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Physical and chemical factors controlling carbon gas emissions and organic matter transformation in coastal wetlands

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PHYSICAL AND CHEMICAL FACTORS CONTROLLING CARBON GAS EMISSIONS AND ORGANIC MATTER TRANSFORMATION IN COASTAL WETLANDS

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by
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ABSTRACT

Wetlands are important sinks for the atmospheric carbon (C) and play a major role in the global carbon cycle. However, factors impacting wetland soil C transformations and C gas production are not yet well understood. Elucidating these influences is especially important to Louisiana as wetlands are being impacted by salt water intrusion, subsidence and Mississippi river water diversion activities. This study evaluates, the effects of salinity, ions in river and sea water (K^+, Ca^{2+}) , clays and electron acceptors (NO_3^-, SO_4^{2-}) on soil C transformations as well as C gas production from Louisiana coastal wetlands. Wetland soils were collected from forest swamp (FS), freshwater marsh (FM), and saline marsh (SM) and various characterizations were carried out. Aerobic incubations showed that addition of either K^+ or Ca^{2+} chloride salts significantly increased CO_2 production from FS soil, but had little effect on CO_2 production from FM soil. Clay addition (2 and 5%) to FM soil significantly decreased CO_2 production compared to unamended soil ($P < 0.02$). A combination of 5% clay and 5 mM Ca^{2+} further decreased the CO_2 production in this soil. Increase in salinity decreased CO_2 production from both FS and FM soils.

In anaerobic incubations, addition of alternative electron acceptors, NO_3^- decreased CO_2 production significantly whereas SO_4^{2-} had little effect. Nitrate and SO_4^{2-} decreased CH_4 production but the NO_3^- almost completely inhibited CH_4 production (>99%). Among the three wetland soils, FM exhibited the greatest denitrification potential (PDR), but it also tends to yield more N_2O as compared to FS and SM soils. Soil organic C has significant effect on regulating PDR ($P< 0.007$). Among the different organic C mioties, polysaccharides positively influenced PDR ($P< 0.003$) while phenolics had negative effect ($P < 0.03$). Labile organic C as measured by aerobically mineralizable C was positively correlated with polysaccharides and carboxylic C. Further, characterization of humic acids (HA) in these wetland soils showed that FM HA had

greater aromaticity whereas FS HA contained more aliphatic C. Increasing salinity tended to cause an increase in crystalline nature of aliphatics and guaicyl structural units in HA, suggesting more resistant HA formation.

CHAPTER 1

INTRODUCTION

1.1 Introduction

Organic matter tends to accumulate in the sediments of wetland soils. While wetlands cover only about 4 to 5% of the land area of the globe, they are estimated to contain up to 450 Gt C or approximately 20% of the carbon in the terrestrial biosphere and thus play an important role in global carbon cycles (Amthor et al., 1998, Roulet 2000; Zhang et al., 2002; Chmura et al., 2003). High net primary production coupled with slow organic matter decomposition allows wetlands to serve as net carbon sinks for atmospheric CO_2 (Gorham, 1991). The main factors leading to slow decomposition in northern wetland ecosystems are unfavorable environmental conditions, such as low oxygen, cold, acidic peat, and a deficiency of resources, such as nutrients and readily decomposable substrates (Bridgham and Richardson, 2003; Yavitt et al., 2004). Soil carbon sequestration in the wetlands is estimated to be about 20 to 30 g C m^{-2} yr^{-1} (Chmura et al., 2003). In addition to being to being net C sinks, wetlands are also sources of greenhouse gas emissions. Even though wetlands are currently a small, persistent sink for CO_2, they are a large source of CH_4 (Moore and Dalva, 1997; Roulet, 2000). Wetlands, including rice paddies, contribute between 15 and 45% of global methane emissions (Segers, 1998). In addition to CH_4, wetlands are also a source of N_2O in the atmosphere, a potent greenhouse gas that is highly efficient in trapping emergent radiation and long-lived in the atmosphere (Inubushi et al., 2003). Though the relative amounts of CH_4 and N_2O emissions are lower than CO_2 emissions, their global warming potentials are estimated to be 23 and 296 times that of CO_2, respectively (100 yr perspective) and are considered significant contributors to climate change (IPCC, 2001).

Soil organic matter (SOM) is composed of a complex mixture of decayed plant and soil matter, and the polymeric arrangements produced by the random combinations of these materials with other organic substrates (Wilson et al., 1983). The various compounds in SOM have differing degrees of resistance to biological attack under aerobic or anaerobic conditions (Dai et al., 2002; White et al., 2002). The rate and extent of degradation of SOM under either oxic or anoxic conditions are dependent on many biological controls, including nutrient availability, temperature, oxidation/reduction potential, clay content, salinity, and SOM quality (Zech et al., 1997; Baldock and Skjemstad, 2000; Blodau, 2002; Bertrand et al., 2007).

The effects of water table levels, temperature and pH effects on SOM mineralization in both upland and wetland soils have been extensively investigated (Moore and Dalva, 1993; Updegraff et al., 1995; Trumbore et al., 1996; Knorr et al., 2005). Studies of SOM transformation in upland soils show that clay plays an important role in protecting SOM from degradation (Gregorich et al., 1991; Bottner et al., 1998; Baldock and Skjemstad, 2000) where cations play a central role in binding the organic matter with soil mineral matter that provides protection from microbial degradation (Varadhachari et al., 1991; Brynhildsen and Rosswall, 1997; Zech et al., 1997; Cruz-Guzman et al., 2003; Nguyen et al., 2004; Wang and Wang, 2007). There is little or no information on how different cations and clays affect the SOM mineralization in wetland soils. In view of the major contribution of wetlands to total C pool, a thorough understanding of the various controls on SOM degradation is required to understand relationship to the current global carbon cycle and be able to anticipate future changes in carbon transformation/ sequestration.

Louisiana's coastal area contains one of the largest expanses of coastal wetlands in contiguous United States with variety of wetlands ranging from forested swamps, freshwater marsh, intermediate marsh, and brackish marsh to saline marshes (US Army Corps of Engineers, 2004). However these wetlands are disappearing at an alarming rate due to both natural and human induced factors (DeLaune et al., 2003). It was reported that Louisiana accounts for about

90 % of total coastal marsh loss in the US (US Army Corps of Engineers, 2004). Louisiana coastal wetlands are being impacted by continuous rise in eustatic sea level, sediment subsidence, and human activities such as creation of water ways and oil and gas recovery operations. Mississippi River fresh water has been diverted to wetlands aimed at restoring wetlands by lowering of salinity, and providing sediment and nutrients (DeLaune et al., 2005). These processes may have appreciable effect on carbon transformation, storage, and greenhouse gasses emissions of these wetlands. Moreover, the inclusion of some terrestrial carbon sinks in the Kyoto Protocol and the provision for the possible future inclusion of other terrestrial sinks such as wetlands underscore the need to examine the implications of various changes occurring to wetlands may have on the national carbon sinks/source inventories (Roulet, 2000).

In addition, both created and natural wetlands of including those coastal wetlands has been used to treat the pollutant rich waters such as for removal of nitrates from agricultural run-off and river sources (Poe et al., 2003). To better understand the long-term role of wetlands in removing pollutants from water bodies, a more complete understanding of chemical structure of organic matter/ quality is needed because of its role in controlling the microbial activity as well as removing pollutants by adsorption (Bridgham and Richardson, 2003; Fukushima et al., 2006) needs to be explored.

1.2 Objectives

The overall goal of this study was to understand various factors including the possible effects of salt water intrusion and Mississippi river water diversion on mineralization and transformation of soil organic matter and release of greenhouse gasses from Louisiana coastal wetlands. The specific objectives were to (I) evaluate the effects of cations, sediment and salinity on soil carbon mineralization and transformation in forest swamp and freshwater marsh; (II) evaluate carbon gas production in a freshwater marsh soil under different electron acceptors; (III) assess the nitrate removal capacity (denitrification potential) and its relationship to soil organic carbon quality in a swamp forest, and freshwater and saline marshes; (IV) characterize labile organic carbon fractions (LOC) of coastal wetlands using different methods; and (V) characterize humic acids of coastal wetland soils.

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CHAPTER 2

LITERATURE REVIEW

2.1 Introduction

Wetlands are areas where saturation with water is the dominant factor determining the nature of soil development and the types of plant and animal communities living in the soil and on its surface (EPA). In other words, wetlands are transitional areas between aquatic and terrestrial ecosystems that are inundated or saturated for long enough periods to produce hydric soils and support hydrophytic vegetation. In earlier days wetlands were treated as wastelands and many of them were converted for agricultural and industrial purposes. In recent decades their environmental importance has been recognized. Ecologically wetlands are important for protecting coastal areas from hurricanes, floods, intrusion of sea water and submergence. They provide habitat to a variety of wildlife and fisheries and are widely used for amusement and recreation. Wetlands are the most productive systems among the terrestrial ecosystems with a net primary production of up to 1300g C m^{-2} yr^{-1} compare to the 800g C $m^{-2}yr^{-1}$ in tropical forests (Schlesinger, 1997; Keddy, 2000). The high rate of primary production coupled with slower rate of soil organic matter (SOM) decomposition turns wetlands as net carbon sinks for atmospheric carbon dioxide (Gorham, 1991). On global basis wetlands are estimated to have a potential of sequestering 0.1 to 0.2 Gt C yr^{-1} (Metting et al., 2002). Because of their capacity to serve as net carbon sinks, wetlands form an important component of the terrestrial landscapes that exert a great influence over global carbon cycle and climate change (Zhang et al, 2002). In spite of relatively small proportion of land area (4 to 5 % of total land area), wetland soils are projected to contain about 450 Gt C or approximately 20 to 25% of the terrestrial carbon and thus play an important role in global carbon cycles (Amthor et al., 1998, Roulet 2000; Zhang et al., 2002; Chmura et al., 2003).

Regardless of currently being net C sinks, wetlands are also one of the major sources of greenhouse gas emissions (Roulet, 2000). Wetlands, including rice paddies, contribute between 15 and 45% of global methane emissions (Segers, 1998). Wetlands also contribute to significant amount of N_2O to the atmosphere, another major greenhouse gas (Inubushi et al., 2003). Though the amounts of these gases released to the atmosphere are small compared to CO_2, CH_4 and N_2O have global warming potentials of 23 and 296 times greater than that of CO_2 respectively (100 yr perspective) indicates their significant role in climate change (IPCC, 2001).

Continuous inundation renders wetland soil anoxic below a few millimeters from the surface. The diffusion rate of oxygen into deeper layers of soil occurs slower than the rate of consumption mainly due to the slower rate of diffusion through water column. Because of their large reserves of organioc C, wetland soils are microbiologically very active and consume available oxygen quickly. Lack of oxygen availability causes the microbial community in these soils to resort to alternative pathways for C consumption. Under anaerobic conditions complex organic polymers are initially degraded by fermenting bacteria to yield a few simple products, which are subsequently used by methanogens to produce CH_4 (Keller and Bridgham, 2007). In freshwater wetlands, the dominant methanogenic substrates are considered to be dihydrogen (H_2) and CO_2, which are used by autotrophic methanogens, and acetate, which is converted to CH_4 and CO_2 by aceticlastic methanogens (Keller and Bridgham, 2007). Any changes to these wetland soils may affect their biogeochemistry and C dynamics. Intrusion of sea water, NO_3^- rich polluted waters, sediment deposition and rise in temperatures could significantly affect the microbiological activity and could possibly turn these wetlands into net C emitters. The present concerns about the effect of increasing concentrations of greenhouse gases in the atmosphere on global climate have increased interest in soil carbon cycle with focus on potential for increasing soil C sequestration. Despite the fact that soil C in wetlands is recognized as being an important component of global C budget and future climate change scenario, very little work has been done to consider the role of wetland ecosystems in managing the C sequestration (Lal 2001; Metting 2001).

2.2 Wetlands and Louisiana

Louisiana’s coastal zone contains one of the largest expanses of coastal wetlands in contiguous United States with variety of wetlands ranging from forested swamps, freshwater marsh, intermediate marsh, and brackish marsh to saline marshes (US Army Corps of Engineers, 2004). Louisiana has an estimate of more than 729,000 ha (2848 Sq.miles) of wetlands of which 723, 500 ha are marsh (NOAA, 1991). About 40% of coastal wetlands in the U.S. are present in Louisiana (Coast-2050, 1998). Louisiana Coastal Wetlands Conservation and Restoration Taskforce estimated that the value of Louisiana’s coastal wetlands exceeds $100 billion. Every year as much as $680M yr^{-1} is generated from fishing and recreational activities (Coast2050, 1998). Coastal wetlands also provide habitat for a variety of flora and fauna.

These valuable coastal wetlands are disappearing at an alarming rate of 6400 to 8960 ha yr^{-1} (25 to 35 square miles yr^{-1}). The loss is especially severe in Barataria and Terrebonne basins (Britsch and Dunbar, 1993). Moreover, 80% of the national coastal wetland loss is occurring in Louisiana. It was estimated that Louisiana has lost 1900 square miles of wetland between 1932 and 2000 and projected to lose another 700 square miles between 2000 and 2050 (USGS 2003). The major causes responsible for the loss of wetlands in Louisiana are subsidence, intrusion of seawater and lack of sediment deposition (Delaune et al, 2005). Subsidence is due to the natural tendency of wetlands to compact and sink, which is generally offset by the deposition of new organic matter and sediments. Vertical growth through deposition is necessary to keep up with increasing sea level due to global warming. It is estimated that coastal marshes must vertically accrete at more rapid rate than the present to offset the projected 1 cm yr^{-1} increase of sea level for the next hundred years (Titus and Narayanan, 1995; Nyman and DeLaune, 1999). Increased sea levels due to global warming and construction of intra-coastal waterways have led to the intrusion of sea water into the freshwater marshes of coastal Louisiana. The increased salinity of these marshes has a detrimental effect on both flora and fauna. Construction of more than1600 miles of levees along the Mississippi river has stopped the seasonal flooding of the river into these wetlands and there by preventing natural flushing and sediment deposition (Kesel, 1988).

There has been a reduction in sediment load of the Mississippi River in excess of 70% as a result of land use changes and the construction of dams and levees along the Mississippi River (Kesel, 1988). The resultant changes in sediment and water distribution are considered major causes of wetland loss along the Louisiana Gulf Coast (Salinas et al., 1986). Recent intervention by state and federal agencies has provided an opportunity to restore wetlands in some areas by constructing large Mississippi river diversion projects in the coastal zone. Six diversions are currently in operation and several others are being planned or are under construction. The diversions at Bonne Carre Spillway, Caernarvon, and Davis Pond are the largest of these projects, capable of diverting a total of 1376 cms at maximum discharge or 1% of mean Mississippi River flow (Rabalais et al., 1995; Iwai, 2002). During episodic flooding, however, up to 16% of the Mississippi flow has been diverted through the Bonne Carre Spillway into Lake Pontchartrain (Day et al., 1999). The Caernarvon and Davis Pond diversions have the potential to release a maximum discharge of 230 and 300 cms into Breton Sound and northern Barataria Basin estuaries, respectively.

Nutrient-rich discharge from diversions likely affect the ecology of receiving basins in both beneficial and harmful ways. Added sediment and nutrients from the river has been shown to stimulate macrophyte production and contribute to soil stability and marsh accretion (Mendelssohn and Kuhn, 2003). Diversions also may stimulate freshwater fisheries and enhance wildlife in the receiving basins. On the other hand, high levels of nutrient increase the potential for eutrophication and its consequences (Iwai, 2002). The nitrate concentration was found to be 20-50 times higher in the Mississippi River during spring peak discharge than the average in the Barataria Basin estuary, though concentrations in the river vary considerably with differences in seasonal rainfall and river flows (Rabalais 1995). The Mississippi River receives high levels of nutrients over its entire course. During the past century, nutrient levels have increased steadily primarily due to agricultural practices in the Corn Belt region of the northern United States (Turner and Rabalais, 1991; Goolsby et al., 2000). The heavy use of fertilizers along with current crop management practices contributes to large amounts of nutrient runoff and leaching directly into the Mississippi River and its tributaries (Iwai2002). Nitrate concentrations in the Mississippi River and some of its tributaries have increased by 2 to 5 times since the early 1900s, and almost tripled in the past thirty years (Goolsby et al., 2000). As a result of human influences, historic oligotrophic flows have been replaced by eutrophic conditions that threaten to permanently alter the ecology and economy of the Mississippi River delta and coastal zone (Turner and Rabalais, 1994). Eutrophication of the receiving waters can lead to deleterious consequences both ecologically and economically. The impacts may be short-term, such as the algal blooms in Lake Pontchartrain at the Bonne Carre Spillway that have resulted from singular pulsed events (Day et al., 1999). Lack of sediment deposition forces these marshlands to rely solely on the addition fresh organic matter to raise height from the continuous compaction and subsidence. Various studies have showed that organic matter could be the main determinant in the vertical growth rate of marshes in the Mississippi river deltaic plain (Salinas et al., 1986; Nyman and DeLaune, 1999). Addition of organic matter is believed to greatly contribute to increased structural strength of marsh (Hatton et al., 1983).

Wetlands in Louisiana are generally high in organic matter content with the presence of peat layers as much as 2 m deep and containing as much as 80% organic matter (Cahoon, 1994). In most of the wetlands, soil organic matter occupies the major volume rather than soil mineral matter (Nyman et al., 1993). This signifies the importance and the significant role of Louisiana wetlands in C sequestration. However, they are also major sources of greenhouse gas emissions such as CO_2, CH_4 and N_2O (Nyaman and Delaune, 1991; Segers, 1998). Any change that affects C mineralization in these wetlands likely results in changes in the emission of CO_2, CH_4 and therefore sequestration rates.

Intrusion of sea water due to the construction of intra water canals and annual mean sea level rise adding in lot of salts, especially those containing SO_4^-, Cl^-, Na^+, Ca and Mg^{+2}, to these wetlands. On the other hand, recent diversion of Mississippi river aimed at decreasing the salinity, addition of sediments and nutrients is contributing to appreciable raise of nitrate concentrations in these coastal marshes. Intrusion of sea water and nutrient-rich discharge from diversions of Mississippi river into these wetlands can affect the ecology of receiving basins in both beneficial and harmful ways. Like many estuarine systems, the coastal zone of the Mississippi River delta is nutrient limited, particularly in nitrogen, and thus large inputs of nutrients can have major ecological effects on the receiving waters (Turner and Rabalais, 1991). The development of a large seasonal hypoxic zone in the Gulf of Mexico has been a concern in recent years (Iwai, 2002). Hypoxia of the offshore bottom waters occurs each summer because of blooms of phytoplankton from river borne nutrients. The disturbance of trophodynamics and changes in water quality affects important Gulf coast fisheries and may have long-term impacts that remain unknown (Turner and Rabalais, 1994; Iwai, 2002). As sources of high amounts of C reserves, it is very important to know how these changes could affect the mineralization of organic matter, release of greenhouse gases and overall stability of these Louisiana wetlands.

2.3 Mineralization and Transformation of Soil Organic Matter

The turnover or mineralization rate of soil organic matter depends on several factors such as climate, quantity and quality of plant residues, water table, salinity, cations and those affecting microbial activity and soil properties, in particular pH and clay content (Bottner et al., 1998; Baldock and Skjemstad, 2000; Blodau, 2002). In submerged soils, low oxygen diffusion limits the oxic zone to a thin uppermost layer of sediment where as the remaining portion stay anoxic. Aerboic microorganisms are believed to be capable of decomposing organic matter completely whereas mutualistic consortia of microorganisms accomplish anaerobic decomposition because no single type of anaerobic bacteria seems capable of complete mineralization (Kristensen sen and Holmer, 2001). Presence of submerged conditions for prolonged period of time leads to very slow organic matter mineralization in wetland soils as compared to upland soils. Previous work showed that under anoxic conditions degradation of organic matter was 5 to 10 times lower than that of oxic conditions (Kristensen and Holmer, 2001). Anaerobic conditions for prolonged periods prevent complete microbial decay of organic matter (Stevenson, 1994). Following the depletion of oxygen, some respiratory microbes can utilize alternative electron acceptors and continue to mineralize the soil organic carbon. In anoxic conditions alternative electron acceptors such as SO_4^{2-}, NO_3^-, Fe^{3+}, and Mn^{4+}, likely play major role in the mineralization and transformation of organic carbon. Air and soil temperature, water table level, and the quality of organic substrates could be also the main local controls of CO_2 emissions from peatlands (Bridgham et al., 1995; Mendelssohn et al., 1999). Warmer air and soil temperatures stimulate microbial activity resulting in higher CO_2 emissions (Silvola et al., 1996). The temperature response of CO_2 is modified by substrate quality, with higher substrate quality resulting in greater temperature responses (Valentine et al., 1994; Updegraff et al., 1995). A more complete understanding of the factors controlling rganic C mineralization and the fluxes of CH_4 and CO_2

production from wetlands will aid us in assessing the impact of environmental change on C mineralization and the implications for atmospheric concentrations of CO_2 and CH_4 (Yavitt et al., 2004).

2.3.1 Role of Cations and Clays

Cations play an important role in the chemistry and organic matter stability of wetland soils. Presence of exchangeable cation could affect the rate of organic matter turnover in soils. Most of the cations in wetland soils are provided by the mineral matter present in it and the sediment that deposits from different sources such as rivers and runoff waters. Presence of cations could help in buffering system by forming complexes with the organic acids produced during decomposition of organic matter. Cations also form complexes with toxic compounds and may turn them to nontoxic compounds. The presence of Fe^{2+} under anaerobic conditions helps in decreasing the H_2S toxicity, formed as a result of SO_4^{2-} reduction, by precipitating it as insoluble FeS. Declining Fe/S ratio has been reported as one of the factors that affect the stability of wetlands (Nayman and DeLaune, 1999).

In general, the longevity of chemically stabilized SOM has been attributed to binding with exchangeable or structural cations especially to Ca^{2+}, Fe^{3+} and Al^{3+} (Nguyen et al., 2004). Different studies on upland soils had showed that the soil organic matter content is significantly and positively correlated with cation exchange capacity of soils (Zech et al., 1997; Wang and Wang, 2007). Furthermore, various studies have demonstrated the important role of different cations especially divalent and trivalent cations in associating clays with organic C, and therefore, enhancing the protection of organic C by soil clays (Brynhildsen and Rosswall, 1997; Cruz-Guzman et al., 2003). Exchangeable cations such as Al, Ca, Mg and Fe promote interaction between clay and humic substances and molecular aggregation of humic compounds (Varadhachari et al., 1991; and Wershaw, 1999). In addition, the three-dimensional orientation of organic macromolecules containing carboxyl functional groups may be altered in the presence of multivalent cations. The structures would become more condensed and the orientation of functional groups could be altered (Oades, 1987). Such changes may alter the efficiency of microbial enzymatic attack. Organic particles bonded to Fe^{2+} and Al^{3+} bonds lead to the formation relatively stable, polymerized complexes. In contrast, Ca^{2+} saturation causes rapid precipitation of soluble organic compounds and inhibits the polymerization process leading to less condensed and more labile material (Bottner et al., 1998). In addition, Duchafour (1983) reported that complexes linked to clay minerals by Ca^{2+} are less stable than Fe and Al bonds. Also exchangeable Ca^{2+} found to be well correlated with many of the microbial properties (Bottner et al., 1998). Moreover, increased C mineralization was expected due to the slight increase in soil pH by the addition of K and Ca (Motavalli et al., 1995). It is due to the fact that in soils with basic reaction microbial and faunal communities become more diversified, leading to the increased performance of enzymatic activity (Bottner, 1998). On the other hand, clay minerals containing Ca such as smectites show more association with organic matter than the non calcium containing clays as kaolinite (Furukawa, 2000). Addition of Ca containing salts to the soil causes the formation of Ca –organic linkages, decrease the organic carbon solubility and leads to the stabilization of organic matter (Muneer and Oades, 1989a, b). The degree of humication was found to be positively associated with exchangeable polyvalent cations such as Ca^{2+} and Mg^{2+} while negatively associated with exchangeable K, suggesting an influence of exchangeable cations on the turnover of soil organic matter (Nguyen et al., 2004). Baldock and Oades (1989) found that addition of Ca^{2+} cations to soil did not alter the rate of glucose decomposition and assumed that probably Ca^{2+} forms linkages with the products of microbial decomposition processes rather than the undecomposed precursors. Also, addition of Ca containing salts to the soil was found to decrease the solubility and mineralization of organic C

(Baldock and Skjemstad, 2000). Gaiffe et al., (1984) reported that replacement of Ca^{2+} cations in an organic soil with K^+ led to a threefold increase in the C mineralization. In addition, ca2+ has been found to enhance the formation of intermolecular bondings in humic acids (Brukert et al., 1994). Furthermore, removal of Ca from fulvic acid-activated C mixture strongly increased the desorption of fulvic acid from activated carbon (Reemtsma et al., 1999). Feng et al., (2005) indicated that cation bridging contributed 41 % of total peat humic acid adsorbed on to the clay while ligand exchange was estimated to account for approximately 32% and van der Waals 22 % when the Ca^{2+} was background electrolyte.

Aluminum ion has been shown inhibitory effect on the decomposition of citric acid that was inoculated to the soil (Brynhildsen and Rosswall, 1997). The formation of Al-organic complexes has been postulated to provide a mechanism for stabilizing OM against biological attack. Schwesig et al., (2003) reported that increasing initial Al/C ratios up to 0.1 led to a considerable decrease in organic matter mineralization, up to 50% compared with control samples. The ability of Al^{3+}/Al-hydroxy cations to form biologically stable Al-organic complexes has been demonstrated in incubation experiments where oxygen consumption and C mineralisation rates from a variety of Al and Fe saturated organic compounds were measured (Martin et al., 1966, 1972). The presence of iron oxides at the mineral surfaces was found to increase sorption of organic matter through ligand exchange process and led to an increase in the strength of organic matter by causing flocculation and become hard to breakdown by microorganisms (Chi and Amy, 2004). In addition, Zunino et al. (1982) reported that the chemical structure of the substrate contributed to the degree of biological stabilization imparted by the presence of allophone, an aluminum containing clay. The presence of amorphous Fe minerals and Fe^{3+} cations can also offer protection against biological attack, but the effects are not as great as those noted with the corresponding forms of Al (Boudot et al., 1989).

In general, mineral matter especially clays can protect the organic C from mineralization through both physical and chemical mechanisms. Many field and laboratory studies have been demonstrated that clay minerals or fine textured soils generally lower the decomposition of organic matter (Gregorich et al., 1991; Bottner et al., 1998; Baldock and Skjemstad, 2000). Organic carbon held with in clay aggregates get physical protection from any microbial attack (Krull et al., 2003). In addition organic C chemically bond to the clay particles and become more recalcitrant. Sorption of organic matter (OM) to clay minerals is an important process in the natural environment and believes to stabilize soil organic carbon in the global C cycle (Feng et al., 2005). Collins et al., (1995) stated that sorption of organic matter onto clay is believed to be a possible mechanism for the preservation of the labile biopolymers. Addition of mineral matter especially in presence of cations such Ca may enhance the association of organic matter and mineral matter by acting as cross linking agents between clay and organic functional groups such as carboxylic and phenolic groups (Lu and Pignatello, 2004). Formation of organo-mineral complexes protects the degradation of less recalcitrant organic matter such as polysaccharides from microbial degradation. Organic matter can be adsorbed to clay surfaces through six mechanisms: ligand exchange, cation bridges (including water bridges), anion exchange, cation exchange, van der Waals interactions and hydrophobic bonding, with ligand exchange at an electrified interface being the dominant reaction of humic acid adsorption to clay (Feng et al., 2005). More processed decomposition products having intimate association to soil minerals classified as heavy fraction and believed to be having longer turnover time (Alvarez and Alvarez, 2000). Organic matter in marine sediments accounts for approximately 20% of total carbon burial and in that more than 90% of the carbon is intimately associated with mineral surfaces (Keil et al., 1994). In addition, soil organic matter can be physically covered within soil aggregates or chemically adsorbed onto clay surfaces, therefore protected from degradation by microbes (Baldock and Skjemstad, 2000). A study based on analysis of organic C in different size fractions has showed that significant amounts of O-alkyl C (polysaccharides) are likely found in the clay fraction, suggesting the protection of easily degradable organic C such as polysaccharides by clays (Baldock et al., 1990). Ladd et al. (1985) monitored the mineralization of carbon from ^{14}C labeled plant materials added to four cultivated soils having similar mineralogies but different clay contents (5±42%, and found that the amounts of residual labeled plant carbon and residual native soil organic C remaining after the incubation were proportional to soil clay content. Sorption studies in marine sediments also showed that the mineralization of organic matter sorbed on to mineral surfaces was decreased by up to five orders of magnitude by preservation of labile molecules such as amino acids and simple sugers (Keil et al., 1994). Li et al., (2007) found that in addition to significant increase in mineral-associated organic C with increase in clay content of soil, pH-stimulated mineralization of organic C was mainly occurred in particulate but not in mineral-associated organic matter fractions. Rochette et al., (2006) found that, during the first year, mineralization of manure organic C was almost 31 % lower in the clay than in the loamy soil, presumably as a result of less favorable aeration conditions and greater physical protection of organic substrates in the clay. Gonzalez and Laird (2003) found that the coarse clay fractions typically contain more aromatic and recalcitrant or humified organic matter, whereas fine clay fraction contain more labile or less humidified organic matter. Also soils with high clay content contain less water extractable organic carbon, which is used as one of the measures of labile organic C. Wang and Wang (2007) reported that the amount of hot water extractable C, was significantly and negatively correlated with the amount of clay in the soil. However, addition of clay initially leads to increased C mineralization due to the sudden rise in microbial activity as the clay surfaces lead to change in cell membrane processes and pH buffering by ion exchange (van Loosdrecht et al., 1990).

Though the importance of cations and clay minerals in protecting organic C in upland soils has been established, there is not much information available on their role in protecting the carbon in wetland soils. Understanding the effect of cations and clay minerals content on carbon mineralization in wetland soils will be helpful as they are influenced by different biological and environmental conditions. The possible effects on carbon sequestration due to the addition of more nutrients and sediment interpret by the diversion of Mississippi river to the sediment deprived Louisiana coastal wetlands.

2.3.2 Effect of Salinity

Increasing salinity in the historically freshwater wetlands due to the intrusion of seawater is a serious wetland deterioration issue worldwide. An understanding of the effects of salinity and sodicity on soil carbon (C) stocks and fluxes is critical to environmental management, as the areal extents of salinity and sodicity are predicted to increase. Effect of overall concentration of ions on the decomposition of organic matter may differ from the effect of individual ions. High salinity could have detrimental effect on the growth of less tolerant plant species as well as lead to the slower rate of organic matter mineralization due to its detrimental effect on type and amount of microorganisms (Rietz and Haynes, 2003). Yuan et al. (2007) reported that negative exponential relationship exists between soil EC and microbial biomass carbon. The inhibitory effect on soil enzyme activities may vary with the type of enzymes and the nature and amounts of salts present (Frankenberger Jr. and Bingham, 1982). The high salinity causes osmotic desiccation of microbial cells releasing intracellular enzymes and makes them vulnerable to be attacked by soil proteases, a "salting-out". It modifies ionic conformation of the active site of the enzyme-protein. Wong et al., (2008) studied the effect of salinity on soil respiration and microbial population and revealed that the soil respiration rate was highest in the low-salinity treatment (0.5 dS m^{-1}) whereas respiration was lowest in the mid salinity treatment (10dS m^{-}

^{1})and lowest (1-5mg CO_2-C kg C kg $^{-1}$ soil) in the mid-salinity treatments. On the other hand, soil microbial biomass (SMB) was found highest in the high-salinity treatments (30dS m^{-1}) whereas the lowest in the low-salinity treatments. This observation attributed to the increased substrate availability with higher salt concentrations due to either increased dispersion of soil aggregates or dissolution or hydrolysis of soil organic matter, which may offset some of the stresses placed on the microbial population from high salt concentrations. The apparent disparity in the trends of the respiration and the SMB may be due to an induced shift in the microbial population, from one dominated by more active microorganisms to one dominated by less active microorganisms (Wong et al., 2008). In contrast, Wichern et al. (2006) showed that an increase in salinity of an upland soil led to a decrease in both microbial respiration and microbial biomass. They were also noticed an increase in ratio of ergosterol/ microbial biomass which could be an evidence for fungal dominance under high salinity. In addition, elevated salinity level from 1 to 17 ppt decreased the woody litter decomposition as well as grass litter degradation (Steinke and Charles, 1986; Mendelssohn et al., 1999). Wu et al., (2008) reported a 20% drop in dissolved organic carbon mineralization when the salinity of a mangrove soil increased from 0 to 30 ppt. Further, Pathak and Rao reported as much as 50% drop in the amount of C mineralized when the salinity as measured by EC of soils increased from 1 to 97 dS m^{-1}. Frankenberger Jr. and Bingham (1982), stated that the relationship between C mineralization and EC (Salinity) appeared to be independent of salt type and total EC was the dominant factor influencing C mineralization. On the other hand, Li et al. (2006) said that the effect of salinity on C mineralization depends on the soil water content and period of incubation. Monitoring of field carbon gas emissions, which is a indirect measure of soil carbon mineralization, revealed lower CO_2 and CH_4 emissions from salt marsh soils as compared to those from freshwater marsh soils (DeLaune et al., 1983).

Contrary to above reported findings, other researchers reported, for example, Weston et al. (2006) reported that salinity intrusion into a previously freshwater riverine sediments increased overall mineralization rate as much as two times compared to no salinity intruded soil. They also found that the salinity intrusion led to increased nutrient release, decreased methanogenesis and a rapid shift to sulfate reduction. This indicates that in wetland soils, which tend to be anoxic even few millimeters below the surface, increased availability of electron acceptors such as SO_4^{2-} with increased salinity may offset the detrimental effect of salinity.

Salinity of wetlands may also affect the ratio of release of different greenhouse gases. Methane emission was found varied among salt, brackish and fresh marsh, which are differing in salinity. This could be due to availability of SO_4^- as an electron acceptor and also detrimental effect of high salinity on microbial activity. It was estimated that 4.3, 73 and 160g CH_4-C m^{-2} releases from salt, brackish and fresh marsh respectively, indicating the negative correlation of salinity with the amount of CH_4 emission (DeLaune et al., 1983).

On the whole, salinity may play a major role in soil carbon mineralization by affecting microbial population and dissolution of soil organic carbon and is very important to understand in order to assess the implication of seawater intrusions into freshwater wetlands on soil carbon sequestration.

2.3.3 Role of Alternative Electron Acceptors

Availability of alternative electron acceptors allows the microbial activity under anoxic conditions. In wetlands, the aerobic layer is limited in depth by the slow diffusive property of dissolved oxygen in submerged sediment and rapid reduction of the small amounts of diffused oxygen by benthic organisms. In anoxic sediments, a diverse consortium of microorganisms that hydrolyze, ferment, and terminally oxidize organic compounds mediates anaerobic organic matter mineralization. The presence of alternative electron acceptors such as NO_3^-, SO_4^{2-}, Fe^{3+}, and Mn^{4+} play major role in the anaerobic mineralization and transformation of organic carbon. As the oxygen availability deplete, microorganisms start utilizing alternative electron acceptors such as Mn^{4+}, NO_3^-, Fe^{3+} and SO_4^{2-} to oxidize organic matter. Thus, increasing loads of nutrients that contain NO_3^- and SO_4^{2-} may significantly change the biogeochemistry of the anoxic wetland soils. In Louisiana, appreciable amounts of NO_3^- are being added to the freshwater marshes from the diversion of Mississippi river due to long-term fertilizer application and run-off into upstreams (Lane and Day, 1999). Also wetlands have been widely used for the protection of water bodies from point and non-point source of pollution particularly for nitrate removal (Hunt et al., 2007). In this case, availability of alternative electron acceptors like NO_3^- and SO_4^- likely lead to change in the pathways of organic matter decomposition by microorganisms and could further affect the overall rate of organic matter decomposition in wetland soils (Liikanen et al., 2002).

In general, the presence of N could affect the organic matter mineralization in two different ways that by affecting the C: N ratio and acting as electron acceptor in the form of NO_3^- . Presence of nitrogen in the form of NH_4^+ may decrease the SOM decomposition rate by decreasing C: N ratio. The presence of nitrogen in the form of NH_4^+ was found to decrease CO_2 and CH_4 emissions from a carex dominated peat land and paddy soils (Aerts and Toet, 1997; Huang et al., 2002). Other studies have showed that application of N fertilizers to upland soils decreased soil respiration and overall soil C mineralization (Soderstrom et al., 1983; Willams and Silcock, 1997; Al-kaisi et al., 2008). The decrease of CO2 production by N fertilizer has been attributed to the pH decrease which affects diversity of microorganisms (Aerts and Toet, 1997). However, Al-kaisi et al., (2008) found that while the application of N fertilizer decreased CO_2 emission, it was not accompanied by a significant change in soil pH. In contrary to CO_2 emission they found higher microbial biomass C in highest N fertilized soil than no N applied soil. However, the impact of N fertilization on such microbial activity and CO_2 release has been reported inconsistent, with decrease or increase. Even though various studies have reported negative effect of N application on C mineralization, the exact nature of the reactions was not yet understood (Willams and Silcock, 1997; Al-kaisi et al., 2008). Sulfate is one of the major alternative electron acceptor in many reduced soils especially in soils that undergo seawater intrusion as in coastal wetlands. Based on thermodynamics, SO42- reduction is expected to occur after NO_3^- reduction (Ponnamperuma, 1972). However, Kristensen and Holmer (2001) reported that organic matter oxidation rates under SO_4^{2-} were similar or up to twice faster than that under NO_3^-. In addition, although electron acceptors could play important role in C mineralization, a large percentage of C gas production was found not to be accounted for by the known reduction processes of major electron acceptors including Mn^{4+}, NO_3^-, Fe^{3+}, and SO_4^{2-} (Keller and Bridgham, 2007). Clearly conflict results exist in current literature. In general, the presence of either SO_4^- or NO_3^- decreased the release of CH_4 from methanogenic soil (D'Angelo and Reddy, 1999).

