Mn. The lakes in the Itasca and Red Lake Region grouped together opposite to the Windom sites indicating that the former sites were lower in Ca, Si, chlorophyll-*a* and turbidity. Some of the lakes in Grant County grouped together in the lower left of the graph and were related to high Mg, Na, S and Sr concentrations.

Results of a pRDA showed that percent woodland and percent agriculture cover were significant sources of variance explaining 58.3% of the variation in element concentrations in the lake waters (Figure 5.8, Table 5.7). Region as a covariable explained 6.1% and covariance (shared variance) explained 3% of the variation while the remaining 32.6% was unexplained. Percent woodland was inversely associated with Ca, Mg, Na and Si. Percent agriculture was positively associated with S and Sr and inversely associated with Mn. Percent woodland was associated with lakes in the Itasca and Red Lake regions while percent agriculture was associated with lakes in the Grant County and Windom regions.

5.4.3. Sediment chemistry

The LOI (estimate of organic matter content) varied among regions and among lakes within regions (Table 5.9a). Lakes in the Red Lake region had significantly greater LOI than Grant County, Itasca and Windom. Lakes in the Windom region had the lowest LOI of the four regions. The *f*<63 µm varied significantly among regions but was similar among lakes within regions. Lakes in Grant County and Windom had significantly higher *f*<63 µm in their sediments compared to lakes in Itasca. Lakes in the Red Lake region had significantly lower *f*<63 µm in their sediments compared to lakes in Windom. The LOI and *f*<63 µm in the sediment were not significantly different between lakes of low and high macrophyte abundance.

Table 5.8. Average chlorophyll-*a* concentrations (chl-*a*, µg l^{-1}), turbidity (NTU), pH and element concentrations (mg l^{-1}) for the water of 45 shallow lakes in Minnesota (average ± standard deviation, n = 5-10, * indicates data collected by the MN DNR in July of the same year).

Lake	*chl-*a*	turbidity	pH	Ca	Mg	Mn	Na	S	Si	Sr
GC01	1.9	2.1±1.0	9.3±0.1	15.5±1.2	22.5±1.5	0.21±0.01	3.7±0.3	0.63±0.10	4.20±0.77	0.05±0.004
GC02	164	44±4	8.8±0.1	21±1.8	31.5±2.3	0.23±0.02	5.4±0.4	2.23±0.10	16.6±0.73	0.09±0.008
GC03	7.6	2.6±0.6	8.3±0.1	73±4.0	183±7.9	0.24±0.05	105.8±4.9	314.4±2.5	2.42±0.25	0.4±0.025
GC04	134	41±2	8.4±0.1	78.4±5.2	85.2±3.7	0.28±0.08	43.6±2.3	142.1±1.1	22.3±0.73	0.4±0.027
GC05	13	3.9±0.3	8.8±0.1	40.1±0.7	92.8±1.8	0.2±0.01	38.8±0.5	75.3±0.4	12.4±0.41	0.2±0.005
GC06	6.8	2.6±0.8	8.2±0.6	25.7±7.8	44.1±3.3	0.27±0.13	13.3±1.0	9.34±1.10	1.46±0.88	0.09±0.022
IT01	1.9	1.2±0.3	5.7±0.1	1.4±0.1	1.0±0.5	0.20±0.00	0.5±0.0	0.20±0.03	0.07±0.02	0.04±0.000
IT02	16	10.1±1.2	9.2±0.3	3.1±0.1	1.5±0.3	0.20±0.001	0.6±0.0	0.31±0.05	0.28±0.02	0.04±0.000
IT03	14	2.4±0.4	6.8±0.02	2.8±0.3	1.6±0.2	0.20±0.00	0.5±0.0	0.24±0.10	0.07±0.03	0.04±0.000
IT04	3.1	1.2±0.5	7.2±0.2	3.8±0.4	1.4±0.3	0.20±0.00	0.7±0.2	0.27±0.10	0.13±0.01	0.04±0.000
IT05	1.5	0.9±0.2	8.0±0.1	13.1±2.0	7.6±0.5	0.20±0.00	1.1±0.1	0.22±0.04	0.41±0.10	0.04±0.000
IT06	5.3	4.1±0.2	7.6±0.2	28.7±2.2	6.8±0.3	0.20±0.002	0.9±0.3	0.20±0.03	1.91±0.07	0.04±0.000
RL01	7.8	6.1±0.8	7.6±0.04	36.9±0.9	11.7±0.2	0.20±0.00	1.3±0.1	0.26±0.03	2.96±0.04	0.04±0.001
RL02	3.4	1.2±0.2	7.8±0.2	12.0±1.2	6.5±0.5	0.20±0.00	0.5±0.0	0.17±0.01	1.01±0.04	0.04±0.00
RL03	2.0	0.8±0.2	8.1±0.3	37.3±3.4	13.8±1.0	0.20±0.003	1.4±0.3	0.33±0.10	4.69±0.17	0.04±0.002
RL04	2.8	0.8±0.1	7.6±0.1	29.2±2.5	12.2±0.9	0.20±0.003	1.2±0.2	0.26±0.03	9.49±0.79	0.05±0.004
RL05	4.4	1.2±0.3	7.7±0.1	12.6±1.2	4.2±0.3	0.20±0.00	0.5±0.1	0.25±0.10	0.59±0.04	0.04±0.00
RL06	2.6	2.9±0.7	7.6±0.1	33.5±3.2	15.4±1.0	0.20±0.00	1.7±0.2	0.22±0.03	8.66±0.31	0.04±0.002
RL07	3.2	2.2±0.4	------	13.6±1.1	6.1±0.5	0.22±0.01	2.9±0.5	0.35±0.05	5.57±0.22	0.04±0.001
RL08	3.6	3.3±0.6	------	15.3±1.2	5.6±0.4	0.25±0.04	2.2±0.2	0.34±0.10	6.65±0.22	0.04±0.002
RL09	4.6	2.6±0.7	------	27.0±0.2	7.1±0.2	0.29±0.05	2.4±0.1	0.82±0.10	8.25±0.13	0.07±0.001
RL10	6.0	4.3±0.6	------	19.5±1.4	6.7±0.2	0.27±0.02	2.4±0.6	0.38±0.04	4.55±0.13	0.05±0.004

Table 5.8 (continued)

RL11	1.3	0.9±0.3	------	29.4±2.5	8.4±0.7	0.20±0.01	2.5±0.7	0.77±0.10	7.45±0.38	0.07±0.006
RL12	2.4	1.2±0.1	------	10.3±0.7	4.8±0.2	0.21±0.01	2.1±0.4	0.3±0.1	2.56±0.11	0.04±0.000
W01	68	8.6±1.7	7.9±0.2	44.1±2.6	29.2±1.6	0.04±0.02	4.0±0.5	7.6±0.5	13.2±0.99	0.20±0.010
W03	81	19±3	9.0±0.1	32.3±8.3	28.0±4.5	0.06±0.06	6.5±1.1	16.2±6.9	9.59±4.1	0.20±0.05
W04	63	38±3	9.5±0.1	31±7.8	25.6±2.9	0.04±0.02	5.5±0.6	12.7±5.2	15.0±6.45	0.19±0.04
W05	14	5.7±3.0	9.3±0.6	35±9	29.6±3.1	0.06±0.04	5.9±0.7	6.6±3.0	13.3±6.79	0.19±0.02
W06	99	94±17	8.7±0.1	44±9	28.5±3.4	0.05±0.02	9.9±3.4	17.1±1.5	15.6±5.85	0.23±0.01
W07	63	60±13	9.1±0.2	35±3	30.2±4.3	0.03±0.02	6.7±1.4	14.4±3.9	18.5±5.17	0.21±0.05
W08	32	16±2	8.7±0.1	34±1	20.7±3.3	0.04±0.03	3.9±0.8	4.04±1.2	5.61±3.56	0.10±0.03
W09	119	65±6	9.1±0.2	35±2	32.7±1.8	0.05±0.04	7.3±0.7	8.04±0.4	15.1±1.52	0.19±0.01
W10	13	3.6±0.9	7.9±0.1	45±17	33.5±12.2	0.07±0.02	7.0±2.9	10.6±4.9	11.0±4.06	0.21±0.08
W11	76	32±2	8.7±0.1	35±2	29.8±1.9	0.07±0.04	4.7±0.7	2.02±0.1	13.8±0.77	0.16±0.01
W12	109	27±2	9.2±0.1	32±2	39.4±3.8	0.04±0.03	5.9±0.9	8.65±2.3	13.4±1.11	0.21±0.02
W13	15	7.2±5.5	8.9±0.2	42±9	45.4±4.2	0.07±0.04	9.9±3.4	30.8±19	9.47±3.91	0.34±0.11
W14	4.9	3.8±3.2	9.0±0.2	76±13	44.9±2.9	0.05±0.03	8.3±2.7	36.1±7.9	12.8±3.18	0.44±0.02
W15	37	6.9±2.5	10.0±0.3	32±2	34.3±1.5	0.05±0.02	5.7±0.4	22.2±0.5	7.45±1.74	0.20±0.01
W16	72	28±2	8.8±0.04	32±3	27.9±2.8	0.03±0.02	4.3±0.9	9.66±6.6	6.61±0.57	0.18±0.01
W17	143	27±2	8.7±0.1	43±6	27.7±1.2	0.55±0.37	4.2±0.2	2.86±2.7	11.3±3.22	0.19±0.01
W18	3.1	3.5±1.2	9.4±0.1	38±8	27.8±2.5	0.05±0.02	4.0±0.6	1.75±3.5	25.6±5.51	0.16±0.03
W19	49	67±15	8.8±0.1	50±4	32.6±2.0	0.06±0.04	5.6±1.0	11.2±2.0	20.1±4.42	0.24±0.03
W20	386	56±5	9.0±0.1	61±6	33.2±1.4	0.09±0.04	5.1±0.6	13.3±1.9	26.7±5.11	0.25±0.02
W21	16	2.9±1.0	9.2±0.4	35±15	35.2±2.5	0.32±0.35	5.4±0.5	5.47±3.3	5.0±7.95	0.14±0.05

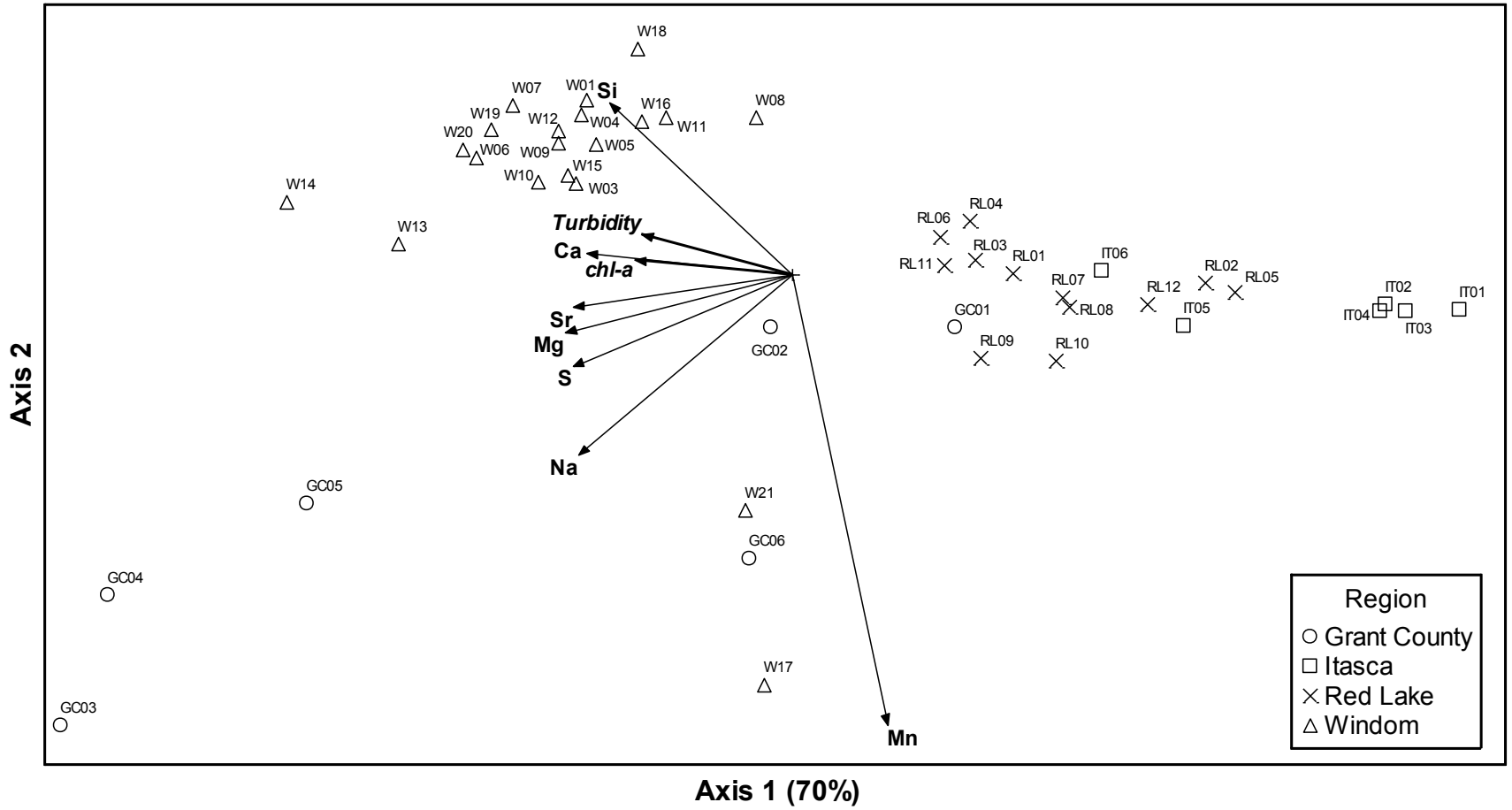

Figure 5.7. PCA ordination diagram showing how shallow lakes of different regions group out according to element concentrations in the water with turbidity and chlorophyll-*a* concentrations (chl-*a*) as overlays from the explanation matrix (Vectors indicate the direction of increasing concentrations or turbidity and their length reflects the magnitude of the association with the ordination axes).

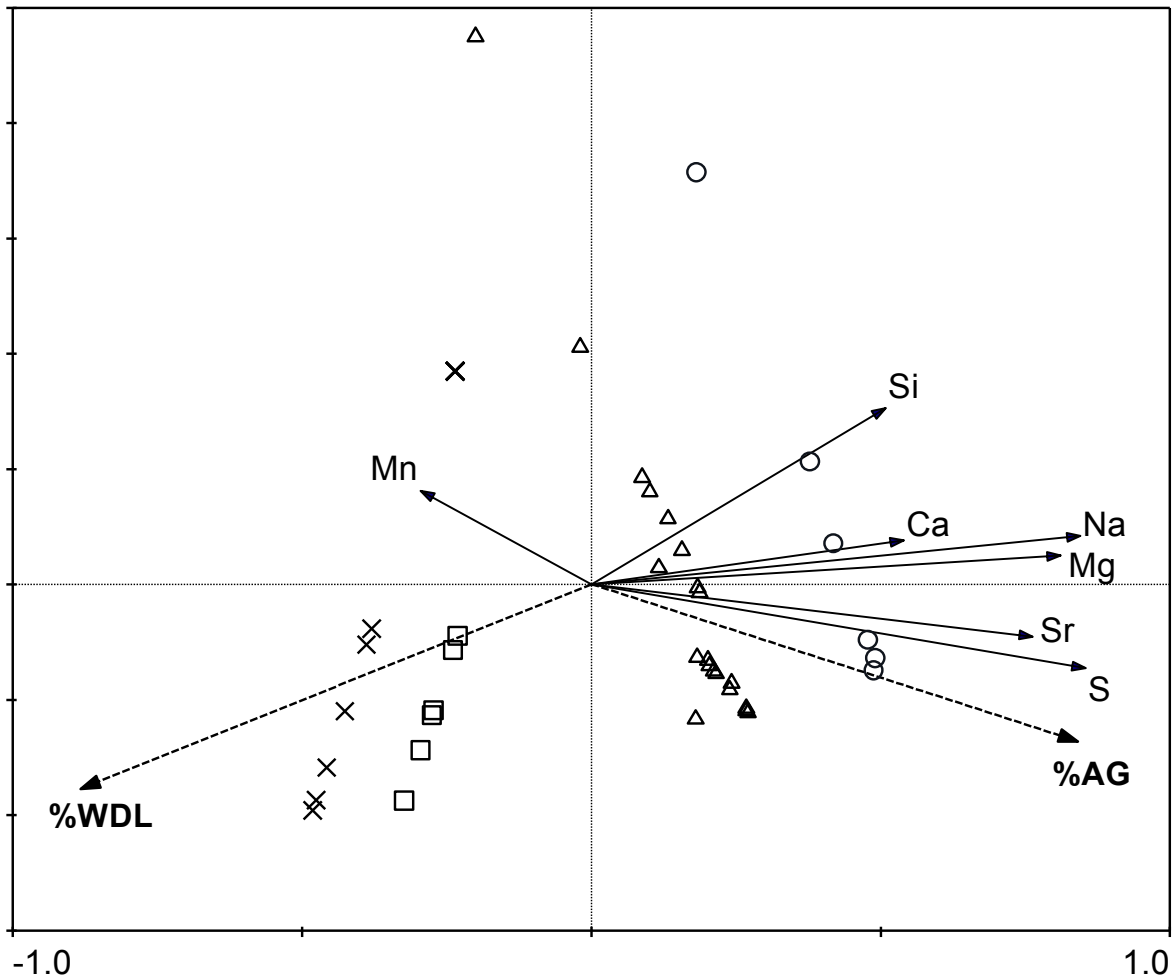

Figure 5.8. Ordination plot of the partial RDA for water chemistry variables constrained by environmental variables while controlling for region (Regions: Grant County (o), Itasca (□), Red Lake (X), Windom (Δ); Environmental variables (in bold): percent woodland (%WDL), percent agriculture (%AG); water chemistry variables: Ca, Mg, Mn, Na, S, Si, Sr concentrations).

Concentrations of Al, As, B, Ba, Ca, Cd, Ce, Co, Cr, Cs, Cu, Dy, Fe, Ga, Gd, K, La, Li, Mg, Mn, Mo, Na, Nd, Ni, P, Pb, Pr, Rb, S, Sc, Sm, Sr, Th, U, V, Y, Zn and Zr varied significantly among regions and among lakes within regions (Table 5.9). Concentrations of As, Cd, Cu, Fe, K, P, Rb, U were significantly greater in Grant County and Windom compared to the Itasca and Red Lake regions. Lakes in Grant County had the highest concentrations of B, Ca, Mg, Mn, Mo, Na, S and Sr while lakes in Windom had the highest concentrations of Ce, Co, Cr, Dy, Ga Gd, La, Li, Nd, Ni, Pb, Pr, Sc, Sm, Th, V, Y and Zn. Lakes in Itasca had the highest Ba concentrations compared to Grant County and Windom. The highest concentrations of Cs occurred in Grant County, Windom and Itasca while the highest concentrations of Zr occurred in Windom and Red Lake. The lowest concentrations of Al, As, Co, Mg, Rb, Sr, Th and U occurred in Itasca and Red Lake and the lowest concentrations of Mo and Na in Windom, Itasca and Red

Lake. The lowest concentrations of Ce, Cr, Cs, Cu, Dy, Fe, Ga, Gd, K, La, Li, Nd, Ni, Pr, Sc, Sm, V and Y occurred in the Red Lake region while the lowest concentrations of B, Ca, Cd, Mn, P, Pb, S, Zn and Zr occurred in the Itasca region. A comparison of lakes with low and high macrophyte abundance revealed that lakes with high macrophyte abundance had significantly higher B, Ba, Ca, Mo, P, S, Sr, U and Zr in the sediment than lakes with low macrophyte abundance.

Principal components analysis grouped lakes of different regions based on element concentrations in the sediment (Figure 5.9). Variance explained by PCA ordination axis 1 was 65%, while axis 2 explained an additional 14%. Both axis 1 and axis 2 were significant ($p<0.01$). Axis 1 was generally related to higher Al, As, B, Cd, Ce, Co, Cr, Cs, Cu, Dy, Fe, Ga, Gd, K, La, Li, Mg, Nd, Ni, P, Pb, Pr, Rb, Sc, Sm, Th, U, V, Y, Zn, Zr and LOI while Axis 2 was related to higher Ca, Mn, Mo, Na, S, Sr and $f<63$ µm. Lakes in Grant County and Windom grouped together and were related to high concentrations of most of the elements measured. Most of the Windom lakes were associated with high concentrations of the Lanthanoids (Ce, Dy, Gd, La, Nd, Pr, Sm) and other elements (Al, Co, Cr, Cs, Cu, Fe, Ga, K, Li, Ni, Pb, Rb, Sc, Th, V, Y, Zn). Most of the Grant County lakes are associated with high concentrations of As, B, Ca, Cd, Mg, Mo, Mn, Na, P, Sr, S, U, Zr and $f<63$µm. Most of the lakes of the Red Lake region are associated with high LOI.

Results of a pRDA showed that percent woodland, lake watershed area, $f<63$ µm and LOI were significant sources of variance which collectively explained 61.5% of the variation in element concentrations in the sediments (Figure 5.10). The covariable, region explained 2.1% and covariance explained 4.4% of the variation while the remaining 32% was unexplained. Percent woodland was positively related with Ba and was associated with lakes in the Itasca and Red Lake regions. Percent woodland was also inversely associated to $f<63$ µm and most of the major elements (B, Ca, K, Na, Mg, P, S) and several metals (As, Cd, Mn, Mo, Pb, Sr, U, Zn, Zr). Watershed area had a strong positive association with LOI and was inversely related to most elements in the sediment including the rare-earth metals (Ce, Dy, Gd, La, Nd, Pr, Sm) and several other metals (Al, Co, Cr, Cs, Cu, Fe, Ga, Li, Ni, Rb, Sc, Th, V, Y). Lake watershed area and LOI proportions was greatest for most lakes in the Red Lake region. Most of the elements were positively associated with lakes in the Grant County and Windom regions.

Table 5.9a. Mean loss-on-ignition (LOI, % dry soil), soil particles smaller than 63 µm (f<63µm, % dry soil) and element concentrations (in µmol g^{-1}, except where indicated by † for nmol g^{-1} and ‡ in mmol g^{-1}) of sediments for 45 shallow lakes in Minnesota (mean±standard deviation, *n* = 5-10).

Lake	LOI	f <63	Al	†As	B	Ba	‡Ca	†Cd	†Ce	†Co	Cr	†Cs	Cu
GC01	25±3	95±4	350±79	42±21	1.8±0.1	0.35±0.21	1.24±0.19	3.0±0.8	147±21	90±15	0.23±0.06	5.5±1.0	0.18±0.02
GC02	16±7	85±24	280±100	47±22	2.1±1	0.34±0.14	1.88±0.50	2.7±1.4	130±28	75±22	0.20±0.07	4.7±2.2	0.16±0.08
GC03	6±2	77±27	360±110	79±41	3.2±1	0.36±0.11	1.52±0.37	3.5±1.3	164±16	96±24	0.29±0.10	5.8±1.8	0.22±0.08
GC04	8±2	85±17	390±130	137±45	3.6±1.1	0.30±0.17	1.53±0.57	4.3±1.3	173±27	104±23	0.32±0.08	6.1±1.2	0.24±0.07
GC05	14±3	87±9	330±20	47±23	2.7±0.3	0.30±0.03	1.90±0.12	3.4±0.4	143±6	95±9	0.25±0.02	5.5±0.4	0.2±0.02
GC06	18±8	93±3	420±75	80±15	2.0±0.4	0.19±0.09	0.95±0.31	3.8±1.0	167±25	90±11	0.29±0.05	5.0±0.9	0.24±0.02
IT01	32±6	43±23	540±180	7±10	0.5±0.2	0.69±0.21	0.08±0.01	4.0±1.7	244±77	95±54	0.48±0.19	7.7±2.3	0.31±0.15
IT02	22±17	71±31	520±170	10±12	0.3±0.2	0.51±0.21	0.08±0.02	1.9±1.1	212±48	71±36	0.45±0.15	6.8±2.0	0.22±0.10
IT03	18±9	50±15	490±150	25±9	0.4±0.2	0.58±0.19	0.10±0.02	3.3±1.9	214±67	93±34	0.50±0.13	7.0±2.5	0.28±0.12
IT04	3±3	34±13	230±56	5±8	0.17±0.04	0.20±0.09	0.06±0.02	1.6±2.4	109±22	46±9	0.24±0.05	3.0±1.1	0.06±0.03
IT05	21±8	95±6	83±61	118±71	0.8±0.7	1.19±0.33	5.48±1.59	1.7±1.1	42±35	31±15	0.08±0.07	1.7±1.2	0.06±0.03
IT06	4±4	24±10	180±49	5±5	0.2±0.1	0.20±0.08	0.08±0.02	0.9±0.4	103±23	46±13	0.22±0.14	2.6±1.0	0.05±0.01
RL01	42±19	57±20	240±87	17±8	1.4±0.6	0.56±0.22	0.44±0.27	6.0±4.2	126±61	62±32	0.19±0.07	4.8±1.6	0.16±0.06
RL02	33±16	65±16	340±80	21±15	1.1±0.2	0.55±0.11	0.21±0.05	4.5±3.5	179±48	75±19	0.32±0.10	5.8±1.5	0.18±0.02
RL03	35±10	81±22	100±33	23±20	0.8±0.3	0.68±0.56	2.50±2.15	2.2±1.1	51±22	42±19	0.10±0.04	2.2±0.8	0.07±0.02
RL04	30±11	67±22	220±71	37±28	1.2±0.2	0.46±0.13	0.39±0.14	3.3±0.9	135±28	67±30	0.23±0.09	4.2±0.5	0.19±0.05
RL05	42±17	55±30	210±32	26±15	1.0±0.2	0.55±0.17	0.97±1.34	4.2±2.1	110±17	96±22	0.19±0.08	4.3±0.6	0.14±0.03
RL06	17±8	99±1	32±19	32±21	0.4±0.2	1.87±0.19	6.58±1.08	0.8±0.4	14±10	25±13	0.02±0.01	0.9±0.5	0.02±0.02
RL07	50±34	88±9	140±28	23±16	1.1±0.2	0.18±0.10	0.34±0.03	3.4±1.1	51±11	45±7	0.10±0.02	3.6±0.7	0.07±0.01
RL08	51±4	88±67	160±19	19±19	1.2±0.3	0.19±0.07	0.28±0.02	4.1±0.5	57±4	51±3	0.10±0.01	4.3±0.7	0.08±0.01
RL09	48±4	79±15	130±14	25±15	1.2±0.3	0.06±0.04	0.56±0.22	3.1±0.6	47±3	43±4	0.08±0.03	3.5±0.2	0.07±0.01
RL10	51±5	78±22	140±14	34±26	1.5±0.2	0.09±0.02	0.33±0.05	3.1±0.8	58±17	50±16	0.12±0.03	3.5±0.3	0.09±0.01

Table 5.9a (continued)