2.4 Denitrification and Its Relationship with Organic C Quality

Increased nitrogen inputs and subsequent export of nitrogen are placing increasing pressure on sensitive ecosystems. Nitrate is one of the nutrients considered to be a major cause for eutrophication in riverine systems. As Mississippi river water has been diverted to Louisiana coastal marshes in order to help restore these impaired wetlands, it brings in appreciable amounts of nitrate into these wetlands (DeLaune et al., 2005). The nitrate concentration, for example, has been found to be 20-50 times higher in the Mississippi River during spring peak discharge than the average in the Barataria Basin estuary (Rabalais et al., 1995). Approximately 90, 000 metric tons of nitrate in Mississippi River water was estimated to be added to Gulf of Mexico between 1991 and 1992 alone (DeLaune et al., 2001). The impact of elevated NO_3^- levels in the diverted

Mississippi River water on the health of these coastal wetlands and their capacity to assimilate NO_3^- has become an increasingly important topic of study. With the presence of both aerobic and anaerobic conditions, wetlands are especially effective in NO_3^- removal through denitrification (White and Reddy, 2003; DeLaune et al., 2005). Denitrification a microbially-mediated reduction of nitrate to gaseous end products, and represents a major pathway for the removal of nitrogen in wetland and aquatic ecosystems. In anoxic conditions NO_3^- is a high energy yielding alternative electron acceptor (3030 kJ/ M glucose). Seitzinger (1990) reported that denitrification in aquatic sediments can remove up to 75% of nitrogen loading. The high mobility and solubility of nitrate allows the rapid diffusion of nitrate from the water column down into anaerobic sediment layers where active denitrifying organisms interact with the substrate. Heterotrophic bacteria such as *Psuedomonas* spp. use nitrate as an electron acceptor in the absence of oxygen to carry out the oxidation of organic compounds to CO_2 (Germon, 1985). Of the intermediates produced, NO is evolved in minor quantities because of its highly reactive nature, while the evolution of N_2O may be substantial. The evolution of N_2, however, exceeds that of the other species being the final reduced species in denitrification. The relative proportion of the gases evolved could depend on nitrate, carbon, and oxygen availability (Weier et al., 1993). Denitrification rates for aquatic sediments span a wide range, varying by ecosystem type (e.g. marine and freshwater, emergent marsh and forested swamp).

Various factors such as soil hydrology, soil chemistry and vegetation type affect the denitrification process (Cornwell et al., 1999). Generally, in wetlands denitrification rate is limited by the availability of NO_3^- and organic C (Cornwell et al., 1999; Greenan, et al., 2006). Although nitrate limitation in most natural wetlands results in pseudo-first-order reductions, large site-to-site variations in apparent denitrification rates cannot be easily explained by water quality parameters such as pH, Temp, DOC or plant productivity (Hume et al., 2002). Various studies have shown that when sufficient NO_3^- is available to induce the denitrifying activity, an increase in soil organic matter (SOM) increases soil denitrification rate (Bijay-Singh et al., 1988; Gale et al., 1993). Few studies reported that, water soluble carbon, mineralizeable carbon, and to a lesser degree, total carbon in sediment were found to be good indices of denitrification potential for a broad range of soils incubated under anaerobic conditions (Burford and Bremner, 1975; Stanford et al., 1975). Pfenning and McMahon (1996) found that in carbon limited riverbed sediment, the type of organic carbon (i.e., acetate, fulvic acid) added to the sediment greatly influenced denitrification rates. Few earlier studies have related denitrification rate to physical and chemical fractions of organic C such as dissolved organic C (DOC) (Hill et al., 2000; Stow et al., 2005), anaerobically or aerobically mineralizable C (Davidson et al., 1987), and acid soluble carbohydrates (Bijay-Singh et al., 1988; Hume et al., 2002; Hill and Cardaci, 2004). Such studies have suggested a complex relationship between C transformation and denitrification. For instance, DOC was previously believed to be labile and easily utilized by microbes based on positive relationship between N_2O emissions and DOC in the stream water (Stow et al., 2005). Other evidence indicated that the differences in DOC levels of wetland soils and streams had no significant influence on denitrification rates (Davidsson and Stahl, 2000; Bernhardt and Likens, 2002). Recently, Sirivedhin and Gray (2006) reported that sediments treated with two different pore waters collected from different sites but having similar DOC concentrations exhibited significantly different denitrification rates. Although various strains of denitrifying bacteria are able to use aromatic C (Nozawa and Maruyama, 1988), plant litter bearing high concentration of soluble phenolics and low holocellulose quotient (holocellulose / lignocellulose) was found to inhibit the decay of overall organic matter (Bridgham and Richardson, 2003). Under sub-oxic condition where NO_3^- was an alternative electron acceptor, it was shown that denitrifying microbes preferentially consumed nitrogen-rich organic C (Van

Mooy et al., 2002). All these results indicate that chemical structure, rather than the solubility, may determine organic C quality and availability which may in turn govern the denitrifying bacteria in soils.

Labile organic C (LOC) fraction is deemed to include all organic C of SOM, regardless of solubility, that are able to be used by microbes, and its determination, however, has often been limited to a specific short-term incubation process (Davidson et al., 1987; McLauchlan and Hobbie 2004). It is unlikely that this short-term LOC can reveal the types or molecular structures of organic C within SOM that are preferred by microbes such as denitrifying bacteria. While addition of simple organic C such as glucose to organic C-limited soils drastically increases denitrification rates (Garcia-Montiel et al., 2003), addition of glucose to a forest soil, despite high organic matter content, increased denitrification rate as compared to a grassland soil containing substantially lower amounts of total organic matter (Groffman et al., 1991). Ganjegunte et al., (2006) found that the addition DOC with higher amounts of polyphenols led to very small increase in soil respiration as compared to the soil amended with low levels of polyphenols. In addition, denitrification potential in vegetative filter strips was found to vary depending on vegetation type (Groffman et al., 1991). Studies of riparian wetlands showed that differences in organic carbon supply from leaf litter and root exudates between woody and grassy sites produced different denitrification rates (Martin et al., 1999).Microcosm studies by Hume et al., (2002) found that acid soluble carbohydrates to NO_3^- ratio explain more variability in denitrification potential (DNP) than total carbon to NO_3- ratio. Sirivedhin and Gray (2006) found a positive relationship between DNP and biodegradable organic carbon concentration and identified chemical markers illustrating that biodegradability is promoted by a combination of polyhydroxyaromatic and polysaccharide parent structures. Laursen and Seitzinger (2002) also showed that denitrification was not related to the organic content of the sediment suggesting that quality of organic matter, rather than simply quantity, was important in controlling denitrification in continental shelf sediments. Despite the importance of chemical structure, there is little information available on how molecular composition of organic matter affects denitrification in wetland soils (Van Mooy et al., 2002).

2.5 Characterization of Soil Organic Matter

Soil organic matter consists of continuum of materials ranging in mean residence time from less than a few weeks to greater than several thousand years (Collins, et al., 2000; Cheng et al., 2007). Based on the resistance to microbial degradation SOM has been divided into different pools (McLauchlan and hobbie, 2004). In three pool model SOC has been divided into labile organic carbon (LOC), slow pool (SL) and recalcitrant pool (RC) (Collins et al., 2000). LOC has been defined as carbon with turnover time of less than few years (Zou et al., 2005). Availability of LOC in a given soil affects the microbial activity and governs the eventual release of CO_2 into atmosphere (Zou et al., 2005). On the other hand, recalcitrant C is a more resistant to microbial attack and represents the C sequestered in the soil. Various physical, chemical and biological methods have been proposed to separate and characterize soil C pools. Physical fractionation methods include particle size fractionation and density fractionation (Cheng et al., 2007). In density fractionation, SOC is divided into light and heavy fraction where the light fraction is believed to represent LOC whereas heavy fraction represents RC pool (Alvarez and Alvarez, 2000). Other methods proposed to separate labile C from recalcitrant C pools include water extractable C (WEOC), hot water extractable C (HWEC), salt water extractable C (SEOC), aerobically mineralizable C (AMC), digesting with permanganate, and acid hydrolysis (Bijay-Sing et al;., Paul et al., 2001, Weil et al., 2003; McLauchlan and Hobbie, 2004; Fang et al., 2005; Rovaria and Vallejo, 2007; Wang and Wang, 2007). Unfortunately, none of those fractionation methods can satisfactorily and practically separate different soil carbon pools on the basis of stability, for example, particle size and density fractionations have been shown to yield pools of SOC with different properties regarding chemical composition and turnover (Cheng et al., 2007). In addition, WEOC, which is believed to represent LOC fraction, from different forest floors have different effects on microbial degradation (Ganjegunte et al., 2006). This suggests that chemical structure/ quality of the organic matter play major role in controlling the microbial degradability, which is difficult to be separated using wet chemistry. Nonetheless, wet chemical methods are widely used for its simplicity. Advanced methods like Pyrolysis-GC/MS, and ^{13}C NMR techniques provide important information on SOM structural features which can be used as quantitative measure for SOM quality (Rovira and Vallejo, 2007).

In order to understand specific recalcitrant pools, humic substances (HS), especially humic acids (HA), are often characterized to assess long-term storage of C in soil (Cheng et al., 2007). Very high heterogeneity and complex structure of HAs could be the main reason for their increased resistance against microbial decomposition (MacCarthy 2001). In addition, HAs along with fulvic acids (FAs) play a major role in regulating transport and retention of contaminants (Fukushima et al., 2006). Their amphipathic nature enables them to interact with a wide variety of inorganic and organic pollutants including heavy metals and charged organic pollutants via chemical bonding and with non polar organics through nonspecific physical interactions (Li et al., 2003). Interaction of humic compounds and inorganic mineral phases leads to the formation of organic coatings on mineral phases and a change in surface charge (Schafer et al., 2007). Further, different types of organic matter could have different influences on mineral colloid coagulation (Schafer et al., 2007). Therefore understanding chemical and structural composition of HAs in the given soil could be helpful to interpret its reactions with mineral surfaces as well as its possible role in contaminant transport.

Environmental conditions such as redox status, sedimentation rate, vegetation, type of microorganisms, and biomass productivity control the rate of diagenesis along with the nature of the source material (Golding et al., 2004). Lu et al., (2000) stated that the proportion of the various sources of organic matter in humic substances is related to maturity, depositional environment, and degree of degradation. Although lot of research has been done on characterizing humic compounds in upland soils (Hatcher and Clifford, 1994) and aquatic systems (Deshmukh et al., 2001), there was little work done on humic compounds in wetland soils. In fact, little is known about coastal wetland SOM structure (Alongi, 1998; Keddy, 2000). There is limited organic geochemical characterization of wetland soils (Hornibrook et al., 2000; Cocozza et al., 2003). In addition, limited understanding of organic matter transformation in wetland ecosystems has hindered efforts to elucidate relations among different C pools (Stevenson and Cole, 1999).

Various spectroscopic techniques such as nuclear magnetic resonance (NMR), Fourier transform infrared (FTIR), Pyrolysis GC/MS, and Electron paramagnetic resonance (EPR) are available for identification of functional groups and molecular structures of SOM. The identification of functional groups and molecular structures provides a better understanding of decomposition pathways of OM and qualitative alterations induced by different biogeochemical conditions of soils (González Pérez et al., 2004).

Solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization magic angle spinning (^{13}C CPMAS NMR) is widely used for the characterization of humic acids and bulk SOM (Alarcón-Gutiérrez et al., 2008). Recent advances in solid-state ^{13}C NMR for examining the chemical structure of organic materials in natural environments has allowed non-destructive and potentially quantitative examinations of organic carbon chemistry in-situ, without prior extraction and/or degradation procedures (Cook et al., 1996; Nelson and Baldock, 2005). The method allows assignment of broad classes of organic chemical structure to specific NMR resonances based on chemical shift (Golding et al., 2004).

Pyrolysis-GC/MS is another popular and useful technique for the characterization of natural organic polymers and their composite materials (Moldoveanu 1998). The technique involves the degradation of organic sample in an inert atmosphere at high temperatures for few seconds followed by the product separation by a GC and eventual detection by a mass spectroscopy. However the recent research showed that highly polar oxygenated compounds such as polyhydric phenols, benzenecarboxylic acids could be under estimated (Hatcher and Clifford, 1994). The development of new methodology, where hydroxyl and carboxylic functional groups are methylated using tetra methyl ammonium hydroxide (TMAH), increases the effective gas chromatographic analysis of polarized compounds (Chefetz et al., 2000). Although this complexity of pyrolytic reactions inevitably causes some limitations in the pyrolytic studies of SOM, Py-GC/MS technique has been widely applied to characterize these heterogeneous molecular assemblages obtained from aquatic environments (Wilson et al. 1983, Schulten et al. 1987, Lehtonen, 2005). The major advantages of Py-GC/MS includes low sample requirements, reproducible results, and provides information about most potential SOM precursors such as carbohydrates, lignin, amino acids and lipids (Lehtonen, 2005).

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CHAPTER 3

EFFECT OF ABIOTIC FACTORS ON SOIL CARBON MINERALIZATION AND TRANSFORMATION IN COASTAL LOUISIANA SWAMP FOREST AND FRESHWATER MARSH

3.1 Introduction

Wetlands constitute one of the major sources of global terrestrial carbon (C) pool and play an important role in global carbon cycle (Chmura, 2003). In spite of relatively small proportion of land area, wetland soils are projected to contain about 20 to 25% of the total terrestrial carbon (Amthor et al., 1998). They are also the most productive systems among the terrestrial ecosystems with a net primary production of up to 1300 g C m^{-2} yr^{-1} as compared to 800 g of C m^{-2} yr^{-1} in tropical forests (Schlesinger, 1997; Keddy, 2000). Organic C accumulates in wetlands as the net primary production (NPP) exceeds decomposition rate (DeBusk and Reddy, 2003). On a global basis wetlands are estimated to have a potential of sequestering 0.1 to 0.2 Gt C yr^{-1} (Metting et al., 2002). On the other hand, they are also major sources of greenhouse gas emissions such as CO_2, CH_4 and N_2O (Aerts and Ludwig, 1997; Segers, 1998). Any change that affects C mineralization in these wetlands likely results in changes in the emission of CO_2, CH_4 and therefore C sequestration rates.

Louisiana is rich in wetlands consisting of more than 729,000 ha with the majority marshlands (NOAA, 1991). Soils in these wetlands are very rich in organic matter as high as 80 % and low in mineral matter content (Cahoon, 1994). Organic matter accretion along with sediment has been identified as a key to stabilize these wetlands (Salinas et al., 1986; Nyman and DeLaune, 1999). Increased sea level rise along with high rates of subsidence has led increased submersion and salt water intrusion into these coastal wetlands, which contain freshwater marshes and coastal swamp forest. Marsh dieback due to high salinity, submergence has resulted in destruction of wetlands. To avert the loss of the wetlands, Mississippi river is being diverted into these wetlands with an aim to decrease salinity, provide sediments and nutrients (Lane at al., 1999). All these factors could lead to changes in bio-geochemistry of these wetlands and therefore C dynamics.

Various factors can affect organic C mineralization in soils. They include temperature, water table, soil clay content, soil pH, cations, and salinity (Baldock and Skjemstad, 2000; Blodau, 2002; Bertranda et al., 2007). Mineral matter especially clays can protect soil organic C from mineralization through physical protection and chemical stabilization. Soil organic C can be physically coasted within soil aggregates or chemically adsorbed onto clay surfaces, therefore protecting from microbial degradation (Baldock and Skjemstad, 2000). A study based on analysis of organic C in different size fractions has showed that significant amounts of O-alkyl C (Polysaccharides) present in the clay fraction, can protect easily degradable organic C such as polysaccharides from degradation (Baldock et al., 1990). Various studies also have demonstrated the role of various cations especially divalent and trivalent cations associated with clays and organic matter can protect organic C from degradation (Brynhildsen and Rosswall, 1997; Cruz-Guzman et al., 2003). Exchangeable cations such as Al, Ca, Mg and Fe not only promote interactions between clay minerals and humic substances but also increase molecular aggregation of humic compounds which limits degradation (Varadhachari et al., 1991; and Wershaw, 1999). In addition, cations promote buffering ecosystems by forming complexes with the organic acids produced from organic matter decomposition.

Presence of iron oxides at the allumino-silicate mineral surfaces has been shown to increase sorption of organic matter through ligand exchange process (Chi and Amy, 2004). This can lead to an increase in the strength of organic C causing flocculation and making it harder to be broken down by microorganisms (Boudot and Brahim, 1989). Cruz- Guzmán et al., (2003) reported that Ca^{2+} an interlayer cation promotes the interaction between humic acid and clay.

They also reported that ≥ 80 % and 52% of humic acid respectively was absorbed by freshly precipitated ferrihydrite and Ca-montmorillonite. This suggests that iron oxides along with allumino-silicate clays can also play a major role in protection of organic matter from degradation. Also, clay minerals containing Ca, such as smectites, showed more association with soil organic matter than the non-calcium containing clays such as kaolinite (Furukawa, 2000). Zech et al., (1997) reported that the amount of carbon in upland soils was positively correlated with the cation exchange capacity of soils, indicating the important role of cations in protecting the soil carbon. However the effects of cations and mineral fractions on C dynamics of wetland soils have not been fully evaluated.

The predicted effects of global warming in accelerating sea level rise are likely to further impact coastal wetlands by promoting salinity intrusion and submergence (Titus, 1988; Mendelssohn et al., 1999). In addition to impact on vegetation, increases salinity and flooding may also affect the mineralization of soil organic C thus impacting greenhouse gas production. Increase in overall concentration of salts/ salinity could lead to the slower rate of organic matter mineralization due to the detrimental effect of high salinity on type and number of microorganisms (Rietz and Haynes, 2003). An elevated salinity level between 1 to 17 ppt was showed to decrease woody litter decomposition as well as grass litter degradation (Steinke and Charles, 1986; Mendelssohn et al., 1999). Also, lower CO_2 and CH_4 emissions were observed in Louisiana salt marsh soils as compared to those from freshwater marsh soils (DeLaune et al., 1983). Such facts could infer that the increased salinity may decrease carbon mineralization and eventual release of greenhouse gases.

Louisiana wetlands are experiencing wetland loss due to coastal submergence including salt water intrusion. Several Mississippi river freshwater diversion sites are introducing freshwater and sediment to slow the rate of deterioration of these wetlands. The processes wetland loss, river diversion, coastal submergence, saltwater intrusion will all have impact on carbon mineralization rates including CH_4 and CO_2 production, two important greenhouse gases. These interactions on carbon transformation effects have not been fully evaluated in Louisiana coastal wetlands.

Assessing the factors controlling soil carbon storage and turnover of carbon reservoir in coastal wetlands is essential for understanding the impact on the global carbon cycle. The present study was aimed at determining carbon mineralization, CH_4 and CO_2 gas production and organic C transformation in a Louisiana freshwater marsh soil and a forested woody swamp soil.

3.2 Materials and Methods

3.2.1 Site and Soil Characteristics

Two wetland ecosystems, namely bottomland forested woody swamp (FS) and freshwater marsh (FM), located in the Louisiana Barataria Basin estuary were selected for this study. The major vegetation types in FS and FM are *Taxodium distichum* and *Sagittaria lancifolia*, respectively. Both the wetlands are seasonally flooded, but, stay water saturated throughout the year. The soils at the FS and FM sites were classified as Barbary muck (Very-fine, smectitic, nonacid, hyperthermic Typic Hydraquents) and Allemands muck (Clayey, smectitic, euic, hyperthermic, Terric Haplosaprists), respectively. Composite soil samples were collected from the top 0 – 50 cm depth at each site, placed into zip-lock bags and transported to the lab in an ice chest. The collected soil samples were thoroughly mixed removed any visible plant parts and stored in a refrigerator until they were used. Soil pH, electric conductivity (EC) and bulk density was determined using field moist soil samples whereas other properties were determined using freeze-dried samples. Bulk density was measured by gravimetric method. Total C and N were determined by dry combustion at $900^{o}C$ using a TruSpec CN analyzer (LECO, St. Joseph, MI). The remaining fresh samples were used for incubation experiments.

3.2.2 Laboratory Microcosm Study

A series of 100-g moist field soil samples were placed in 400 mL ISCO jars and treated with 100 mL of various treatment solutions that consisted of two levels of Ca (1 and 5 mM) as $CaCl_2$, K (2 and 10 mM) as KCl, clay (2 and 5 %) as montmorillonite, salinity (15 and 30 ppt), and a control (DI water). To evaluate the effect of clay in the presence of cations, an additional treatment of 5% clay mixed with 5 mM Ca as $CaCl_2$ was also established in similar fashion. The clay treatments were only applied to the FM soil which is low in mineral matter content. All the treatments were replicated three times. During the entire incubation period, de-ionized water was periodically added to the jars to replenish any water loss by evaporation. The amount of water added was based on the weight loss of jar with the treatment. The jars were aerobically incubated for a period of 528 days in the dark at room temperature (~20^oC). Production rates of CO_2 and CH_4 were periodically monitored during the entire study period. For monitoring CO_2 and CH_4 production rates, at each sampling period sample jars were capped for 24 hours and headspace gases were collected through a septum in the lid after 0 and 24 h (Robertson et al., 1999; McLauchlan and Hobbie, 2004). The concentrations of $CO_2$and CH_4 were determined simultaneously using Tremetrics 9001 gas chromatograph (GC) instrument (Tremetrics, Eden Prairie, MN) equipped with a flame ionization detector. The GC was equipped with stainless steel columns packed with Porapak-Q. The system had a back-flush mechanism operated by 10-port Valco valves to prevent moisture in the samples entering into the detectors. Operating temperature was 50^oC for the oven, and 150 ^oC for the FID. Gas samples were injected through a 0.5 ml sample loop connected to the Valco valves for FID detection. A methanizer catalyst column was installed in the GC after a sample separation column, which reduced the CO_2 to CH_4 for detection. All GC analytes were calibrated individually using certified standards of known concentration (Scott Specialty Gases, Inc., Plumsteadville, PA, USA). At the end of incubation, soil samples were freeze-dried and characterized by pyrolysis- gas chromatography/mass spectrometry (Py- GC/MS) and 13C NMR for organic C structure to evaluate the changes in organic C structure under different treatments.

3.2.3 Pyrolysis-GC/MS

Organic C structure of FS and FM soils in the initial soil and after the incubation under different cations, clay and salinity treatments were determined by Py–GC/MS. The GC/MS analysis of pyrolysis products evolved from the thermal degradation of OM provides information on the chemical nature of different bio-precursors, and it has been extensively used for obtaining molecular level information on SOM and humic substances (Chefetz et al., 2002). Pyrolysis-GC/MS was performed using a Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer (Varian Inc., Palo Alto, CA) and a CDS 5000 pyroprobe platinum heated filament pyrolyser (Chemical Data System, Oxford, USA). Pyrolysis was carried out at 620^{o}C for 20 seconds. A Varian factor FOUR VF-5MS capillary column coated with poly (5% diphenyl/95% dimethyl) siloxane stationary phase (30 m, 0.25mm i.d., 0.25 µm film thicknesses) was used for separation of pyrolysis products. The column temperature was ramped from 40^{o}C (held for 2 min) to 300^{0}C at 5^{o}C min^{-1} and hold for 10 minutes, using helium as carrier gas. The temperature of injector and transfer line was kept at 300 and 240^{o}C respectively. Mass spectra analyses were recorded in the electron impact mode (70 eV) at 1 scan s^{-1} in the 45–600 *m*/*z* range. The identification of pyrolysis products was based on a comparison of their mass spectra with those of standard compounds, NIST 2005 mass spectral library, literature data and GC–MS characteristics. The relative quantities of the products were estimated using the peak areas of the total ion current (TIC) pyrograms. Pyrolysis products are grouped into compound classes according to their origin.

3.2.4 Solid State Nuclear Magnetic Resonance Spectroscopy

Organic C structure of selected FM samples that had significant amount of gaseous C production during the incubation were also characterized using solid state ^{13}C nuclear magnetic resonance (NMR) spectroscopy. All the NMR spectra were collected on a Bruker Avance wide bore 400 MHz Nuclear Magnetic Resonance Spectrometer. The resonance frequencies of 1H and 13C are 400 and 100 MHz, respectively. Cross polarization total sideband suppression magic angle spinning (CP-TOSS-MAS) was used to acquire the ^{13}C NMR spectra. Samples were loaded into 4mm Zirconium rotors and analyzed using a double resonance CP-MAS probe. Spectra were collected at a spinning speed of 5 kHz, with 1 ms contact times, 2 s recycling times, 72,000 scans, and spectra were referenced to the 176.03 ppm C=O peak of glycine. Within the 0-230 ppm chemical shift range, C atoms were assigned to various molecular groups according to Malcolm (1989) in to aliphatic C (0-50 ppm), methoxy C (50-60 ppm), polysaccharides (60-108 ppm), aryl C (108-140 ppm), phenolic (140-165) carboxyl C (165-190 ppm) and carbonyl (190-230 ppm). All other assignments have either been assigned based on reference literature or calculated via ChemDraw Ultra 9.0 by CambridgeSoft. Carbon percent distribution was summarized based on spectral peak area for all C groups, and the amount of each C functionality per unit of soil mass was calculated based on their relative distribution and total organic C.

3.3 Results and Discussion

3.3.1 Physical and Chemical Characteristics of Study Sites

Selected physic-chemical characteristics of study soils are presented in Table 3.1. The top 0-50 cm of the FS soil representing the A1 horizon contained 81.5 g C kg^{-1} soil with a molar C: N ratio of 12.3. On the other hand, the FM soil top 0-50 cm representing the Oe horizon contained 342 g C kg^{-1} soil with a molar C: N ratio of 15.3. The difference in amount of soil organic matter was reflected in soil bulk density which was 0.24 g cm^{-3} for FS soil as compared to 0.07 g cm^{-3} for FM soil. Both the soils were slightly acidic in reaction.

Table 3.1 Sampling site info and physico-chemical properties of selected wetland soils.

Site	Location	Depth	Horizonation	pH	EC†	BD‡	Total C	Total N	C:N
		cm			dS m^{-1}	g cm^{-3}	-------g kg^{-1}-----		
Forest swamp	29.91165°N 90.72812°W	0-50	A1	6.2	1.1	0.24	81.5	6.7	12.3
Freshwater marsh	29.77195°N 90.29026°W	0-50	Oe	5.5	0.6	0.07	342	22.3	15.3

†EC, electrical conductivity.
‡BD, bulk density.

3.3.2 Effect of K and Ca on CO_2 Production

The addition of both K and Ca led to a significant increases in CO_2 production from the FS soil as compared to control (Figure 3.1 a). The total amount of CO_2 of all the measurements during the entire incubation were in the order of 1 mM Ca > 2 mM K = 5 mM Ca > 10 mM K > control. Even though both K and Ca treatments produced higher CO_2 production than the control soil, the increase was highest at lower K and Ca levels as compared to higher concentrations. The higher CO_2 production in K and Ca treated soils could be due to the increased C availability for mineralization from the replacement of high valency cations such as Mn, Fe and Al with K and Ca. Organic particles bonded to the Fe and Al bonds lead to the formation relatively stable, polymerized complexes. In contrast Ca^{2+} saturation causes rapid precipitation of soluble organic compounds and inhibits the polymerization process leading to less condensed and relatively more labile material (Bottner et al., 1998). Similarly, Duchafour (1983) reported that organic complexes linked to clay minerals by Ca^{2+} are less stable than Fe and Al bonds. To confirm the assumption of replacement of high valency cations by K and Ca we conducted an additional 3

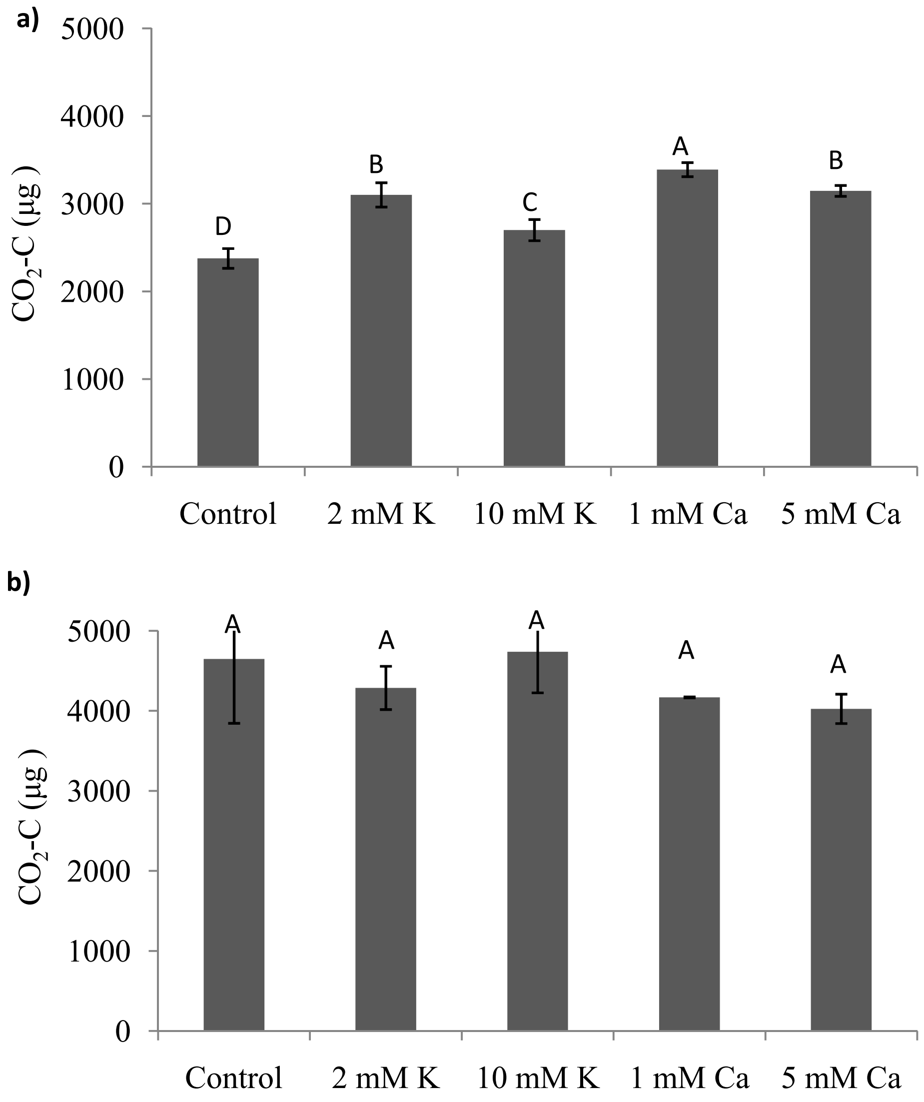

Fig 3.1 Effect of K and Ca on cumulative CO_2 production from a) forest swamp and b) freshwater marsh soils. Different uppercase letters indicate significant differences among treatments at the $p<0.05$ level.

day incubation study which revealed that addition of both K and Ca led to the release of Mn from FS soil which was not detected in the control. Moreover it was reported that, presence of Mn is beneficial to some fungi that have Mn containing phenol oxidase enzymes that degrade lignin (Yavitt et al., 2004). Also exchangeable Ca^{2+} has been reported to be correlated with many microbial properties (Bottner et al., 1998). Additionally, increased C mineralization can be expected due to a slight increase in soil pH by the addition of K and Ca (Motavalli et al., 1995). This is due to the fact that in basic soil reaction in soils microbial and faunal communities become more diversified leading to the increased enzymatic activity (Bottner, 1998). It could be also possible that the added K and Ca and desorbed cations such as Mg, Fe, and Mn might have served as nutrients resulting in enhanced microbial growth (Yavitt et al., 2004). However, in contrast to the lower levels of K and Ca treatments the low CO_2 production at higher levels of K and Ca may be due to lower C mineralization with the increased organic C condensation due to higher salt concentrations (Sparks, 2003).

In contrast to the FS soil, the addition of either K or Ca to the FM soil did not show any significant effect on CO_2 production rates as compared to control (Figure 3.1 b). However, even though not significant, cumulative gas production for all measurements during the period of incubation showed that the addition of Ca led to a decrease in overall CO_2 production. This could be due in part to the role of Ca in enhancing the binding of organic matter with other organic material and with clay present in the system. Polyvalent metal ions such as Ca can function as cross-linking agents though their ability to coordinate multiple functional groups (mainly carboxyl and phenolic functional groups) which can enhance aggregation of organic matter (Lu and Pignatello, 2004). A three day incubation of FM soils with K and Ca did not release any detectable amount of Mn, Fe or Al which indicated that the FM soil contained only limited amounts of such multivalent cations. Also in contrast to the FS soil in the FM soil, the addition of Ca led to the replacement of monovalent cations such as Na and K. Further, the lower CO_2 production from the 5 mM Ca treatment compared to the 2 mM Ca treatment support the role of Ca in protecting the organic matter. This signifies that added Ca was adsorbed onto the FM soil and could enhanced bonding between organic C and clay resulting in the condensation of organic C.

Overall the results indicate that addition of Ca may be beneficial in decreasing carbon mineralization in the FM soils which were low in mineral matter and low in multivalent cations such as Fe^{3+}, and Al^{3+}. However addition of Ca to soils rich in multivalent cations such as Fe^{3+}, and Al^{3+}, may lead to their release which could decrease the relative stability of soil organic carbon.

3.3.3 Effect of Clay on CO_2 Production

Coastal Louisiana freshwater low bulk density marsh soils of are very high in organic matter content and low in mineral sediment which allows the opportunity to assess the role of clay in tying up organic compounds, reducing mineralization and decreasing the carbon gas production. Cumulative CO_2 production data showed that all the clay treatments resulted in a significant decrease in CO_2 production as compared to control (Figure 3.2). However, the decrease was not significant between 2 and 5 % clay treatments. On average, addition of clay at 2 and 5 % level decreased CO_2-C production by 20 % while the decrease was 28 % at 5 % clay with 5 mM Ca. Further examining the kinetics of CO_2 production showed that addition of 5% clay led to the increased CO_2 productions for the initial days of incubation. This could be expected since the addition of clay could cause sudden rise in microbial activity as the clay surfaces lead to change in cell membrane processes and pH buffering by ion exchange (van Loosdrecht et al., 1990). CO_2 production decreased gradually in all clay treatments over time as compared to control. This was likely due to the increasing association of organic matter with clay with over time can physically and/ or chemically protects the organic matter from degradation. These results are supported with the findings of Wang and Wang (2007) who reported that the amount of hot water extractable C, which is used as one measure to estimate labile organic carbon, was significantly and negatively correlated with the amount of clay in the soil. The results also confirm that the effect of clay on decreasing the CO_2 production was further

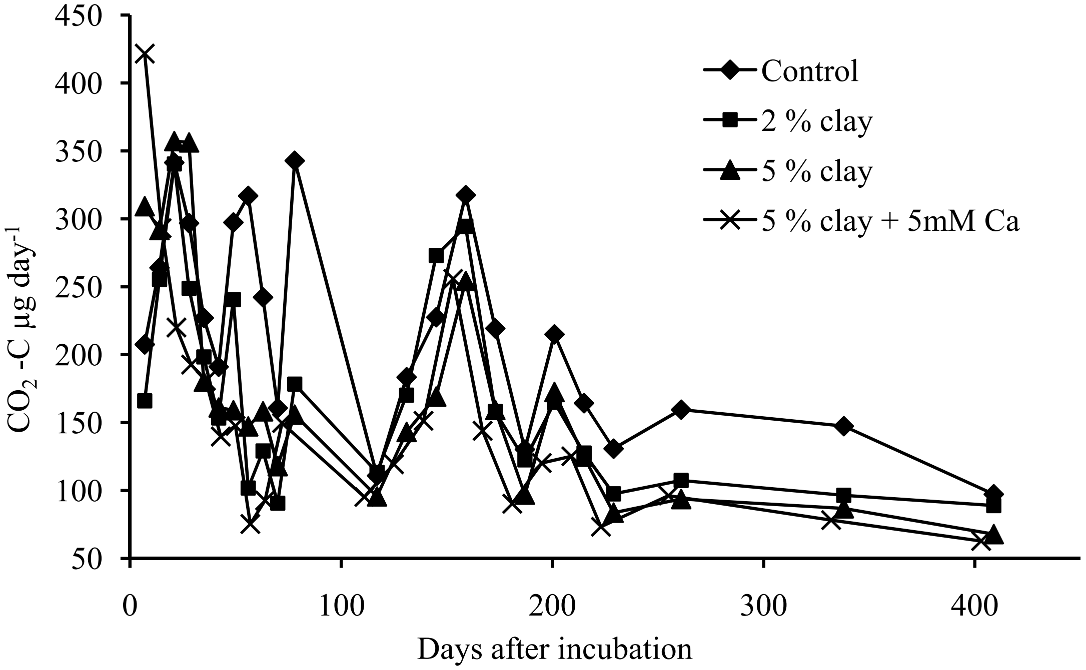

Fig. 3.2 Kinetics of CO_2 production in a freshwater marsh soil treated with different levels of clay

enhanced when clay was added along with Ca. The latter supports the assumption that the polyvalent cations such as Ca^{2+} plays a major role in bonding organic matter to clay minerals through enhancing the association of organic matter to mineral matter by acting as cross linking agents between clay and organic functional groups such as carboxylic and phenolic groups. Furukawa, (2000) reported that clay minerals containing Ca such as smectites had more association with organic matter than the non calcium containing clays such as kaolinite. Formation of organo-mineral complexes protects the degradation of less recalcitrant organic matter such as polysaccharides from microbial degradation. In addition to the chemical stabilization of organic matter by clay, organic carbon held with in clay aggregates provides additional physical protection from microbial attack (Krull et al., 2003). Our results show the role of mineral matter (clay) in protecting soil organic C from degradation in freshwater marsh soils thus increasing C sequestration.

3.3.4 Effect of Salinity on CO_2 Production

Salinity did not show any significant effect on decreasing CO_2 production from the FS soil at both the 15 and 30 ppt level. However it should be noted that the ultimate salinity of soils treated with 15 and 30 ppt saline water was only 7.1 and 13.8 ppt. The total amount of CO_2-C measured at different intervals during the entire period of incubation in the FS control, for the 15 ppt and 30 ppt salinity treatments were 2376 (± 112.5), 2071(± 77) and 2311(69) µg CO_2-C respectively. Even though there was significant difference among treatments, further examination of at the kinetics showed that the CO_2 production decreased significantly in both the 15 and 30 ppt treatments as compared to control beginning from the 166 days incubation (Figure 3.3). These results were similar to those reported by Mendelssohn et al., (1999) on salinity effect on mangrove leave degradation which reported significant decrease in decomposition of mangrove leaves at 30 ppt salinity and no significant decrease in decomposition at 15 ppt salinity. These results shows that salinity failed to show enhance degradation by microorganisms under high clay conditions.