RL11	47±4	89±12	130±13	15±8	1.8±0.2	0.07±0.04	0.68±0.31	3.3±0.5	49±2	57±3	0.09±0.02	3.3±0.3	0.08±0.01
RL12	59±12	80±30	110±42	17±20	1.2±0.1	0.27±0.18	0.39±0.10	3.2±1.5	44±14	32±15	0.08±0.03	2.7±1.2	0.06±0.02
W01	9±3	94±12	330±52	81±11	1.7±0.4	0.14±0.03	1.78±0.27	5.3±1.8	169±21	115±10	0.32±0.06	4.6±1.0	0.22±0.03
W03	7±1	99±1	550±120	108±7	2.1±0.4	0.21±0.09	1.79±0.21	6.0±1.3	235±26	141±6	0.47±0.09	6.0±0.7	0.27±0.01
W04	7±2	93±13	420±62	92±17	1.8±0.2	0.16±0.02	1.79±0.15	4.7±0.5	213±14	131±16	0.39±0.03	5.5±1.0	0.25±0.06
W05	9±1	94±16	610±83	112±19	2.1±0.3	0.37±0.28	1.32±0.21	5.0±0.5	244±17	141±7	0.51±0.07	5.9±0.8	0.31±0.05
W06	6±1	97±2	570±61	101±14	2.2±0.2	1.05±0.38	1.32±0.25	4.8±0.3	253±21	148±9	0.50±0.06	5.7±0.4	0.27±0.03
W07	19±3	99±1	390±130	95±30	3.0±0.5	0.27±0.20	1.31±0.32	4.9±2.3	162±24	103±9	0.34±0.09	4.8±1.2	0.22±0.01
W08	4±3	70±13	250±140	29±10	0.7±0.2	0.47±0.23	1.31±0.21	1.3±0.8	171±37	62±18	0.26±0.10	3.4±1.7	0.09±0.05
W09	5±3	78±17	290±180	58±27	1.1±0.5	0.22±0.14	0.55±0.22	2.4±2.0	174±50	100±27	0.28±0.12	4.1±2.1	0.15±0.10
W10	11±1	99±1	530±92	58±12	2.0±0.4	0.27±0.16	0.55±0.31	4.8±0.5	221±26	110±11	0.44±0.09	4.4±1.0	0.25±0.03
W11	11±2	96±6	600±140	88±10	1.9±0.5	0.57±0.33	0.55±0.22	5.9±4.4	254±58	139±28	0.48±0.09	5.7±1.4	0.24±0.07
W12	3±3	66±22	190±160	48±45	0.8±0.4	0.39±0.30	0.55±0.05	1.0±1.3	132±61	76±39	0.22±0.11	2.8±1.7	0.08±0.10
W13	6±1	96±1	870±200	103±20	2.4±0.5	0.76±0.41	0.51±0.15	5.9±1.3	313±39	152±16	0.68±0.13	6.6±0.9	0.36±0.04
W14	9±1	98±1	680±67	85±18	1.9±0.3	0.18±0.05	0.51±0.70	4.7±0.3	229±15	112±11	0.52±0.04	6.1±1.8	0.31±0.02
W15	10±4	90±12	360±95	102±27	1.8±0.5	0.26±0.17	0.96±0.33	3.9±1.7	193±21	116±25	0.35±0.07	4.8±1.0	0.22±0.10
W16	15±5	96±7	470±110	86±13	1.8±0.2	0.23±0.14	0.96±0.54	3.6±0.4	196±35	110±22	0.38±0.08	5.3±1.2	0.26±0.05
W17	9±2	99±1	690±110	121±25	1.9±0.2	0.19±0.04	0.77±0.22	4.4±0.6	287±39	169±14	0.56±0.08	6.9±0.7	0.28±0.05
W18	11±2	97±1	470±95	107±24	1.9±0.4	1.08±0.26	0.77±0.34	4.2±0.5	227±39	142±17	0.40±0.08	5.7±1.3	0.24±0.03
W19	8±3	90±13	480±150	73±34	1.4±0.4	0.35±0.25	0.52±0.22	4.2±1.5	235±51	124±52	0.42±0.11	5.6±1.4	0.26±0.14
W20	12±2	99±1	480±83	106±30	2.2±0.3	0.18±0.04	0.52±0.50	4.2±0.3	198±23	131±7	0.42±0.07	6.4±1.9	0.24±0.04
W21	7±1	94±5	620±130	82±14	1.7±0.3	0.93±0.23	0.70±0.42	4.6±0.9	276±43	143±14	0.53±0.09	5.8±2.1	0.29±0.05

Table 5.9b. Mean element concentrations (in μmol g^{-1}, except where indicated by † for nmol g^{-1} and ‡ in mmol g^{-1}) of sediments for 45 shallow lakes in Minnesota (mean±standard deviation, *n* = 5-10).

Lake	†Dy	Fe	†Ga	†Gd	K	†La	Li	Mg	Mn	†Mo	Na	†Nd	Ni
GC01	7.8±1.2	220±45	35±10	11.2±1.7	39±7	77±11	1.0±0.2	270±33	13.1±4.2	15±5	8±3.9	66±10	0.24±0.03
GC02	6.5±1.7	210±65	30±12	9.2±2.1	37±20	67±11	1.0±0.2	550±350	14.0±6.5	18±12	15±10	57±12	0.22±0.07
GC03	9.5±1.8	230±74	40±11	12.8±2.2	59±20	83±9	2.3±0.7	580±85	10.1±4.3	15±7	25±4	76±10	0.31±0.10
GC04	10±1.7	250±77	46±15	13.6±2.1	61±20	88±13	2.0±0.5	510±140	10.2±2.5	30±17	15±2	80±14	0.33±0.08
GC05	8.5±0.7	220±23	34±2	11.3±0.7	63±6	75±3	1.6±0.3	490±43	16.3±2.4	19±9	19±2	64±3	0.27±0.03
GC06	9.4±1.4	230±31	45±8	13.1±2.0	39±7	87±13	1.1±0.2	230±34	8.4±1.3	34±17	6.5±2.7	77±12	0.29±0.03
IT01	9.0±3.2	140±51	56±17	13.5±4.4	30±10	121±39	1.3±0.5	86±31	2.4±0.3	14±9	9.4±7.8	101±36	0.32±0.14
IT02	8.0±2.3	160±50	52±16	12.2±2.8	31±10	111±25	1.3±0.4	89±28	1.9±0.5	4.5±4.9	6.5±2.8	88±19	0.26±0.11
IT03	8.8±3.0	160±37	53±17	13.2±4.7	26±10	111±33	1.3±0.3	110±21	2.1±0.5	8.0±4.8	8.6±2.8	97±31	0.31±0.11
IT04	3.9±1.2	100±16	30±4	6.2±1.5	12±3	60±11	0.8±0.1	79±15	1.5±0.1	1.1±1.0	6.2±2.4	48±10	0.14±0.02
IT05	1.8±1.5	230±200	5.5±7.3	2.6±2.2	7.2±3.6	21±16	0.3±0.2	190±29	5.7±2.8	25±10	8.5±4.6	17±15	0.12±0.03
IT06	3.5±0.5	120±26	25±6	5.3±0.6	11±2	54±9	0.8±0.2	81±16	1.3±0.4	0.4±0.1	4.7±3.6	42±7	0.13±0.05
RL01	4.9±2.1	140±33	26±10	7.6±3.5	21±6	65±29	0.8±0.3	130±27	2.3±1.0	15±10	8.2±6.1	52±24	0.17±0.07
RL02	7.6±1.8	150±25	38±8	11.7±2.9	35±7	90±24	1.1±0.3	120±14	2.5±0.5	7.5±3.5	6.6±4.5	78±20	0.25±0.07
RL03	2.2±0.8	70±22	8.7±6.3	3.3±1.1	9.5±3.5	27±11	0.3±0.1	110±48	2.4±1.3	16±9	7.4±5.9	22±9	0.13±0.04
RL04	6.1±1.4	100±44	25±9	8.9±1.8	23±7	70±15	0.7±0.2	110±33	2.9±0.4	34±17	4.4±3	60±13	0.22±0.07
RL05	4.7±0.8	140±12	18±3	6.7±1.2	18±2	56±9	0.6±0.1	140±33	2.9±0.5	20±6	11±7	45±7	0.20±0.04
RL06	0.8±0.3	47±16	0.3±0	1.2±0.5	5.8±2.3	7.9±4.1	0.1±0.1	210±26	4.1±1.1	13±8	7±4.7	7±3	0.10±0.01
RL07	2.5±0.5	63±6	12±2	3.6±0.7	15±3	26±5	0.4±0.1	97±7	7.1±1.5	6.5±2.3	17±1	22±5	0.09±0.01
RL08	2.8±0.3	62±6	13±1	4.2±0.3	17±3	30±2	0.39±0.04	85±2	5.6±0.3	7.6±0.7	12±3	25±2	0.11±0.01
RL09	2.6±0.1	94±13	11±1	3.7±0.3	13±2	24±1	0.33±0.04	100±7	12.0±1.1	8.9±1.3	12±4	21±1	0.10±0.01
RL10	3.0±0.6	86±20	14±3	4.2±1.3	16±1	30±8	0.4±0.1	100±6	9.5±2.3	9.1±2.9	15±3	26±7	0.11±0.04

Table 5.9b (continued)

RL11	2.6±0.1	130±13	12±1	3.6±0.3	13±2	24±1	0.3±0.1	110±10	17.6±1.5	5.9±2.4	12±3	21.6±0.6	0.11±0.01
RL12	2.3±0.8	76±21	9.6±3.1	3.4±1.2	12±5	22±8	0.3±0.2	100±11	14.8±7.7	4.6±2.0	11±4	20±7	0.07±0.03
W01	10±1	240±55	40±7	13.5±1.2	35±6	81±11	1.4±0.3	310±30	12.4±1.8	15±3	8.5±2.4	79±10	0.32±0.06
W03	15±2.1	410±47	60±9	19.7±2.7	47±7	115±16	2.1±0.4	310±35	14.3±1.2	10±2	9±1.9	115±17	0.41±0.02
W04	13±0.8	330±59	49±9	17.6±1.1	45±4	102±7	1.7±0.3	320±36	12.5±1.4	12±3	16±21	102±7	0.35±0.05
W05	15±0.6	390±35	67±8	20±1.5	48±7	122±11	2.1±0.3	250±30	11.5±1.0	8.5±2.4	9.9±3.1	118±10	0.42±0.04
W06	14±1.1	370±35	55±3	19.5±1.9	43±5	127±11	2.1±0.3	290±47	13.4±1.6	8.6±4.7	11±3	118±12	0.42±0.03
W07	10±1.6	230±62	41±10	13.2±2.3	31±7	81±12	1.4±0.4	160±59	8.7±2.1	35±11	8.7±1.9	79±14	0.3±0.03
W08	7.5±2.6	140±92	28±14	11±3.4	20±10	84±19	0.8±0.3	130±120	3.9±4.2	2.1±1.1	6.9±1	77±20	0.15±0.06
W09	9.5±4.1	190±130	34±20	13.1±5.5	31±17	87±23	1.2±0.7	240±86	4.8±3.0	8.5±7.6	7.2±1.9	80±25	0.24±0.13
W10	13±1.4	250±65	57±8	17.2±2.0	42±8	109±12	1.8±0.4	220±69	7.9±3.0	20±9	9.9±1.3	104±12	0.39±0.04
W11	15±3.2	340±93	62±18	20.2±3.8	49±12	126±29	2.1±0.6	200±49	8.5±2.6	18±8	7.3±0.8	121±26	0.39±0.09
W12	6.4±3.4	120±110	24±21	9.0±5.1	20±16	65±30	0.8±0.5	210±72	2.7±2.0	3.9±6.4	6.6±0.7	59±31	0.14±0.1
W13	19±3.3	450±78	91±17	26±4.8	62±13	160±22	2.8±0.5	260±61	6.9±2.0	6.7±2.2	9.5±1.4	155±23	0.47±0.06
W14	15±1.2	330±61	75±5	20.1±1.7	44±6	119±9	2.6±0.4	240±22	3.7±1.4	20±9	9.6±2	118±8	0.37±0.03
W15	12±1.9	260±99	42±13	15.4±1.8	39±11	93±9	1.5±0.4	280±40	9.1±3.8	13±5	7.3±1	90±12	0.31±0.1
W16	12±2.1	280±77	53±13	16.4±3.2	38±5	96±17	1.8±0.4	220±54	8.0±4.1	15±3	6.3±1	95±19	0.31±0.04
W17	17±2.2	460±66	76±11	23.2±3.6	57±10	139±21	2.5±0.5	220±54	11.6±4.0	13±4	7.1±1.7	135±20	0.45±0.06
W18	13±1.7	260±70	50±10	16.9±2.4	40±9	111±21	1.6±0.4	200±54	9.9±2.7	16±5	7.9±2.6	105±20	0.38±0.04
W19	13±2.4	270±100	53±17	18.3±2.5	45±14	113±21	1.7±0.5	210±90	5.8±2.6	10±4	7.2±1.4	110±20	0.4±0.23
W20	12±1.2	390±52	53±10	15.6±1.7	44±5	96±11	1.9±0.3	260±13	18.4±2.6	17±6	7.1±0.7	94±11	0.37±0.03
W21	16±3	360±86	64±13	21.6±4.0	54±14	139±24	2.1±0.4	230±73	10.5±4.4	9±7	7.4±1.7	130±22	0.42±0.06

Table 5.9c. Mean element concentrations (in μmol g^{-1}, except where indicated by † for nmol g^{-1} and ‡ in mmol g^{-1}) of sediments for 45 shallow lakes in Minnesota (mean±standard deviation, *n* = 5-10).

Lake	P	†Pb	†Pr	Rb	S	†Sc	†Sm	Sr	†Th	†U	V	†Y	Zn	†Zr
GC01	29±2	48±20	18±3	180±35	140±23	34±7	12±2	0.73±0.13	7±1	7.1±0.3	0.49±0.12	74±11	0.77±0.11	45±3
GC02	23±10	38±20	16±3	160±73	250±165	33±10	10±2	1.12±0.59	7±1	11±6	0.45±0.19	65±21	0.94±0.68	39±10
GC03	21±4	51±20	20±2	190±54	240±80	48±11	15±2	2.42±0.97	9±2	26±10	0.74±0.22	92±15	0.81±0.27	41±13
GC04	21±4	42±20	22±4	210±61	350±152	53±11	15±3	1.68±0.43	11±1	18±8	0.82±0.18	100±17	0.98±0.38	54±11
GC05	25±2	51±9	17±1	180±16	300±16	37±5	12±1	2.26±0.14	8±2	12±3	0.55±0.06	77±4	0.84±0.08	42±6
GC06	30±2	49±7	21±3	170±25	210±84	46±9	15±2	0.73±0.15	10±2	35±12	0.70±0.10	87±13	0.92±0.11	56±10
IT01	23±4	37±9	29±10	180±73	59±17	28±14	17±6	0.25±0.06	2±1	2.8±0.6	0.60±0.22	83±33	1.09±0.90	22±17
IT02	17±6	36±8	25±6	180±71	33±16	37±13	15±3	0.22±0.06	5±2	2.4±0.6	0.5±0.21	66±20	0.63±0.25	22±18
IT03	16±4	33±10	27±9	160±63	45±18	49±14	17±6	0.21±0.04	4±2	2.4±0.9	0.55±0.15	83±30	0.88±0.39	37±19
IT04	8±2	18±5	14±3	66±21	9.4±6.3	25±6	8.1±1.8	0.16±0.02	5±2	1.3±0.4	0.27±0.05	34±9	0.26±0.08	9±5
IT05	13±4	9±7	5±4	31±21	120±21	11±7	3.1±2.6	1.25±0.30	3±2	2.6±0.6	0.16±0.08	18±14	0.21±0.17	30±8
IT06	7±1	19±7	12±2	66±19	7±3	27±9	6.9±1.2	0.13±0.02	7±4	1.6±0.5	0.26±0.09	31±5	0.25±0.09	25±9
RL01	23±8	43±30	15±7	150±38	79±38	21±12	9.2±4.5	0.2±0.07	7±4	4.6±2.4	0.39±0.15	46±18	1.28±0.86	41±22
RL02	22±4	62±70	22±6	160±37	49±21	33±10	14±4	0.18±0.01	8±2	3.2±0.6	0.52±0.12	71±15	0.97±0.38	55±19
RL03	14±4	14±10	6±3	47.3±23	160±48	10±4	3.9±1.6	0.52±0.35	3±2	3.8±1.1	0.15±0.06	22±7	0.46±0.24	27±7
RL04	27±4	26±7	16±3	200±53	110±24	26±11	11±2	0.29±0.07	7±3	7.1±2.0	0.32±0.11	57±15	1.0±0.35	40±18
RL05	22±4	17±4	13±2	110±12	110±11	23±2	7.8±1.3	0.32±0.31	6±1	3.7±1.3	0.35±0.08	47±8	0.83±0.19	52±7
RL06	7±4	2.6±2	2±1	18.1±10	100±26	5±2	1.2±0.6	1.33±0.15	1±2	1.2±0.6	0.04±0.02	8±3	0.19±0.14	23±2
RL07	17±1	39±20	6.1±1	87±21	240±63	12±3	4.3±0.8	0.4±0.030	3±1	3.3±0.2	0.14±0.05	24±5	0.61±0.17	63±71
RL08	19±1	63±20	7±1	98±8	180±7	12±1	4.8±0.6	0.34±0.03	3±1	3.3±0.4	0.16±0.02	26±2	0.79±0.10	31±1
RL09	22±1	46±20	5.8±0.3	83±8	440±17	12±2	4.1±0.3	0.50±0.13	3±1	4.9±0.4	0.13±0.03	24±2	0.76±0.18	30±3
RL10	19±3	45±20	7±2	93±14	270±23	13±2	4.7±1.2	0.41±0.04	4±1	3.7±1.3	0.18±0.03	29±6	0.82±0.07	32±4

Table 5.9c (continued)

RL11	19±2	34±1	5.8±0.3	87±7	640±62	13±2	4±0	0.63±0.14	3±1	5.3±1.3	0.12±0.01	27±1	0.75±0.06	31±3
RL12	15±3	49±20	5±2	62±30	220±82	8±6	3.7±1.4	0.51±0.11	2±1	2.2±0.5	0.12±0.03	23±7	0.68±0.30	22±11
W01	23±2	54±10	21±3	180±38	380±48	43±7	15±2	1.58±0.34	10±1	14±3	0.62±0.13	92±15	0.93±0.15	40±9
W03	23±1	64±10	30±4	230±24	420±37	75±14	22±3	1.36±0.15	15±1	14±1	1.03±0.21	114±3	1.10±0.03	37±3
W04	24±1	60±10	27±2	210±33	370±74	56±9	20±1	1.05±0.12	13±2	13±2	0.85±0.09	103±16	1.06±0.23	43±14
W05	23±2	71±2	32±3	240±37	220±38	72±11	23±1	1.26±0.21	13±2	20±3	1.10±0.12	130±21	1.22±0.12	48±10
W06	22±1	70±8	32±4	210±28	83±24	74±11	23±2	1.23±0.29	15±2	13±3	1.07±0.11	120±22	1.19±0.09	55±16
W07	29±6	59±10	21±4	180±28	490±94	39±19	15±3	1.18±0.18	7±3	46±11	0.71±0.15	78±8	0.92±0.11	44±10
W08	16±6	29±20	21±5	98±60	15±6	33±15	14±4	0.19±0.05	9±2	4.5±2.0	0.46±0.21	59±21	0.44±0.22	15±2
W09	21±9	40±20	21±6	140±85	250±177	38±21	15±5	0.45±0.20	10±3	12±8	0.52±0.26	77±31	0.70±0.42	32±12
W10	25±7	71±20	28±3	170±26	280±43	63±13	20±2	0.87±0.21	12±2	32±11	0.88±0.17	95±11	1.11±0.12	54±12
W11	24±3	72±9	32±7	210±59	160±73	71±19	26±5	0.62±0.12	14±3	17±6	0.95±0.22	114±37	1.03±0.25	50±13
W12	17±2	26±30	16±8	87±72	44±22	26±19	11±6	0.32±0.14	7±4	3.2±1.7	0.36±0.26	55±31	0.43±0.41	21±8
W13	24±4	73±9	40±6	270±30	110±41	100±20	30±5	0.85±0.16	17±2	31±7	1.47±0.28	147±15	1.43±0.19	52±4
W14	18±1	68±5	31±2	260±28	320±33	79±5	23±2	1.78±0.72	17±2	45±25	1.08±0.06	133±16	1.21±0.12	81±16
W15	27±5	79±60	24±3	170±52	320±173	49±11	18±2	0.83±0.31	11±1	13±6	0.74±0.19	94±21	0.95±0.39	37±13
W16	22±1	120±100	25±5	190±31	350±117	52±13	19±4	1.05±0.57	11±2	16±2	0.74±0.15	100±19	1.01±0.11	50±7
W17	24±2	74±10	36±6	270±54	290±79	87±16	26±4	0.72±0.12	16±3	14±2	1.23±0.18	137±34	1.17±0.19	51±18
W18	32±1	66±8	28±6	200±45	53±8	47±10	20±3	0.80±0.24	8±1	18±4	0.83±0.17	113±21	1.12±0.13	37±6
W19	29±9	62±20	29±5	210±67	200±88	63±19	21±4	0.56±0.20	14±3	16±10	0.88±0.27	104±27	1.07±0.40	46±17
W20	25±1	69±20	25±3	230±44	520±102	58±12	18±2	1.58±0.22	11±2	12±1	0.89±0.16	102±20	1.06±0.18	36±7
W21	27±8	70±10	34±6	230±66	60±20	78±18	25±5	0.59±0.17	15±4	10±3	1.20±0.24	125±25	1.13±0.21	48±21

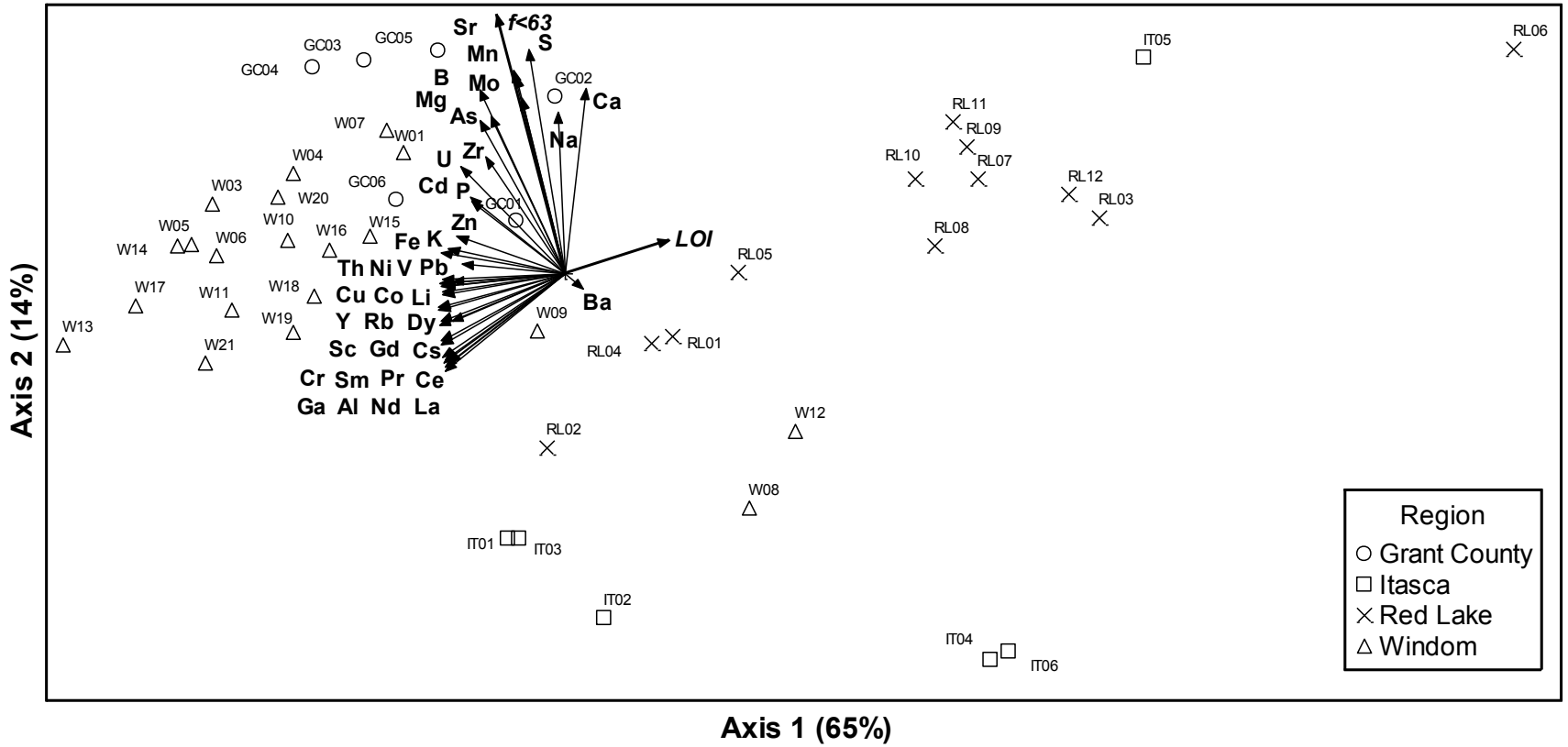

Figure 5.9. PCA ordination diagram showing how shallow lakes of different regions group out according to element concentrations in the sediments with LOI and *f*<63µm as overlays from the explanation matrix (Vectors indicate the direction of increasing element concentrations, LOI or *f*<63µm (scaled to 70% to fit graph) and their length reflects the magnitude of the association with the ordination axes).

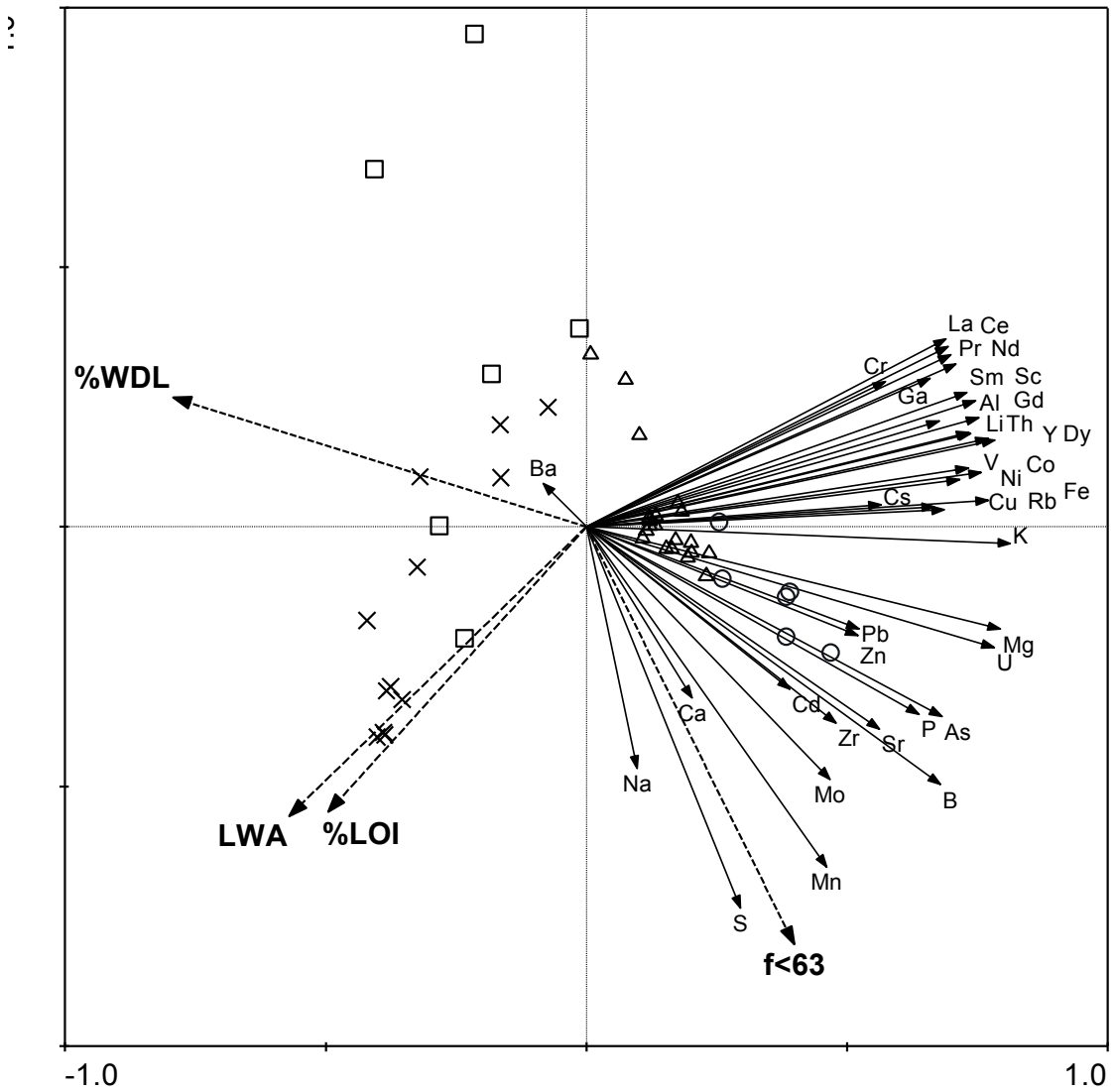

Figure 5.10. Ordination plot of the partial RDA for sediment chemistry variables constrained by environmental variables while controlling for region (Regions: Grant County (o), Itasca (□), Red Lake (X), Windom (Δ); Environmental variables (in bold): *f*<63 μm; %LOI, lake watershed area (LWA), percent woodland (%WDL); Sediment chemistry variables: multiple element concentrations).