However, in freshwater marsh salinity had a significant effect in decreasing the CO_2 production at 30 ppt whereas the decrease was not significant at 15 ppt (Figure 3.4). The ultimate salinity of FM soils treated with 15 and 30 ppt was 7.4 and 14.5 ppt. The total amount of CO_2-C measured from FM control, 15 ppt and 30 ppt salinity treatments for all the intervals during the period of incubation were 4649, 3932 and 3145 µg CO_2-C respectively. It has been reported that an increase in overall concentration of salts/ salinity can lead to the slower rate of organic matter mineralization and lower CO_2 production due to the detrimental effect of high salinity on type and amount of microorganisms (Rietz and Haynes, 2003). In addition, Yuan et al., (2007) showed that a negative exponential relationship exists between soil salinity and microbial biomass carbon. It has also been reported that at higher salt concentration organic

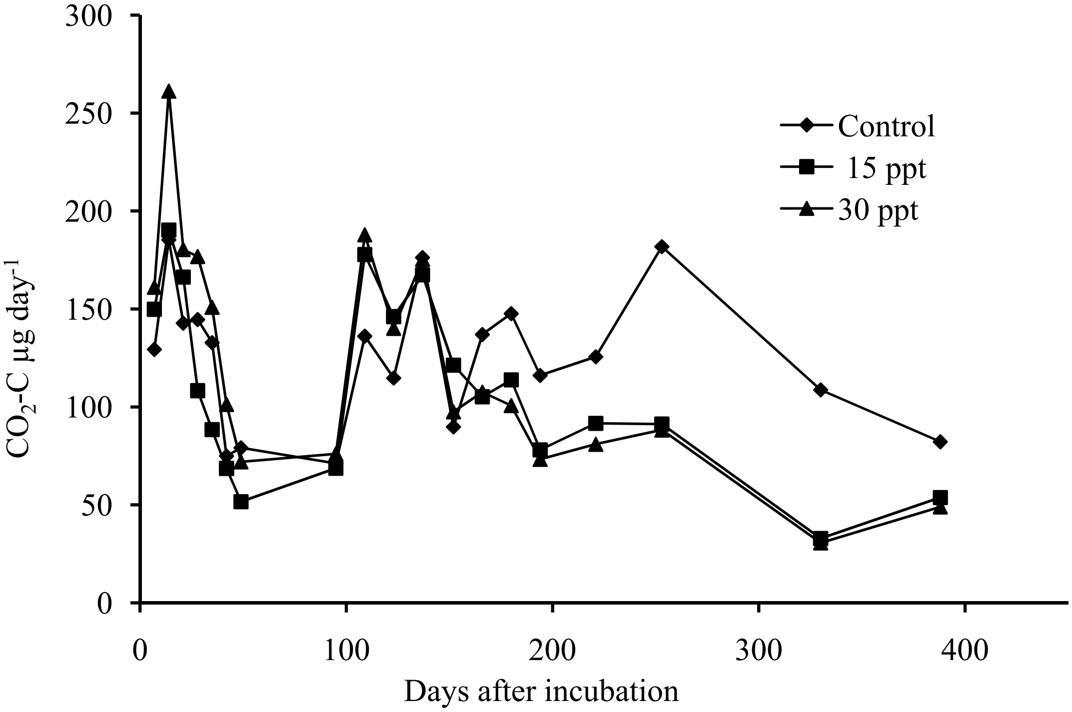

Fig. 3.3 Kinetics of CO_2 production in forest swamp soil treated with different levels of salinity

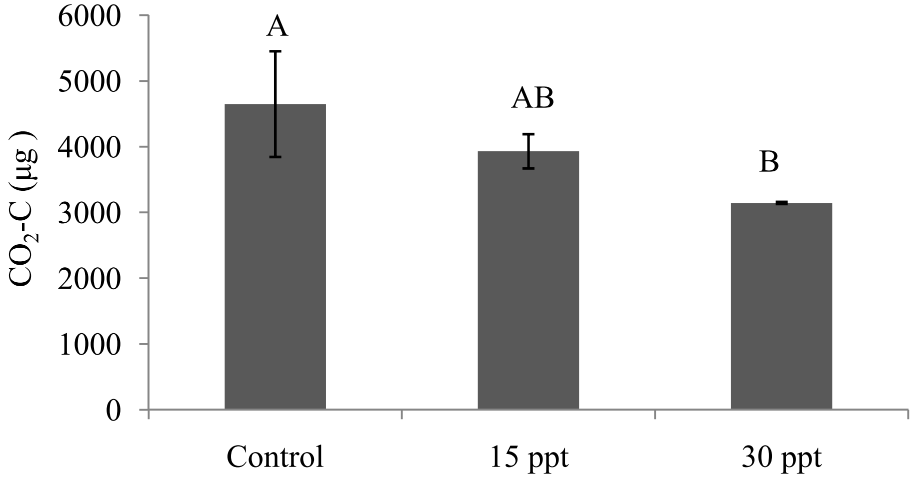

Fig. 3.4 Effect of salinity on cumulative CO_2-C production for 528 days from a freshwater marsh soil. Different uppercase letters indicate significant differences among treatments at the $p<0.05$ level.

carbon is present in more condensed form (Sparks, 2003) which lower microbial mineralization. Our data indicate that high salinity level as a result of salt water intrusion would likely cause a decrease in CO_2-C production from both FS and FM soils. However, high amounts of clay present in FS soil may lessen decrease the salinity impact on microbes with little influence on C mineralization rate.

3.3.5 Methane Production

Methane productions from FS soil were generally small and were not affected by any of the treatments. The cumulate amount of CH_4- C measured at different times of entire incubation period ranged from 45 to 61 µg CH_4- C. Methane production from FM soil were higher than measured in the swamp soil. The cumulative amount of CH_4-C measured over the entire incubation was ranged from 79 to 508 µg CH_4- C. Among all the treatments only salinity showed a significant effect in decreasing CH_4 production as compared to control whereas no significant effect was observed in other treatments as compared to control. Also, the decrease in CH_4 production was similar at both the 15 and 30 ppt treatment indicating even the low salinity was as effective as higher treatment salinity level in decreasing CH_4 production. Methane produced under the two salinity treatments was at least 60 % lower than for the control soil. The lower CH_4 productions from the 15 and 30 ppt salinity treatments can be explained by the presence of high SO_4^{2-} concentration. Though no toxic effect of SO_4^{2-} was found on methanogens both sulfate reducers and methanogens compete for their common substrate hydrogen, where more efficient sulfate reducers dominate the methanogens, and decrease the CH_4 emissions (Klüber and Conrad, 1998). The relative low CH_4 emissions from both the forested swamp and freshwater marsh soil treatment indicate that the soil may not have been highly reduced. Sulfate tends to buffer redox at levels above which CH_4 is produced.

3.3.6 Organic C Transformation - Pyrolysis-GC/MS Analysis

At the end of incubation soil samples were freeze dried, to avoid any possible changes to organic C by air drying. The freeze dried samples were ground, passed through a 250 µM sieve and analyzed for the C structural changes by Py-GC/MS. All the identified compounds in the Py-GC/MS analysis were classified based on the expected origin into alkyl compounds (Al), lignin-derived compounds (L), N-containing compounds (N), poly phenols/ other aromatic compounds (Pp) and polysaccharides (Ps). The amount of each compound was quantified semi-quantitatively based on relative peak area of each peak as compared to total peak area.

3.3.6.1 Forest Swamp Soil

Main compounds identified in the Py-GC/MS analysis of FS soils were grouped based on the origin is presented in Table 3.2. Also, the relative abundance of main compounds in each treatment soil is also summarized in Table 3.3. The major alkyl compounds found among all the treatment soils were nonadecane, tetradecene (Z)-, Hexadecene, pentadecanoic acid 14-methyl methyl ester and 11- octadecanoic acid methyl ester. Overall distribution of compounds revealed that the relative amount of alkyl C increased in all the treatments as compared to initial soil. However, the increase of alkyl compounds was significantly higher in both the 15 and 30 ppt salinity treatment. This is finding is similar to that of FM soil where salinity treatment led to relatively higher amount of alkyl compounds. The major lignin derived compounds found among the treatments were 2-methoxy phenol, 2-methoxy-4-methyl- phenol, 2-methoxy vinylphenol, and 1-(3-hydroxy-4-methoxyphenyl)- ethanone. The contribution of lignin was significantly lower in salinity treatments while the change was not significant in other treatments as compared to control. However the amount of poly phenolic/ other aromatic compounds were significantly higher in both salinity treatments with no significant difference was found in other treatments. The relative amount of polysaccharides was appreciably lower in all treatments as compared to

Table 3.2 Main Pyrolysis- GC/MS compounds identified in Forested swamp soil under different treatments

Compound	
Alkyl/ Fatty acids	
7 –Tetradecene	A1
3-Dodecene (E)	A2
9-Heptadecanol	A3
Nondadecane	A4
7-Tetradecene	A5
Hexatricontane	A6
Heptacosane	A7
Pentadecanoic acid, 14- methyl methyl ester	A8
Ascorbic acid, 2,6- dihexadecanoate	A9
Oleic acid	A10
11-octadecanoic acid methyl ester	A11
Lignin derived compounds	
Phenol	L1
2-methoxy Phenol	L2
2-methoxy-4-methyl Phenol	L3
2-methoxy-4-ethyl Phenol	L4
2-methoxy-4-Vinylphenol	L5
2,6-dimethoxy Phenol	L6
2-methoxy-6-(2-propenyl)- Phenol	L7
2-methoxy-4-(1-propenyl)- Phenol	L8
2-methoxy-4-(1-propenyl)- Phenol (E)	L9
1-(-3-hydroxy-4methoxyphenyl)-Ethanone	L10
2,6-dimethoxy-4-(2-propenyl)-Phenol	L11
Other aromatic/ poly phenol derived compounds	
Toluene	Pp1
Ethylbenzene	Pp2
p-Xylene	Pp3
1,3,5-trimethyl Benzene	Pp4
1-ethyl-3-methyl Benzene	Pp5
1-propynyl Benzene	Pp6
2-methyl Phenol	Pp7
2,4-dimethyl Phenol	Pp8
2-ethyl Phenol	Pp9
2,3-dimethyl Phenol	Pp10
1,3-dihydro-2H-Inden-2-one	Pp11
4-methyl 1H-Indole	Pp12
2,3,6-trimethyl Naphthalene	Pp13
Nitrogen- containing compounds	
Pyridine	N1
3-methyl Pyridine	N2

3-methyl- 1-H- Pyrrole	N3
Caprolactum	N4
Benzyl nitrile	N5
Benzenepropanenitrile	N6
2-Propenenitrile, 3-phenyl- (E)	N7
Indole	N8
Polysaccharide derived compounds	
2-methyl Furan	Ps1
Acetic acid	Ps2
2,5-dimethyl Furan	Ps3
2-Propenoic acid, 2-methyl-, methyl ester	Ps4
3-Furaldehyde	Ps5
2-Cyclopenten-1-one, 2 methyl	Ps6
5-methyl, 2-Furancarboxaldehyde	Ps7
2-hydroxy-3-methyl- 2-Cyclopenten-1-one	Ps8
2,3-dihydro- Benzofuran	Ps9

Table 3.3 Qualitative changes in organic C distribution under different treatments in a forested swamp soil determined by Py-GC/MS

Treatment	Alkyl	Lignin	N-compounds	Poly phenols	Polysaccharides
			%		
Original	19.5	16.0	12.4	26.7	11.4
Control	20.4	14.3	9.9	28.5	7.6
Cations					
2 mM K	21	16.7	10.1	25.2	10.1
10 mM K	24.4	15.2	9.0	25.4	9.2
1 mM Ca	19.1	20.2	6.9	26.5	10.1
5 mM Ca	22.0	14.3	10.1	28.7	8.2
Salinity					
15 ppt salinity	24.6	4.7	7.8	31.8	8.9
30 ppt salinity	27.5	7.2	9.5	29.0	6.9

initial soil. This is in accordance with the various previous findings who reported decrease in the relative amount of polysaccharides in humified fractions (Ussiri and Johnson, 2003).

3.3.6.2 Freshwater Marsh Soil

The main compounds identified in the treated soils are presented in Table 3.4. The relative abundance for the main group of compounds in each treatment is also summarized in Table 3.5. Among all the treatment soils (including initial soil sample) lignin derived compounds were the dominant fraction. The major lignin derived compounds identified were phenol, mequinol, 2-methoxy-4-methyl phenol, 2-methoxy-4-vinyl phenol, and 2-methoxy-5-(1-propenyl) phenol. Most of the lignin derived compounds identified in these samples were also observed by other researchers in the peat soils characterized by pyrolysis- GC/MS (Chefetz et al., 2002). The relative amount of lignin derived compounds was slightly higher in control, 2 mM K and 2 % clay treatments as compared to initial soil. In contrast, amount of lignin was lower in 5 % clay +5 mM Ca, 15 ppt and 30 ppt treatments. Observation of the distribution of individual compounds derived from lignin in each soil treatment showed that the relative amount of 2-methoxy-4-methyl phenol significantly increased in all the treatments as compared to the initial soil. The ratio of syringyl (S) type of lignin compounds to guaiacyl (G) type lignin compounds was also estimated for all the treatments. The ratio of S/G serves as an indicator of lignin degradations was based on the fact that condensed guaiacyl units are less prone to biological degradation than the lignin with larger proportion of methoxyl groups such as syringyl type (Chefetz et al., 2000). The S/G ratio was 0.67 in control treatment as compared to 0.7, 1.2 and 0.77 in initial, 5 % clay + 5mM Ca and 30 ppt salinity treatments respectively. In contrast to lignin derived compounds, the amount of alkyl C increased significantly in 5 % clay +5 mM Ca, 15 ppt and 30 ppt treatments. A decrease was observed in the control, 10 mM K and 5 mM Ca treatment. On the other hand, the amount of alkyl C remained unchanged in 2 mM K, 1 mM Ca, and 2 % clay

Table 3.4. Main Pyrolysis- GC/MS compounds identified in Freshwater marsh soil under different treatments

Compound	
Alkyl/ Fatty acids	
7 –Tetradecene	A1
7-Heptadecene	A2
1-Dodecanol, 3,7, 11- trimethyl	A3
Nonadecane	A4
Pentadecanoic acid , 14-methyl methyl ester	A5
n- Hexadecanoic acid	A6
n-Hexadecanoic acid, 9-hexadecenyl ester	A7
8, 11- Octadecanoic acid methyl ester	A8
1-Decanol, 2-hexyl	A9
Lignin derived compounds	
Phenol	L1
Mequinol	L2
Phenol, 2-methoxy-4-methyl-	L3
1,2-Benzenediol, 3-methoxy-	L4
Phenol, 4-ethyl-2-methoxy-	L5
2-Methoxy-4-vinyl phenol	L6
Phenol, 2,6-dimethoxy-	L7
Benzaldyhyde,3-hydroxy-4methoxy-	L8
Phenol, 2-methoxy-5-(1-propenyl)-	L9
Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-	L10
Phenol,26-dimethoxy-4-(2-propenyl)-	L11
Other aromatic/ poly phenol derived compounds	
Toluene	Pp1
Phenol, 2-methyl	Pp2
Phenol,4-methyl	Pp3
Phenol, 2,4-dimethyl-	Pp4
Phenol, 2,3-dimethyl-	Pp5
Phenol,3-ethyl	Pp6
Resorcinol	Pp7
Indole	Pp8
1,2-Benzenediol, 4-methyl-	Pp9
1-H-Indole, 2-methyl-	Pp10
5-tert-Butylpyrogallol	Pp11
Nitrogen- containing compounds	
1-Tetrazole-2-ylethanone	N1
Acetohydroximic acid	N2
1-H-Pyrrole, 1-methyl-	N3
Pyridine	N4
Isoserine	N5

1-H-Pyrrole, 3-methyl-	N6
1-H-Pyrrole, 2,5-dimethyl	N7
3-Pyrazolidinine, 1,4-dimethyl	N8
Oxazolidine, 2,2-diethyl-3-methyl	N9
Benzyl nitrile	N10
Benzenepropanenitrile	N11
Polysaccharide derived compounds	
Acetic acid	Ps1
Furan, 2,5-di methyl	Ps2
2-cyclopenten-1-one, 2-methyl	Ps3
2(5H)-Furanone	Ps4
1,2-Cyclopentanedione	Ps5
2-Furancarboxaldehyde, 5-methyl-	Ps6
1,2-cyclopentanedione, 3 methyl	Ps7
Maltol	Ps8
Benzofuran, 23-dihydro-	Ps9

Table3.5 Qualitative changes in organic C distribution under different treatments in a freshwater marsh soil determined by Py-GC/MS

Treatment	Alkyl	Lignin	N-compounds	Poly phenols	Polysaccharides
	--%			--	
Original	18.7	40.8	7.7	15.2	12.6
Control	15.7	44.4	5.7	13.8	12.1
Cations					
2 mM K	19.2	45.6	4.7	14.4	11.5
10 mM K	14.2	41.0	8.3	16.7	11.7
1 mM Ca	18.7	42.2	5.3	14.8	10.2
5 mM Ca	15.4	41.3	7.7	18.2	11.5
Clay					
2 % clay	18.8	43.3	7.4	14.2	10.4
5 % clay	15.1	41.6	10.1	15.6	12.4
5 % clay +5 mM Ca	25.8	34.1	7.0	16.4	11.7
Salinity					
15 ppt salinity	21.9	35.4	8.6	16.8	11.0
30 ppt salinity	25.4	34.3	8.2	14.4	13.7

treatments. The increase in relative amount of alkyl compounds under high salt conditions was similar to our findings in another study on humic acid fractions where we found higher amount of alkyl compounds in salt marsh humic acid than freshwater marsh humic acid.Even though the relative contribution of lignin derived compounds was highest in all the treatments, presence of high amounts of salts may lead to increase in relative amounts of alkyl compounds. The major poly phenolic/ other aromatic compounds identified were toluene, 2-methyl phenol, 4-methyl phenol, and 2, 3- dimethyl phenol. Results showed that there was a decrease in the relative amount of alkyl C and N-containing compounds in control sample with an increase in the amount of lignin derived compounds derived compounds. However, the relative amount of polysaccharide C showed little change among the treatments. In all the treatments the amounts of N containing compounds were not significantly different from that of initial soil.

3.3.7 Organic C Transformation - ^{13}C NMR Analysis

Selected treatments from FM soil were analyzed by ^{13}C CPMAS NMR technique for identifying changes in organic C functional groups distribution as compared to initial soil. Soil samples from control, 5 % clay + 5 mM Ca, and 30 ppt salinity treatments were analyzed along with the original soil (Table 3.6). The overall results indicate that there was no significant change in relative distribution of organic C functionalities as compared to the initial soil. Even though not significant, all the soils contained relative increased amount of aromatic C and carboxylic C while there was a decrease in polysaccharide carbon. The relative contribution of aliphatic C did not show any change as compared to initial soil. However, observation of peaks at 28 ppm which represent amorphous polymethylenic (CH_2) n chains (Kögel-Knabner et al., 1992; Shechter et al., 2006; Mao et al., 2008) showed that the peak subdued in the 30 ppt salinity treatment. However there was little difference control and 5 % clay + 10 mN Ca treatment as compared to original soil. This is similar to our findings in another study where we found more crystalline

Table 3.6 Distribution of ^{13}C in selected treatments of freshwater marsh soil determined by CP-MAS ^{13}C NMR spectroscopy

	Percentage distribution of carbon within indicated ppm. regions						
	0 – 45	45 – 60	60 – 110	100 – 145	145 – 160	160 – 185	188 – 220
Sample	Aliphatic	Methoxyl	Polysaccharide	Aryl	Phenolic	Carboxyl	Carbonyl
Initial soil	17.85	9.56	36.44	17.29	9.57	7.58	1.71
Control	17.71	9.68	35.41	18.11	9.23	8.31	1.56
5 % clay + 5 mM Ca	16.95	9.25	33.64	19.05	9.57	9.13	2.40
30 ppt salinity	17.57	8.98	35.28	19.15	9.11	8.18	1.73

polymethylenic (CH_2) n chains in salt marsh humic acid as compared to freshwater marsh humic acid fraction.

In spite of the significant variations observed in gaseous C productions among the treatments in FM soils, low or no significant differences was noticed in the organic C functional group distribution. This could indicate that under oxidized or moderately reducing conditions microbes may mineralize most forms of carbon.

3.4 Conclusions

The study revealed that cations, clay and salinity play major role in controlling organic C mineralization in wetland soils. Addition of multivalent cations such as Ca^{2+} to low bulk density highly organic to mineral deficient wetland soils and mineral soils low in multivalent cations decreases soil organic matter mineralization. However addition of Ca^{2+} to wetland soils rich in multivalent cations such as Fe^{3+} and Mn^{4+} may enhance the soil organic matter degradation since organic particles bonded to the Fe and Al bonds lead to the formation relatively more stable, polymerized complexes than those found with Calcium. Addition of clay to the mineral deficient freshwater marshes was found to be very effective in decreasing organic matter mineralization especially when multivalent cation such as calcium was also added. Salinity effect on decreasing C mineralization was more effective in freshwater marsh soils. Inspite of the significant decrease in total CO_2 production, salanity effect was slow on the heavy clay forested swamp soils. On the other hand, salinity was found to be very effective in significantly decreasing CH_4 productions from both freshwater marsh and forested swamp soils. Py-GC/MS characterization of organic C before and after treatments showed that salinity conditions may lead to relatively high levels of alkyl C in the soils.

Results suggest that increases in coastal submergence as result of global sea level rise and /or coastal restoration projects (river diversion) which directly or indirectly influence salinity, sedimentation and other processes will likely impact soil carbon mineralization. Such would also influence carbon sequestration and greenhouse gas production from coastal marsh soils.

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CHAPTER 4

CARBON GAS PRODUCTION UNDER DIFFERENT ELECTRON ACCEPTORS IN A FRESHWATER MARSH SOIL

4.1 Introduction

Carbon (C) gas emissions from wetlands are an important part of global C budget. Carbon gas production from wetland soil, primarily carbon dioxide (CO_2) and methane (CH_4) depends upon the rate of carbon deposition and the rate of mineralization (Smith et al., 2003). The rate of mineralization depends on a series of both biological and environmental factors and their interactions (Mackin and Swider, 1989; Canfield et al., 1993; Kristensen and Holmer, 2001). While almost all aerobic microorganisms have the enzymatic capacity to perform a total mineralization of complex organic substrates, the degradation of organic materials under anoxic conditions such as in wetland soils occurs by mutualistic consortia of bacteria and no single type of anaerobic bacterium seems capable of complete mineralization (Kristensen and Holmer, 2001). Soil temperature, water table level, availability of electron acceptors and the quality of organic substrates are the main local controls of CO_2 productions from wetlands (Keil et al., 1994; Mayer, 1994; Updegraff et al., 1995). Some of these environmental controls over microbial respiration in wetlands have been extensively studied (reviewed by Segers, 1998). For example, the importance of water table level, temperature, pH, soil carbon quality, and nutrients in controlling anaerobic mineralization of organic C to CO_2 and/or CH_4 in peat lands has been well established (Aller, 1994; Blodau, 2002). Warmer air and soil temperatures stimulate microbial activity resulting in higher CO_2 productions (Silvola et al., 1996). An increase in depth of water table above the soil decreases CO_2 production (Chimner and Cooper, 2003). In addition, an increase in soil pH enhances C mineralization and CO_2 production due to more

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Reprinted by permission of “Chemosphere” diversified microbial and faunal communities which, in turn, lead to the increased performance of enzymatic activity (Bottner, 1998). However, the relative importances of the various microbial pathways that contribute to anaerobic carbon mineralization in wetlands [i.e., denitrification, Fe (III) reduction, Mn (IV) reduction, sulfate reduction, and methanogenesis] have received considerably less attention (Keller and Bridgham, 2007).

The presence of alternative electron acceptors such as NO_3^- and SO_4^{2-} influences the mineralization and transformation of organic C under anoxic conditions, and affect the biogeochemistry of wetlands (Hunt et al., 2007; Keller and Bridgham, 2007). The very few studies have been conducted to assess organic matter mineralization under the availability of different electron acceptors reported conflicting results (Aerts and Toet, 1997; Kristensen and Holmer, 2001; Liikanen et al., 2002; Dettling et al., 2006). While thermodynamics predicts that NO_3^- is a more efficient electron acceptor than SO_4^{2-}, various studies showed little or no significant difference between the two in the mineralization in organic matter of lake sediment (Conrad, 1989; Liikanen et al., 2002). Moreover Kristensen and Holmer (2001), based on a microcosm study of fresh and aged diatoms and barley hay, reported that organic matter oxidation rates under SO_4^{2-} were similar to or even faster by up to two times than that of NO_3^- depending on the stage of studied diatoms and barley hay. On the other hand, addition of NO_3^- in addition to glucose, a labile C source, increased C mineralization in soils of minerotrophic fens but not in soils of ombrotrophic bogs (Dettling et al., 2006). Clearly the exact nature of CO_2 production and control in wetland soils is unclear. In addition, past studies have focused primarily on northern peat soils and observations that were based on shorter incubation periods of less than 30 d.

Louisiana is rich in wetlands, consisting of more than 729,000 ha with almost 90% being marshlands (NOAA, 1991). These wetlands, located on the Mississippi delta plain, are high in organic matter (as much as 80 %) and low in mineral matter content (Cahoon, 1994). The wetlands have been subsiding due to the compaction of recent alluvial sediments (Baumann et al., 1984). As a result, freshwater marshlands have been increasingly under the influence of seawater intrusion. The Mississippi river water is currently being diverted into these wetlands with the aim to decrease salinity, provide sediments and nutrients to combat the sea level rise (Lane et al., 1999). While the seawater intrusion brings in SO_4^{2-} and other ionic species, the diverted Mississippi river water carries elevated levels of NO_3^-, coming primarily from runoff of agricultural fields in the Mississippi valley (Donner, 2004). Previous studies conducted with Louisiana coastal wetlands showed that major C gas fluxes in saline marshes were lower than those in freshwater marshes whereas net apparent C accumulation rates were not much different between the two (DeLaune and Smith, 1984). Since freshwater marsh generally has a larger biomass production, this could imply that a certain degree of salinification of freshwater marshes with SO_4^{2-}-rich seawater might actually increase C sequestration. However, the specific role of NO_3^- and SO_4^{2-} in controlling these Louisiana freshwater marshes has not been evaluated. Since wetlands generally function as a long-term sink for atmospheric CO_2 and a source for atmospheric CH_4, it is important, via estimation of net changes in CO_2 and CH_4 production under anticipated conditions, to determine whether these wetlands would continue to act as net C sinks or sources of atmospheric C for future predictions (Moore, 2002; Dettling et al., 2006). Therefore, the objective of the present study was to evaluate the effect of NO_3^- and SO_4^{2-} on major C gas productions (CH_4 and CO_2) from a freshwater marshland that has been under the influence of Mississippi river diversion.

4.2 Materials and Methods

Coastal freshwater marsh soil located in the Louisiana’s Barataria basin was selected for this study. Barataria basin is one of the places where the Mississippi river water diversion project is taking place to avert the wetland loss due to sea water intrusion. The soil of the site was classified as Allemands muck (Clayey, smectitic, euic, hyperthermic, Terric Haplosaprists). Composite samples were collected from top 50-cm soil using a McCauly augur. Collected soil samples were immediately placed into zip-lock bags and transported to the lab on ice. Samples were thoroughly mixed and distinguishable plant roots, stems and leaves were removed. Selected physical and chemical properties of these soils are presented in Table 4.1. Soil pH, electric conductivity (EC) and bulk density, pore-water ionic concentrations were determined using moist field soil samples whereas other properties were determined using freeze-dried samples. Bulk density was measured by gravimetric method. Total C and N were determined by dry combustion using a TruSpec CN analyzer (LECO, St. Joseph, MI). Nitrate and SO_4^{2-} concentrations in pore water were analyzed using a Dionex ICS-2000 ion chromatography (Dionex, Sunnyvale, CA). In addition, total free Fe oxides and Mn oxides were estimated using citrate-bicarbonate-dithionite extraction (Phillips et al., 1998).

Anaerobic incubation was carried out to evaluate impact of electron acceptors on C gas production from the freshwater marsh soil. Ten-gram field moist soil samples (containing about 200 mg organic C) were weighed into a series of 400 mL glass jars followed by 130 mL of deionized water. Sample slurries in the jars were flushed with N_2 gas (99.999 % purity) for 30 min to remove any dissolved/ trapped oxygen. The sample jars were then immediately closed with lids and sealed with silicone. Additional flushing of headspace of each jar was carried out with N_2 gas for 20 min by inserting an inlet and outlet needles through the rubber septa in the lid.

Treatment solutions containing NO_3^- or SO_4^{2-} were then added through a syringe after they are degassed and followed by adding deionized H_2O to bring the added overall volume to 150 mL. The final treatment concentrations consisted of 2 levels of nitrate (3.2 and 5 mM) and sulfate (2 and 5 mM) along with a control (deionized H_2O). Both sulfate and nitrate were added as salts of potassium (KNO_3 and K_2SO_4). The effect of NO_3^- and SO_4^{2-} was evaluated at similar molar concentrations (5 mM) as well as similar equivalent concentrations. For the latter, the 3.2 mM NO_3^- treatment was made to compare to 2 mM SO_4^{2-} based on equal oxidation capacity to transform NO_3^- to N_2 and SO_4^{2-} to H_2S, respectively. In addition, KCl was added to the jars to bring a final salt concentration of 0.87 g L^{-1} so that the effect of electron acceptors was compared at similar ionic strength (salinity). Higher soil to treatment solution ratio of 1:150 was used to ensure sufficient availability of added electron acceptors over the entire period of incubation. Each treatment of incubation was carried out in triplicates, and all incubations were conducted in dark at room temperature (22±0.5 ^{o}C) for 214 d.

Table 4.1. Phyico-chemical characteristics of soil

Parameter	Mean
Organic matter	747.6 g kg^{-1}
Organic C	377.5 g kg^{-1}
Total N	22.3 g kg^{-1}
C/ N ratio	17.1
Bulk density	0.04 g cm^{-3}
Water content	95.3 %
pH	5.5
Electric conductivity	0.6 mS m^{-1}
Pore water SO_4^{2-}	66.7 μM
Pore water NO_3^-	1.6 μM
Fe[†]	730 mg Kg^{-1}
Mn[†]	21 mg Kg^{-1}

[†] Estimated by Citrate-Bicarbonate-Dithionate digestion

During the incubation, the headspace of each jar was sampled periodically and analyzed for CO_2, and CH_4 concentrations. To sample gasses in the head space of the jar, 2 mL of O_2- free N_2 was injected into the jar and same amount was taken out for the analysis. The measured concentrations in the gas samples were corrected for this amount of added N_2 gas. In addition, prior to sampling, each sample jar was shaken vigorously before injection to remove gas bubbles trapped within sample slurry. All sample jars were intermittently flushed for 10 min using ultrapure N_2 gas flowing at ~ 100 mL per min throughout the incubation period to avoid the excessive accumulation of CO_2 and CH_4 gasses in the headspace. Gas samples were analyzed simultaneously for CO_2, and CH_4 concentration by gas chromatography (Varian 3800, Varian Inc., Palo Alto, CA) equipped with a flame ionization detector (for CO_2 and CH_4) using a 27.5 m x0.53 mm long capillary column coated with poraplot-Q maintained at 60 ^{o}C to separate the gasses. The injector, methanizer, and detector temperatures were kept at 60 ^{o}C, 450 ^{o}C, and 250 ^{o}C, respectively. The GC was calibrated using the CH_4, and CO_2 standards purchased from Scotty specialty gases (Scotty Gas, Plumsteadville, PA). The CO_2 and CH_4 productions were expressed as μg L^{-1} based on the measured concentrations in the headspace with the correction of Bunsen coefficient for the dissolved gases (Bodelier and Frenzel, 1999). Headspace concentrations of N_2O were also periodically determined during the incubation for different treatments. Nitrous oxide was analyzed using gas chromatography equipped with electron capture detector. At the end of the incubation, sample of soil solution from each jar was collected by passing through a 0.45 μm membrane filter under N_2 conditions was analyzed for SO_4^{2-}, NO_3^- and CH_3COO^- remaining in soil solution using a Dionex ICS-2000 ion chromatography (Dionex, Sunnyvale, CA) (Carvalho et al., 1995). Nitrate, SO_4^{2-} and CH_3COO^- were separated on an anion exchange column (Dionex AS18 IonPac column, 4 x 250 mm) fitted with AG 18 guard column. All the anions were eluted under isocratic conditions using KOH (32.5 mM).

In addition, the amount of carbon mineralized under each treatment was fit to an exponential decay model of the form $y = a+e^{-kt}$ (Liao et al., 2006) and the mineralization rate constant (k) was derived. All statistical analysis of the collected data was carried out using the SAS software package version 9.1 (SAS Institute, 2004).

4.3 Results and Discussion

The cumulative productions of CO_2 and CH_4 from freshwater marsh soil at each sampling event throughout the incubation are presented in Fig. 4.1 and 4.2, respectively. The total productions of CO_2 and CH_4 and C mineralization rate constant (k) for the entire incubation under SO_4^{2-} and NO_3^- treatments are compared in Table 4.2. In general, CO_2 production from freshwater marsh soil was little influenced by SO_4^{2-} treatment (Fig. 4.1a) but significantly reduced by NO_3^- treatment especially at 3.2 mM NO_3^- as compared to that of the untreated sample (control) (Fig. 4.1b). At both equal equivalent (2 mM SO_4^{2-} vs. 3.2 mM NO_3^-) and equal molar (5 mM SO_4^{2-} vs. 5 mM NO_3^-) concentrations, NO_3^- repressed CO_2 production whereas SO_4^{2-} showed no impact or a slightly stimulated production of CO_2. These results were reflected in both cumulative and total productions (Fig. 4.1 and Table 4.2). On the other hand, more significant reduction of CO_2 production at 3.2 mM NO_3^- than 5 mM could arise from substrate shock due to the relatively high concentrations of these electron acceptors (Mavinic et al., 2005). The similar inhibitory effect of NO_3^- on CO_2 production was also observed in a 65 d anaerobic incubation of similar freshwater marsh soil at 1:10 soil to solution ratio and equivalent concentration of 1 mM NO_3^- under a continuous flow of N_2 gas system with CO_2 trapped using NaOH (the cumulative amount of CO_2-C released was 3099±222 $\mu g\ L^{-1}$ for the control whereas it was only 2252±94 $\mu g\ L^{-1}$ for 1 mM NO_3^-). These results strongly indicate negative influence of NO_3^- on CO_2 production from the freshwater soil under anaerobic condition. Due to the strong NO_3^- impact on CO_2 reduction, overall organic C degradation was also significantly low as indicated by the smaller mineralization rate constant (k) in the presence of NO_3^- especially at 3.2 mM (Table 4.2).

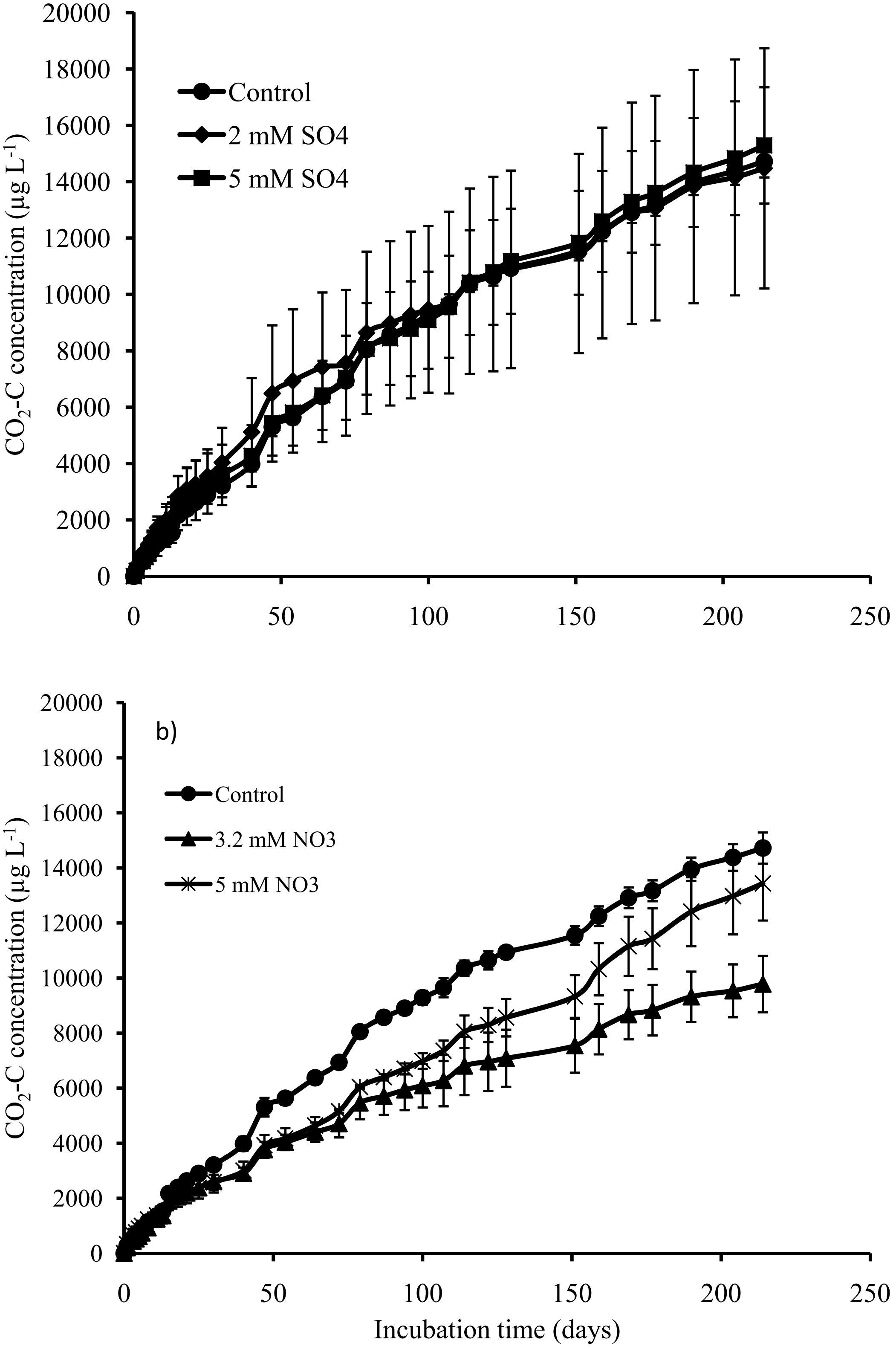

Fig. 4.1 CO$_2$ production under different concentrations of a) SO$_4^{2-}$ and b) NO$_3^-$

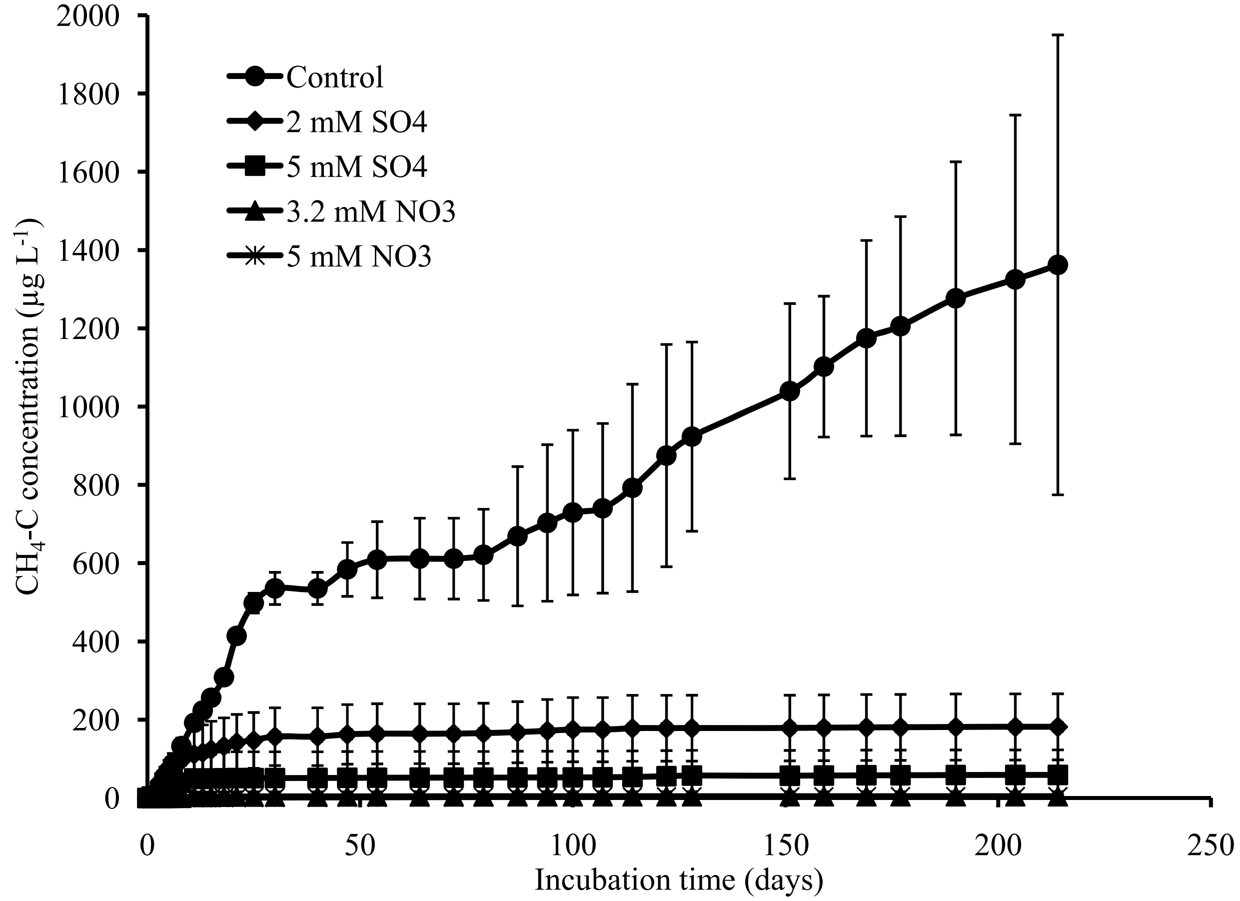

Fig. 4.2 CH_4 production under different SO_4^{2-} and NO_3^- concentrations

Table 4.2. Total productions of CH_4 and CO_2 and C mineralization rate constants treated with different electron acceptors

Treatment	CO_2-C[†]	CH_4-C[†]	k[‡]
	--------------------- μg L^{-1}	----------------------	----- mg Kg^{-1} day^{-1}----
Control	14723 (566)a	1362 (588)a	0.455a
2 m M SO_4^{2-}	14477 (4263)a	182 (84)b	0.435a
5 mM SO_4^{2-}	15293 (2062)a	59 (64)b	0.455a
3.2 mM NO_3^-	9782 (1026)b	4 (5)b	0.290b
5 mM NO_3^-	13437 (1348)ab	3 (2)b	0.365ab

[†] Values in parenthesis are standard deviations; Values in columns, followed by the small same letter, are not significantly ($\alpha = 0.05$) different as determined by the LSD test.
[‡] k, Carbon mineralization rate constant.

Different results have been reported in limited studies on how NO_3^- along with other electron acceptors affects CO_2 productions under anoxic conditions (Liikanen et al., 2002; Dettling et al., 2006; Keller and Bridgam, 2007). The evaluation of electron acceptors on C mineralization in lake sediments of a Finland hyper-eutrophic lake under a short-term incubation showed that there was no significant difference between NO_3^- and SO_4^{2-} in influencing C gas productions (Liikanen et al., 2002). Nitrate and SO_4^{2-} were also found to have little impact on CO_2 production from northern bog soils but they increased CO_2 production from New York fen soils (Dettling et al., 2006). Studies on anaerobic C mineralization rates of diatoms and hay particulates with alternative electron acceptors indicated that SO_4^{2-} generated either a similar mineralization rate as NO_3^- or twice that of NO_3^- (Kristensen and Holmer, 2001). In comparing different soils, both NO_3^- and SO_4^{2-} were found to slightly elevate CO_2 production from a Louisiana saline marsh soil but no statistical difference between the two electron acceptors were observed (D'Angelo and Reddy, 1999). Clearly our results are different from those reported for wetland soils in the current literature in that NO_3^- decreased CO_2 production under anoxic conditions. Moreover, in a mechanistic study, NO_3^- was found to inhibit glucose turnover and reduce acetate production in rice soils under anaerobic incubation whereas SO_4^{2-} and ferric ion were not (Chidthaisong and Conrad, 2000). The same study showed, however, overall CO_2 production resulting from glucose turnover was similar among NO_3^-, SO_4^{2-}, and the untreated samples but enhanced from acetate turnover by both electron acceptors especially SO_4^{2-} as compared to the untreated control (Chidthaisong and Conrad, 2000). In our study, we did not add simple sugars to the soil and monitor their conversions since the focus was on the transformation of existing organic C in the freshwater marsh soil. However, note the much lower interstitial concentration of acetate at the end of the incubation observed with NO_3^- treatment than with SO_4^{2-} treatment (Table 4.3), which could indicate acetate inhibition. This could suggest that, in our study, the presence of NO_3^- reduced the CO_2 production that would otherwise be normally associated with fermentation process especially acetogensis. It should be pointed out that N as a nutrient control has been found to reduce CO_2 production from field application of varying forms of N fertilizer and in aerobic incubations, and reasons behind have been suggested as lowering pH impact due to acidity generated from the transformation of NH_4^+-N as well as NO_3^- impact on .OH radicals that likely affect decompositions of recalcitrant substrates such as lignin (Fog, 1988; Aerts and Toet, 1997; Al-Kaisi et al., 2008). In our study, however, It is unlikely that pH factor was the cause for CO_2 production reduction as result of NO_3^- addition since the pH in treatment-soil suspensions was increased slightly (<0.3 pH unit) rather decreased throughout experiments. While we did not measure NO_3^- impact on .OH radical production, our previous study did indicate that lower denitrification rates were associated with phenolics especially phenolic aldehydes and ketonics in these wetland soils (Dodla et al., 2008). Phenolics have been shown to be toxic or inhibitory to other decomposer microorganisms (Fog, 1988).

Table 4.3 Initial added and final Concentrations of SO_4^{2-} and NO_3^- and CH_3COO^-

Treatment	Initial added concentration		Final concentration[†]		
	SO_4^{2-}	NO_3^-	SO_4^{2-}	NO_3^-	CH_3COO^-
	-------- µM--------- -----------		------------------------- µM --------------		
Control			11.5 (3.1)	ND	17.5 (0.1)
2 mM SO_4^{2-}	2000		1561 (115)	ND	130 (8.0)
5 mM SO_4^{2-}	5000		4549 (207)	ND	116 (9.7)
3.2 mM NO_3^-		3200	96.9 (11.5)	989 (96.9)	30.7 (0.5)
5 mM NO_3^-		5000	245 (168)	2455 (166)	21.0 (0.7)

[†] values in parenthesis are standard error of means

Methane production was significantly reduced by additions of NO_3^- or SO_4^{2-} beginning from day 2 of the incubation as compared to the control (Fig. 4.2). Specifically, the NO_3^- decreased CH_4 production by more than 99 % at both 3.2 and 5 mM concentrations whereas the SO_4^{2-} treatments decreased it by 78 % and 90% at 2 mM and 5 mM SO_4^{2-} respectively, as compared to the control (Fig. 4.2 and Table 4.2). The complete inhibition of CH_4 production by NO_3^- could be due to the inhibitory effect of denitrification products such as nitrite, NO and N_2O on the methanogens in addition to the competitive suppression effect of NO_3^- on their activity (Klüber and Conrad, 1998). Periodical measurements of N_2O concentrations in headspace during the incubation indicated that appreciable amount of N_2O was released in NO_3^- treatments, confirming NO_3^- reduction. Therefore, the toxicity effect would be expected. On the other hand, since acetate production was likely inhibited by the presence of NO_3^- (Chidthaisong and Conrad, 2000), the substrate source for acetoclastic methagenesis would be much reduced besides the toxicity to methanogens. Although both toxicity and suppression were likely at work, toxicity impact was probably the dominant cause in yielding a complete and immediate inhibition of CH_4 production. On the other hand, SO_4^{2-} was not found to be toxic to methanogens (Klüber and Conrad, 1998). Sulfate reducers would likely compete with methanogens for their common substrate hydrogen and efficiently dominated the methanogens, leading to the decreased CH_4 productions (incomplete inhibition) as seen in Fig. 2. The fact that acetate levels were significantly higher under SO_4^{2-} than under the NO_3^-treatment and the control suggest that metabolism of acetate under SO_4^{2-} was slow or somewhat inhibited (Table 4.3). While the low acetate level in the control could be due to the consumption through efficient acetoclastic methanogenesis as indicated by significant CH_4 productions and the low acetate level in NO_3^- -treated samples due to inhibition as previously discussed, the higher level of acetate under SO_4^{2-} treatment likely suggest inefficient use of acetate in sulfate reduction in these freshwater marsh soils. By calculation, this amount of acetate accumulated under SO_4^{2-} treatment was found to account for the equivalent amount of CH_4 emitted in the control. We did not characterize microbial community under these electron acceptors but addition of SO_4^{2-} to soils with low populations of sulfate reducers relative to methanogens could result in the reduction in CH_4 without causing a drastic decrease in acetate (Chidthaisong and Conrad, 2000). Acetate-utilizing sulfate reducers have been reported present only as spores and are not active in acetate consumption (Achtnich et al., 1995). On the other hand, the accumulation of acetate under sulfate was also linked to the inhibition of syntrophic acetate oxidation coupled via interspecies H_2 transfer between acetoclastic methanogens and H_2-utilizing sulfate reducers (Achtnich et al., 1995). Since acetate-utilizing sulfate reduction would produce CO_2 and it was a fact that in this study the CO_2 production under SO_4^{2-} was not inhibited as opposed to that produced under the control (Fig. 4.1 and Table 4.2), the likely cause for acetate accumulation under SO_4^{2-} could be the inhibition of syntrophic acetate oxidation with coupling interspecies H_2 transfer. Further study is needed to confirm this phenomenon.

The reduction in CH_4 production under SO_4^{2-} would have important implication to climate change since global warming potential (GWP) of CH_4 is 21 times more than CO_2. Although SO_4^{2-} has less effect on the inhibition of CH_4 production as compared to NO_3^-, the reduction of SO_4^{2-} could be preferred over NO_3^- in wetlands since the reduction of NO_3^- could likely release appreciable amounts of N_2O if denitrification is not complete and GWP of NO_3^- is 300 times of CO_2 (Dodla et al., 2008).

The molar ratio of CO_2/CH_4 productions during the incubation ranged from 7 to 17 with an overall total average of 13. This ratio was smaller than 16-28 generally reported for northern peat soils (Dettling et al., 2006), suggesting that these freshwater marsh soils of the Gulf Coast would, in general, favor slightly higher CH_4 productions from methanogenesis as compared to those of northern peat soils under anaerobic conditions. Higher CO_2/CH_4 ratio would suggest favorable and vigorous CO_2 production from the combined process of fermentation, methanogenesis itself, and terminal metabolism with electron acceptors (Dettling et al., 2006). It is interesting to note that based on the initial NO_3^-, SO_4^{2-}- as well as total free Fe and Mn as estimated using citrate bicarbonate dithionite digestion, the potential contribution from the uses of these alternative electron acceptors were accountable for only 5% of C mineralized in the control sample (Tables 4.1 and 4.4). This suggests that as much as 95% of CO_2 productions were unexplained in these freshwater marsh soils. By contrast, based on difference between the initial added and final NO_3^- and SO_4^{2-} concentrations (Table 4.3), it was found that the actual consumption of NO_3^- would potentially explain 100% and 53% of CO_2 productions for 3.2 mM and 5 mM NO_3^- treatments, respectively but the actual SO_4^{2-} consumption would account for only 33 % and 32% of CO_2 productions for 2 and 5 mM SO_4^{2-} treatments, respectively (Table 4.4). These results suggested that other processes may also be involved in the production of CO_2 especially in the control sample.

Table 4.4 Estimated maximum contribution of NO_3^- and SO_4^{2-} to the total respiration in different treatments

Treatment	Estimated		Actual Consumption		Maximum % contribution	
	NO_3^-	SO_4^{2-}	NO_3^-	SO_4^{2-}	NO_3^-	SO_4^{2-}
	-------μM--------		---------μM---------			
Control	2490	1556	1.6	55.2	0.1	3.5
2 mM SO_4^{2-}	2448	1530	1.6	505	0.1	33.0
3.2 mM NO_3^-	1655	1034	1668		101	0.0
5 mM SO_4^{2-}	2587	1617	1.6	518	0.1	32.0
5 mM NO_3^-	2273	1421	1198		52.7	0.0

Thermodynamically, nitrate, which has higher redox potential under anaerobic condition, is expected to be reduced first and followed by MnO_2, Fe(III), SO_4^{2-}, and finally the direct reduction of CO_2 to CH_4 (Ponnamperuma, 1972; Zehnder and Stumm, 1988). However, various studies have shown that these processes could occur simultaneously due to the heterogeneity of wetland systems (Yavitt and Lang, 1990; Keller and Bridgham, 2007). Our results imply that the major reduction processes of alternative electron acceptors were not able to account for the overall observed C mineralization particularly CO_2 production under clearly anaerobic conduction as indicated by methagenesis. A similar situation has been also reported for northern peat soils. For instance, as much as 85% of carbon mineralized in northern bog and fen soils was unexplained by known and measured processes such as reductions of NO_3^-, Mn (IV), Fe(III), SO_4^{2-} as well as methanogenesis (Keller and Bridgham, 2007). Such unexplained C mineralization was speculated to results from fermentation or possibly from the use of organic molecules as other alternative electron acceptors besides those mentioned above (Keller and Bridgham, 2007). Quinones extracted from peat humic substances have been found to be able to oxidize H_2S and acetate (Coates et al., 1998; Scott et al., 1998). Further Lovely et al. (1996) showed that humic substances help microorganisms to utilize less soluble electron acceptors such as iron oxides by acting as electron shuttles between microorganisms and iron oxides. We did not test the impact of humic acids as electron acceptor on carbon mineralization in these soils but could not rule out the possibility, and further work is needed to elucidate the mechanisms of alternative electron acceptors including possible role of humic acids on C mineralization. On the other hand, even though the amount of carbon mineralized was varied among the treatments all of them were fitted well with simple exponential decay model.

4.4 Conclusions

This study demonstrated that under anaerobic conditions the presence of NO_3^- (at 3.2 mM) significantly decreased CO_2 productions from freshwater marsh soil whereas SO_4^{2-} did not. It was speculated that the presence of NO_3^- reduced the CO_2 production that would otherwise be normally associated with fermentation process especially acetogensis. The latter was based on observed lower acetate concentration in interstitial water under NO_3^- as compared to acetate accumulation under SO_4^{2-} presence. On the other hand, both NO_3^- and SO_4^{2-} treatments decreased CH_4 production but the NO_3^- almost completely inhibited CH_4 production (>99%) whereas the SO_4^{2-} treatments reduced CH_4 production by 78-90%. Between CO_2 and CH_4 productions, the fresh marsh soil showed CO_2/CH_4 molar ratio of 13 indicating favor of CO_2 production under anaerobic conditions. Nonetheless, 95% C mineralized in the soil was unexplained by the potential contributions of known reduction processes of electron acceptors, which require further elucidation.

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CHAPTER 5

DENITRIFICATION POTENTIAL AND ITS RELATION TO ORGANIC CARBON QUALITY IN THREE COASTAL WETLAND SOILS

5.1 Introduction

Denitrification is an important tool in processing nitrate rich effluents and reducing the contribution of nitrogen to eutrophication in wetlands and aquatic environments. With the presence of both aerobic and anaerobic conditions, wetlands are especially effective in NO_3^- removal through denitrification and plant uptake (White and Reddy, 2003; DeLaune et al., 2005). Various factors such as soil hydrology, soil chemistry and vegetation type affect the denitrification process (Cornwell et al., 1999). Generally, in wetlands denitrification rate is limited by the availability of NO_3^- and organic C (Cornwell et al., 1999; Greenan, et al., 2006). Various studies have shown that when sufficient NO_3^- is available to induce denitrifying activity, an increase in soil organic matter (SOM) increases soil denitrification rate (Bijay-Singh et al., 1988; Gale et al., 1993). Addition of simple organic C such as glucose to organic C-limited soils drastically increases denitrification rates (Garcia-Montiel et al., 2003). Total SOM does not necessarily indicate the capacity of a soil to denitrify. Addition of glucose to a forest soil, despite high organic matter content, increased denitrification rate compared to a grassland soil containing substantially lower amounts of total organic matter (Groffman et al., 1991). In addition, denitrification potential in vegetative filter strips was found to vary depending on vegetation type (Groffman et al., 1991). These results suggest that not all the organic carbon present in soil can be utilized by the denitrifying bacteria, and both quantity and quality could be important in governing the denitrification process (Hill and Cardaci, 2004).

Past studies have related denitrification rate to physical and chemical fractions of organic C such as dissolved organic C (DOC) (Hill et al., 2000; Stow et al., 2005), anaerobically or

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aerobically mineralizable C (Davidson et al., 1987), and acid soluble carbohydrates (Bijay-Singh et al., 1988; Hume et al., 2002; Hill and Cardaci, 2004). Such studies have suggested a complex relationship between C transformation and denitrification. For instance, DOC was previously believed to be labile and easily utilized by microbes based on positive relationship between N_2O emissions and DOC in the stream water (Stow et al., 2005). Other evidence indicated that the differences in DOC levels of wetland soils and streams had no significant influence on denitrification rates (Davidsson and Stahl, 2000; Bernhardt and Likens, 2002). Recently, Sirivedhin and Gray (2006) reported that sediments treated with two different pore waters collected from different sites but having similar DOC concentrations exhibited significantly different denitrification rates. Although various strains of denitrifying bacteria are able to use aromatic C (Nozawa and Maruyama, 1988), plant litter bearing high concentration of soluble phenolics and low holocellulose quotient (holocellulose / lignocellulose) was found to inhibit the decay of overall organic matter (Bridgham and Richardson, 2003). Under sub-oxic condition where NO_3^- was an alternative electron acceptor, it was shown that denitrifying microbes preferentially consumed nitrogen-rich organic C (Van Mooy et al., 2002). All these results indicate that chemical structure, rather than the solubility, may determine organic C quality and availability which may in turn govern the denitrifying bacteria in soils. On the other hand, labile organic C (LOC) fraction is deemed to include all organic C of SOM, regardless of solubility, that are able to be used by microbes, and its determination, however, has often been limited to a specific short-term incubation process (Davidson et al., 1987; McLauchlan and Hobbie 2004). It is unlikely that this short-term LOC can reveal the types or molecular structures of organic C within SOM that are preferred by microbes such as denitrifying bacteria. Despite the importance of chemical structure, there is little information available on how molecular composition of organic matter affects denitrification in wetland soils (Van Mooy et al., 2002).

Coastal Louisiana has experienced rapid wetland losses due primarily to subsidence and sea level rise (Baumann et al., 1984). Mississippi river water has been diverted to Louisiana coastal marshes to help restore these impaired wetlands through reducing elevated salinity levels (caused by salt water intrusion) and providing a new sediment source (DeLaune et al., 2005). Wetlands within the Barataria Basin estuary have been receiving diverted water since 2000 (LDNR, 2005). The diverted river water contains elevated nitrate levels coming primarily from agricultural run-off. Approximately 90,000 metric tons of nitrate in Mississippi River water was added to Gulf of Mexico between 1991 and 1992 alone (DeLaune et al., 2001). The elevated NO_3^- levels in the Mississippi River water are considered one of the main causes for hypoxia in the Gulf of Mexico (Rabalais et al., 1996). The impact of elevated NO_3^- levels in the diverted Mississippi River water on the health of these coastal wetlands and their capacity to assimilate NO_3^- has become an increasingly important topic of study. Understanding the relationship between the denitrification rate and the organic C quality could further elucidate the response of different wetlands to increased nitrogen loadings. In addition, denitrification and its kinetics over the depth of soil/ soil profile are poorly documented (Elmi et al., 2005). Therefore, the objectives of this study were to determine potential denitrification rate (PDR) and to characterize the relationship between denitrification rate and molecular C composition of SOM in three selected wetland soils in the Louisiana Barataria Basin estuary.

5.2 Materials and Methods

5.2.1 Study Sites and Soil Analysis

Three different wetland ecosystems, namely, bottomland forest swamp (FS), freshwater marsh (FM), and saline marsh (SM) located in the Louisiana Barataria Basin estuary were selected for this study (Fig.5.1). Mississippi River water has periodically diverted in to the northern region of the Basin as part of effort to restore these wetlands (LDNR, 2005). The major vegetation types in forest swamp, freshwater marsh and saline marsh are *Taxodium distichum*, *Sagittaria lancifolia*, and *Spartina alterniflora* respectively. The soils at the FS, FM and SM sites were Barbary muck (Very-fine, smectitic, nonacid, hyperthermic Typic Hydraquents), Allemands muck (Clayey, smectitic, euic, hyperthermic, Terric Haplosaprists), and Timbalier muck (Euic, hyperthermic, Typic Haplosaprists), respectively.

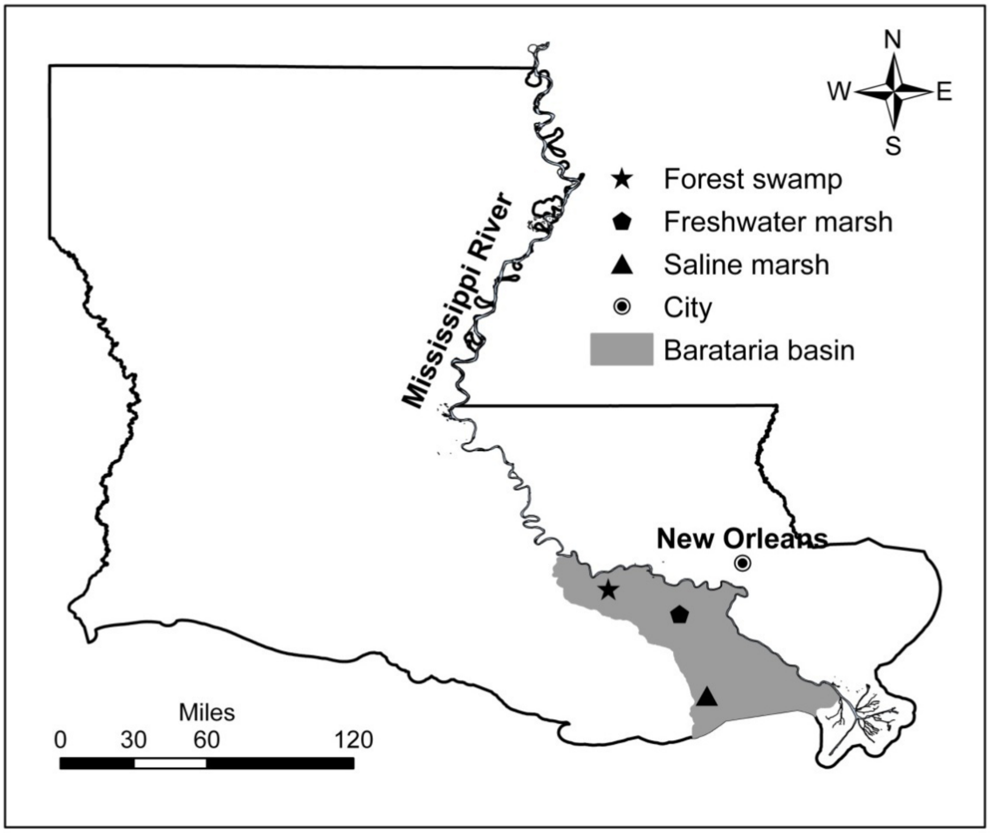

Fig. 5.1 Location of sampling sites selected for this study

Fifteen composite soil samples from different soil horizons at each site were collected. Collected soil samples were immediately placed into zip-lock bags and transported to the lab on ice. Samples were thoroughly mixed, a portion was freeze-dried and the reminder was stored frozen or directly analyzed. Selected physical and chemical properties of these soils are presented in Table 5.1. Soil pH, electric conductivity (EC) and bulk density were determined using field moist soil samples whereas other properties were determined using freeze-dried samples. Bulk density was measured by gravimetric method. Total C and N were determined by dry combustion at 900°C using a TruSpec CN analyzer (LECO, St. Joseph, MI). Preliminary experiment based on 1 N HCl treatment of samples indicated that there was little or no inorganic C present in these soils. Therefore, the measured total C was considered as total organic C (TOC). Pore waters were separated from field moist samples by centrifuging, and major anions in pore waters were analyzed using ion chromatography (DX-100 model, Dionex Corporation, Sunnyvale, CA). In addition, labile organic carbon (LOC) was estimated by determining the amount of organic C mineralized under an anoxic incubation of 10 g field moist soil with 10 mL of 10 mg L^{-1} NO_3^- -N for 5 days.

5.2.2 ^{13}C NMR Characterization

Molecular C composition of wetland SOM was determined using solid-state ^{13}C nuclear magnetic resonance (NMR). The solid-state ^{13}C NMR technique was chosen over other structural characterization techniques for its non-destructive and potentially quantitative examinations of the chemical structural composition of SOM in-situ, without prior extraction and/or degradation procedures (Nelson and Baldock, 2005; Alarcon-Gutierrez et al., 2008). Freeze-dried soil samples were ground to pass through a 125µm sieve before NMR analysis. NMR spectra were obtained using a Bruker Avance wide bore 400 MHz instrument with a double resonance 4mm CP-MAS probe. The cross polarization total sideband suppression magic angle spinning (CP-TOSS-MAS) technique was used to acquire the ^{13}C NMR spectra. The prepared soil samples were packed into a cylindrical zirconium rotor and spun at 5 kHz. The spectra were collected with a contact time of 2 msec, and a recycling time of 1 second. A total of 72k scans were collected for each sample. Chemical shifts were expressed relative to tetramethylsilane (TMS). (See Appendix A for NMR spectra of FM soil profile). The peaks between 0 -230 ppm chemical shift range were assigned to various C moiety groups according to Malcolm (1989) as aliphatic C

Table 5.1 Physico-chemical properties of selected wetland soils[†‡].

Site	Location	Depth	Horizonation	pH	EC	BD	TOC	Total N	Molar C:N
		cm			dS m^{-1}	g cm^{-3}	-------g kg^{-1}-----		
Forest swamp	29.91165°N	0-25	A	6.2	1.1	0.24	75.4	5.5	16.0
	90.72812°W	25-50	Cg1	6.1	1.3	0.44	56.3	3.7	17.8
		50-75	Cg1	6.1	1.5	0.49	41.7	2.5	19.5
Freshwater marsh	29.77195°N	0-25	Oe	6.1	2.7	0.07	371	24.5	17.7
	90.29026°W	25-50	Oa1	6.4	3.5	0.19	212	11.8	21.0
		50-100	Oa2	6.6	4.5	0.16	312	18.6	19.5
Saline marsh	29.25549°N	0-50	Oa1	7.3	38.9	0.16	159	8.7	21.3
	90.15680° W	50-75	Oa2	7.4	42.5	0.20	119	6.7	20.7
		75-100	Oa3	7.3	34.6	0.11	255	14.3	20.8

[†] Standard errors were ≤ 5% for all measured parameters.

[‡] EC, electrical conductivity; BD, bulk density; TOC, Total organic carbon.

(0-50 ppm), polysaccharides (50-108 ppm), aromatic C (108-165 ppm), carboxyl C (165-190 ppm), and aldehydes and ketonic C (190-230 ppm). Chemical shift range 108-165 ppm was further divided into aryl C (108 -140 ppm) and phenolic C (140 – 165 ppm) (Malcolm, 1989; Cook and Langford, 1998). According to Wong et al. (2002), ^{13}C CPMAS NMR spectra are intrinsically semi-quantitative and the peak areas represent the relative amount of each functional group. Therefore relative percentages of different C moieties of SOM for each sample were calculated based on integrations of specific spectral regions that correspond to a particular C of structural coordination against the sum of overall spectra as 100 %. Using this data, the amounts of different types of C compounds in the soil were calculated from TOC.

5.2.3 Denitrification Experiment

Denitrification rates were determined using the acetylene blockage technique (Tiedje, 1994). It has been reported that PDR measured using disturbed soils by the acetylene blockage technique correlated well with that determined using core samples by the same method (Hunt et al., 2003). Samples of 10 g field-moist soils were placed in 44-mL vials and treated with 10 mL solutions containing different concentrations of NO_3^- -N, 0 (control), 2 and 10 mg L^{-1}as KNO_3. All treatments were replicated six times of which three replicates were treated with acetylene and three without. The use of acetylene allowed estimation of the potential denitrification whereas non acetylene replicates were for estimating incomplete denitrification (Hunt et al., 2007). Vials were closed and mixed thoroughly. The head space of each sample vial was then flushed with N_2 to create anaerobic conditions. For the acetylene treated samples, about 15% of the head space volume was replaced with acetylene to stop the reduction of N_2O to N_2 so that N_2O could be quantified. Sample vials were kept in the dark at room temperature and incubated for 5 days since the added acetylene gas can become a source of carbon after 7 days (Hill et al., 2004). The relatively longer incubation times (5 days) were employed so that the kinetics of denitrification process could also be evaluated. The head space was sampled 50 µL each time at intervals of 0, 6, 12, 24, 48, 72 and 120 h of incubation.

The N_2O concentration in head space was determined using a Shimadzu GC-14A gas chromatography (GC) equipped with a 10-mCi ^{63}Ni electron capture detector (ECD). The GC injector, column and detector temperatures were 65, 40, and 340^{o}C, respectively. The amount of N_2O present in the headspace of a given vial with acetylene treatment represents the potential for the amount of NO_3 that can be denitrified. The amount of N_2O in the head space of non acetylene-treated vials represents the potential for incomplete denitrification. Potential denitrification rate (PDR) was estimated from the steepest portion of the curve plotted between total amount of N_2O released (with acetylene treatment) and incubation time (White and Reddy, 2003).

5.2.4 Statistical Analysis

All statistical analysis of the collected data was perfomed using the SAS software package version 9.1 (SAS Institute, 2004). Simple linear regression and multiple regression analyses were performed to relate PDR with LOC and different carbon moieties, whereas GLM and t-test were carried out on PDR and other soil property parameters among different wetlands.

5.3 Results and Discussion

5.3.1 Physical and Chemical Characteristics of Sites

Soils collected from the Barataria Basin exhibited substantially different physico-chemical properties among the different wetland sites as well as within each soil profile (Table. 5.1). Saline marsh soil generally had a higher pH (7.3-7.4) than the FM (6.1-6.6) and FS (6.1 to 6.2) soil. Within each profile, soil pH varied only slightly. Soil electric conductivity (EC) differed among the three sites with a maximum of 42.5 dS m^{-1} in SM soil to a low of 1.06 dS m^{-1} in FS soil, but within a given profile, there was little variation in EC. Soil bulk density was similar in the FM and SM soils but was higher in the FS soil profile. Among the three ecosystems, FM soil contained the highest TOC (212 to 371 g kg^{-1}), followed by SM (119 to 255 g kg^{-1}) and FS (41.7 to 75.4 g kg^{-1}). Total organic carbon varied appreciably within soil profiles and there was no trend with depth except for the FS where total C decreased with depth. These variations could be attributed to different interactions between sediment deposition and vegetation during the development of these wetland soils. The C: N ratios in these profiles were generally higher in SM (17.7-18.2) as opposed to FM and FS profiles (15.1-17.9 and 13.7-16.8, respectively). These differences in chemical and physical properties among and within soil profiles could influence denitrification characteristics.

5.3.2 Denitrification within Soil Profile and among Wetlands

Figures 5.2 and 5.3 shows total N_2O-N levels in the headspace during the denitrification incubation experiment of wetland soils treated with 2 and 10 mg L^{-1} NO_3^--N, respectively. Potential denitrification rate (PDR) was estimated from the steepest portion of the curve between total amount of N_2O and the incubation time (White and Reddy, 2003). Considering the large variation in the bulk density within and among the sites, PDR was evaluated on the basis of both unit dry weight and unit field volume (Table 5.2).

Large differences existed in denitrification potential and kinetics among and within the wetland soil profiles (Figs. 5.2 and 5.3). For both NO_3^--N treatment concentrations, FM showed greater denitrification potential at all profile depths than for the SM and FS soils. The FM soil also exhibited faster kinetics of denitrification, especially at the 2 mg L^{-1} NO_3^--N concentration. The higher potential and faster kinetics of denitrification exhibited by the FM soil profile than those by the SM and FS soil profiles on dry weight basis are reflected in the estimated PDR values (Table 5.2).