Results of an RDA showed that Ca, Mg, Na, S and Sr concentrations in the water explained 33% of the variation in macrophyte community composition of the shallow lakes (Figure 5.11a). Filamentous algae, Potamogeton pusillus and Lemna minor were positively associated with S and Sr concentrations while Potamogeton richardsonii, Stuckenia pectinata and Ceratophyllum demersum were positively associated with Na, Mg and Ca concentrations in the water. Concentrations of Al, Li, Nd, Pr, Sc and U in the sediment explained 39.2% of the variation in macrophyte community composition (Figure 5.11b). Filamentous algae, Stuckenia pectinata and Potamogeton richardsonii were positively associated with Al, Li, Nd, Pr, Sc and U concentrations in the sediment.

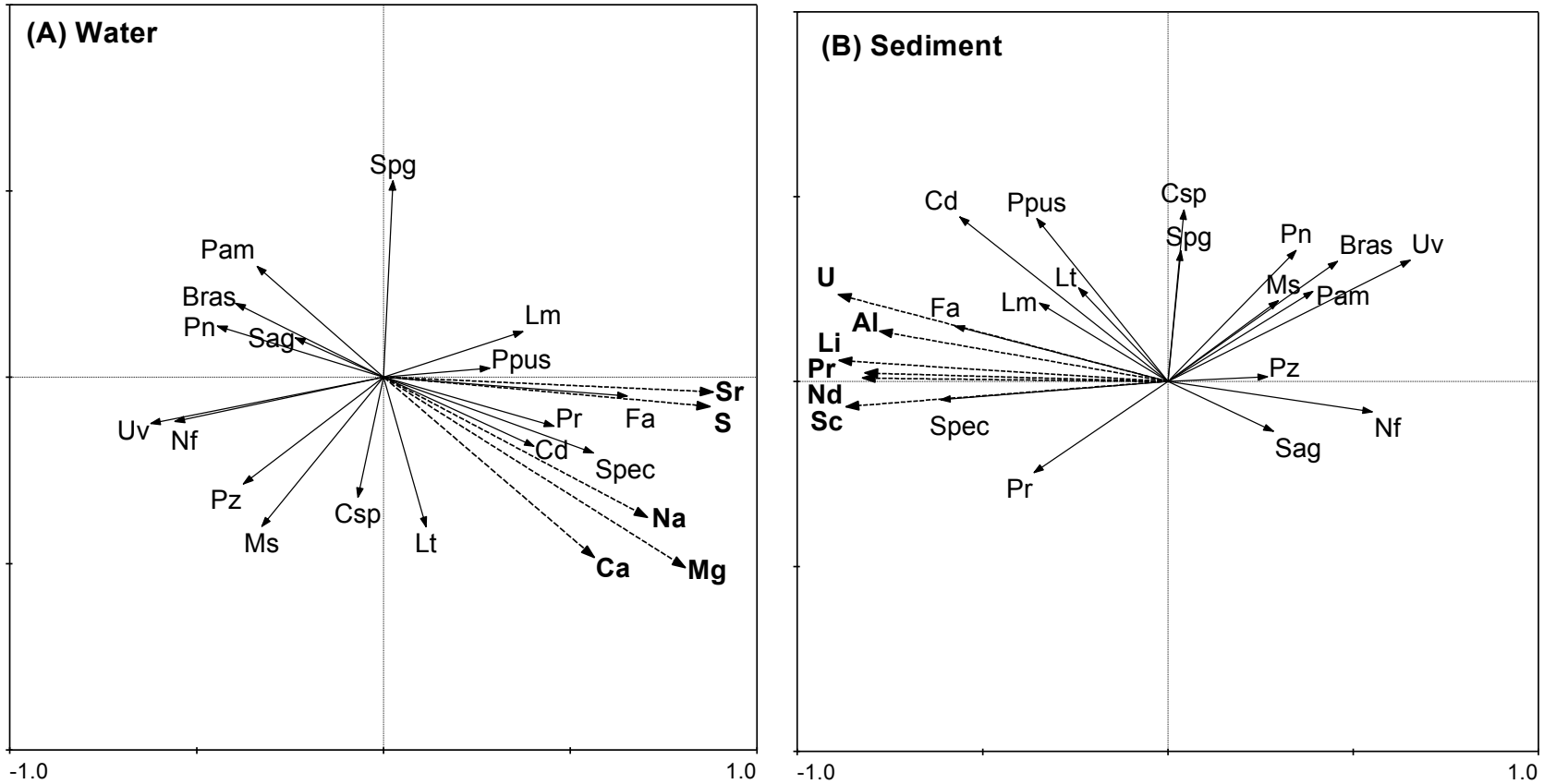

Figure 5.11. Ordination plots of the RDA of macrophyte community composition constrained by (A) Ca, Mg, Na, S, Sr concentrations in the water and (B) Al, Li, Nd, Pr, Sc and U concentrations in the sediments (in bold); common macrophyte or algae species detected by rake and viewer method combined (occurred in >7% of the shallow lakes sampled): *Brasenia schreberi* (Bras), *Ceratophyllum demersum* (Cd), *Chara* spp. (Csp), filamentous algae (Fa), *Lemna minor* (Lm), *Lemna trisulca* (Lt), *Myriophyllum sibiricum* (Ms), *Najas flexilis* (Nf), *Potamogeton amplifolius* (Pam), *Potamogeton natans* (Pn), *Potamogeton pusillus* (Ppus), *Potamogeton richardsonii* (Pr), *Potamogeton zosteriformis* (Pz), *Sagittaria cristata* (Sag), *Sparganium americanum* (Spg), *Stuckenia pectinata* (Spec), *Utricularia vulgaris* (Uv)).

5.4.4. Relationships between elements, plant cover and biomass and other variables

Turbidity showed positive correlations with chlorophyll-*a*, Si and Sr in water and negative correlations with LOI, plant biomass and cover (Table 5.10a). Chlorophyll-*a* showed negative correlations with plant biomass and cover and positive correlations with Mg, S, Si, and Sr concentrations in water. Negative correlations were observed between LOI and chlorophyll-*a*, Mg, S and Sr concentrations while the *f*<63µm showed positive correlations with Ca, Mg and Si in water. Positive correlations were observed between Ca, Mg, Na, S, Si and Sr in water. Turbidity showed positive correlations with As, Co, Dy, Fe, Gd, Li, Sc, Sm, Th and V in sediment (Table 5.10b). Chlorophyll-*a* showed positive correlations with As, Ce, Co, Dy, Fe, Gd, K, La, Li, Mg, Nd, Ni, Pr, Rb, Sc, Sm, Th, V and Y in sediment. LOI showed negative correlations with Ce, Cr, Dy, Fe, Gd, La, Li, Nd, Ni, Pr, Sc, Sm, Th, V and Y in sediment while the *f*<63µm showed positive correlations with As, B, Mg, Mn, Mo, P, S, Sr, U and Zr. Positive correlations were also observed between several elements in the sediment and in the water (Ca, Mg, S, Si and Sr).

Table 5.10a. Pearson correlations for turbidity, LOI, *f*<63 µm, chlorophyll-*a* concentrations and element concentrations in the water of shallow lakes in MN. Correlations with r ≥ 0.500 (that explain 25% or more variation) are shown (*p*<0.001).

	Turbidity	chl-*a*	LOI	*f*<63 µm	Ca	Mg	Na	S	Si
chl-*a*	0.921								
LOI	-0.504	-0.554							
Plant Biomass	-0.617	-0.546							
Plant Cover	-0.597	-0.559							
Ca				0.528					
Mg		0.509	-0.513	0.581	0.866				
Na					0.671	0.886			
S		0.529	-0.570		0.653	0.848	0.926		
Si	0.635	0.583		0.624	0.786	0.701	0.523	0.504	
Sr	0.508	0.537	-0.589		0.697	0.81	0.831	0.934	0.592

Table 5.10b. Pearson correlations for turbidity, LOI, *f*<63 µm, chlorophyll-*a* concentrations and element concentrations in water and sediment of shallow lakes in MN. (Correlations with r ≥ 0.500 (that explain 25% or more variation) are shown (*p*<0.001), element_w indicates elements in water, element_s indicates elements in sediment).

	chl-*a*	Turbidity	LOI	*f*<63 µm	Ca_w	Mg_w	Na_w	S_w	Si_w	Sr_w
Al_s								0.502		0.558
As_s	0.565	0.511		0.774	0.668	0.783	0.635	0.667	0.644	0.700
B_s				0.684	0.677	0.791	0.788	0.735	0.641	0.709
Ce_s	0.537		-0.547							0.530
Co_s	0.605	0.527				0.531		0.586		0.641
Cr_s			-0.541							0.548
Cu_s								0.54		0.599
Dy_s	0.608	0.536	-0.571			0.537		0.617		0.669
Fe_s	0.592	0.521	-0.551			0.585		0.618		0.665
Ga_s										0.540
Gd_s	0.588	0.516	-0.566					0.585		0.635
K_s	0.567					0.638	0.643	0.707		0.706
La_s	0.523		-0.552							0.525
Li_s	0.572	0.501	-0.618			0.582	0.564	0.708		0.771
Mg_s	0.560			0.545	0.621	0.866	0.812	0.799	0.552	0.734
Mn_s				0.719		0.541	0.562		0.586	
Mo_s				0.571						
Na_s							0.524			
Nd_s	0.557		-0.567					0.521		0.570
Ni_s	0.535		-0.513			0.519		0.590		0.661
P_s				0.533						
Pr_s	0.545		-0.566					0.506		0.556
Rb_s	0.501							0.532		0.571
S_s				0.731					0.538	
Sc_s	0.602	0.521	-0.641			0.528		0.62		0.672
Sm_s	0.573	0.501	-0.574					0.557		0.61
Sr_s				0.618	0.602	0.736	0.748	0.75	0.514	0.715
Th_s	0.644	0.586	-0.669		0.599	0.689	0.544	0.665	0.505	0.713
U_s				0.597	0.658	0.786	0.72	0.761	0.605	0.788
V_s	0.564	0.504	-0.578			0.572		0.639		0.711
Y_s	0.578		-0.525			0.525		0.614		0.658
Zr_s				0.523						

5.5. Discussion

5.5.1. Differences in water and sediment chemistry

Sediments are good indicators of past events and represent a longer time period compared to water analyses, which represent shorter time frames or single situations (Håkanson and Jansson 1983). Elements and nutrients can be exchanged or transported across the sediment-water interface (De Laune et al. 1981; Jaynes and Carpenter 1986; Weis and Weis 2004; Nurminen and Horppila 2009). Rooted submerged macrophytes are a link between the sediment and water column and thus play major roles in nutrient cycling within freshwater ecosystems (Carignan and Kalff 1980; Carpenter and Lodge 1986; Barko and James 1998; Fritioff and Greger 2006). Chemistry of sediments thus influences composition, chemistry and water quality of overlaying water (Håkanson and Jansson 1983). Previous studies have found that local geology and land use within watersheds play key roles in the sediment and water chemistry of surface waters (Moyle et al. 1945; Newton et al. 1987; Nilsson and Håkanson 1992; Fraterrigo and Downing 2008). Lakes in western and southwestern Minnesota tend to have harder water, higher pH ranges and higher alkalinity compared to lakes in the central and northern counties (Moyle 1945). These differences in the chemical characteristics of Minnesota lake waters can be attributed to geology and climate (Moyle 1945; Ojakangas and Matsch 1982). The surface and underlying geology and land use patterns varied across the regions of this study and appeared to influence the distribution of elements in the water and sediment of shallow lakes. PCA ordination plots showed that the shallow lakes clustered together according to region, indicating that element concentrations in water and sediment were variable, but variation among regions exceeded that within geographical regions across the state. However, results of pRDAs showed that region explained less than 10% while land use/cover variables within the watershed explained more than 40% of the variation in water and sediment element concentrations.

The results identified significant associations between several environmental variables and, 1) macrophyte cover, 2) macrophyte biomass, 3) macrophyte community composition, 4) water element concentrations, and 5) sediment element concentrations in shallow lakes. While controlling for geographic region, I found that percent woodland and percent agriculture were significant sources of variation of water element concentrations, while percent woodland, lake watershed area, LOI and *f*<63μm

were significant sources of variation of element concentrations in the sediment. In both cases, the percent woodland was positively associated with lakes of the Red Lake and Itasca regions, which are located within MN counties dominated by forested land (Minnesota Geospatial Information Office Staff 1999). The incidence of cultivated land increases as forested land decreases from north to south in Minnesota, which may account for the low element concentrations in the northern study regions, compared to the higher element concentrations in the central and southern study regions.

A gradient of increasing concentrations of dissolved minerals has been reported from northeast to southwest portions of Minnesota (Moyle 1945). Uplands dominated by forests are less prone to sedimentation whereas developed uplands are more prone to surface runoff and sedimentation due to the absence of constant vegetation cover (Lougheed et al. 2001). The tree roots of the forested areas stabilize soils, reduce erosion and decrease nutrient inputs into streams and lakes (Wood et al. 1984; Qualls et al. 1991; Lombi et al. 2001), which could explain why shallow lakes in the Red Lake and Itasca regions have clearer lakes and lower element concentrations in their waters and sediments. Runoff from agriculture contributes to small/fine grain particles in wetlands and thus agriculture-impacted wetlands tend to be dominated by finer grain particles such as silts and clays which are more prone to disturbance and resuspension in surface waters (Hamilton and Mitchell 1997) and keeps the water column turbid, limiting macrophyte growth. Lougheed et al. (2001) reported that wetland sediments of forested watersheds had larger particle size and organic content compared to wetland sediments of agricultural watersheds.

The LOI (indicative of organic matter content) and the *f*<63 μm (estimate of clay and silt content) of the sediments appear to play important roles in the mobility of elements in the water and sediment of shallow lakes in MN. Negative correlations observed between elements in the water and LOI may indicate that those elements may be tightly bound to organic matter in the sediment (Davies 1994; Jackson 1998) and hence are less mobile in the water column of high LOI lakes. Negative correlations observed between elements in the sediments and LOI may indicate that these elements are bound to the inorganic fraction of the sediments or are depleted or leached from the upper sediments due to low pH of the high LOI lakes (Yanes et al. 2006; Das et al. 2008). High LOI may be due to inputs of organic material from the surrounding woodland, high lake productivity and reduced conditions preventing

decomposition (Håkanson and Jansson 1983). The positive correlations observed between *f*<63 μm and several elements (in water: Mg, S, Si, Sr; in sediment: As, B, Mg, Mn, Mo, P, S, Sr, U, Zr) indicate that this fraction of small particles is a source of these elements in the water and sediment of the shallow lakes. Smaller particles (clays and silts) tend to bind elements since the active binding area of particles increases with decreased particle size (Håkanson and Jansson 1983). Sandy sediments have a low potential for the attenuation of elements due to the large pore spaces (Håkanson and Jansson 1983) and hence tend to have low nutrient availability (Barko and Smart 1986).