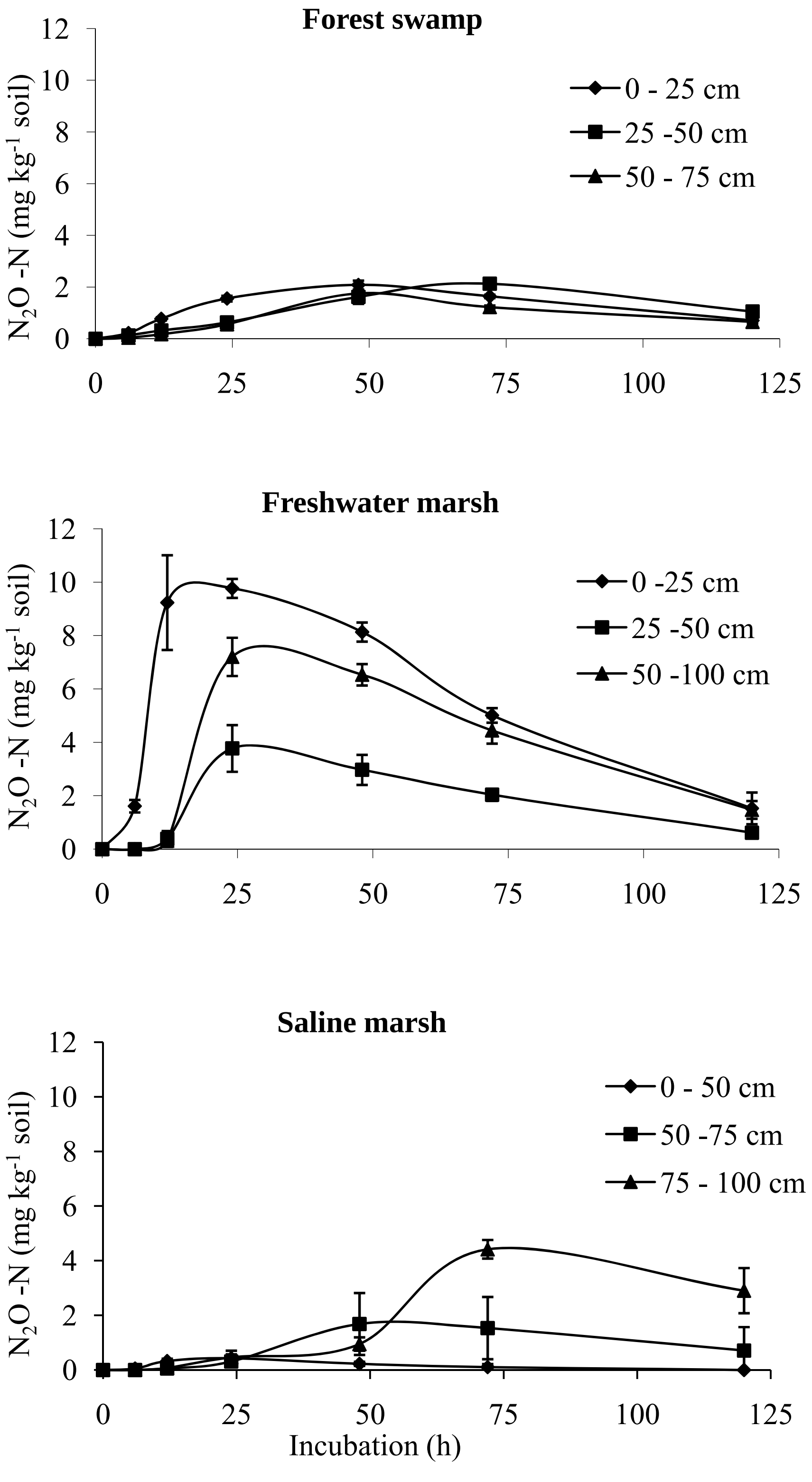

Fig. 5.2 Total N_2O-N released during the incubation of three wetland soils from different depths treated with 2 mg L^{-1} NO_3^- -N

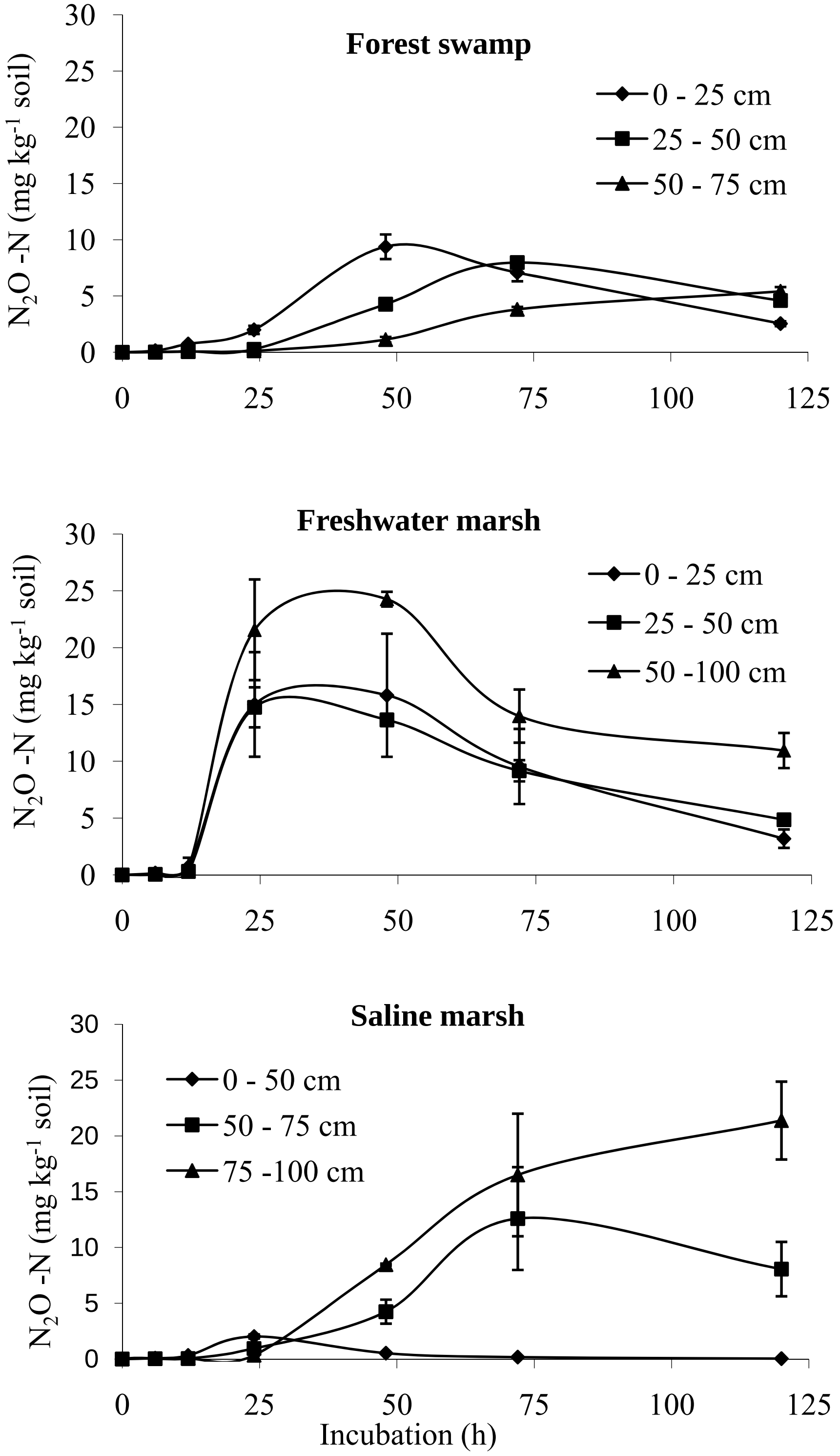

Fig. 5.3 Total N_2O-N released during the incubation of three wetland soils from different depths treated with 10 mg L^{-1} NO_3^- -N

The averages of PDR across all depths on unit dry weight basis for FM profile were appreciably higher than those estimated for SM and FS profiles amended with both 2 and 10 mg L^{-1} NO_3^--N incubations (9.16 and 20.50 vs. 1.42 and 5.10, and 1.24 and 4.70 mg N $kg^{-1}d^{-1}$). It should be pointed out that due to bulk density variation among these soils, although still higher the differences in the averages of PDR across all depths on unit volume basis between FM and SM or between FM and FS soil profiles were not as large as those on unit dry weight basis (Tables 5.1 and 5.2). The findings are consistent with Van Hoewyk et al. (2000) who found similar PDR values for mineral and peat soils on volume basis. The SM soils exhibited the lowest measured PDR (on volume basis) among the three wetlands across the studied profiles, a result which could be attributable to high salinity effect. It has been reported that microbial biomass C decreased exponentially with the increasing EC levels (Rietz and Haynes, 2003). Across the three depths, on a volume basis, FS, FM and SM soils denitrified 43-51%, 48-55% and 4-24% of added NO_3^--N, respectively, under 2 mg L^{-1} NO_3^--N incubation and decreased slightly at 10 mg L^{-1} NO_3^--N when the denitrification reached maximum. The denitrification rates in the surface horizons of these Barataria Basin wetland soils were similar PDR as those reported for Florida wetland soils as determined under comparable conditions (Gale et al., 1993).

It is interesting that similar or slightly greater PDR values (on volume basis) were observed for deeper horizons of the three wetlands, suggesting that these wetland soils were capable of removing nitrate in deeper horizons. According to Venterink et al. (2003), denitrification potential in the deeper horizons would be regulated primarily by the diffusion of NO_3^- from surface waters to deeper horizons. Due to the fact that wetland soils, especially marshlands, often have low bulk density and high water table, this result would have significant field implications as these systems receives pulses of high nitrate concentration.

Table 5.2 Potential denitrification rates (PDR) in selected Louisiana wetland soils[†].

		PDR (Weight basis)			PDR (Volume basis)		
		----------------------------$\mathrm{NO_3^-}$ -N added			--------------------------		
Site	Depth	Control	2 mg L^{-1}	10 mg L^{-1}	Control	2 mg L^{-1}	10 mg L^{-1}
	cm	------mg N kg^{-1} d^{-1}------			------g N m^{-3} d^{-1}------		
Forest swamp	0-25	0.09 (0.005)	1.55 (0.11)	7.39 (1.28)	0.02 (0.001)	0.37 (0.03)	1.77 (0.31)
	25-50	0.10 (0.005)	0.99 (0.14)	4.01 (0.39)	0.05 (0.002)	0.44 (0.06)	1.76 (0.17)
	50-75	0.09 (0.004)	1.19 (0.17)	2.69 (0.18)	0.05 (0.001)	0.58 (0.08)	1.32 (0.09)
Freshwater marsh	0-25	0.47 (0.024)	18.48 (3.55)	24.12 (2.98)	0.03 (0.002)	1.29 (0.25)	1.69 (0.21)
	25-50	0.04 (0.002)	3.77 (0.87)	14.74 (2.52)	0.01 (0.000)	0.72 (0.17)	2.80 (0.48)
	50-100	0.21 (0.01)	7.20 (0.71)	18.94 (2.13)	0.03 (0.001)	1.15 (0.11)	3.45 (0.39)
Saline marsh	0-50	-	0.43 (0.22)	2.02 (0.31)	-	0.07 (0.04)	0.32 (0.05)
	50-75	-	1.37 (1.01)	8.35 (3.10)	-	0.27 ((0.20)	1.67 90.62)
	75-100	0.12 (0.006)	3.47 (0.14)	8.01 (0.49)	0.01 (0.000)	0.38 (0.02)	0.88 (0.05)

[†] Values are the mean (standard error) of 3 replicates.

Denitrification kinetics differed within each soil profile. In general, slow denitrification was observed in the deeper horizons within each soil profile. For instance, for FM soil at 2 mg L^{-1} NO_3^--N incubation, denitrification reached the maximum at about 12 h for 0 - 25 cm depth whereas it reached the maximum around 24 h for 25-50 and 50-100 cm depths. The delay in maximum denitrification could be attributed to low initial populations of denitrifying bacteria which would be often caused by low substrate (NO_3^-) concentration (Cornwell et al., 1999; Hunt et al., 2006), though it is doubtful that significant growth within an inactive denitrifying population could occur in 24 hrs. More likely, more time was required to induce the denitrifying pathway within a population less accustomed to significant concentrations of this alternative electron acceptor.

Higher denitrification activity at higher NO_3^- levels was also evident in these soil profiles as the loading rate was changed from 2 to 10 mg L^{-1} NO_3^--N (Figs. 5.2 and 5.3). As shown in Table 5.2, the jump in the PDR between the two treatments ranged from about 2 to 6 fold. The impact of increasing NO_3^- levels on PDR was greater in the lower soil horizons of FM but greater in the surface horizons for FS and SM soils. These variations could reflect the difference in carbon quality and other properties in the respective horizons of each soil profile.

In order to further examine the efficiency of denitrification, headspace concentration of N_2O-N without acetylene treatment was determined and headspace concentration of N_2-N was calculated from the N_2O-N difference between vials with and without acetylene treatment (see Appendix B). In general, the combined N_2-N concentration in the headspace of each vial was higher than the combined N_2O-N concentration during the 120 h incubation in all profile depths of the three soils except for the top 25 cm of FM soil, suggesting that in most cases denitrification was complete with all NO_3^- converting to N_2 for both 2 and 10 mg L^{-1} NO_3^--N treatments. For the top 25 cm of FM soil, there was very high N_2O-N concentration relative to

N_2-N concentration, especially at 10 mg L^{-1} NO_3^--N treatment, suggesting potentially incomplete denitrification from NO_3^- to only N_2O. This incomplete denitrification at the top of FM soil profile indicates a potential emission of greenhouse gas N_2O especially during the early stage of denitrification. Although the PDR was relatively smaller in the SM and FS profiles than that in FM soil profile, the ratio of N_2-N/N_2O-N in the incubation vial over 120 h was generally larger based on detectable amounts of N_2-N and N_2O-N, suggesting that the emission of N_2O from SM and FS soils was likely insignificant. While FM wetlands could play a role in removing nitrate from wastewater effluents and other sources through denitrification, our study suggests that a significant amount of N_2O, a global warming gas, could also be potentially released into the atmosphere.

Overall the above results have important implications in assessing nutrient removal function of wetlands. While both freshwater marshes and bottomland forest swamps may be used as a more effective tool for NO_3^- removal than saline marshes from contaminated waters, the removal efficiency could be further enhanced by facilitating the NO_3^- movement to deeper wetland horizons. On the other hand, a balance may need to be maintained in utilizing wetland functions as it is also potentially possible that a significant amount of N_2O from NO_3^- could be released into the atmosphere due to incomplete denitrification.

5.3.3 Relation between Denitrification and Organic Carbon

Regression analysis was performed to relate PDR to soil properties across these wetland soil profiles. There was no significant relationship between PDR and pH or EC (data not shown), although both EC and pH could have negative effects on denitrifying population of microbes (Gale et al., 1993; Rietz and Haynes, 2003). Of the soil parameters listed in Table 5.1, TOC content was the only soil property shown to correlate significantly and positively with PDR (Fig. 5.4). These results suggest that in these ecosystem systems organic C was the dominant factor

controlling potential denitrification. To further evaluate the potential denitrification in relation to soil organic carbon characteristics in these wetland systems, the labile organic C (LOC) was approximated from the total C gas released (CO_2-C plus CH_4-C) at 10 mg NO_3^- -N L^{-1} during the entire incubation (5 days), and distribution of different organic C moieties was estimated based on solid–state ^{13}C NMR of whole soils and TOC. These results are shown in Table 5.3.
Labile organic C exhibited a similar trend to TOC for these soil profiles (Tables 5.1 and 5.3) and the two were significantly related (R^2= 0.65, P<0.01). Labile organic C was also significantly related to PDR, especially at 2 mg NO_3^--N L^{-1} treatment (y = 256.7x + 1.46, R^2= 0.94, P<0.001). Significant and positive correlation between LOC and PDR supports that majority of the LOC was evolved from the denitrification activity. Polysaccharides were the dominating C of TOC in all the three soil profiles followed by alkyl, aryl, carboxyl, phenolic and Aldehyde/ Ketonic C moieties (Table 5.3). This distribution of major C moieties in the order of polysaccharides, alkyls, total aromatics (aryl and phenolic), and carbonyls (carboxyl, and aldehyde and ketonic) was similar to that observed for the colder prairie wetland soils of Saskatchewan, Canada (Jokic et al. 2003) and for 300 various forest and cultivated agricultural soils (Mahieu et al., 1999). The similarity of our results with those reported for other soils suggests that SOM transformation in coastal wetland ecosystems is likely controlled by similar processes or mechanisms. On average, there were no consistent differences in relative distribution of the C moieties among the three soils but in all three, the surface horizons tended to have a higher percent of polysaccharides-C than the deeper horizons whereas the opposite was true for aryl C (Table 5.3). The latter indicated an increased humification of older plant materials with depth into the soil profile (Kögel-Knabner, 1997).

Potential denitrification rate, especially at 2 mg L^{-1} NO_3^--N, was significantly and positively correlated to amount of polysaccharides C in the soil (Fig. 5.5). Additional multiple

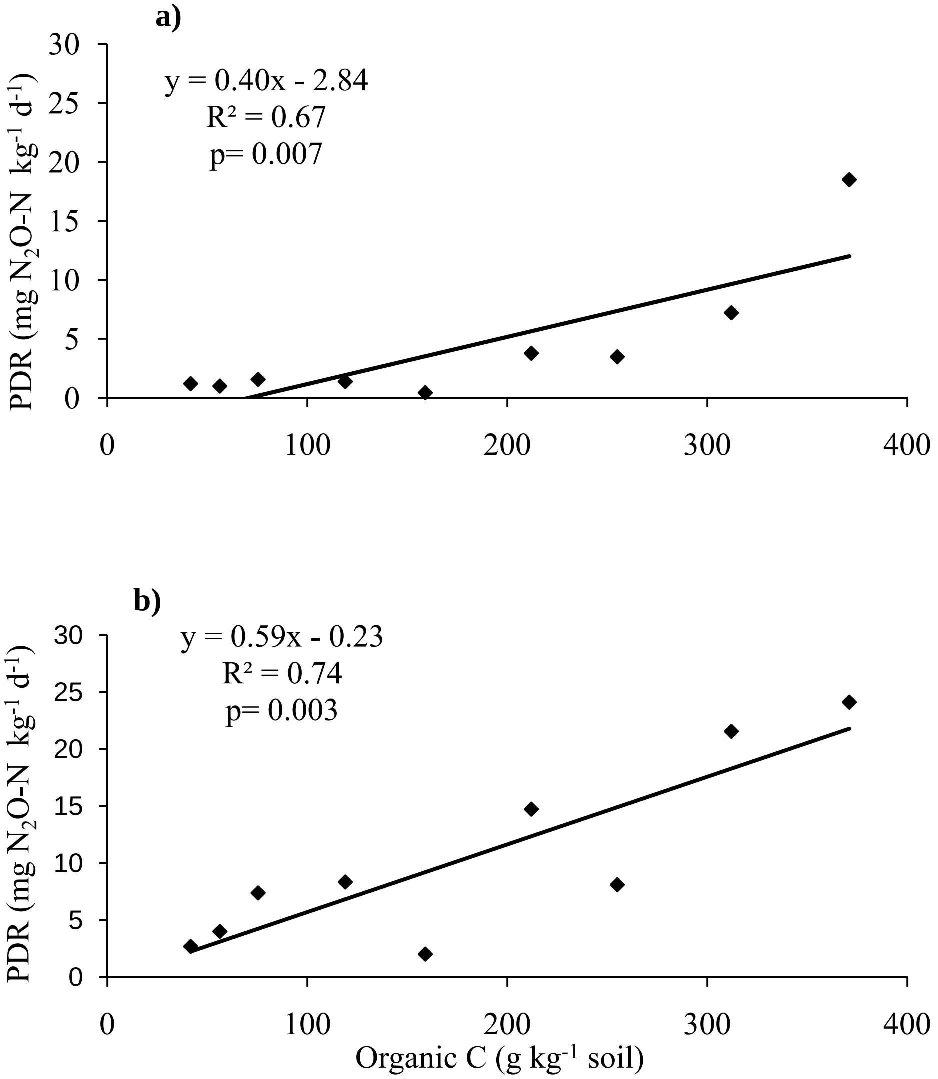

Fig. 5.4 The relationship between the potential denitrification rate (PDR) and soil organic C under the treatments of a) 2 mg L^{-1} NO_3^--N and b) 10 mg L^{-1} NO_3^--N.

Table 5.3 Distribution of major C moieties in the organic matter of selected Louisiana wetland soils.

Site	Depth	LOC[†]	Polysaccharides	Aryl	Phenolic	Alkyl	Carboxyl	Aldehyde / Ketonic
	cm	g C kg^{-1} soil	------------------------------------		% TOC[‡]		------------------------------------	
Forest swamp	0 – 25	0.0158	38.2	14.4	8.3	25.6	10.1	3.4
	25 – 50	0.0070	29.8	14.4	9.7	29.4	12.1	4.6
	50 – 75	0.0035	32.3	17.8	10.4	25.4	9.6	4.5
Freshwater marsh	0 – 25	0.0774	48.8	12.3	6.7	20.9	10.0	1.4
	25 – 50	0.0185	31.8	15.4	8.4	27.8	12.2	4.3
	50 – 100	0.0281	36.9	16.7	8.3	26.1	10.8	1.3
Saline marsh	0 – 50	0.0185	40.5	14.5	8.6	20.5	11.0	4.8
	50 – 75	0.0152	39.8	17.9	8.5	20.5	10.9	2.4
	75 – 100	0.0172	32.2	16.2	7.7	31.6	9.4	2.9

[†] LOC, labile organic carbon under 10 mg L^{-1} $NO_3 – N$.

[‡] TOC, total organic carbon.

regression analysis between LOC and different C moieties also showed that the amount of polysaccharides in soil had significant positive effect on LOC ($p < 0.0001$). These results would be expected since polysaccharides were the dominant organic C within these soil profiles (Table 5.3). Polysaccharides are highly labile and can be utilized by wide range of microorganisms (Krull et al., 2003).

Regression analysis between PDR and non-polysaccharide C moieties in organic C was also performed. In this case, fraction of C functionalities relative to TOC was used as the independent variable to minimize the impact of bulk TOC on the regression relations since most of these C moieties were small and tended to increase with TOC. Significant ($p \leq 0.04$) but negative relationships existed between PDR and percent phenolic C in TOC (Fig. 5.6) and between PDR and percent Aldehyde/ Ketonic C in TOC (Fig. 5.7). Linear relations of PDR with percent alkyl C, aryl C, and carboxyl C in TOC were also negative but not statistically significant ($P \geq 0.11$) (data not shown). Similar relations between percent LOC and percent C moieties in the organic C pool were also observed. These results likely indicated that phenolics and Aldehyde/ Ketonics are important in influencing potential denitrification rates in these wetland soils. The significant negative relationship between PDR and phenolic C in TOC suggested a non-preference in using phenolic C by denitrifying bacteria in these ecosystems. This is consistent with an earlier report showing that addition of pore water containing high level of phenolic C led to decreased denitrification rates as compared to the pore water containing low level of phenolic C (Sirivedhin and Gray, 2006). The presence of phenolic moieties indicated the presence of lignin from plant materials. Various studies have shown that certain strains of bacteria were able to denitrify NO_3^- using aromatic carbons (including both phenolic and aryl moieties) under anaerobic condition (Nozawa and Maruyama, 1988). The energy required for the breakdown of lignin was shown to be much larger than for other biochemical compounds of

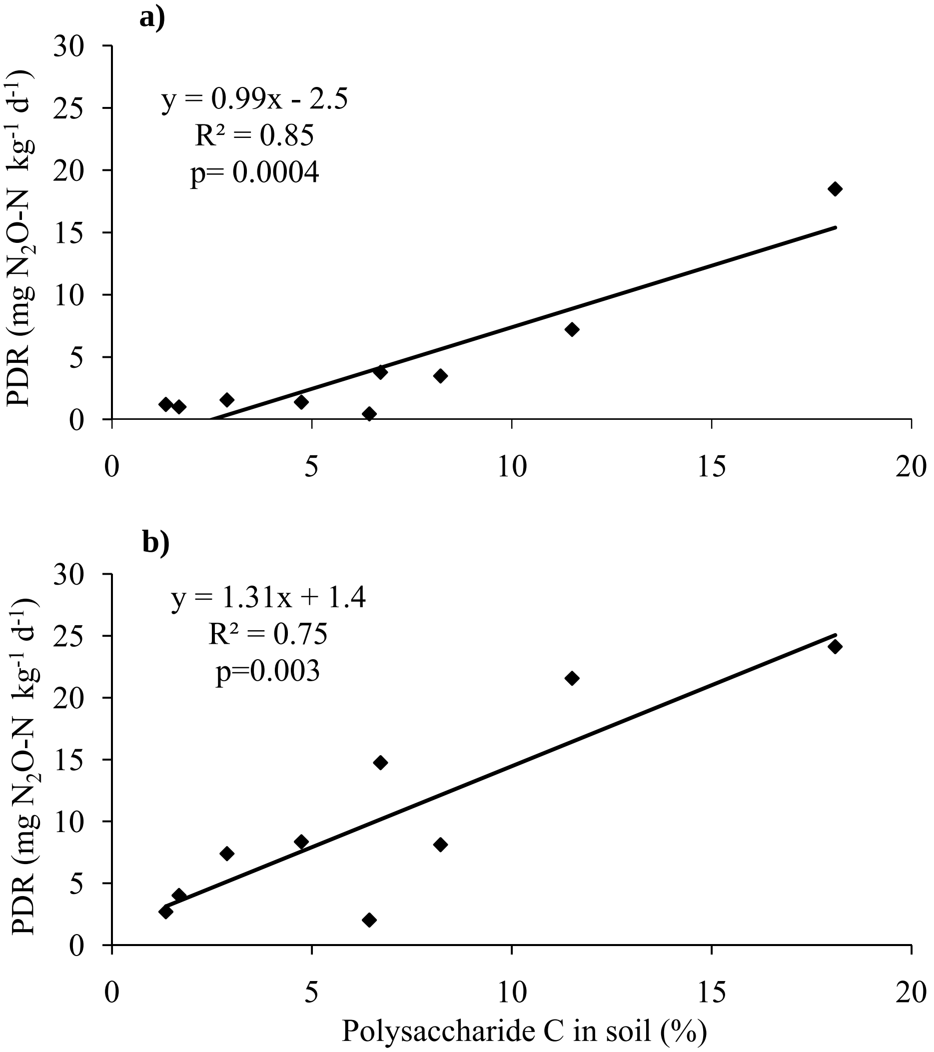

Fig. 5.5. The relationship between potential denitrification rate (PDR) and percent polysaccharide C in soil under the treatments of a) 2 mg L^{-1} NO_3^--N and b) 10 mg L^{-1} NO_3^- -N.

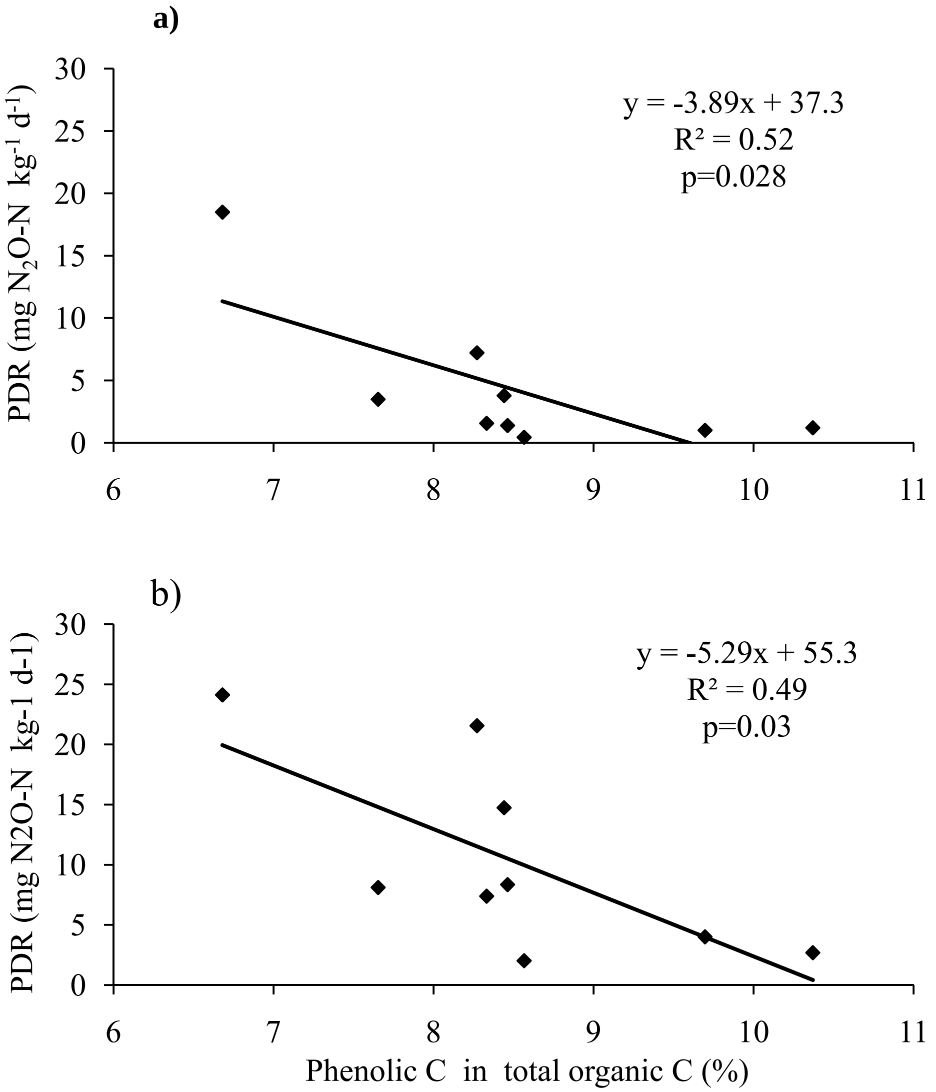

Fig. 5.6. The relationship between potential denitrification rate (PDR) and percent phenolic C in organic C under the treatments of a) 2 mg L^{-1} NO_3^--N and b) 10 mg L^{-1} NO_3^- -N.

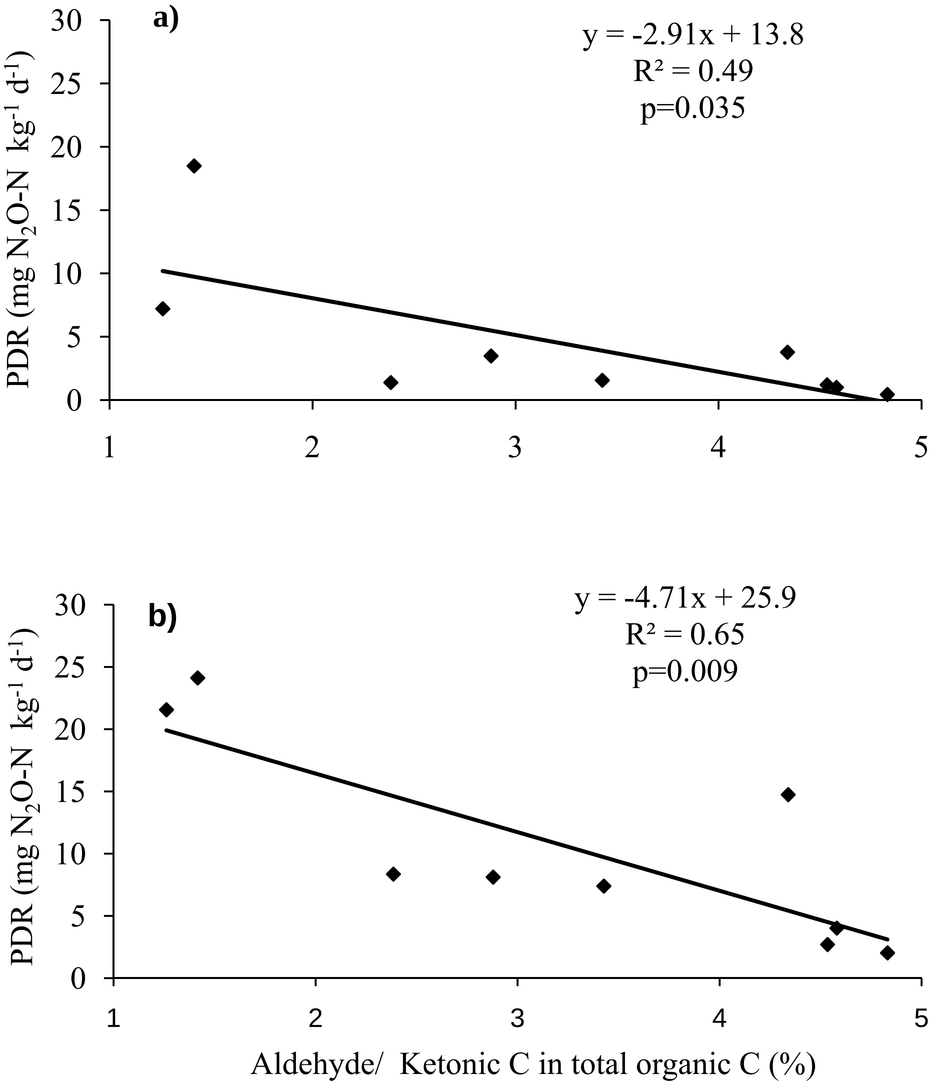

Fig. 5.7 The relationship between potential denitrification rate (PDR) and percent aldehyde/ ketonic in organic C under the treatments of a) 2 mg L^{-1} NO_3^--N and b) 10 mg L^{-1} NO_3^--N.

plant material and molecular oxygen was often necessary to keep the phenolic oxidizing enzyme active (Freeman et al., 2001; Hume et al., 2002). Recently, it was suggested that phenolics could act as antioxidant, by scavenging reactive free radicals and terminating the oxidative chain reaction responsible for soil organic matter degradation (Rimmer, 2005). Our study indicated that phenolics not aryls, possibly of lignin, were likely associated with significantly reduced denitrification in these wetland soils. It should be pointed that in addition to aromatics (aryls and phenolics), alkyls have been also shown to be recalcitrant to biodegradation (Baldock et al., 1997). Along with aromatics and carboxyls, alkyls are often concentrated as a result of the decomposition and humification of plant residues in soils (Kögel-Knabner, 1997). Alkyl C is primarily due to aliphatic lipids. Recent research showed that addition of an alkyl C-rich soybean oil to wood chips led to significant increase in denitrification rate over woodchips alone (Greenan et al., 2006). However, our study suggested that alkyl C played a lesser role in regulating denitrification in these wetland soils.

The significant negative influence of aldehyde and ketone C on denitrification rate as shown in Fig. 5.7 was interesting. These C functionalities were significantly and positively related to phenolics (R^2 =0.47, P <0.03). There was no significant relation between these and other C moiety. This result suggests that some of the aldehydes and ketones in these wetland systems could be phenolic. Phenolic aldehydes have been proposed as intermediates of lignin in the transformations of plant residues that would turn into humic substances (Stevenson, 1994). Our results suggest that phenolic aldehydes and ketones could be the primary C source that inhibits denitrification in these wetland systems.

5.4 Conclusions

This study clearly demonstrated that on both a dry weight and volume basis, the FM soil profile had greater PDR than the SM and FS soils in the Louisiana Barataria Basin estuary.

While there was little difference in PDR between SM and FS profiles on dry weight basis, SM profile showed the smallest PDR among the three soil profiles on a volume basis. The FM profile yielded the highest NO_3^- removal or denitrification rate followed by the FS and SM profiles. The FM soil profile also tended to yield the highest amount of N_2O, a global warming gas, especially at the earlier stage of denitrification, which could have significant implication in assessing the over-all benefit of using this wetland to process nutrient-rich wastewaters, nonpoint source agricultural runoff, and nitrate in diverted Mississippi River water. On the other hand, it should be noted that the actual denitrification rates in the field may be lower than these found under the reported laboratory situations due to less reduced conditions in the field caused by bioturbation and fluctuations in water table especially at surface soil horizons. In addition, the functioning of deeper horizons in contributing to NO_3^- removal would likely be dependent upon actual downward diffusion of NO_3^- in the field. Across these three wetland systems, organic C was the dominant factor regulating PDR. Of the different C moieties, polysaccharides positively influenced denitrification rate whereas phenolics, likely phenolic adehydes and ketonics, significantly inhibited denitrification rate in these wetland soils.

5.5 References

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Chapter 6

Evaluation of Selected Methods for Characterizing Labile Organic Carbon in Wetland Soils: Relationships to Carbon Functionalities

6.1 Introduction

Soils contain at least 1500 Pg C as soil organic matter (SOM), representing one of the largest near surface pools in the global carbon cycle (Schlesinger et al., 2005). Because of the amount of C sequestered in this dynamic pool, increases and decreases in its size can have a significant influence on atmospheric concentrations of CO_2 (Trumbore et al., 1996). A more complete understanding of the factors influencing the stability of SOM is needed to more accurately elucidate its impact on the gaseous composition of the atmosphere and to anticipate its response to climate change. Soil organic matter is a continuum of heterogeneous substances with variable and molecular structure and elemental composition in various stages of decomposition and reformation (Oades, 1988; McLauchlan and Hobbie, 2004). Based on resistance to mineralization, SOM has been divided into three different pools which include labile, intermediate and recalcitrant organic matter. Soil labile organic carbon pool is the most active fraction of soil organic carbon with rapid turnover rates, and governs production and flux of CO_2 from soils to atmosphere (Zou et al., 2005; Silveria et al., 2008). Parton et al. (1987) defined soil labile carbon as the fraction of soil organic carbon with a turnover time of less than a few years as compared to recalcitrant carbon with a turnover time of several hundred or thousands of years. The relative distribution of labile and recalcitrated fractions govern mineralization rates of soil organic matter. The LOC fraction more readily mineralizes to carbon dioxide and increasing emission of CO_2 to the atmosphere. Hence LOC may not contribute significantly to the long-term C sequestration in soils.

Wetland soils are estimated to contain about 20 – 25 % of the terrestrial soil carbon, despite comprising a relative small portion of the total land area occupied and thus play a major role in global carbon cycle (Amthor et al., 1998). High rates of organic C input coupled with slow mineralization rate cause wetland soils to serve as an important C sink for atmospheric CO_2 (Gorham, 1991). The major factors causing slow mineralization of SOM in the wetland soils are low oxygen, acidic peat, lack of nutrients and poor substrate/ organic matter quality (Bridgham and Richardson, 2003; Yavitt et al., 2004). The amount of C could be sequestered in these soils may be impacted by the amount of labile organic C (LOC) pool available for microbial mineralization. The different SOM pools vary in chemical characteristics and the degradability of SOM is determined by the relative amounts of recalcitrant and labile C pools (Rovira and Vallejo, 2007; Silveria et al., 2008). The lability of SOM depends on both chemical recalcitrance and physical protection from microbial attack (McLauchlan and Hobbie, 2004). Under oxic conditions, compounds with simple structure such as polysaccharides followed by proteins and lipids are rapidly utilized by microbes (Harvey et al., 1995) while compounds with high molecular weight, irregular structure, and aromatic structure are recalcitrant (Krull et al., 2003). Labile fractions of organic matter can respond rapidly to changes in C supply and are considered to be important indicators of soil quality (Zhang et al., 2006). As a result, researchers have designed various methods to estimate the amount of LOC in a given soil. Several scientists have questioned whether chemical extractions of SOM can provide fractions that clearly define turnover rates and contributions to nutrient cycling (Ladd et al., 1977; Cadisch et al., 1996). On the other hand, Haevey et al., (1995) stated that organic matter in soil does not degrade as a single pool but rather as a composite of multiple rates among the various classes of organic matter. Nonetheless, majority of studies on labile organic carbon were conducted on upland soils. Not much or very little work was done on LOC in the wetland soils who contribute a significant amount of carbon to the terrestrial C reserves.

Several different methods that include chemical, physical and biological approaches have been used to estimate the amount of LOC in soils. McLauchlan and Hobbie (2004) reported more than nine different types of methods have been used/ proposed by different researchers for separating SOM into labile and recalcitrant pools. Chemical fractionation methods rely on the solubility of organic carbon in oxidizer, acid or base, producing soluble organic carbon (Zou et al., 2005). On the other hand, physical fractionation methods separate SOM into heavy and light carbon (Tisdall, 1996). One physical fractionation method for estimating LOC is density fractionation (Gregorich and Janzen, 1996; Alvarez and Alvarez, 2000). LOC by chemical methods are reproducible, whereas physical fractionation methods do not produce reproducible results because data vary with the mineral composition and density found in different soil types, with the size and density that differ among plant materials (Sollins et al., 1999; Zou et al., 2005). Among different LOC fractionation methods, aerobically mineralizable carbon (AMC) by incubation, a biological method, has been extensively used for estimating soil LOC (Robertson et al., 1999; Alvarez and Alvarez 2000). Simple chemical methods such as cold water extractable C and salt-extractable C of SOM has also been proposed as a method for determining LOC fraction (Gregorich et al., 2003; Fang et al., 2005; Jinbo et al., 2006). Acid hydrolysable C has been used to approximate both labile and slowly-degradable C consisting primarily of young SOM (Sollins et al; 1999; Ran et al., 2007). Other techniques used are hot water extraction (Wang and Wang, 2007; Silveira et al., 2008), and permanganate digestion (Ettema et al., 1999; Weil et al., 2003; McLauchlan and Hobbie, 2004).