In the case of element concentrations in the lake waters, percent agriculture cover on surrounding land was negatively associated with lakes in Red Lake and Itasca regions and positively associated with lakes in Windom and Grant County regions. Shallow lakes in Grant County and Windom regions occurred in areas where dominant land cover is cultivated land (>79%) and soils are well drained. Very well drained soils in adjacent cultivated fields could facilitate transport of nutrients from fertilizer or animal waste into shallow lakes resulting in the elevated element concentrations detected in lakes of the southern most regions of this study (Grant County and Windom). These lakes, which are mostly impacted by agricultural activities, may be considered nutrient-rich compared to the low nutrient lakes in the northern most regions of this study (Itasca and Red Lake). Similarly, Atkinson et al. (2011) found the water chemistry of wetlands impacted by agriculture were very different from wetlands not influenced by agriculture. They reported that suspended solids, pH, alkalinity and soluble reactive phosphorus were significantly higher in agricultural compared to non-agricultural wetlands. Wetlands within forested watersheds were found to have significantly different water and sediment chemistry as well as different macrophyte community composition compared to wetlands within agricultural watersheds (Lougheed et al. 2001). Nilsson and Håkanson (1992) found a relationship between agricultural land cover and water chemistry which may be due to the soil characteristics of the land (dominated by clay-rich soils) and agricultural activities that involve plowing which leads to exposure, erosion and runoff and the addition of fertilizer which leads to nutrient loading.

Nutrient loading into lakes is dependent on the land use of the lake watershed, the size of the lake watershed and the potential for the transport of the nutrients within the watershed (Fraterrigo and Downing, 2008). In this study, lake watershed size was positively associated with LOI and negatively

associated with most elements in the sediments, which indicates that lake watershed size may play a role in nutrient inputs into lakes. The relationship between watershed size and LOI is confounded due to the larger watersheds that occurred in the Red Lake region. Land use within the watersheds appears to play a more important role in the water and sediment chemistry, which in turn influenced the abundance and composition of macrophyte communities in the shallow lakes.

5.5.2. Differences in macrophyte abundance and community composition

Plant community composition and distribution are influenced by geology, land cover, turbidity, pH, nutrient status, and water and sediment chemistry (Moyle 1945; Stewart and Kantrud 1972; Barko and Smart 1986; Barko et al. 1991; Bini et al. 1999; Koch 2001; Lougheed et al. 2001; Hansel-Welch et al. 2003; Bayley et al. 2007; Del Pozo et al. 2011). Hansel-Welch et al. (2003) found that changes in macrophyte community structure were influenced by water clarity and filamentous algae abundance. The results indicated that macrophyte cover, biomass and community composition in MN shallow lakes are influenced by several factors such as turbidity, watershed land cover, sediment physical characteristics, and water and sediment chemistry. Many studies have shown that widespread submerged macrophyte growth coincided with clear conditions in freshwater lakes (Mjelde and Faafeng 1997; Blindow et al. 1998; Faafeng and Mjelde 1998; Scheffer and Jeppesen 1998; van den Berg et al. 1998; Horppila and Nurminen 2003). Negative relationships between plant abundance and lake turbidity have been reported in a number of studies (Lougheed et al. 2001; Hansel-Welch et al. 2001; Zimmer et al. 2003). I also found negative correlations between turbidity and chlorophyll-*a* with macrophyte cover and biomass. Bayley et al. (2007) reported that clear lakes had <18 µg l^{-1} chlorophyll-*a* while turbid lakes had >18 µg l^{-1} chlorophyll-*a*. The clear lakes in their study had significantly lower P, S and Si concentrations in the water, lower turbidities and higher submerged vegetation cover compared to turbid lakes. Zimmer et al. (2009) reported that clear lakes had higher macrophyte biomass and <22 µg l^{-1} chlorophyll-*a* while turbid lakes had lower macrophyte biomass and >31 µg l^{-1} chlorophyll-*a*. Similarly, high macrophyte lakes in my study had lower turbidities, chlorophyll-*a* (averaged <16 µg l^{-1}), and Si concentrations compared to low macrophyte lakes. In general, the high macrophyte lakes were clearer than the low macrophyte lakes. Clear lakes allow more light penetration, which is essential for macrophyte growth (Philips et al. 1978). Positive correlations were observed between turbidity and chlorophyll-*a*, Si and Sr in the water, which

indicates that these variables may be contributing to the turbidity of the shallow lakes. Both chlorophyll-*a* and turbidity showed negative correlations with plant biomass and cover, which indicates that macrophyte growth in shallow lakes is influenced by light availability. Turbidity and chlorophyll-*a* also showed positive correlations with As, Ce, Co, Dy, Fe, Gd, K, La, Li, Mg, Nd, Ni, Pr, Rb, Sc, Sm, Th, V and Y in the sediments which may indicate that turbid lakes are enriched with these elements.

Certain macrophyte and algae species are indicators of specific types of shallow lakes and are indicative of particular chemical, environmental or habitat conditions associated with those lakes (Mackie 2004). One species, *Stuckenia pectinata* inhabits turbid lakes because it has a canopy growth form that allows for the concentration of most biomass close to the water's surface where it obtains maximum light (Scheffer 2004). I observed *Stuckenia pectinata* in lakes with high macrophyte abundance as well as lakes with low macrophyte abundance where the turbidity and chlorophyll-*a* concentrations tend to be greater than high macrophyte abundant lakes. Some macrophyte species can tolerate and are thus indicators of saline and alkaline conditions (Moyle 1945; Stewart and Kantrud 1972). One lake in this study (GC03) had Na concentrations at levels considered to be slightly to moderately brackish (Stewart and Kantrud 1972) and was the only lake in which *Ruppia occidentalis* was observed. *Ruppia occidentalis* and other *Ruppia* species are known to inhabit brackish, saline or very alkaline lakes (Moyle 1945; Stewart and Kantrud 1972; Husband and Hickman 1989; Borman et al. 2001).

Natural resource managers often use macrophytes as biological indicators to determine the nutrient status of freshwater ecosystems (Melzer 1999; Lougheed et al. 2001; Albert and Minc 2004; Mackie 2004; Perleberg and Loso 2009). Lakes with turbid waters and nutrient-rich sediments tend to be dominated by emergent vegetation and some submersed species that can tolerate low light and high nutrient levels (Stuckey 1975). Species such as *Potamogeton richardsonii* and *P. pectinatus* have been classified as possible indicators of nutrient enriched, turbid lakes (Stuckey 1975), and occurred in both low and high macrophyte lakes in this study. Filamentous algae and *Potamogeton richardsonii* are indicative of excess nutrients in lakes (Mackie 2004) and were significant indicators of lakes within Grant County where the concentrations of most elements were the highest compared to lakes in other regions of this study. *Chara* species are also indicators of enriched conditions and hard water lakes (Mackie 2004). *Chara* spp. prefer hard waters rich in Ca and bicarbonate ions which it uses as a source of carbon

(Kufel and Kufel 2002). *Chara* spp. were dominant in lakes of the Red Lake and Itasca regions which, though not enriched in nutrients, may have hard water lakes. The Red Lake and Itasca regions are within central and northern Minnesota where the lakes formed over calcareous glacial till and tend to have hard, alkaline waters (Moyle 1945).

Other indicators of nutrient-rich conditions include diatoms such as *Cyclotella cryptica* and *Gomphonema parvulum*, which are also major sources of silica in lakes (Mackie 2004). Silicon is derived from the weathering of rocks (Håkanson and Jansson 1983; Mackie 2004) and is a major limiting nutrient for diatom growth (Martin-Jézéquel et al. 2000). Silicon uptake is dominated by diatoms and sponges as well as blue-green algae (Fraústo da Silva and Williams 2001; Quiroz-Vazquez et al. 2008). Dissolved silica concentrations in the water column are primarily controlled by benthic macroalgae (Sigmon and Cahoon 1997). Silicon may be released or excreted from diatom grazers and decomposing diatom frustules (Parker et al. 1977; Mackie 2004). Ahlgren (1970) and Egge and Aksnes (1992) reported that increases in diatom biomass were associated with increases in the silicon or silicate concentrations in water.

Silicon concentrations were higher in low macrophyte lakes and showed significant positive relationships with turbidity and chlorophyll-*a* concentrations. Similarly, Mi et al. (2008) reported that Si concentrations were significantly higher in the water column when plants were absent compared to when they were present in a lake. Diatoms or diatom grazers may dissolve, leak or excrete Si (Mackie 2004) and thus contribute to high Si, chlorophyll-*a,* and turbidity, hence decreasing light availability and the potential for abundant macrophyte growth in the low macrophyte lakes. In contrast, Mn concentrations were lower in the low macrophyte lakes. Interactions between Mn and Si concentrations in the lake water may play a role in phytoplankton biomass. Silicon has antagonistic effects on Mn uptake and toxicity in plants (Horst and Marschner 1978; Kabata-Pendias and Pendias 2001; Maksimović et al. 2012). In the high macrophyte lakes Si may be taken up by plants and phytoplankton and this may counter Mn uptake and toxicity. After uptake by plants and phytoplankton, the remaining Si in the water may not be enough to sustain diatom growth. Manganese mobility in the environment is influenced by pH and redox conditions and can be derived from the weathering of rocks and forest litter (Heal 2001). Manganese concentrations in surface waters of Scotland were reportedly related to conifer cover of the surrounding

land (Heal 2001). Lakes of the Itasca and Red Lake regions are within the coniferous forest biome of Minnesota and the acidic nature of the conifer litter and soils (Binkley and Sollins 1990) may promote Mn mobility and subsequent leaching into surface waters of some lakes in these regions. Soils with greater clay content tend to have higher sorption of Mn than sandy soils (Radwan et al. 1979) hence lakes with clay-dominated sediments may have lower Mn concentrations compared to lakes with sandy sediments.

Particle size influences sedimentation processes and the capacity to bind elements (Håkanson and Jansson 1983) and thus impacts macrophyte communities (Lougheed et al. 2001). Although the low and high macrophyte lakes had similar *f*<63 μm, the Grant County and Windom regions had significantly higher *f*<63 μm and therefore higher amounts of clay which may be responsible for the lower Mn concentrations in the water column of these lakes. Poor macrophyte growth was observed in sandy sediments due to the low nutrient availability, low organic matter and low rates of nutrient diffusion and exchange (Barko and Smart 1986). The low macrophyte cover (<5%) that we observed in some clear lakes (<6 μg L^{-1} chl-*a*) in Itasca may be due to their sandy sediments (*f*<63 μm: <35%) and/or low nutrients.

The macrophyte community structure and macrophyte abundance were influenced by land cover variables as well as water and sediment chemistry. Land cover variables and sediment physical characteristics (LOI and *f*< 63μm) influenced the water and sediment chemistry. Differences in geology across the regions play a role in the sediment physical characteristics and element concentrations detected in the lake waters and sediments. Further studies are needed to confirm these findings. Studies are also needed to determine if macrophytes influence the water and sediment chemistry of shallow lakes. This can be addressed by comparing the water and sediment chemistry of open water and within macrophyte beds.

5.6. Conclusions

This study found that land cover, lake turbidity, chlorophyll-*a*, sediment characteristics, water and sediment chemistry play important roles in macrophyte abundance and community composition. Land cover also influenced water and sediment chemistry of MN shallow lakes. Land cover uses, chlorophyll-*a* concentration, and open water area were significant predictors of macrophyte cover while sediment characteristics (LOI and *f*< 63μm), land cover, and turbidity were significant predictors of macrophyte

biomass. Element concentrations in the water and sediments were significant predictors of macrophyte community composition. The percent woodland and agriculture were associated with element concentrations in the water while the percent woodland, lake watershed area and sediment physical characteristics were associated with elements in the sediments. Shallow lakes with high element concentrations in their water were associated with agriculture-dominated watersheds while those with high element concentrations in their sediments were inversely associated with woodland-dominated watersheds. High macrophyte abundant lakes had higher Mn and lower Si concentrations in their waters and higher B, Ba, Ca, Mo, P, S, Sr, U and Zr in their sediments compared to high macrophyte abundant lakes.

CHAPTER 6. GENERAL DISCUSSION AND FINAL CONCLUSIONS

The two sections of this thesis focused on processes in the root zone and variation in shallow lake ecosystems, which may appear disparate, but through the plants, are closely connected. The question is, is that connection recognizable in the biogeochemical behavior of the elements?

The biogeochemical differences associated with wetland plants at two different spatial scales, the root zone and the shallow lake ecosystem are summarized in Table 6.1. In general, multi-element concentrations were higher in the root zone compared to the bulk zone under flooded and non-flooded conditions. However, plants under flooded conditions took up 2-27 times more amounts of elements which indicated that these plants were exposed to more elements in the root zone. The root zone studies further showed that accumulation of elements in the rhizosphere may be due to differences in moisture content, pH and redox status. In the shallow lake study, lakes with high plant abundance were associated with higher concentrations of elements in the sediments which may be due to inputs of sediments, organic material and elements from the surrounding uplands.

Table 6.1. Summary of the significant biogeochemical differences associated with wetland plants at different spatial scales (↑ = increased; ↓ = decreased; chl-*a* = chlorophyll-*a*; $p<0.01$).

Spatial Scale	Treatments	Element concentrations		Other factors
Root Zone		Root Zone compared to Bulk Zone		
	High moisture	Soil (μmol g^{-1})		
	Rumex crispus	Fe^{2+} (↓); Al, Cr, Fe, K, La, Sr, V, Y, Zn (↑)		Fe-plaque present, ↑Eh, ↓pH
	Typha angustifolia	Fe^{2+} (↓); Be, Cu, Fe, Li, Sr, Zn (↑)		Fe-plaque present, ↑Eh
	Triglochin maritima	no differences		Fe-plaque present, ↑Eh
	Low moisture	Root Zone compared to Bulk Zone		
	Rumex crispus	Al, Ba, Cu, Cr, Fe, K, La, Mg, Na, Sr, V, Y, Zn (↑)		Fe-plaque absent
	Typha angustifolia	Ni, Sr (↑)		Fe-plaque absent
	Triglochin maritima	no differences		Fe-plaque absent
Shallow Lakes	**Plant abundance**	Sediment (μmol g^{-1}, ‡mmol g^{-1}, †nmol g^{-1})	Water (mg L^{-1})	
	Low plant	B, Ba, ‡Ca, †Mo, P, S, Sr, †U, †Zr (↓)	Mn (↓); Si (↑)	Turbid regime (>16 μg L^{-1} chl-*a*)
	High plant	B, Ba, ‡Ca, †Mo, P, S, Sr, †U, † Zr (↑)	Mn (↑); Si (↓)	Clear regime (<16 μg L^{-1} chl-*a*)

6.1. Influences of biogeochemistry at different spatial scales

The results indicate that several factors played important roles in the biogeochemistry of wet ecosystems at the two spatial scales. These factors are highlighted in the modified version of the conceptual model shown in Figure 6.1. The root zone studies indicated that soil pH, redox status, and Fe-plaque, influenced element concentrations in the soil. These factors can be influenced by plants (Jaynes and Carpenter 1986; Alloway 1995) and microbes in the rhizosphere (Trolldeneir 1988; Neumann and Römheld 2002; Neubauer et al. 2007). Soil pH is modified by plants via root exudation, release of H^+ ions, iron oxidation, cation and anion uptake (Hinsinger 2001; Neumann and Römheld 2002; Inderjit and Weston 2003; Kirk 2004). Changes in soil pH can lead to the dissolution and increased mobility of elements such as Al, Cu, Fe, Mn, Mo and Zn in soil (Kirk and Bajita 1995; Jones et al. 1996; Jungk 2002). In the root zone studies, changes in pH were most likely due to iron oxidation reactions at the soil-root interface. It has been shown that decreased pH in the rhizosphere of wetland plants was due to the release of protons and iron oxidation reactions (Begg et al. 1994; Wright and Otte 1999). Changes in redox status and Fe-plaque formation also play a role in the mobility and availability of elements such as As, Cu, Fe, Mn and Zn (De Laune et al. 1981; Otte et al. 1989; Otte et al. 1991; St-Cyr and Campbell 1993; Otte et al. 1995; Mi et al. 2008). Under wetland conditions, redox status is influenced by plant radial oxygen loss which leads to the oxidation of Fe and subsequent Fe-plaque formation in the vicinity of the roots (Mendelssohn 1993; Kirk and Bajita 1995; Hinsinger 2001; Hinsinger and Courchesne 2008). The root zone studies also indicated that soil moisture content influenced element concentrations in the soil as evident by the differences in element concentrations between the high moisture compared to low moisture content soils.

In the shallow lake study, representing the whole plant community effect, land cover uses and watershed size played a role in the distribution of elements in the water and sediments. The land cover variables that were associated with element concentrations in the waters and sediments of shallow lakes included percent agriculture and percent woodland. Watersheds dominated by agricultural or cultivated lands can increase nutrient inputs into lakes and thus impact the water and sediment chemistry (Fraterrigo and Downing 2008; Atkinson et al. 2011). In contrast, watersheds dominated by woodland or forest would have the opposite effect since tree roots and the constant vegetation cover decrease the

impacts of erosion and prevent the release of nutrients into surface waters (Wainwright et al. 1999; Lougheed et al. 2001). The watershed size and the potential for transport of elements within the watershed influence the impact of the land use activities (Fraterrigo and Downing 2008). In the shallow lake study, the lakes in agriculture-dominated watersheds had higher element concentrations while lakes in the woodland-dominated watersheds had lower element concentrations in the waters and sediments.

In the small-scale root zone studies, loss-on-ignition and *f*<63 µm did not vary and hence did not appear to play a significant role in element concentrations, but these two varied among lakes and thus had significant influences in the large-scale shallow lake study. High LOI in lakes could be due to high lake productivity, reduced conditions and inputs of organic materials from the surrounding uplands (Håkanson and Jansson 1983). Complexation with organic matter can decrease the mobility of elements in soil, sediment and water (Antonovics et al. 1971; Guilizzoni 1991; Davies 1994; Doyle and Otte 1997; Jackson 1998) and could result in low element concentrations. Soils or sediments dominated by particles smaller than 63 µm would indicate the presence of proportions of silts and clays. Complexation with clay-sized particles can also decrease the mobility and availability of elements (Håkanson and Jansson 1983; Davies 1994). In the shallow lake study, high LOI lakes were associated with larger watersheds and low element concentrations in the sediments. In contrast, lakes dominated by particles smaller than 63 µm were associated with high element concentrations in the sediments.

In the large-scale shallow lakes study, element concentrations in the waters and sediments, LOI, and *f*<63 µm played significant roles in the abundance and distribution of macrophytes. High LOI lakes contained higher biomass of *Potamogeton natans, Brasenia schreberi*, and *Potamogeton amplifolius* while lakes with high *f*<63 µm contained higher biomass of *Ceratophyllum demersum, Potamogeton richardsonii, Myriophyllum sibiricum, Lemna minor*, and filamentous algae. The species associated with high LOI lakes may be tolerant of the low nutrient conditions of those lakes while the species associated with clay-rich sediments (high *f*<63 µm) may be tolerant of the high nutrient conditions of those lakes. *Lemna minor, Lemna trisulca, Potamogeton pusillus, Potamogeton richardsonii, Stuckenia pectinata, Ceratophyllum demersum*, and filamentous algae have been reported as tolerant of high nutrient conditions (Stuckey 1975; Lougheed et al. 2001; Mackie 2004; Povidisa et al. 2009) and were associated with high element concentrations in the waters and sediments in the shallow lakes study.

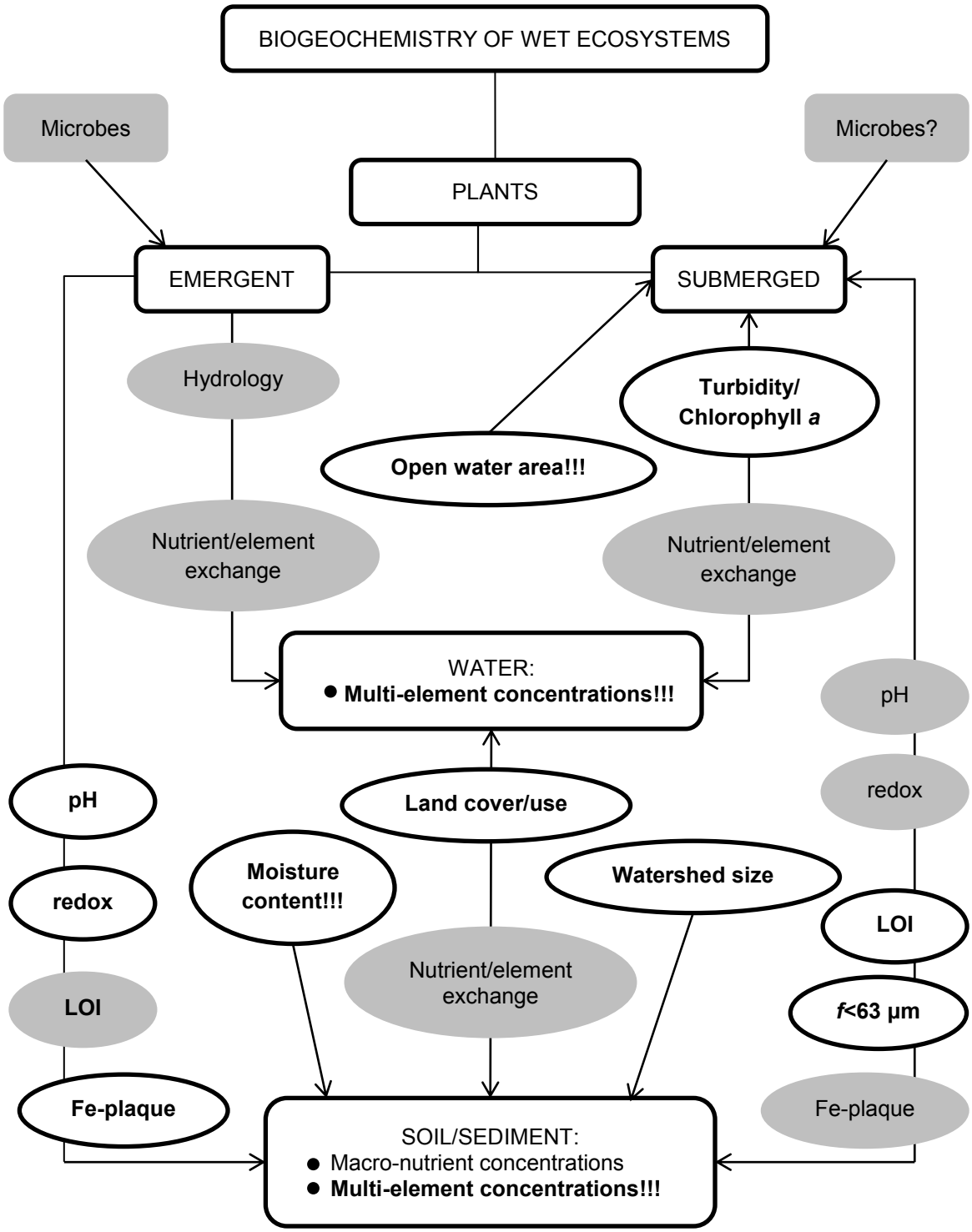

Figure 6.1. Revised conceptual diagram of the influences of submerged and emergent plants on the sediments and water as reported in the literature (rectangles indicate components in wet ecosystems; ovals indicate influencing factors; factors in bold are relevant in these studies; factors in gray did not play a role in these studies; ? indicates gaps in the literature; !!! indicates new findings; LOI=loss-on-ignition; *f*<63 µm=fraction of particles smaller than 63 µm).

6.2. Role of plants in the biogeochemistry of wet ecosystems

The root zone studies indicated that processes in the rhizosphere play an important role in the mobility, availability and subsequent uptake of elements. The plants in these studies influenced the pH and redox status of the soil which may have impacted the distribution of elements (Figure 6.2). These plants also accumulated elements from the soil in their tissues. Studies have shown that wetland plants altered the soil pH and redox status which in turn influenced the mobility of elements in soils or sediments (Jaynes and Carpenter 1986; Jacob et al. 2004b; Kidd et al. 2009). The accumulation of elements in emergent plants was also observed for *Phragmites communis, Typha latifolia, Schoenoplectus lacustris* (Szymanowska et al.1999), *Juncus effusus, Phragmites australis, Phalaris arundinacea, Polygonum hydropiper, Schoenoplectus lacustris,* and *Typha angustifolia* (Samecka-Cymerman and Kempers 2001).

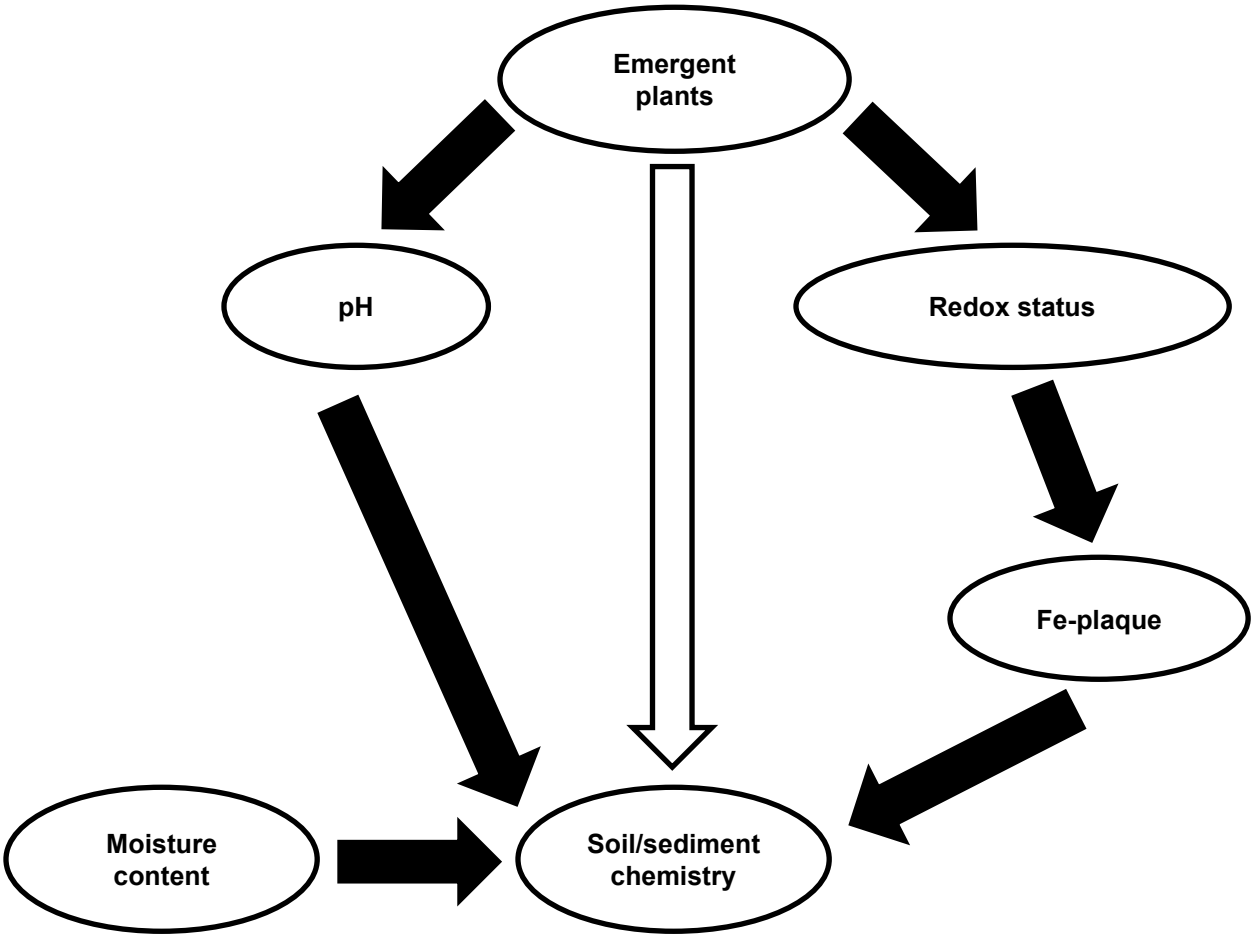

Figure 6.2. Role of emergent plants in the biogeochemistry of soil as determined by the root zone studies (black filled arrows indicate direct relationships, white arrows indicate indirect relationships).