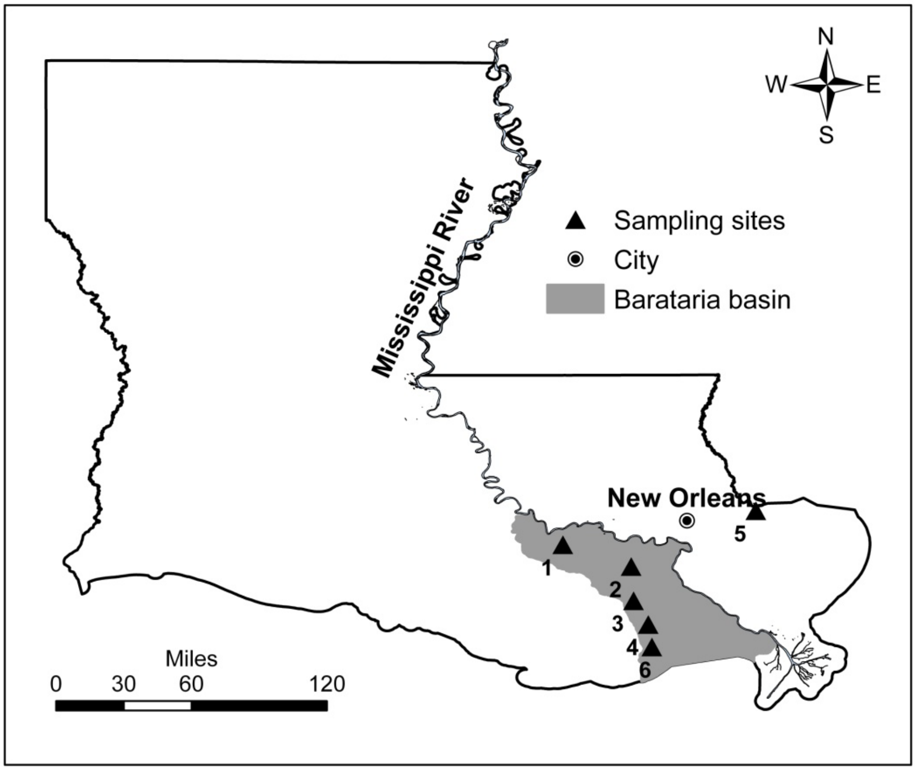
Organic C in the soil is stabilized by various physical and chemical mechanisms and as a result the C extracted by the various fractionation methods can differ. All methods can vary in the amount and type of C extracted from a given soil. Little work has been conducted to identify the relationship between organic C extracted by the various fractionation techniques as related to the distribution of C functional groups. In addition, wetland soils especially in coastal zones are constantly influenced by different environmental factors such as flooded or reduced conditions (anaerobic), salt water intrusions which make them different from upland soils. Therefore, adequate characterization of wetland soil LOC is essential to the understanding the C transformation in those soils. Furthermore estimation of labile C pools and their relationship with C functional groups may enhance the understanding of SOM dynamics as related to changes in bio-geochemistry of wetlands including increases in nutrient and pollutant loading.

Louisiana coastal wetlands are currently experiencing a high rate of relative sea level rise due primarily to subsidence (DeLaune 1978). Marsh must certainly accrete in order to keep pace with the increase in water level. The marshes are currently vertically accretes at a rate of approximately 1 cm yr^{-1} (Hatton et al., 1983). Accretion is strongly dependent on organic matter sequestration in the soil profile (Nyman et al., 1990; DeLaune and Pezeshki, 2003). The net accumulation rate of soil organic matter is affected by decomposition rate.

The objectives of this study were 1) to estimate labile organic carbon fractions in selected Louisiana coastal wetland soils with depth 2) to explore the relationship between the LOC fractions estimated by different methods and the distribution of C functional groups as characterized by ^{13}C solid-state nuclear magnetic resonance (NMR) and 3) to explore potential relations among different C functional groups and LOC in wetland soils to C sequestration.

6.2 Materials and Methods

Soil samples used in this study were collected from different wetland types in Barataria basin along the Louisiana gulf coast which included a forest woody swamp (FS), two freshwater marshes (FM 1and FM 2), a brackish marsh and two salt marshes (SM 1 and SM 2) (Figure 6.1). The wetland sites selected included wetland soils with different levels of organic C and C: N ratio that represented different ecosystems with different vegetative type. The vegetation in both freshwater marshes was dominated by a mixture of *Panicum hemitomon* and *Sagittaria lancifolia,* while the forest woody swamp, brackish marsh and saline marsh were dominated by *Taxodium distichum*, *Spartina patens*, and *Spartina alterniflora* respectively. The soils at the FS, FM, BM and SM sites were Barbary muck (Very-fine, smectitic, nonacid, hyperthermic Typic Hydraquents), Lafitte muck (Euic, Thermic Typic Medisaprists), Allemands muck (Clayey, smectitic, euic, hyperthermic, Terric Haplosaprists), and Timbalier muck (Euic, hyperthermic, Typic Haplosaprists), respectively. All these wetlands are seasonally flooded (little above 6 months in a year), but mostly stay saturated throughout the remaining months. In each wetland site, soils were collected from three to four depths. Overall a total of 21 samples were collected from the selected wetland sites. The depths of soil sampling which were based on the variation in SOM humification level in the soil profile and differed among sites. Composite soil samples from each site and depth were collected, placed in zip-lock bags and transported to the laboratory under ice. In the laboratory all the soils were thoroughly mixed and distinguishable plant roots, stems and leaves removed. A sub-sample of each soil was freeze dried and used for the characterization of the initial organic C distribution while the remaining soil was used for other analyses. Soil pH, electric conductivity (EC) and bulk density were determined using field-moist soil samples whereas other properties were determined using freeze-dried samples. Bulk density was measured by gravimetric method. Total C and N were determined by dry combustion at 900^{o}C using a TruSpec CN analyzer (LECO, St. Joseph, MI). All the soils were checked for the presence of inorganic C by treating with 1 N HCl which showed no fizzing indicating none to contain significant amount of inorganic carbon. Thus the total C determined in all the soils was treated as total soil organic carbon (SOC). Selected physical and chemical properties of these soils are presented in Table 6.1.



[†]1, Forest woody swamp; 2, Freshwater marsh 1; 3, Freshwater marsh 2; 4, Brackish marsh; 5, Saline marsh 1; 6, Saline marsh 2

Figure 6.1 Location of sampling sites selected for this study

Table 6.1 Physico-chemical properties of the selected wetland soils

Site	Depth	pH	EC†	BD‡	C	N	C/N
	cm		dS m^{-1}	g cm^{-3}	------g Kg^{-1} -----		
FS	0-25	6.2	1.1	0.24	75.4	5.5	13.7
	25-50	6.1	1.3	0.44	56.3	3.7	15.2
	50-75	6.12	1.5	0.49	41.7	2.5	16.7
FM1	0-50	5.8	1.9	0.09	413	14.0	15.6
	50-100	6.2	1.4	0.11	311	13.2	14.3
	100-150	6.3	2.2	0.10	348	12.5	14.8
FM2	0-25	6.1	2.7	0.07	371	24.5	15.1
	25-50	6.4	3.5	0.19	212	11.8	17.9
	50-100	6.6	4.5	0.16	312	18.6	16.8
	100-150	6.6	4.4	0.12	317	21.2	14.9
BM	0-75	6.8	2.6	0.08	386	23.0	16.8
	75-125	7.2	4.8	0.07	437	28.0	15.6
	125-150	7.4	6.1	0.08	387	24.0	16.1
	150-175	7.3	7.0	0.11	278	21.0	13.2
SM1	0-50	7.4	8.3	0.25	143	8.0	17.9
	50-100	7.3	13.1	0.22	133	8.9	14.9
	100-150	7.4	13.0	0.49	72.3	5.8	12.5
SM 2	0-50	7.3	38.9	0.16	159	8.7	18.3
	50-75	7.4	42.5	0.2	119	6.7	17.7
	75-100	7.3	34.6	0.11	255	14.3	17.8
	100-150	7.9	42.3	0.76	12.1	0.8	15.1

†EC, electric conductivity; ‡ BD, bulk density.

6.2.1 Solid State Nuclear Magnetic Resonance Spectroscopy

All the NMR spectra were collected using a Bruker Avance wide bore 400 MHz Nuclear Magnetic Resonance Spectrometer. The resonance frequencies of ^{1}H and ^{13}C were 400 and 100 MHz, respectively. Cross polarization magic angle spinning (CPMAS) was used to acquire the ^{13}C NMR spectra. Spinning side bands were eliminated using the total suppression of sidebands

(TOSS). Prior to analysis soils were freeze dried, grounded and passed through a 250 µM sieve. No prior HF treatment was conducted since the soils were low in paramagnetic elements. Samples were loaded into 4mm Zirconium rotors and analyzed using a double resonance CP-MAS probe. The spectra were collected at a spinning speed of 5 kHz, with 1 ms contact times, 2 s recycling times. A total of 72,000 scans were collected for each sample. The ^{13}C chemical shifts were referenced to tetra methyl silane and calibrated with the glycine carbonyl signal, set at 176.03 ppm. Within the 0-230 ppm chemical shift range, C atoms were assigned to various molecular groups in to aliphatic C (0-50 ppm), polysaccharides (50-108 ppm), total aromatic C (108-165 ppm), carboxyl C (165-190 ppm) and carbonyl (190-230 ppm) (Malcolm, 1989; Silveira et al., 2008). All other assignments were assigned based on reference literature or calculated via ChemDraw Ultra 9.0 by CambridgeSoft. Carbon distribution was summarized based on spectral peak area for all C groups. Amount of each functional group carbon per unit soil was calculated based on their relative distribution and the total carbon in soil.

6.2.2 Estimation of Labile Organic Carbon

Labile organic carbon of the soils was estimated by three different methods namely 1) water-extractable carbon, 2) salt extractable carbon and 3) aerobically mineralizable carbon. Acid hydrolysable carbon, which contains both labile and slowly degradable soil C pool, was also estimated. The methodology used for each method is explained below.

Water-Extractable Organic Carbon (WEOC) was obtained using the procedure as described by Bijay Singh et al. (1988). A 10-g soil (wet basis) was mixed with 30-mL deionized water in an Erlenmeyer flask and shaken for 30 minutes. After shaking, the mixture was centrifuged, filtered through 0.45 µm filter, and the filtrate was analyzed for total organic C using a Shimadzu TOC analyzer (Total organic C- V_{CSH} C analyzer, Shimadzu, Kyoto, Japan).

After the extraction the dry weight of the soil was determined by drying the soil at 105°C and the WEOC was calculated based on soil dry weight basis.

Salt-Extractable Organic Carbon (SEOC): Soil organic C was extracted with 0.5 M K_2SO_4 (Fang et al., 2005). Similar to the procedure used for water extraction 10 g of field moist soil samples were weighed into flasks, and following addition of 30 mL of 0.5 M K_2SO_4 the mixture was shaken for 30 min on a rotating shaker. Subsequently the soil samples were centrifuged, filtered through 0.45 µm filter and analyzed for total C using Shimadzu TOC analyzer. Following extraction the dry weight of the soil was determined by drying the soils at 105°C. The SEOC was calculated based on soil dry weight basis.

Aerobically Mineralizable Carbon (AMC) was determined by aerobically incubating 10 g of field moist soil sample in a 250-mL air tight glass jar that had a special septum to collect gas sample. The headspace of the jar was flushed with compressed air and was then incubated in dark for 12 days at room temperature. At the end of incubation period gas samples were collected from the headspace and analyzed for CH_4 and CO_2 using a CP3800 gas chromatography (GC) equipped with an FID detector (Varian Inc., Palo Alto, CA). The GC injector, column, methanizer and detector temperatures were maintained at 60°C, 60°C, 400°C and 250°C respectively. The amount of C mineralized (dry soil weight basis) was based on the total carbon released over 12 days.

Acid Hydrolysable Carbon (AHC): Soil samples were mixed in digestion tubes with 6 *N* HCl (1:10 soil to solution ratio) and refluxed for 16 hours (Sollins et al., 1999; Cheng et al., 2007). The mixture was then cooled to room temperature, and the residual soil separated by vacuum-filtered using Whatman # 50 filter papers. The residue samples were then washed with deionized water and oven-dried at 80°C. After drying, the residual samples were weighed and analyzed for total C by dry combustion using a Thermo CN analyzer. Labile organic C was calculated by difference in total C between soil samples with and without acid hydrolysis.

6.2.3 Data Analysis

Data Analysis was conducted using AS software (SAS 9.1, SAS Institute, and Cary, NC). Least squares regression analysis was used to find the relationship among different methods of LOC measurements and total organic carbon. Multiple regression analysis was conducted to find out the relationship between the amount of different C functional groups in soil and LOC fractions by different methods. Stepwise analysis was used in identifying the most significant factors. The significance level for all statistical analysis was at α equal to 0.05.

6.3 Results

6.3.1 Physico-Chemical Characteristics of Study Sites

The physico-chemical characteristics of the wetland soils used in this study varied among sites and within the soil profile (Table 6.1). The amount of SOC was highly variable among and within the sites. In general, the amount of SOC was higher in freshwater and brackish marsh soils followed by saline marshes and forested swamp soil. Among the soils, SOC ranged from 12 g kg^{-1} soil in bottom most layer of SM-2 to 437 g Kg^{-1} in BM soil, while C: N ratios ranged from 12.5 to 18.3 among the soils. The amount of organic C also varied with depth within each site but no trend was observed except for FS and SM-1 soils where organic C decreased with depth. The pH of the soils tended to increase in alkalinity towards the coast or with the increasing salinity of the soils. The pH of the FS and FM soils was slightly acidic ranging from 5.8 to 6.6. In contrast, brackish and saline soils were slightly alkaline, pH ranging from 7.2 to 7.9 except for the top layer of the BM (0- 75 cm) where the pH was 6.8. The EC of soils was ranged from 1.1 dS m^{-1} in FS soil to 42.5 dS m^{-1} in SM-2 soil. Bulk density of the soils was higher in FS soil followed by SM soils and FM soils and ranged from as low as 0.07 g cm^{-3} in FM soil to 0.76 g cm^{-3} in FS soil.

6.3.2 Labile Organic Carbon among Wetlands

Labile organic C estimated by different laboratory techniques approximates the natural process of microbial degradation (McLauchlan and Hobbie 2004). Soil organic carbon showed significant and positive correlations with the LOC fractions WEOC, SEOC and AHC with an $R^2 > 0.7$ indicating LOC pool increased linearly with increase in SOC (Figure 6.2). However, the rate of increase in LOC fractions with increase in the amount of SOC differed greatly among the sites with highest variation in the acid hydrolysable fraction (Table 6.2). On the other hand correlations between SOC and AMC was poor (R^2 =0.34) due to two outliers. Overall, the amount of AMC found in the surface soil layers of all the wetland sites was high as compared to subsurface layers (Table 6.2). Multiple regression between % C mineralized by AMC and soil physic-chemical characteristics showed that there was a significant negative correlation ($p= 0.03$) between the depth of soil and amount of AMC. This could be due to the fact that the LOC in the deeper layers might be consumed due to the longer residence time of substrate. In addition, presence of more active microorganisms in the surface soil layers than in the deeper layers of soil could also be led to higher amounts of LOC. However, the AMC found in surface layers of FM-2 and BM soils was substantially higher than other layers and the removal of these points significantly improved the correlation with SOC (R^2= 0.62). Multiple regression analysis showed that there was no significant effect of pH, EC and C: N ratio on WEOC, SEOC and AMC.

Three empirical measurements of LOC, WEOC, SEOC, and AMC, and acid hydrolysable carbon were positively correlated with each other (Figure 6.3). Water extractable organic C was positively correlated with SEOC C ($R^2= 0.84$), AMC (R^2=0.50), and AHC (R^2=0.47). Salt extractable Organic C correlated positively with AMC C (R^2=0.63) and AHC (R^2=0.5). Even

Table 6.2 Amount Labile Organic C estimated by different methods

Site		WEOC	SEOC	AMC	AHC
		------------------% Total OC-----------------			
FS	0 - 25	0.38	0.17	0.44	29.8
	25 - 50	0.31	0.15	0.15	35.8
	50 - 75	0.32	0.22	0.11	32.7
FM 1	0 - 50	0.22	0.09	0.13	50.0
	50 - 100	0.31	0.17	0.18	29.4
	100 - 150	0.19	0.12	0.07	32.2
FM 2	0 - 25	0.40	0.28	0.66	31.0
	25 - 50	0.45	0.20	0.25	33.1
	50 - 100	0.32	0.18	0.15	27.7
	100 - 150	0.30	0.18	0.13	29.3
BM	0 - 75	0.34	0.15	0.49	23.3
	75 - 125	0.35	0.14	0.09	19.4
	125 - 150	0.32	0.16	0.18	15.9
	150 - 175	0.37	0.16	0.19	17.4
SM 1	0 - 50	0.20	0.11	0.13	25.6
	50 - 100	0.34	0.16	0.20	28.2
	100 - 150	0.30	0.20	0.06	37.6
SM 2	0 - 50	0.28	0.24	0.26	-
	50 - 75	0.33	0.23	0.18	34.2
	75 - 100	0.23	0.14	0.17	24.5
	100 - 150	2.12	1.40	0.54	49.0

though acid hydrolysable C and AMC were positively correlated with each other, the regression was not significant ($R^2 = 0.24$). However, there were large variations in value of LOC estimates of WEOC, SEOC and AMC when compared with that of acid hydrolysable C which was an order of magnitude higher. The amount of water extractable and salt extractable carbon represented < 1% of total carbon. The amount of WEOC ranged between 0.2 to 0.5 % of total carbon except for the bottom most layer of SM1 where WEOC represented 2.1 % of the total carbon. These values were similar to previously reported value of approximately 0.5 % of total C extractable by cold water in a forest soil (Wang and Wang, 2007). The amount of SEOC was even lower than for WEOC (between 40 to 70% of WEOC). Nonetheless the amount of C extracted by both water and salt solution were highly correlated with each other (R^2=0.84). This supports that both SEOC and WEOC extracted similar type of organic C into the solution. The decrease in the amount of SEOC as compared to WEOC could be due to the coagulation of aromatic compounds under high salt concentration (Dou et al., 2008). However, no significant effect of salinity was observed on WEOC. Similar for the WEOC and SEOC, the amount of aerobically mineralizable C was also < 1% of the total C in soil.

The amount of AHC, that comprises both LOC and slowly mineralizable C pool, was very high ranging between 16 and 50 % of the total carbon. The amount of hydrolysable C determined by AHC contains both labile and slow to turnover carbon pools and resulted in a higher amount of C extracted (MacLauchlan and Hobbie, 2004). In general, there was no relation between AHC and soil depth among the wetland sites except for SM-1 where the amount of AHC increased with soil depth. Few previous studies reported increases in amounts of AHC with depth (Tan et al., 2004). Also, the amount of acid hydrolysable C per unit of SOC showed no significant difference among the wetland sites studied. Overall, there was no significant relationship observed between the amount of AHC extracted and wetland type for any soil depth. The variability in AHC could be due to the differences in the distribution of C functional groups among the soils. However, regression analysis between soil bulk density and AHC showed a positive curve linear relation. This could be due to the physicochemical protection provided by soil mineral matter to the acid hydrolysable carbon compounds. A previous study based on analysis of organic C in different size fractions showed that significant amounts of O-alkyl C (Polysaccharides) in the clay fraction, suggesting the protection of easily degradable organic C such as polysaccharides by clays (Baldock et al., 1990). The amount of AHC in this study were in agreement with the amount of acid hydrolysable C previously reported for different upland and wetland soils (Xu et al., 1997; Paul et al., 2001; Silveria et al., 2008). Determination of total

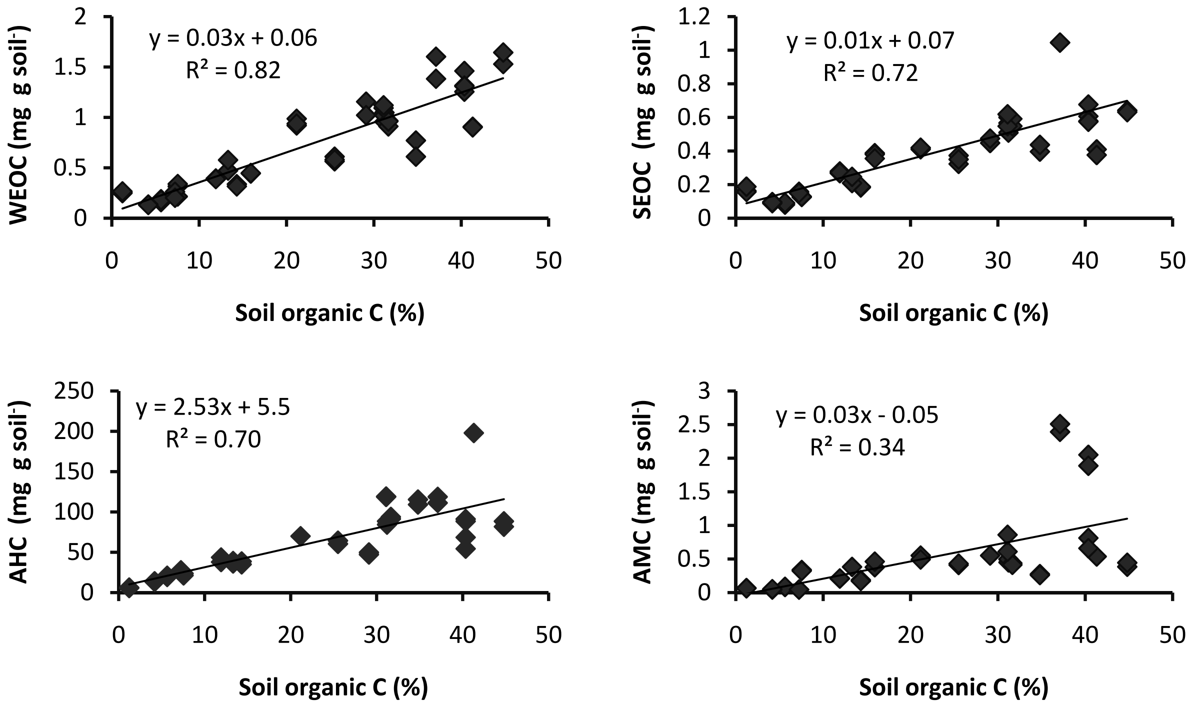


Figure 6.2 Linear regressions between labile organic C pool by different methods and soil organic carbon

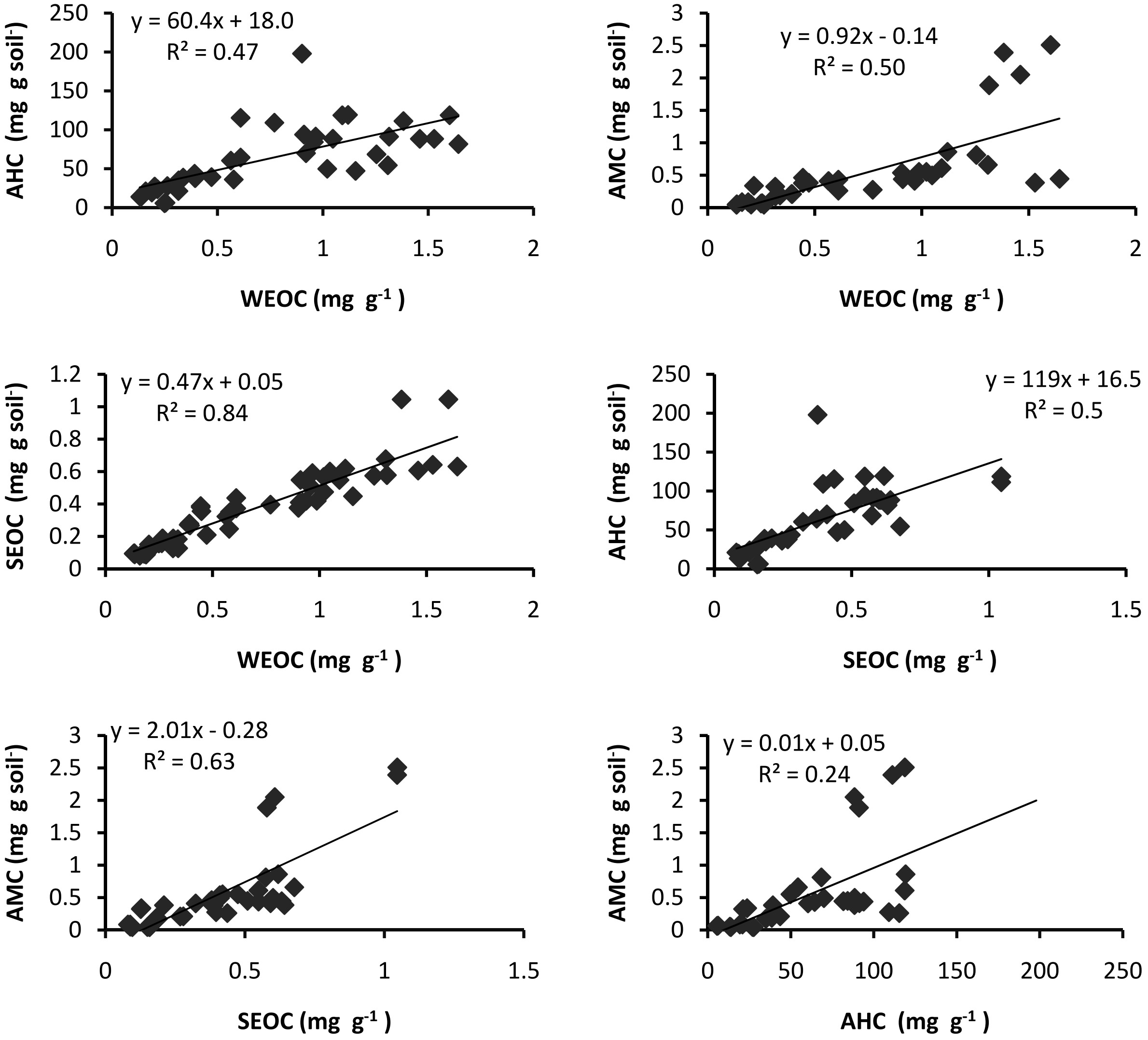


Figure 6.3 Regressions between different techniques to measure labile organic C pools

carbon and nitrogen following acid hydrolysis revealed that the C: N ratio of the soils remains non-hydrolyzed almost doubled in all the soils as compared to the initial soil C: N ratio. The increase in C: N ratio signifies that the majority of N containing compounds such as amino sugars was hydrolyzed (MacLauchlan and Hobbie, 2004). Nonetheless the amount of LOC determined by different methods and AHC were positively correlated except between AMC and AHC (R^2=0.24) (Figure 6.3).

6.3.3 Relationship between LOC and Carbon Functional Groups

Presence of appreciable differences in the amount of total C and distribution of C functional groups in these soils allows for showing the relationship between the relative distribution of C functional groups in organic matter (Table 6.3) and LOC fraction by the different methods. Multiple regression analysis was conducted to determine the relationship between the different C functional groups and LOC determined by the various methods (Table 6.4). Multiple regression between the amount of WEOC and amounts of different C groups showed that carboxyl C had a significant and positive relationship to the amount of C extracted (p = 0. 0007) while the alkyl C had a significant negative effect (p= 0.028). Even though an increase in the amount of aromatic C in soil with a decrease in WEOC, was found the relationship was not significant (p=0.081). However, Otto and Simpson (2007) reported that after extraction with water, the composition of OM in the soil residue did not differ markedly. This could be due to the fact that the amount of WEOC is generally extracted was < 1% of total soil carbon, which could be an insufficient amount for change to be detected by NMR spectroscopy. Similar results were observed with SEOC where carboxyl C had a significant and positive correlation with the amount of C extracted (p =0.014) while a significant but negative correlation was observed for Alkyl C (p=0.044). These results signify that hydrophilic and polar compounds such as carboxyl C are more soluble and represent the major portion of the dissolved organic carbon (DOC) in the

Table 6.3 Relative distribution of C functional groups in different wetland soils

Site	Soil depth	Alkyl	Polysaccharide	Aromatic	Carboxyl	Carbonyl
	cm	-------------------------------------%---				
FS	0-25	25.56	38.16	22.78	10.08	3.43
	25-50	29.42	29.85	24.10	12.05	4.58
	50-75	25.38	32.31	28.20	9.59	4.53
FM 1	0-50	22.72	50.58	17.27	8.13	1.30
	50-100	20.13	50.46	20.89	7.36	1.15
	100-150	21.42	47.04	22.07	7.64	1.83
FM 2	0-25	20.91	48.75	18.96	9.96	1.42
	25-50	27.84	31.77	23.83	12.22	4.34
	50-100	26.10	36.90	24.94	10.80	1.26
	100-150	27.68	35.34	24.66	11.21	1.12
BM	0-75	19.37	47.75	21.86	9.66	1.36
	75-125	23.50	39.13	24.41	11.27	1.69
	125-150	24.82	36.39	25.96	11.13	1.70
	150-175	27.16	32.58	25.78	12.57	1.91
SM 1	0-50	26.87	43.51	21.09	7.51	1.01
	50-100	26.46	41.96	21.95	8.17	1.46
	100-150	29.95	37.79	22.51	8.66	1.09
SM 2	0-50	20.53	40.49	23.09	11.05	4.83
	50-75	20.50	39.85	26.33	10.94	2.39
	75-100	31.63	32.22	23.85	9.42	2.88
	100-150	.	.	.	.	.

Table 6.4 Relationships between labile carbon and carbon functionalities for selected wetland soils

Method		R^2	p
WEOC	= 3.39 - 0.033 alkyl C - 0.019 aromatic C + 0.037 carboxyl C	0.55	0.004
SEOC	= 2.10 - 0.029 alkyl C + 0.056 carboxyl C	0.4	0.02
AHC	= 342.8 + 2.719 alkyl C + 1.287 polysaccharide C – 6.1 aromatic C	0.65	0.001
AMC	= 2.29 – 0.094 alkyl C + 0.017 polysaccharide C + 0.148 carboxyl C	0.36	0.06

wetland soils studied. This could be due to the polarity of COOH group which makes it more soluble in water. Similarly, Said-Pullicino et al., (2007) reported that WEOC consists mainly of hydrophilic fraction of C which includes low molecular weight organic acids, carbohydrates, and amino sugars. Similarly, Herbert and Bertsch (1995) reported that cold-water extractable organic matter in soils was composed mainly of low molecular substances such as organic acids and amino acids and high molecular substances such as fulvic acid. However, no significant relationship was found between WEOC and amount of polysaccharides in the soil. In this study both water and salt extractable C did not show any correlation with the amount of polysaccharides. This is in contrast to the results reported in previous work where majority of the WEOC was polysaccharides (Fröberg et al., 2003).

Aerobically mineralizable carbon, which represents the actual portion of labile C pool available to the microorganisms, showed a positive correlation with polysaccharides (p= 0.039) and carboxyl C (0.024). In contrast, though not significant, a negative correlations were found with the alkyl C (p=0.091) and aromatic C (p= 0.12) functional groups. The simple structure and low molecular weight polysaccharides make them more susceptible to microbial attack than the complex and higher molecular weight compounds like aromatic and alkyl C compounds. Also, several C structural studies for different types of soil showed that highly mineralized organic matter and humified compounds consisted mainly of aromatic and alkyl C groups and were low in polysaccharides (Baldock et al., 1997). On the other hand, the more degradability of carboxylic C could be attributed to their greater solubility in water which makes compounds more susceptible to microbial attack. The results are in confirmation with the generally accepted opinion that polysaccharides are more labile than any other C groups (Silveira et al., 2008). AHC, which is believed to hydrolyze polysaccharides and nitrogenous compounds (Otto and Simpson, 2007), was positively correlated with the polysaccharides (p= 0.003) while negatively correlated with the aromatic C groups in soil (p= 0.001). AHC did not show any significant correlation with alkyl, carboxyl and carbonyl groups. Structural analysis of non hydrolysable carbon by Ran et al., (2007) also showed that the material mainly consists of aromatic and alkyl C groups. Presence of significant and positive correlation of carboxylic C with AMC, WEOC and SEOC supports the use of WEOC or SEOC as a useful tool for predicting microbially degradable carbon (Sollins et al., 1999). The poor correlation between AHC and AMC irrespective of their positive correlation with the amount of polysaccharides could be attributed to the extraction of carbon compounds more than labile organic C, which is slower to decomposition.

6.4 Conclusions

The amount of LOC fractions in the wetland soils determined by different methods increased with increase in total C regardless of wetland type or soil depth. Comparison among soils by normalizing the amount of organic C in soil showed there was no significant difference in the amount of LOC fractions in different wetland soils including depth of soil determined as WEOC, SEOC and AMC. Labile organic carbon represented < 1 % of total soil organic carbon whereas AMC was represented as high as 49 percent. The AMC measured in soils collected at different depths may not be comparable due to their variability in initial number of active microorganisms. Our findings showed that most of the WEOC and SEOC consist mainly of organic C fraction with carboxyl C. Aerobically mineralizable carbon significantly increased with the increase in the amount of polysaccharide and carboxylic C in the soil organic matter. This supports the use of WEOC and SEOC as quick method to assess the amount of labile organic C in a given soil. These results show the relationship between LOC fraction estimated by different methods and C distribution in soil organic matter. Results could be useful in predicting which carbon compound or fraction which are degraded or sequestered in Louisiana wetland soils which vertically accrete primarily through organic carbon accumulation in order to keep pace with water level increase due to subsidence and sea level rise.

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CHAPTER 7

CHARACTERISTICS AND STRUCTURAL COMPOSITION OF HUMIC ACIDS IN COASTAL WETLAND SOILS OF DIFFERENT SALINITY GRADIENTS

7.1 Introduction

Humic substances, formed as a result of the humification process, are the most recalcitrant fractions of soil organic matter (SOM). They play an important role in controlling carbon (C) cycles and biogeochemistry of soils (Swift, 2001). Besides being resistant to microbial degradation, humic acids enhance soil C sequestration through hydrophobic protection (Spaccini et al., 2002) and stabilizing soil aggregates (Hayes and Edward, 2001). The stabilized aggregates can, in turn, protect easily degradable C such as polysaccharides (Bronick and Lai, 2005). While humic acids or humic substances in general have been traditionally believed as relatively large-molecular-mass polymers formed through secondary synthesis (Stevenson, 1994), recent evidence has suggest that they are more of collections of diverse, relatively low molecular mass components forming dynamic associations stabilized by hydrophobic interactions and H bonds (Sutton and Sposito, 2005). The molecular C moieties in humic substances reflect the sources of organic C inputs and are related to maturity, depositional environment, and degree of degradation (Lu et al., 2000; Golding et al., 2004). In addition, humic substances have been shown to contain both amorphous and crystalline domain characteristics, which are expected to have different resistivity to environmental attacks (Hu et al., 2000). Humic compounds affect transport and retention of contaminants (Fukushima et al., 2006). Their amphipathic nature enables them to interact with a wide variety of inorganic and organic pollutants including heavy metals and charged organic pollutants via chemical bonding and with non polar organics through nonspecific physical interactions (Li et al., 2003). Interaction of humic compounds and inorganic mineral phases leads to the formation of organic coatings on mineral phases and a change in surface charge (Schafer et al., 2007). In addition, different types of humic substances could exert contrasting influences on mineral colloid coagulation (Schafer et al., 2007). Hence, understanding the chemical and structural composition of SOM in a given soil will be helpful for elucidating reaction mechanisms of humic substances with mineral surfaces as well as their role in contaminant transport. Much of past research has focused on characteristics of humic substances in upland forest and cultivated soils (Hatcher and Clifford, 1994; Schulten, 1995; Preston, 1996; Koge¨l-Knabner, 2000).

Wetlands are important ecosystems with the highest net primary productivity among terrestrial ecosystems. Wetland soils are estimated to contain about 20-25% of the terrestrial soil carbon (C), despite the relatively small proportion of the total land area occupied (Amthor et al., 1998; Keddy, 2000). The rates of organic matter (OM) decomposition in wetland soils are slow due to anaerobic conditions (Gorham, 1991; Stevenson and Cole, 1999). The high organic input along with slow decomposition rate suggests that wetlands may serve as an important C sink for atmospheric CO_2 (Mitsch, 1994; Turunen et al., 2002). Recently, wetlands of both natural and constructed have been also used for treating waste waters **(**Breaux and Day Jr. 1994; Verhoeven and Meuleman, 1999; Vymazal, 2005).

The nation's richest wetlands are located on and influenced by the Mississippi River deltaic plain. The deltaic plain on a whole is subjected to rapid local and regional subsidence and loss that has been attributed to basement sediment sinking, consolidation of the sediments of the Gulf Coast geosyncline, and local consolidation (Kolb and Van Lopik, 1966). Many of these wetlands, because of their location at the land/ocean interface, are threatened by saltwater intrusion and must maintain elevation within the tidal range or they will cease to function as vegetated wetlands (Salinas et al. 1986). Soil organic C along with sediments is considered as the two most dominated factors in controlling the health and stability of these wetlands (DeLaune and Pezeshki, 2002). Despite ecological and environmental importance as an ecosystem, the organic geochemistry of wetland soils has not been well investigated. Much of structural information of soil organic C in wetlands is largely unknown (Alongi, 1998; Keddy, 2000). Limited studies have focused on peat soils (Hornibrook et al., 2000; Cocozza et al., 2003), which is different from the very heterogeneous immature OM in the surface horizon of wetlands (Hetényi et al., 2006). This limited understanding of organic C transformation in wetland ecosystems has hindered efforts to elucidate relations among different C pools (Stevenson and Cole, 1999). Because of importance of humic substances in understanding the C source origin and fate, its reactivity in biogeochemical processes, and its interactions with living organisms and natural surfaces in ecosystems, chemical and structural characteristics of humic acids of three wetland soils of different salinity gradients from Mississippi river delta plain were determined and compared in the present study.

7.2 Materials and Methods

7.2.1 Study Sites and Sample Collection

Three wetland ecosystems, namely, a forest swamp (FS), freshwater marsh (FM), and salt marsh (SM), from the Barataria basin of the Louisiana coast. The dominant vegetative species in FS, FM and SM are *Taxodium distichum*, *Sagittaria lancifolia*, and *Spartina alterniflora* respectively. The soils at the FS, FM, and SM sites were, Barbary muck (very-fine, smectitic, nonacid, hyperthermic typic hydraquents), Allemands muck (clayey, smectitic, euic, hyperthermic, terric haplosaprists), and Timbalier muck (euic, hyperthermic, typic haplosaprists), respectively. Composite soil samples from top 0 – 50 cm depth were collected at each site. Collected samples were immediately placed into zip-lock bags and transported to the lab on ice. Visible plant parts were removed and samples were thoroughly mixed, used for the analyses. A portion of soil samples was used for soil physico-chemical characterization and the rest was used for humic acids extractions.

7.2.2 Soil Analysis and Humic Acids Purification

Selected physico-chemical properties of the study site soils are presented in Table 7.1. Soil pH, electric conductivity (EC), and bulk density were determined using field moist soil whereas other properties were determined using dried soils. Bulk density was measured by the gravimetric method. Total carbon and nitrogen of the soils were determined by dry combustion using a TrueSpec CN analyzer (Leco, St. Joseph, MI).