The shallow lakes study indicated that water and sediment chemistry, open water area, turbidity, and sediment physical characteristics influenced macrophyte distribution (Figure 6.3). In shallow lakes, rooted submerged macrophytes provide a link between the sediment and water column (Carpenter and Lodge 1986) and thus play an important role in the cycling of nutrients (Barko and James 1998). In the shallow lakes study macrophytes may have less of a chemical effect on the elements and more of a physical effect through the stabilization of sediment bound elements. Wetland plants stabilize sediments, inhibit sediment erosion and suspension and hence prevent loading of nutrients to the water column of lakes (Barko and James 1998). In the shallow lakes study, the plants may play the role of keeping lakes clear by stabilizing sediments and inhibiting phytoplankton growth by blocking nutrient access (Philips et al. 1978; Blindow 1992; Kufel and Kufel 2002). In shallow lakes, uncovered sediments are more vulnerable to disturbance and resuspension by wind, waves, and benthivorous fish, than plant-covered (includes rooted and floating plants) sediments (Faafeng and Mjelde 1998; Scheffer 2004).

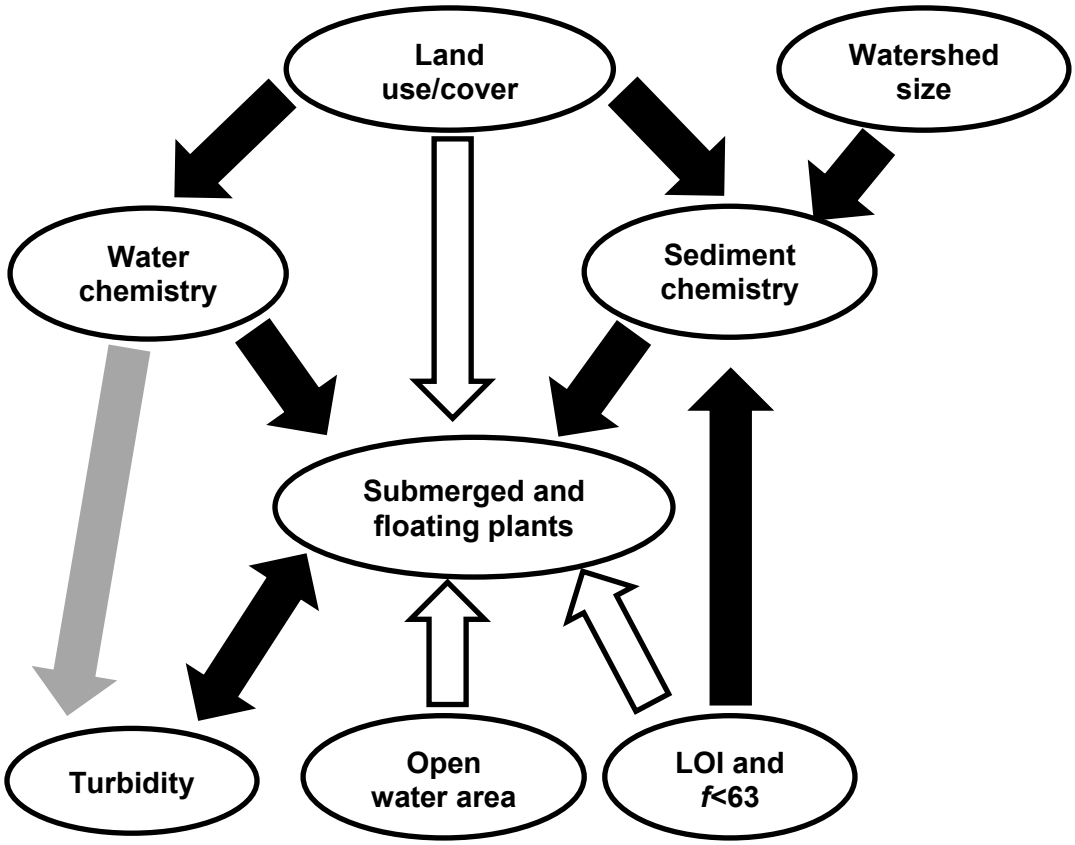

Figure 6.3. Role of submerged and floating plants in the biogeochemistry of soil as determined by the shallow lake study (black arrows indicate direct relationships; empty arrows indicate indirect relationships; grey arrows indicate possible relationships; LOI=loss-on-ignition; *f*<63 μm=fraction of particles smaller than 63 μm).

6.3. Implications and recommendations

The root zone studies indicate that wetland plants can accumulate multiple elements in their root zone and plant tissue which makes them ideal candidates for use in phytoremediation (Salt et al. 1995; Fritioff and Greger 2003) and phytostabilization (Weis and Weis 2004; Azaizeh et al. 2006). Wetland plants such as those used in this study can be used to restore and protect shorelines and act as buffer vegetation for lakes (Sculthorpe 1967; Eggers and Reed 1997; Cronk and Fennessy 2001) particularly those located within agriculture-dominated watersheds. Since these wetland plants effectively accumulate elements in their rhizospheres and tissues they will reduce element inputs into lakes and thus prevent eutrophication and other forms of non-point source pollution (Hoagland et al. 2001; Vymazal 2002; Gottschall et al. 2007; Mitsch and Gosselink 2007). The plant roots will stabilize shorelines, prevent erosion, and subsequent sedimentation into lakes (Odum and McIvor 1990; Brix 1997; Lombi et al. 2001; Horppila and Nurminen 2005).

The shallow lake study in this research indicated that activities of the surrounding watershed play an important role in the element concentrations of the waters and sediments. Lakes within agriculture-dominated watersheds were associated with high element concentrations in the water and sediments which indicate that these shallow lakes may be receiving nutrients from agricultural runoff. The results of the shallow lake study indicated that clear lakes support high plant abundance. These plants may sustain the clear conditions by stabilizing sediments and inhibiting phytoplankton growth (Blindow 1992; Kufel and Kufel 2002). In a study conducted by our research group, we found that lakes dominated by *Typha* spp. fringe had significantly lower turbidities compared to shallow lakes dominated by *Scirpus* spp or tree fringes. This study indicated that the *Typha* fringe may be taking up nutrients from runoff and preventing eutrophication and turbid conditions in the shallow lakes. The root zone studies demonstrated that *T. angustifolia* can take up multiple nutrients under wetland conditions. Based on these findings I recommend the use of wetland plants particularly, *Typha* spp. for phytoremediation and phytostabilization of contaminated soil and water and for use as shoreline vegetation of shallow lakes.

The small-scale root zone studies indicated that wetland plants can be used for phytoremediation and phytostabilization of elements because of their ability to accumulate multiple elements in the root zone soil and plant tissues. The large-scale shallow lakes study also indicated that plants may play a role

in the phytostabilization of elements because of their ability to stabilize sediments and prevent release of nutrients in lakes. In both the small-scale and large scale studies of this research, it is evident that emergent and submerged plants play a vital role in the phytoremediation and phytostabilization of multiple elements in wet ecosystems.

6.4. Future studies

The following studies and experiments would address some of the problems and questions that arose from the research described in this thesis.

6.4.1. Future root zone studies

The root zone studies examined concentrations at different distances to the root surface. One experiment used *Rumex crispus* in a growth column with different mesh compartments of known amounts of soil to quantify the element content of soil at different distances from the root (Figure 6.4). This was to develop a mass balance of multi-element distributions in soil adjacent to plant roots grown under flooded and non-flooded conditions. It was expected that multiple elements would accumulate in the soils closest to the roots (root zone) compared to soils further aware (bulk zone). However, plant growth became limited to the top 10 cm of soil and did not form a rhizoplane by growing a dense mat of roots along the inside of the inner mesh. This may be due to the plant reaching its optimum root length and mass or insufficient nutrients in the soil for growth. The experimental setup needs to be modified so that the plant would produce sufficient roots to create a rhizoplane and so that element content of the soil could be determined at different distances from the roots.

6.4.2. Future shallow lakes studies

Shallow lakes can shift between a clear and turbid regime within one to several years (Blindow et al. 1998; Bayley et al. 2007). Natural resource managers and researchers have studied these regime shifts for years, but still do not understand the mechanisms behind these shifts. Studies by Scheffer and Jeppesen (1998) and Zimmer et al. (2002, 2009) investigated the role of plants, fish, water levels and total phosphorus. I collected data from clear macrophyte-dominated lakes and turbid phytoplankton-dominated lakes to understand the dynamics of shallow lake ecosystems. Studies involving repeated sampling of the same lakes over several years should be conducted to determine the role of the water and sediment chemistry in clear, turbid and shifting regimes. These studies may give an indication of the

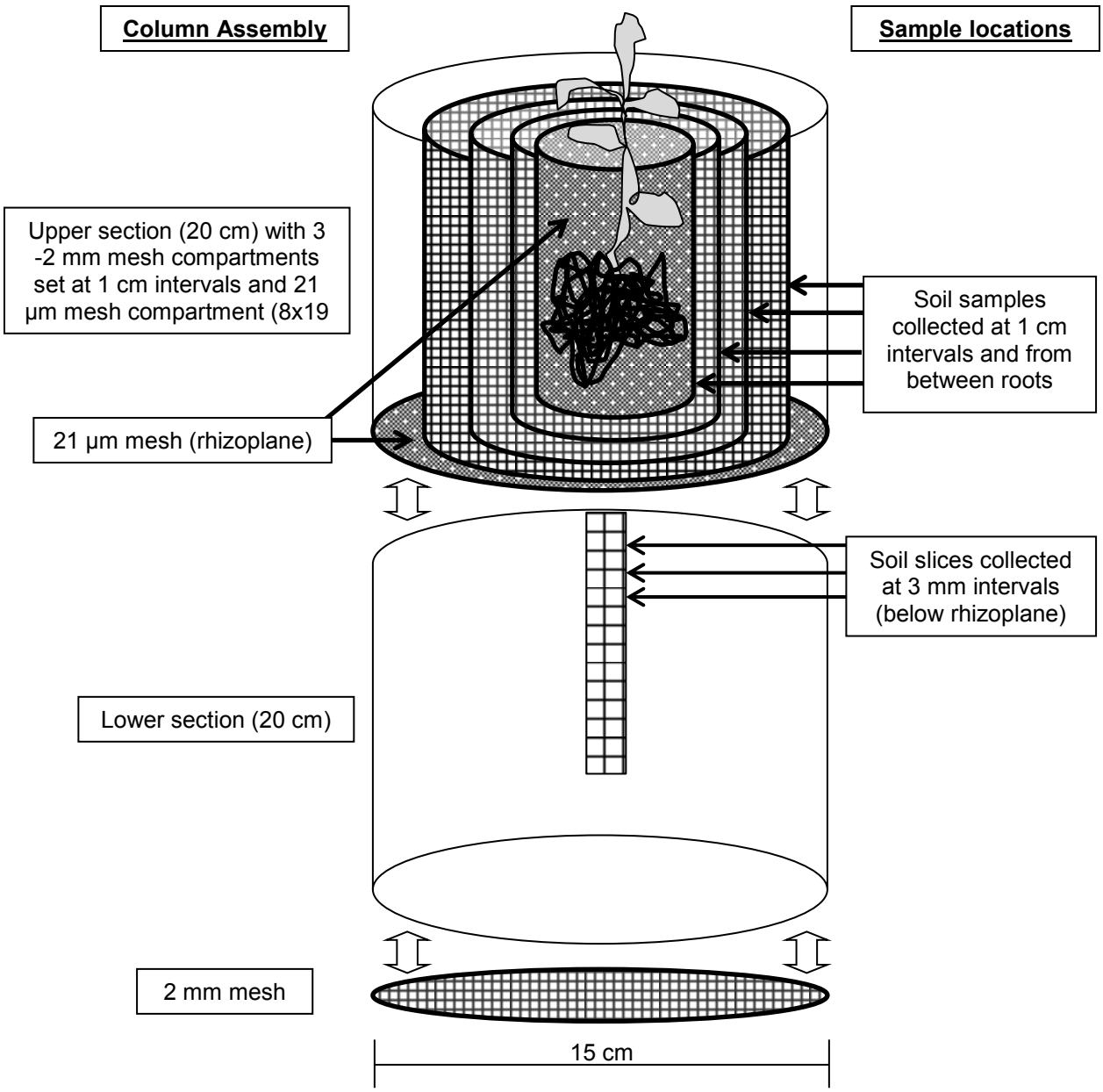

Figure 6.4. Column apparatus for mass balance experiment showing column components on the left and sampling locations on the right.

role of the water and sediment chemistry in the switching of lakes from clear to turbid and vice versa. The scale of my shallow lake study did not focus on the influence of plants on the element concentrations of the water and sediments. To do this, I would need to examine element concentrations within and outside macrophyte beds. These studies should also be carried out in clear, turbid and shifting regimes to determine if macrophytes play different roles.

The shallow lakes study reported in this research found that Si concentrations were higher and Mn concentrations lower in the low macrophyte abundance lakes. The low macrophyte abundance lakes had higher turbidities and higher chlorophyll-*a* concentrations than high macrophyte abundance lakes which indicate high phytoplankton biomass in these lakes (Landers 1982; Bayley and Prather 2003). Research has shown that algal growth is influenced by Si concentrations (Egge and Aksnes 1992; Sigmon and Cahoon 1997; Quiroz-Vázquez et al. 2008), however, the influence of Mn concentrations on phytoplankton growth is unknown. Controlled experiments are needed to further investigate the role of Si and Mn in lake turbidity and phytoplankton growth. The root zone studies indicated that emergent wetland plants can accumulate elements in the root zone and tissues and as constituents of fringe vegetation may also influence lake turbidity and nutrient inputs. Field surveys and shoreline restoration studies are needed to determine the impacts of fringe vegetation on lake turbidity of shallow lakes.

6.5. Final conclusions

Plants in wet ecosystems play important roles in the cycling and sequestering of nutrients. On the small scale, the root zone studies showed that wetland plants influence the mobility and plant uptake of multiple elements. On a larger scale, the shallow lakes study indicated that plants may play the role of stabilizing sediments and minimizing the subsequent release of nutrients from sediments.

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