Humic acids (HA) were extracted and purified from field moist soils following a procedure outlined by International Humic Substances Society (Swift, 1996; Li et al., 2003). Briefly, soil samples were treated with 0.1M HCl in centrifuge bottles to have soil to solution ration ratio of 1:10 (pH 1-2) to remove carbonates, Fe and Mn oxides and hydroxides. The mixtures were centrifuged at 6000 rpm for 15 m, and the supernatant were decanted. The soil residues were neutralized using 1 M NaOH and then extracted using 0.1 M NaOH at a soil to solution ratio of 1: 10 under N_2 atmosphere. Supernatant was collected after centrifugation and the humic acid fraction was separated by acidifying the supernatant with 6 *M* HCl with constant stirring to pH 1.0, followed by allowing the suspension to stand for 12 to 16 h and further centrifugation. The humic acid fraction was re-dissolved by adding a minimum volume of 0.1 M KOH under N_2 and sufficient KCl was added to attain a solution K^+ concentration of 0.3 M. The solution was then centrifuged to remove fine mineral particles. The aqueous HA solution was again acidified, precipitated and supernatant was discarded. Then the HA fraction was treated with 0.1 *M* HCl/0.3 *M* HF solution until the ash content decreased to below 1%. Finally, the obtained humic acids were dialyzed using Spectrapor membrane (size exclusion limit, 12,000–14,000 D) and freeze-dried. The humic acids from the forest swamp, freshwater marsh, and salt marsh are referred as FS-HA, FM-HA, and SM-HA, respectively.

Table 7.1 Physico-chemical characteristics of three Louisiana wetland soils used in the study†

Soil	pH	EC	BD‡	% Clay	Textural class	OC §
		dS M^{-1}	g cm^{-3}			%
Forest swamp	6.2	0.68	0.24	64.5	Clay	6.6
Freshwater marsh	5.83	1.89	0.12	44.2	Silty clay loam	22.6
Salt marsh	7.25	32.8	0.16	33.2	Silty clay	11.6

† EC: Electric conductivity, BD: Bulk density, % Clay: % clay in sediment, OC: Organic carbon

7.2.3 Elemental Analysis

The elemental composition of the freeze dried purified humic acids was determined by dry combustion, followed by gas chromatography using Perkin Elmer 2400 Series II CHNS/O Elemental Analyzer (Waltham, MA). The content of O was estimated as the ash-free mass less C, H, N and S. Ash content of the humic acids was determined by overnight combustion at 550°C in a muffle furnace.

7.2.4 Spectroscopic Characterization of Humic Acids

7.2.4.1 Solid State Nuclear Magnetic Resonance Spectroscopy

Solid state nuclear magnetic resonance is a novel technique to analyze SOM characteristics. Solid-state ^{13}C nuclear magnetic resonance spectroscopy with cross-polarization magic angle spinning (^{13}C CPMAS NMR) is widely used for the characterization of SOM (Alarcón-Gutiérrez et al., 2008). Recent advances in solid-state ^{13}C NMR for examining the chemical structure of organic materials in natural environments has allowed non-destructive and potentially quantitative examinations of organic carbon chemistry in-situ, without prior extraction and/or degradation procedures (Nelson and Baldock, 2005). The method allows assignment of broad classes of organic chemical structure to specific NMR resonances based on chemical shift (Golding et al., 2004).

Solid state ^{13}C NMR spectra were collected on freeze dried humic acids using a Bruker Advance widebore 400 MHz Nuclear Magnetic Resonance Spectrometer. The resonance frequencies of ^{1}H and ^{13}C are 400 and 100 MHz, respectively. Cross polarization magic angle spinning technique (CP-MAS) was used to acquire the ^{13}C NMR spectra. Freeze dried humic acid samples were loaded into 4mm Zirconium rotors and analyzed using a double resonance CP-MAS probe. Spectra were collected at a spinning speed of 12 kHz, with 2.5 ms contact times and 2 s recycling times. The ^{13}C chemical shifts were referenced to tetramethylsilane and calibrated with the glycine carbonyl signal, set at 176.03 ppm for each sample a total of 40,000 scans were collected. Within the 0-230 ppm chemical shift range, C atoms were assigned to various molecular groups (Cook et al., 1996): aliphatic C (0-50 ppm), methoxyl (50-60 ppm), polysaccharides (60-108 ppm), aromatic C (108-140 ppm), phenolic C (140-165), carboxyl C (165-190 ppm) and carbonyl (190-230 ppm). All other assignments were based on reference literature or calculated via ChemDraw Ultra 9.0 by CambridgeSoft. The relative intensity of these regions was determined by integration and carbon distribution was summarized based on spectral peak area for all C groups.

7.2.4.2 Pyrolysis

Pyrolysis is another powerful analytical tool for obtaining molecular level information from complex compounds such as soil organic matter and humic substances (Chefetz et al., 2002). Macromolecular OM can be conveniently analyzed by pyrolysis in combination with gas chromatography and mass spectrometry (Py–GC/MS). The GC/MS analysis of pyrolysis products evolved from the thermal degradation of OM provides information on the chemical nature of different bioprecursors. Pyrolysis was performed at 620°C for 20 s using a CDS 5000 pyroprobe platinum heated filament pyrolyser (Chemical Data System, Oxford, USA), directly connected to a Varian 3900 gas chromatograph coupled to a Varian Saturn 2100T ion trap mass spectrometer. The Py–GC interface and split/splitless 1077 injector (split mode) were kept at 300°C. A Varian factor FOUR VF-5MS capillary column coated with poly (5% diphenyl/95% dimethyl) siloxane stationary phase (30 m, 0.25mm i.d., 0.25 μm film thicknesses) was used for separation of pyrolysis products. The temperature of column oven was ramped from 40°C (held for 2 min) to 300^0C at 8°C min^{-1} and held for 10 minutes, with helium as carrier gas. The temperatures of injector and transfer line were kept at 300 and 240°C, respectively. Mass spectra were recorded in the electron impact mode (70 eV) at 1 scan s^{-1} in the 45–600 m/z range. Pyrolysis–GC/MS was performed on humic acids samples with and without treatment of tetramethylammonium hydroxide (TMAH). The former has been also referred as thermochemolysis-GC/MS. Highly polar oxygenated compounds such as polyhydric phenols, benzenecarboxylic acids, and hydroxybenzenecarboxylic acids require methylation of hydroxyl and carboxylic functional groups for effective gas chromatographic analysis (Hatcher and Clifford, 1994). Various researchers have demonstrated that the TMAH thermochemolysis technique hydrolyzes and methylates esters and ether linkages, assisting depolymerization and methylation, which makes most polar products volatile enough for gas chromatographic analysis (Chafetz et al., 2000). On the other hands, with TMAH, polysaccharides could be underestimated. For this reason, the results of thermochemolysis GC/MS were used for evaluating lignin-derived, aliphatics and nitrogen compounds whereas the results without TMAH treatment were employed to evaluating polysaccharides, and sulfur-containing compounds. The identification of pyrolysis products was based on a comparison of their mass spectra with those of standard compounds and NIST 2005 mass spectral library, literature data and GC–MS characteristics. The corresponding peak areas were calculated from the mass chromatograms using characteristic ions in the mass spectra. The relative quantities of the products were estimated using the peak areas of the total ion current (TIC) pyrograms. Pyrolysis products are grouped into compound classes according to their origin.

7.3 Results and Discussion

Total elemental analysis showed that the amount of C ranged from 53.1 to 55.5 % with only slight difference among the different sites (Table 7.2). There was also little difference in H and O in the humic acids of the different sites. A relatively greater difference was observed in N content and C/N ratio of humic acids among the three sites. Fresh marsh-HA had a smaller total N content than that of FS-HA and SM-HA, leading to a higher C:N ratio in the former than in the latter two humic acids. The amounts of total C and N found in these wetland humic acids were generally similar to that for Pahokee peat humic acid standard (56.4% C, and 3.69 % N) of IHSS (IHSS). Protein- and nitrogen-rich bacteria and healthy marine phytoplankton typically have C/N ratios between 4 and 8, while altered and soil NOM show ratios between 10 and 17 (Mao et al., 2007). The high C/N ratio found in this study (15.9 to 20.5) likely indicates that the most of the organic matter in these wetlands was formed from in-situ vegetation (Six et al., 2001). In addition, the amount of sulfur increased from FS-HA to FM-HA then SM-HA, corresponding to the increase in salinity indicating the increasing role of S in humic acid structure and the effect of salinity on soil humification processes. The H:C ratios of FS-HA (1.04) and SM-HA (1.07) were slightly higher than FM-HA (0.90). Higher H:C ratios in humic acids could suggest more aliphatic (Ussiri and Johnson, 2003). These H:C ratios were also slightly higher than that for IHSS Pahokee peat (0.88). However, they were similar to the H:C ratio for humic acids extracted from a forest soil as reported by Ussiri and Johnson (2003). The ratio of O:C was ranged from 0.48 to 0.5, which is typical to most of the humic acids (<0.6) (Ussiri and Johnson, 2003; Gondar et al., 2005).

Table. 7.2. Elemental composition and elemental molar ratios of purified humic acids from three Louisiana wetland soils

Site	C	H	O	N	S	H:C	O:C	C:N
	--------------------------------	--------	%--------	--------	------------------------------			
FS-HA	54.3	4.8	35.5	3.8	1.7	1.04	0.50	16.7
FM-HA	55.5	4.3	35.2	3.2	2.0	0.92	0.48	20.5
SM-HA	53.1	4.8	34.5	3.9	3.8	1.07	0.49	15.9

7.3.1Cross-Polarization Magic Angle Spinning Carbon 13 NMR

The CPMAS ^{13}C NMR spectra of the humic acids extracted from FS, FM and SM wetland soils are presented in Fig. 7.1. The CPMAS ^{13}C NMR spectra exhibited major peaks at 21, 28, 31 and 35 ppm (Methylene C), 54 ppm (Methoxyl C), 70 ppm (polysaccharides or aliphatic alcohols), 114 and 127 ppm (C substituted aromatic C), 146 and 150 ppm (O-substituted aromatic C or phenolic), and 170 ppm (carboxyl C). Major differences were observed among the NMR spectra of humic acids from the three wetland soils of different salinities (Fig. 1). In general, based relative distribution of overall major C functional groups in humic acids as estimated by integrated areas of the peaks for the specific spectral regions, the humic acid extracted from FM soil was significantly more aromatic followed by SM and FS humic acids (Table 7.3). The FM-HA was high in both C-substituted aromatic C and O- substituted aromatic C. The aromaticity of the humic acids, estimated by % peak area between 108 to 165 ppm to peak area between 0 to 165 ppm (Dai et al., 2001), was 47.2 % for FM-HA as opposed to 35.4% and 39.7% respectively for FS-HA and SM-HA. On the other hand, FS-HA was more aliphatic than SM-HA and FM-HA. Only slight differences were observed in methoxyl, polysaccharide and carboxyl C among the humic acids of different wetland soils.

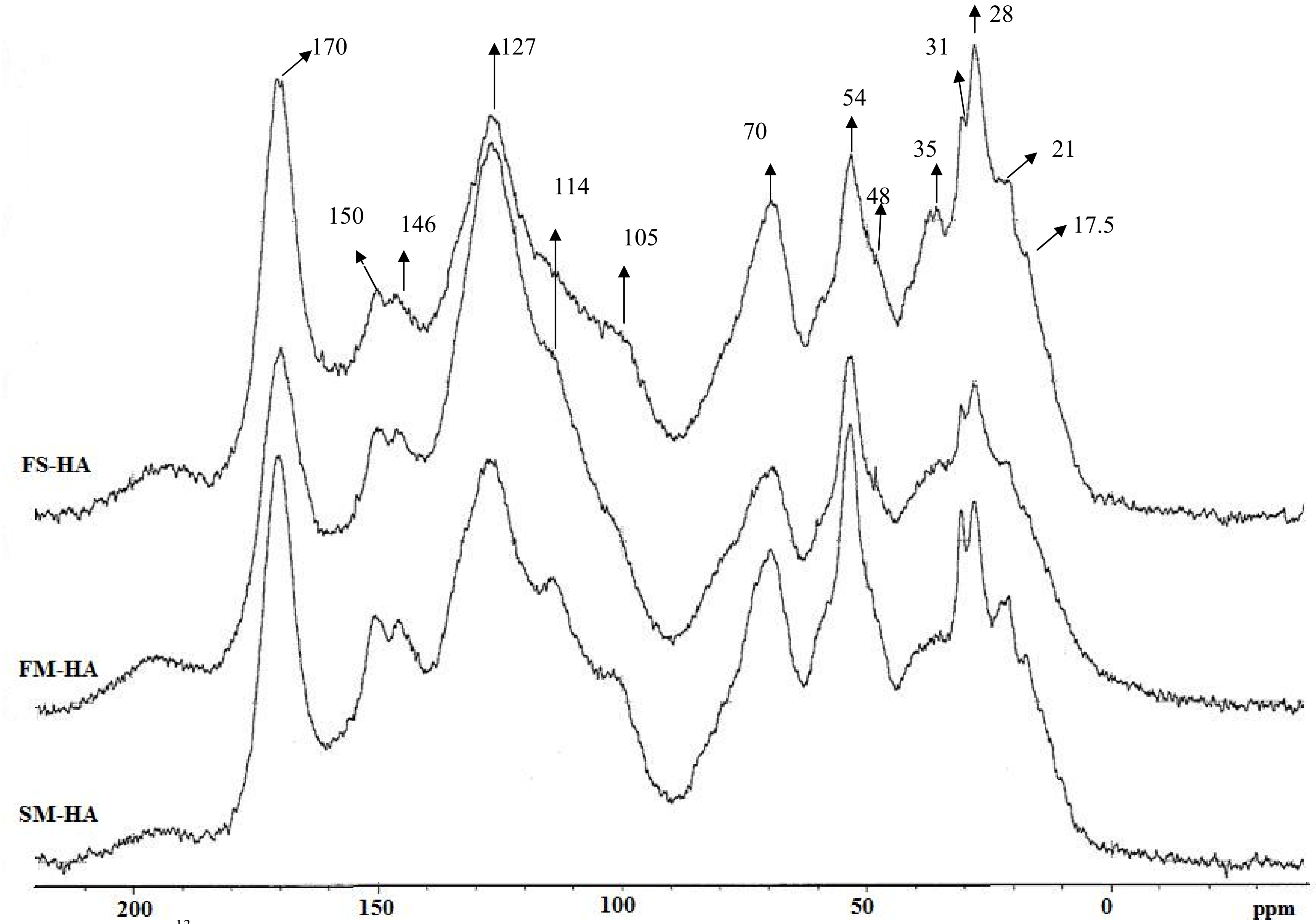

Fig. 7.1. CPMAS ^{13}C NMR spectra of humic acids extracted from different wetlands a) Bottomland hardwood forest swamp b) Freshwater marsh c) Saline marsh

Table 7.3 Molecular C moieties in humic acids determined by CP-MAS ^{13}C NMR spectroscopy

Sample†	Percentage distribution of molecular C within indicated ppm. regions						
	0 – 50 Aliphatic	50 – 60 Methoxyl	60 – 108 Polysaccharide	108 – 140 Aryl	140 – 165 Phenolic	165 – 190 Carboxyl	190 – 230 Carbonyl
FS HA	24.45	11.58	19.27	21.78	8.54	12.14	2.24
FM HA	19.44	9.92	16.57	30.00	11.08	10.74	2.25
SM HA	20.59	12.94	19.42	24.37	10.44	10.56	1.66

† FS HA: forest swamp humic acid, FM HA: freshwater marsh humic acid, SM HA: salt marsh humic acid

Closer examinations of the ^{13}C NMR spectra indicate further differences in the humic acids among the three wetland soils. In the aliphatic region, peaks found between 10 and 25 ppm represents the CH_3 components (Swift, 1996; Mao et al., 2007). SM-HA has more pronounced peak at 17.5 and 21 ppm than FM-HA and FS-HA respectively whereas these peaks were not clearly separated in FM and FS soil humic acids. The peak at 17.5 ppm can be assigned to terminal methyl groups (Swift, 1996), suggesting that SM-HA has more terminal methyl C than the other humic acids and potentially greater reactivity. The sharp peak at around 31 ppm found in all the humic acids represents carbons in long crystalline or condensed polymethylenic $(CH_2)_n$ chains (Kögel-Knabner et al., 1992; Nozirov et al., 2002; Shechter et al., 2006; Mao et al., 2008). This peak is more evident in SM-HA than in FM-HA and FS-HA respectively. Conversely, the amorphous or mobile polymethylenic C peak at 28 ppm was more dominant in FS-HA. Although the amorphous and condensed domains of $(CH_2)_n$ chains have a very similar compositional nature, they exhibit slightly different chemical shifts due to their different conformations. In the condensed state, (CH_2)n chains are all-trans, while in the amorphous state, the chains have both trans and gauche conformations (Hu et al., 2000). The condensed polymethylenic C is expected to be resistant to further degradation and have longer residence times (Hu et al., 2000). This could imply that the alkyl C in humic acids of SM could be more recalcitrant than that of FM and FS respectively. Interestingly, the intensity of 31 ppm peak increased along the salinity gradient of the three wetlands. The result could suggest that the increased salt concentration or associated saline environment enhance the cyrstalinity of alkyl C in humic acids in these soils. Strong and sharp peak at about 31 ppm has been reported for various peat humic acids (Hu et al., 2000; Chefetz et al., 2002). On the other hand, a clear split peak centered at 35 and 38 ppm that represents quaternary C (CR4) and cyclic CH_2-C components was more evident in FS-HA soil than in FM-HA and SM-HA. This may be evidence for the presence of more long chain alkyl compounds in FS-HA than FM and SM humic acids. In addition, a small sharp peak at 48 ppm was seen in FM-HA whereas it is obscure in FS-HA and absent in SM-HA. This peak could be due to the alkyl moieties in lignin (Ussiri and Johnson, 2003). Overall for these humic acids, the ratios of alkyl C (0 to 45 ppm) to carboxyl and carbonyl C (160 to 220 ppm) were 1.5 to 1.7 which indicate that short chain alkyl acids could dominate over long alkyl chains in these humic acids (Knicker et al., 2002). Further, the percentage of aliphatic C appeared to be correlated with the clay content of mineral matter in each wetland soil (64.5%, 33.3% and 44.2% for FS, FM and SM, respectively, Table 7.3). This could suggest a strong role of clay in determining aliphatic C in wetland soils. The result was consistent with the finding of Wang and Xing (2005) who showed that aliphatic fraction of humic acids is preferentially adsorbed by soil clays.

A large, sharp peak at around 54 ppm, which represents methoxyl C and C of amino acids, and the peak at 70 ppm, which can be assigned to secondary alcohols in cellulose and hemicelluloses (Larsson et al., 1999; Mauna 2002), were the major peaks observed in the spectral region of 50-108 ppm for all the three humic acids (Fig. 7.1). The 54 ppm peak was, however, slightly higher in FS-HA and SM HA than FM-HA (Table 7.3). The small shoulder peak at around 59 ppm in all three humic acids is from C in proteins (Kögel-Knabner, 1997). Relatively more contribution of this peak in FS-HA and SM-HA indicates the presence of more proteinacious material, consistent with higher amounts of N found in FS-HA and SM-HA than the FM-HA. In addition, there was a small peak observed at ~ 105 ppm, which is indicative of anomeric C of saccharides (O-C-O and O-CH-O) (Otto and Simpson, 2007). This peak is much small in these humic acids as compared to that in bulk organic C of each corresponding soil (Dodla et al., 2008), likely as a result of preferential loss of carbohydrates such as cellulose and hemicelluloses during the humification process in these wetland soils. Nonetheless, this peak is more evident in FS-HA and SM-HA than in FM-HA, consistent with the overall higher percentage of polysaccharide C in FS-HA and SM-HA than the FM-HA (Fig. 7.1 and Table 7.3). Since both FS and SM soils especially contain more clay or mineral matter in general, this observation was likely a result of humic acids formation as influenced by the presence of more clays (or more mineral matter), which has a major role in chemical and physical stabilization of the less recalcitrant fractions of organic matter (McLauchlan and Hobbie, 2004). The amount of polysaccharide C (16.6- 19.4 %) found in these wetland soil humic acids was similar to those reported for estuarine humic acids (Mao et al., 2007).

In the aromatic and phenolic region (108-165 ppm), an interesting peak at 114 ppm was more evident in SM-HA while slightly evident in FM-HA and no peak in FS-HA. This peak represents H-substituted aromatic C in guaiacyl units which represents lignin (Mahieu et al., 2002). The presence of this peak along with the strong peak at 54 ppm (methoxyl C) in SM-HA suggests the greater presence of lignin moieties in this humic acid than that in FS-HA and FM-HA. The peaks at 146 and 150 ppm observed in all the humic acids were typical for O-substituted carbons of guaiacyl and syringyl units of lignin (Knicker et al., 2002).

7.3.2 Pyrolysis-Gas Chromatography and Mass Spectrometry

Total ion current (TIC) pyrograms of the Py-GC/MS analysis with or without TMAH treatment of the FS-HA, FM-HA and SM-HA are presented in Fig. 7.2 to 7.4 and 7.5 to 7.7, respectively. The major compounds identified in the chromatograms are given in Table7.4 and 7.5 for TMAH-treated and non-treated humic acides. All the compounds identified through pyrolysis were grouped in to alkyl/ fatty acids (A), polysaccharides (Ps), protein/ nitrogen containing compounds (Pr), lignin derived phenols (L), aromatic hydrocarbons/ poly phenols (Pp) and sulfur containing compounds.

The TMAH-treated showed greater improvements in the yield and identification of lignin-derived compounds and alkyl/fatty acid compounds over untreated humic acids (Fig 7.2-

7.4 vs. Figs. 7.5-7.7). Under conditions of TMAH thermochemolysis, alkyl compounds were converted to fatty acid methyl esters (FAMEs) (Hatcher and Clifford, 1994). The major FAMEs detected were butanedioic acid dimethyl ester (A1), 14-methyl-pentadecanoic acid methyl ester (A5), 15-methyl- hexadecanoic acid methyl ester (A6), 16-methyl-heptadecanoic acid methyl ester (A8), and octadecenoic acid methyl ester (A9). The predominance of these fatty acids with $C > 9$ in all humic acids could indicate that they were mainly formed from above-ground plant aliphatic biopolymers such as cutin and cutan, and belowground aliphatic plant biopolymers such as suberin and suberan (Chefetz et al., 2002). Among the three humic acids, alkyl/ fatty acids were higher in FS-HA followed by SM-HA and FS-HA (table 7.6). This result was consistent with that observed with the^{13}C NMR observation in that FS-HA contained more aliphatic compounds.

There were significantly higher lignin-derived compounds in FM-HA than in FS-HA and SM-HA based on the TMAH thermochemolysis which especially enhanced yield and identification of lignin (Figs 7.2-7.4). The major lignin-derived compounds identified in TMAH-treated humic acids were 4-methoxy- benzoic acid methyl ester (L10), 3,4,-dimethoxy benzoic acid methyl ester (L21), 3-(4-methoxyphenyl)-2-propenoic acid methyl ester (L24), and 3,4,-dimethoxy benzoic acid methyl ester (L26). These major peaks are derived from hyp-hydroxyphenyl (L10), syringyl (L21), and guaiacyl (L24 and L26) structures of lignin, respectively (Chefetz et al., 2000). The relative abundance or ratio of syringyl structures (S-type) to guaiacyl residue structures (G-type) has been used as an indicator of stability of lignin due to the assumption that the more condensed G-type structural unit is more resistant to biological degradation than S-type structural unit (Kogel-Knabner, 1997). On the other hand, the relative abundance or ratio of hydroxyphenyl (H-type) to guaiacyl unit indicates the organic residue

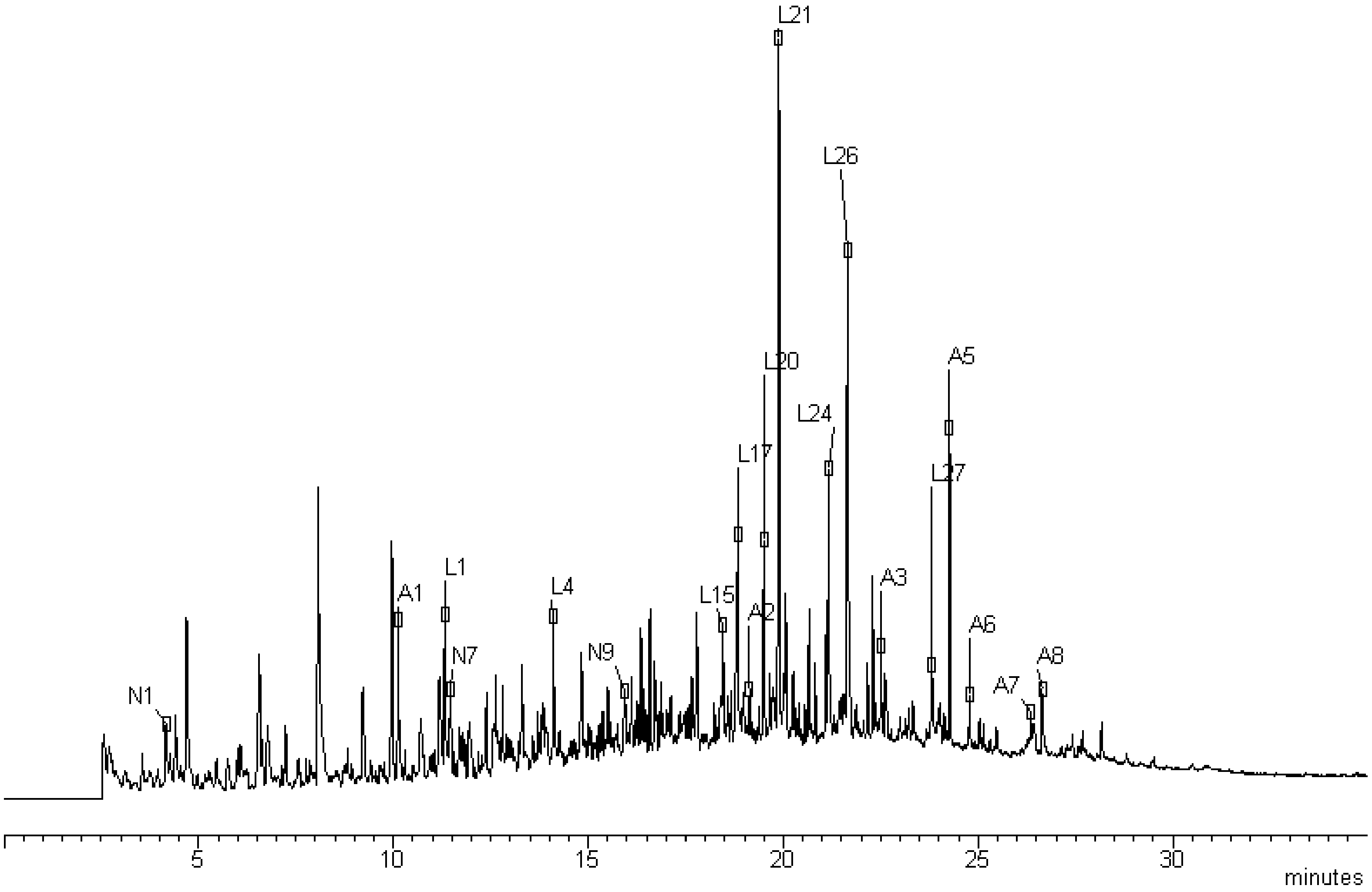

Figure 7.2 Tertamethylammonium hydroxide (TMAH) thermochemolysis-GC/MS chromatogram of Forested swamp humic acid (FS-HA)

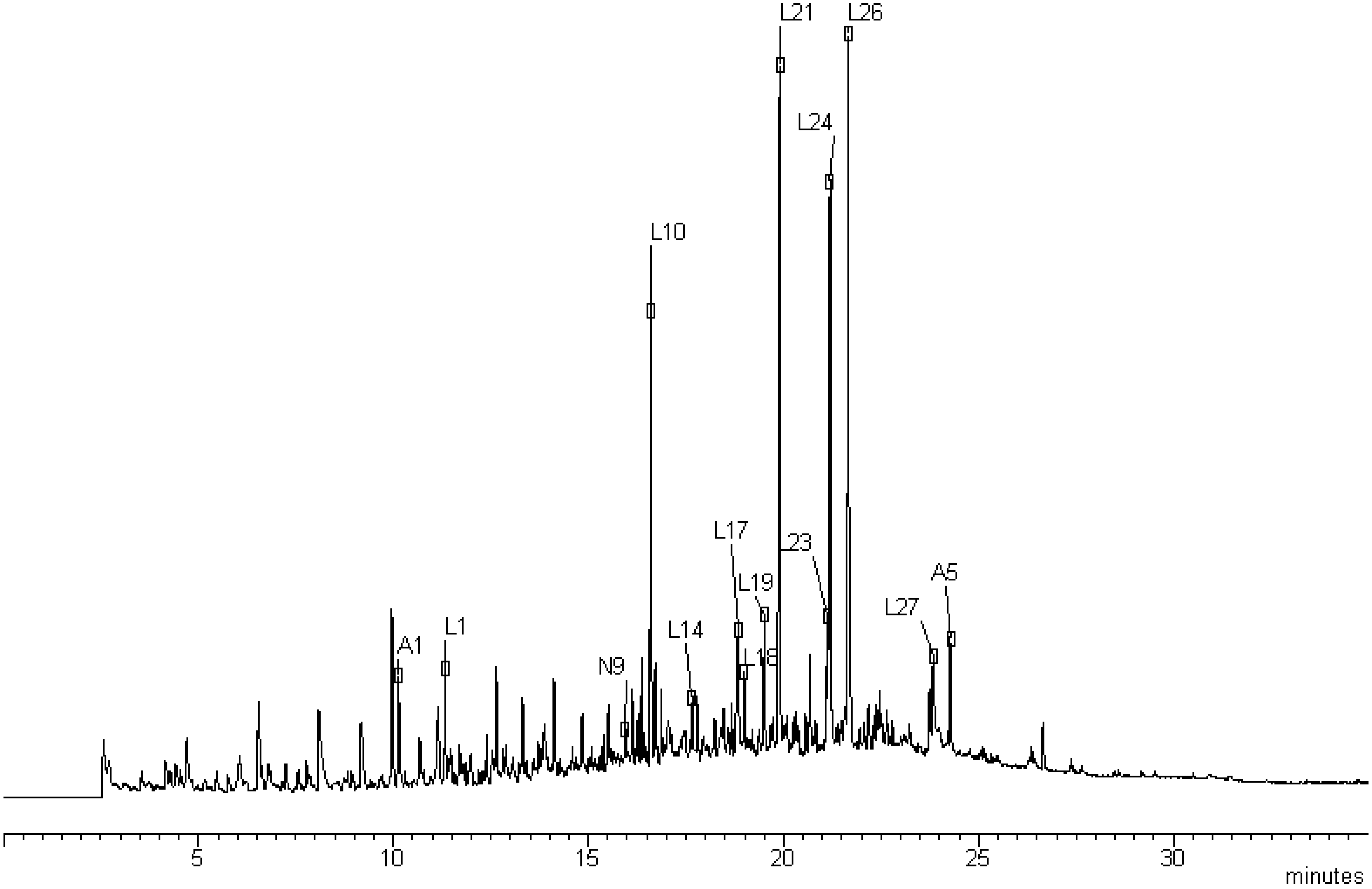

Figure 7.3 Tertamethylammonium hydroxide (TMAH) thermochemolysis-GC/MS chromatogram of Freshwater marsh humic acid (FM-HA)

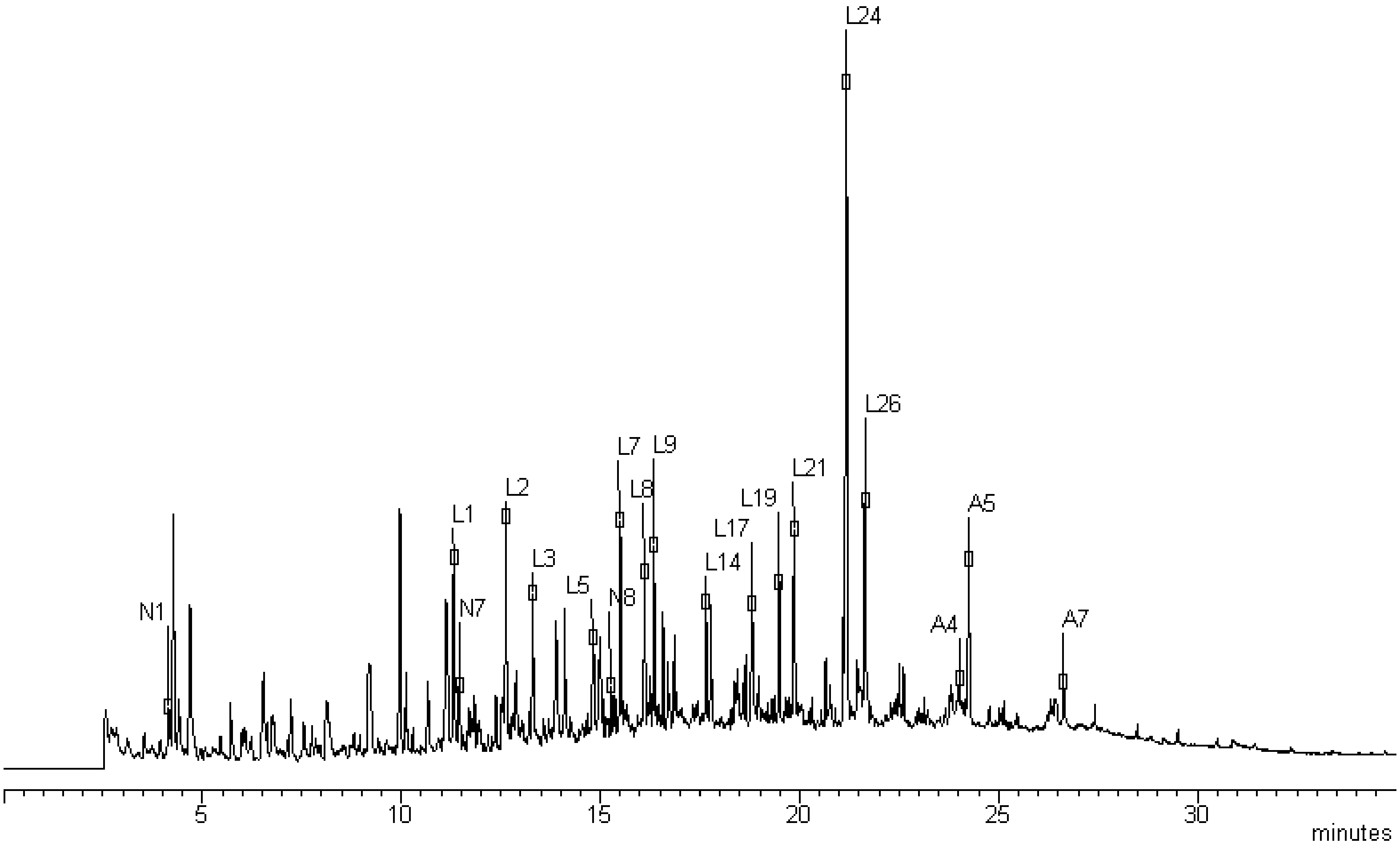

Figure 7.4 Tertamethylammonium hydroxide (TMAH) thermochemolysis-GC/MS chromatogram of Salt marsh humic acid (SM-HA)

Table.7.4. Major pyrolysis products identified by tetramethylammonium hydroxide (TMAH) thermochemolysis-GC/MS in the purified humic acids of three coastal wetland soils

Compound	
Alkyl/ Fatty acids	
Butanedioic acid, dimethyl ester	A1
Nonanedioic acid, methyl ester	A2
Pentadecanoic acid, methyl ester	A3
11- Hexadecanoic acid, methyl ester	A4
Pentadecanoic acid, 14-methyl-, methyl ester	A5
Hexadecanoic acid, 15-methyl, methyl ester	A6
Heptadecanoic acid, 16-methyl, methyl ester	A7
11- Octadecanoic acid, methyl ester	A8
16-Octadecanoic acid, methyl ester	A9
Lignin derived compounds	
Phenol, 2-methoxy-	L1
Benzene, 1-ethenyl-4-methoxy-	L2
Phenol, 2-methoxy-4-methyl-	L3
3,4-Dimethoxytoluene	L4
Phenol, 4-ethyl-2-methoxy-	L5
Estragole	L6
2-Methoxy-4-vinylphenol	L7
Phenol, 2,6-dimethoxy-	L8
Benzene, 4-ethenyl-1,2-dimethoxy-	L9
Benzoic acid, 4-methoxy-, methyl ester	L10
Benzene, 1,2,3-trimethoxy-5-methyl-	L11
2,4,6-Trimethoxybenzoic acid	L12
Benzeneacetic acid, 3-methoxy-, methyl e	L13
1,2,4-Trimethoxybenzene	L14
Benzene, 1,2-dimethoxy-4-(1-propenyl)-	L15
Benzene, 1,4-dimethoxy-2,3,5,6-tetrameth	L16
Benzoic acid, 4-hydroxy-3-methoxy-, methyl	L17
Benzenepropanoic acid, 4-methoxy-, methyl	L18
Ethanone, 1-(3,4-dimethoxyphenyl)-	L19
Benzoic acid, 3,4-dimethoxy-, methyl est	L20
Benzene, 1,2,3-trimethoxy-5-(2-propenyl)	L21
Ethanone, 1-(3,4,5-trimethoxyphenyl)-	L22
2-Propenoic acid, 3-(4-methoxyphenyl)-,	L23
Phenol, 2,6-dimethoxy-4-(2-propenyl)-	L24
Benzoic acid, 3,4,5-trimethoxy-, methyl	L25
2-Propenoic acid, 3-(3,4-dimethoxyphenyl	L26
Nitrogen- containing compounds	
1H- pyrrole, 3-methyl-	N1

1-Methyl-1H-1,2,4 -triazole	N2
1-H Pyrrole, 2, 5 dimethyl	N3
Imidazole, 5-carboxaldehyde, 1-methyl-	N4
4N-Methylcytosine	N5
N-Methyl –L-prolinol	N6
Caprolactam	N7
Indole	N8
Pyrrolidinemethaneamine, 1-ethyl-	N9

contribution of non-woody plants as the H-type residue structure is only a minor component of grass and forage type lignins (Hedges and Mann, 1979). As shown in Figs 7.2-7.4, the major peaks shifted from the presence of predominately L21 (s-type) in FS-HA to both L21 (S-type) and L26 (G-type) in FM-HA and then to predominately the L24 (G-type) in SM-HA. The results clearly suggest that there was an increasing G-type structural dominance of lignin along the salinity gradient, suggesting more stable lignin residue structure in SM-HA even though there was more lignin in FM-HA (more intensity of lignin-derived compounds). This increase in G-structural unit of lignin in SM-HA together with ^{13}C NMR-identified more condensed domain of aliphatic in SM-HA appeared to indicate the more stable nature of this humic acid formed in saline marsh soils. In addition, the strong peak of 4-methoxy- benzoic acid methyl ester (L10, H-type) in the FM-HA could suggest the strong influence of grass and forage residue inputs in the formation of HA in the freshwater marsh soil.

The dominant N-derived compounds from TMAH thermochemolysis were 3-methyl- 1-H-pyrrole (N1), Caprolactam (N7), Indole (N8), and 1-ethyl-pyrrolidinemethaneamine (N9) (Figs. 7.2 –7.4). Indoles may have come from tryptophan-containing peptides while pyrroles could be come from proline-containing peptides (Cheftez et al., 2002). The higher amount of N-containing compounds was observed in FS-HA and SM-HA than that of FM-HA (Table 7.6). This result was consistent with significantly lower C/N ratio in FS-HA and SM-HA than that of FM-HA (Table 7.2).

Pyrolysis-GC/MS analysis of humic acids without TMAH treatment showed that concentration of polysaccharides is dominated in SM-HA than in FS-HA and FM-HA (Table 7.6). The major detected polysaccharides –derived compounds were acetic acid (Ps1), 3-methyl furan (Ps2), 5-methyl -2- furancarboxaldehyde (Ps6), and 2,3-dihydrobenzofuran (Ps8) (Figs. 7.5-7.7). In particular, the benzofuran peak (Ps8) was significantly higher in SM-HA than in FM-HA and FS-HA and contributed up to 7.98 % of the total peak area. These polysaccharide-derived compounds in the humic acids were similar to those found as the characteristic compounds observed in Py-GC/MS analysis of bulk soil organic matter (Chefetz et al., 2002; Fabbri et al., 2005; Dieckow et al., 2006). The higher intensity of polysaccharides-derived compounds as determined by Py-GC/MS in the SM-HA than in the FMHA and FSHA was consistent with the observation by ^{13}C NMR although the latter also showed slightly greater percentage of polysaccharides in the FS-HA. We speculate that the high amounts of polysaccharides in the SM-HA could be derived from the lingocellulose in cell walls of *Spartina alterniflora,* the dominant species in saline marsh. According to Hodson et al. (1984), as much as 75% of total biomass in *Spartina alterniflora* is lignocellulose, which likely influence residue degradation and HA formation. In addition, the presence of uncongenial conditions for microbial activity in the saline marsh could also contribute to the higher concentration of polysaccharide-type of compounds in its humic acid which is normally dominant in fresh plant materials. As expected, sulfur-containing compounds were higher along with the salinity gradient increase with the highest concentration found in the SM-HA followed by FM-HA and FS-HA, which was consistent with the level of total S in each espective HA (Table 7.2). Sulfide in SM soils likely forms sulfur-organic compounds with polysaccharide C which could also increase the stabilization of the latter (Ciglenecki et al., 2000).

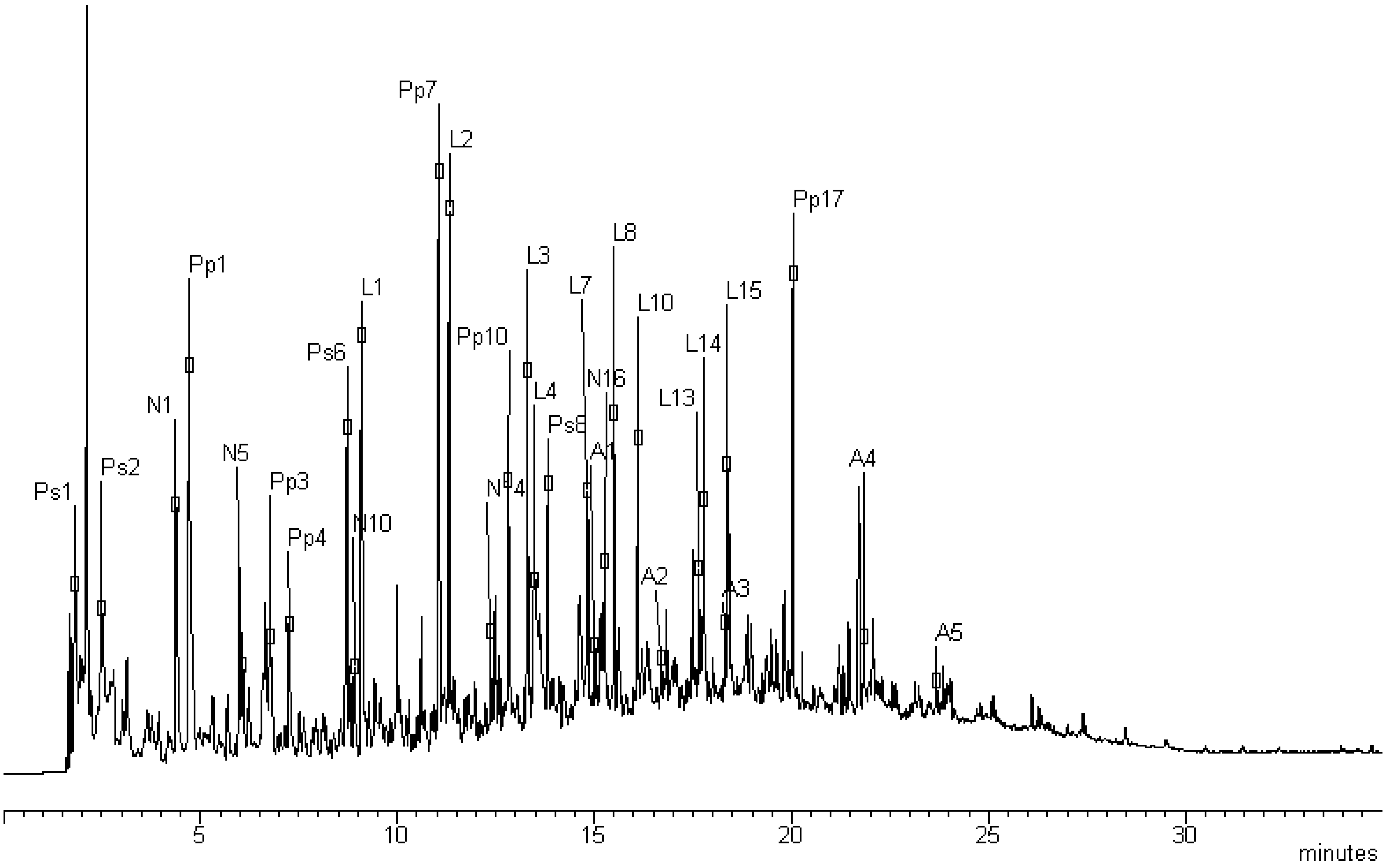

Figure 7.5. Pyrolysis-GC/MS total ion current chromatogram of Forested swamp humic acid (FS-HA)

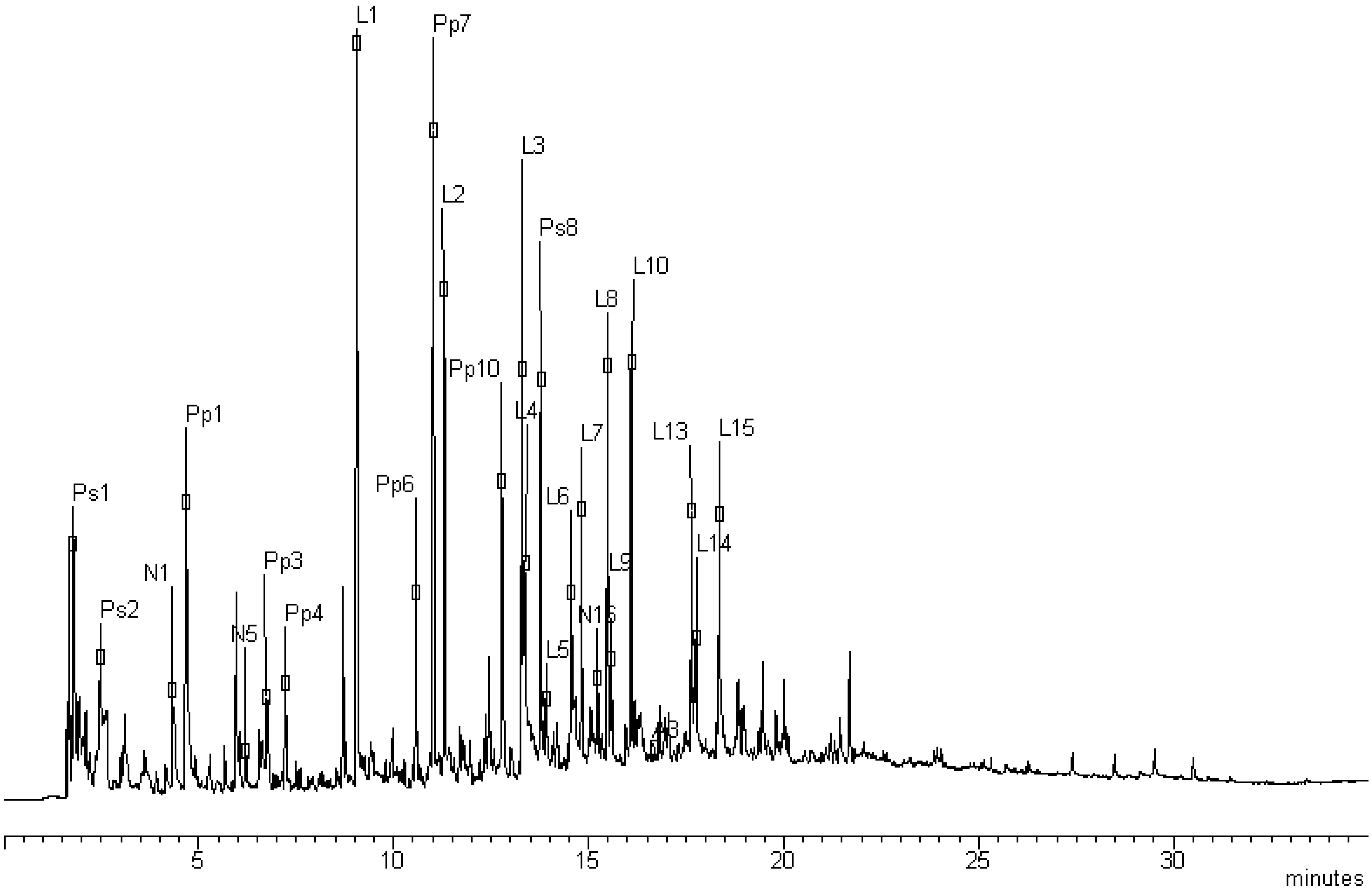

Figure 7.6. Pyrolysis-GC/MS total ion current chromatogram of Freshwater marsh humic acid (FM-HA)

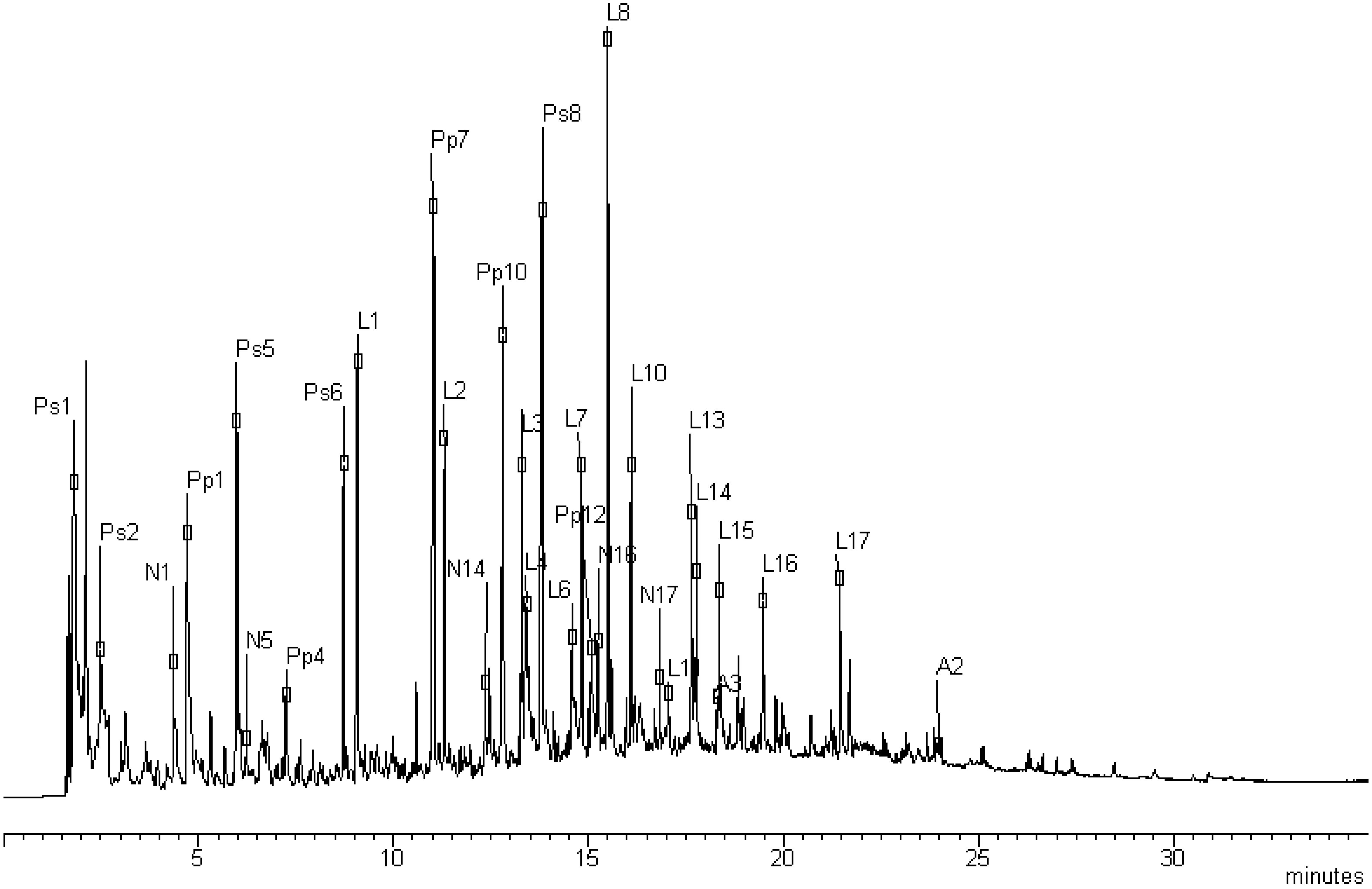

Figure 7.7. Pyrolysis-GC/MS total ion current chromatogram of Salt marsh humic acid (SM-HA)

Table7.5. Main Pyrolysis- GC/MS compounds identified in the purified humic acids of three coastal wetland soils without TMAH

Compound	
Alkyl/ Fatty acids	
Nonadecene	A1
Tetradecene	A2
Heptadecene	A3
1-Dodecanol, 3,7,11-trimethyl	A4
Ethanol, 2-(9-octadecenyloxy)-	A5
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	A6
Hexacosene	A7
Lignin derived compounds	
Phenol	L1
Mequinol	L2
Phenol, 2-methoxy-4-methyl-	L3
1,2-Benzenediol	L4
Phenol, 3-methoxy-	L5
1,2-benzenediol, 3-methoxy-	L6
Phenol, 4-ethyl-2-methoxy-	L7
2-Methoxy-4-vinyl phenol	L8
3-methoxy-5-methylphenol	L9
Phenol, 2,6-dimethoxy-	L10
Benzaldyhyde,3-hydroxy-4methoxy-	L11
Phenol, 2-methoxy-5-(1-propenyl)-	L12
1,2,4- Trimethoxy benzene	L13
Phenol, 2-methoxy-4-(1-propenyl)-	L14
Ethanone, 1-(4-hydroxy-3-methoxyphenyl)-	L15
24-dimethoxyacetophenone	L16
Phenol,26-dimethoxy-4-(2-propenyl)-	L17
Other aromatic/ poly phenol derived compounds	
Toluene	Pp1
Ethyl benzene	Pp2
Xylene	Pp3
Styrene	Pp4
Benzene,13,5-trimethyl-	Pp5
Phenol, 2-methyl	Pp6
Phenol,4-methyl	Pp7
Phenol, 3,5-dimethyl-	Pp8
Benzene, 12,4,5-tetramethyl-	Pp9
Phenol,3-ethyl	Pp10
Phenol,2-ethyl-5-methyl	Pp11
1,2-benzenediol,3-methyl-	Pp12

Phenol,4-(2-propenyl)-	Pp13
Biphenyl	Pp14
Naphthalene, 1,4-dimethyl-	Pp15
5-tert-Butylpyrogallol	Pp16
Naphthalene, 23,6-trimethyl-	Pp17
Nitrogen- containing compounds	
Pyridine	N1
Ethylenediamine	N2
Pyridine, 2-methyl-	N3
Imidazole	N4
1-H-Pyrrole, 3-methyl	N5
Pyrrolidine,1-(1-butenyl)-	N6
1-H-Pyrrole2,5-dimethyl	N7
1-H-Pyrrole, 2-ethyl	N8
Pyridine, 3,4-dimethyl-	N9
Pyridine,3-ethenyl-	N10
2,2-Diallylpyrrolidine	N11
1-H-Pyrrole, 2,3,5-trimethyl	N12
4-Pyridinecarboxamide, N-hydroxy-	N13
Benzene,1-isocyano-2-methyl	N14
Benzenepropanenitrile	N15
m-Aminophenylacetylene	N16
1-H-Indole, 2-methyl	N17
Polysaccharide derived compounds	
Acetic acid	Ps1
Furan, 3 methyl	Ps2
Acetic acid, methoxy	Ps3
Furan, 2,5-dimethyl	Ps4
4,6-O-Furylidene-d-glucopyranose	Ps5
2-Furancarboxaldehyde,5-methyl	Ps6
2-cyclopenten-1-one, 2,3-dimethyl	Ps7
Benzofuran,2,3-dihydro	Ps8
2-Coumaranone	Ps9

Table. 7.6. Qualitative distribution of different compound groups among the three humic acids determined by Pyrolysis GC-MS

Compound Group	FS HA†	FM HA†	SM HA†
TMAH treated			
Alkanes/ Alkenes/ fatty acids	21.9	8.8	16.1
Lignin derived compounds	58.8	79.5	58.4
Poly phenols	10.9	7.6	12.4
Protein/ N- containing compounds	7.8	3.2	5.3
Non TMAH treated			
Polysaccharides	10.2	11.4	16.3
Sulfur containing compounds	3.8	2.2	6.7

†FM HA: Freshwater marsh humic acid, SM HA: Salt marsh humic acid, FS HA: Forest swamp humic acid.

7.4 Conclusion

This study demonstrates significant differences among the humic acids formed in the three different coastal wetland soils of forest swamp, freshwater marsh and saline marsh, reflecting likely effects of different environmental conditions, in-situ biogeochemical conditions and source materials on the humification pathway in a given soil. The CPMAS ^{13}C NMR observation was, in general, consistent with the Pyrolysis-GC/MS characterization of the humic acids. The FS-HA exhibited the highest percentage of aliphatics whereas the FM-HA had the highest percentage of aromatics. On the other hand, along the salinity gradient increase, there was a generally increase in the condensed domain of alkyl C and more stable G-type structural unit of lignin residue as well as the increased contribution of sulfur as a structural component in humic acids, suggesting a stronger or more stable humic acid formation even though a greater aromaticity was found in FM-HA. Overall, the result reveals a clear effect of salinity on structural formation of humic acids.

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CHAPTER 8

CONCLUSIONS

Understanding role of various factors influencing soil organic carbon mineralization and transformation is necessary for assessing the integrated impact of seawater intrusion and Mississippi river water diversion activities on Louisiana coastal wetlands particularly bottomland forest swamp (FS), freshwater marsh (FM) and saline marsh (SM) soils. The focus of this study was first to evaluate the effect of cations, clay and salinity on soil organic carbon (SOC) mineralization and transformation and greenhouse gas emissions in the coastal wetlands. Laboratory microcosm study demonstrated that addition of K^+ and Ca^{2+} significantly increased C mineralization, measured as C gas production, in FS soil. In contrast, addition of K^+ and Ca^{2+} had no significant effect on CO_2 production from FM soil. Clay addition at both 2 % and 5% levels and 5% clay along with 5 mM Ca significantly decreased CO_2 production. Salinity treatment at 15 and 30 ppt led to decreased mineralization in both FS and FM soils. However the decrease was significant only for 30 ppt treatment in FM soil. Methane emissions were not significantly affected by any of the treatments except the salinity treatment which decreased CH_4 emissions significantly. Pyrolysis- GC/MS analysis showed that 5 % clay +5 mM Ca and salinity treatments enriched alkyl compounds in the SOM as compared to more lignin in the SOM of the control

A separate laboratory microcosm study under anaerobic conditions showed that the presence of NO_3^- (especially at 3.2 mM) significantly decreased CO_2 production whereas SO_4^{2-} (2 and 5 mM) did not. On the other hand, both SO_4^{2-} and NO_3^- treatments decreased CH_4 production but the NO_3^- almost completely inhibited CH_4 production (>99%) whereas the SO_4^{2-} treatments reduced CH_4 production by 78 – 90%. In addition, the study revealed that a large proportion (95%) of anaerobic carbon mineralization in the untreated freshwater marsh soil could not be unexplained by the reduction of any of the measured major electron acceptors including NO_3^-, SO_4^{2-}, Fe (III), Mn (IV), suggesting other processes were likely at work to facilitate C gas production under anaerobic environment.

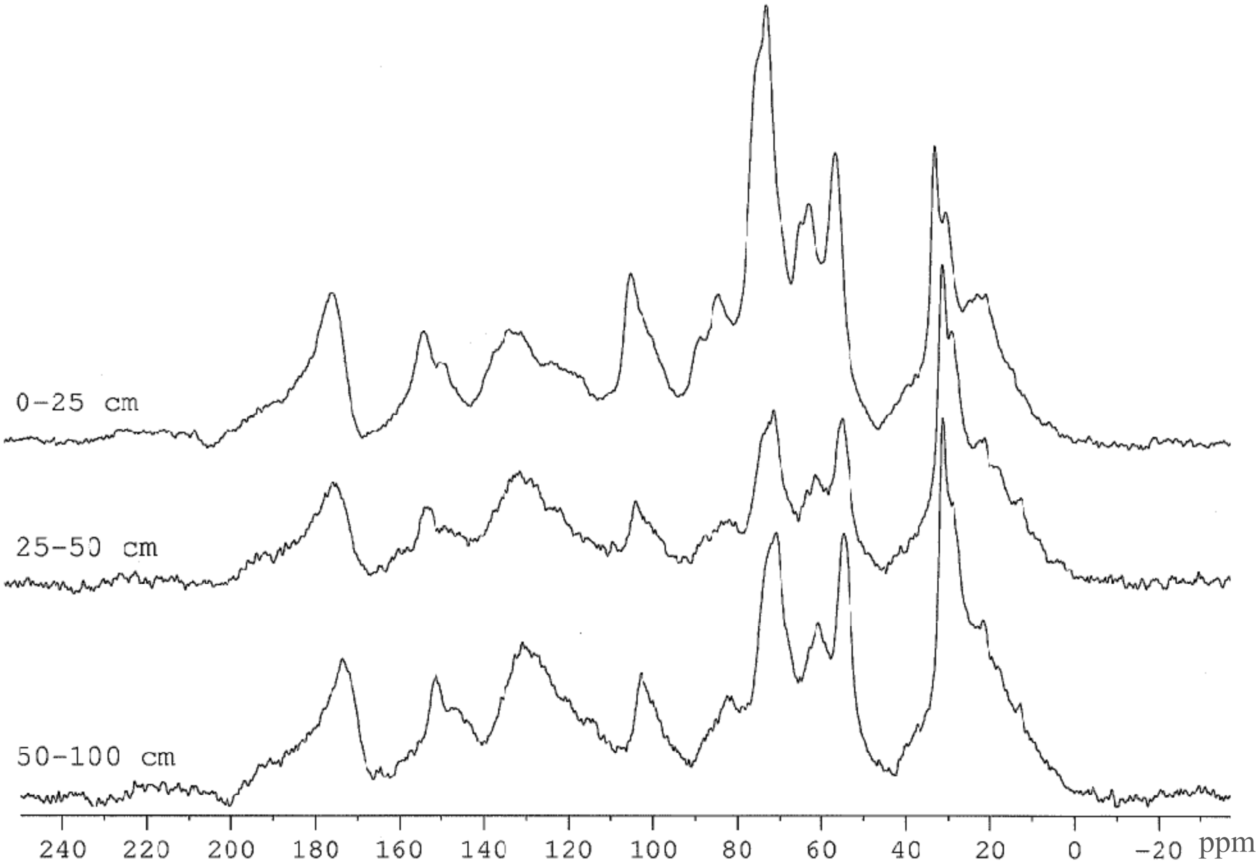
As for the NO_3^- removal capacity of different Louisiana coastal wetland soils, the FM soil profile exhibited the highest potential denitrification rate (PDR) on both unit weight and unit volume basis as compared to FS and SM soils. The FM also tended to yield higher amount of N_2O compared to the FS and SM especially at earlier stages of denitrification, suggesting incomplete reduction of NO_3^- at FM and potential for emission of N_2O. Saline marsh soil profile had the lowest PDR on the unit volume basis. Increasing incubation concentration from 2 to 10 mg NO_3^- -N L^{-1} increased PDR by 2 to 6 fold with the highest increase in the top horizons of FS and SM soils. Regression analysis showed that across these three wetland systems, organic C has significant effect in regulating PDR. Of the compositional molecular C moieties, polysaccharides positively influenced denitrification rate whereas phenolics (likely phenolic adehydes and ketonics) negatively affected denitrification rate in these wetland soils.

In order to better assess C mineralization in these wetland soils, labile organic carbon (LOC) was determined by different methods including water extraction, salt extraction, and aerobic mineralization and compared. In addition, acid hydrolysable carbon (AHC) which includes both labile and slowly degradable C was also determined. The relationships of these LOC fractions to distribution of C functional groups as characterized by ^{13}C NMR were evaluated. In general, the amount of LOC increased with total soil C regardless of wetland soil type. LOC fractions estimated by different methods were positively and significantly correlated with each other. The amount of LOC fraction of soil organic C was <1 % of the total organic carbon (TOC) in all wetlands. In contrast, AHC represented up to 49 % of TOC. The amount of aerobically mineralizable carbon increased significantly with the increase in the amount of polysaccharides and carboxylic C in the SOM.

Further, in order to assess the stable fraction of SOM in each of these wetlands humic acids from 0 – 50 cm horizons of respective soils were fractionated following the procedure outlined by International Humic Substances Society (IHSS). The results of the solid state ^{13}C NMR demonstrated that humic acids from three sites were appreciably different from each other. Humic acids fractionated from forest swamp soil (FS-HA) were high in aliphatic C as compared to those of the saline marsh (SM-HA) and freshwater marsh (FM-HA) soils. On the other hand, FM-HA was more aromatic than SM-HA and FS-HA. The % aromaticity of FS-HA, FM-HA and SM-HA was 35.4, 47.2 and 39.7% respectively. Further 13C NMR indicates that the observation crystalline domine of aliphatics in the humic acids increased along the increasing salinity gradient from FS-HA to SM-HA. Pyrolysis-GC/MS results supported the findings of the ^{13}C NMR analysis, showing that FM-HA is also more aromatic than SM-HA and FS-HA. In addition, Py-GC/MS revealed a trend of increasing guaiacyl structure units (G-type) in the lignin of humic acids along the salinity gradient, suggesting that more stable humic acids formation in saline marsh soils. The amount of N- and S- containing compounds were in line with the trend of total content of elemental N and S in these humic acids.

Overall the results suggest different C mineralization and transformation in these three wetland soils likely reflects the differences in organic residue inputs, biological, and environmental conditions.

APPENDIX A: CARBON 13 CPMAS NMR SPECTRA OF FRESHWATER MARSH SOIL PROFILE

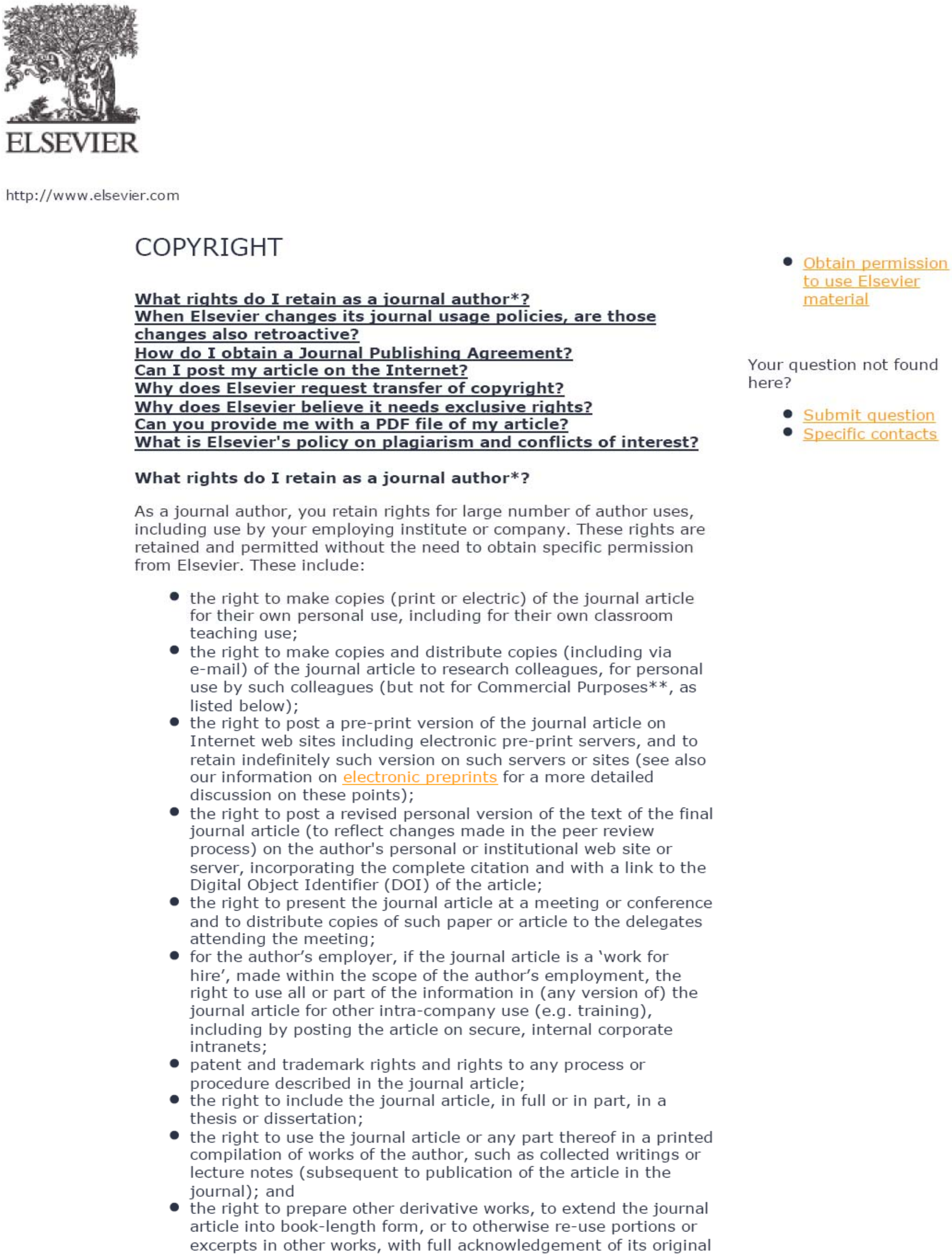


APPENDIX B: RELEASE OF N_2O-N AND N_2-N DURING THE INCUBATION OF SELECTED LOUISIANA WETLAND SOILS.

Site	Incubation Time	Treatment			
		2 mg L^{-1} NO_3^{-1}-N		10 mg L^{-1} NO_3^{-1}-N	
		N_2O -N	N_2-N	N_2O -N	N_2-N
	h	------- mg N kg^{-1} soil ------			
Forest swamp					
0 - 25 cm	6	0.13	0.09	0.04	0.10
	12	0.38	0.39	0.16	0.58
	24	0.78	0.77	0.62	1.38
	48	0.29	1.79	0.41	8.97
	72	0.09	1.55	0.05	7.03
	120	0.02	0.69	0.00	2.54
25 -50 cm	6	0.17	0.13	0.05	-
	12	0.46	0.32	0.10	-
	24	0.61	0.63	0.08	0.19
	48	0.11	1.62	1.72	2.55
	72	0.01	2.13	0.20	7.77
	120	0.00	1.05	0.01	4.59
50 - 75 cm	6	0.02	0.02	0.03	-
	12	0.07	0.10	0.14	-
	24	0.12	0.44	0.18	-
	48	0.07	1.68	0.06	1.06
	72	0.01	1.21	0.05	3.75
	120	0.00	0.65	0.02	5.39
Freshwater marsh					
0 - 25 cm	6	1.34	0.27	0.17	0.01
	12	8.33	0.91	0.71	1.43
	24	8.10	1.67	15.00	9.12
	48	3.15	4.98	15.81	-
	72	0.18	4.83	9.54	-
	120	0.00	1.53	3.19	-
25 - 50 cm	6	0.00	0.00	0.02	0.04
	12	0.24	0.14	0.21	0.10
	24	0.27	3.50	0.06	14.69
	48	0.08	2.89	0.01	13.63
	72	0.00	2.03	0.00	9.16
	120	0.00	0.61	0.00	4.85

50 - 100 cm	6	0.00	0.00	0.06	0.00
	12	0.14	0.17	0.61	0.12
	24	3.40	3.80	0.28	21.29
	48	1.07	5.46	0.01	24.25
	72	0.37	4.08	0.00	13.98
	120	0.02	1.45	0.00	10.94
Saline marsh					
0 - 50 cm	6	0.00	0.05	0.00	0.07
	12	0.00	0.32	0.00	0.31
	24	0.00	0.43	0.00	2.02
	48	0.00	0.22	0.02	0.51
	72	0.00	0.11	0.01	0.16
	120	0.00	0.00	0.01	0.03
50 - 75 cm	6	0.00	0.00	0.02	0.00
	12	0.00	0.06	0.00	0.04
	24	0.00	0.31	0.03	0.91
	48	0.00	1.68	0.00	4.24
	72	0.00	1.53	0.00	12.59
	120	0.00	0.71	0.00	8.06
75 - 100 cm	6	0.00	0.00	0.04	0.02
	12	0.07	0.00	0.04	0.01
	24	0.19	0.27	0.28	0.06
	48	0.14	0.80	0.14	8.32
	72	0.16	4.25	0.88	15.62
	120	0.00	2.90	0.32	21.05

APPENDIX C: PERMISSIONS



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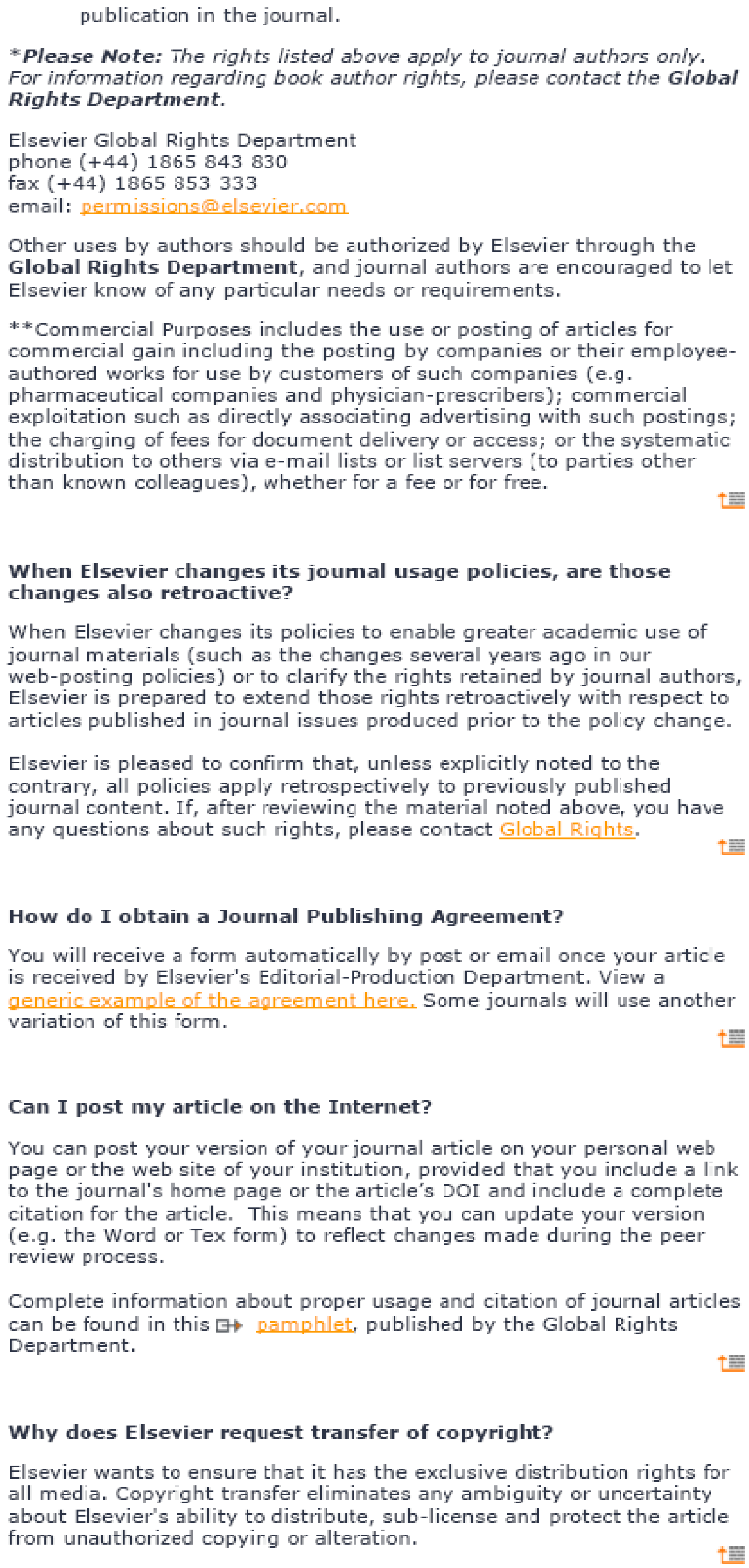

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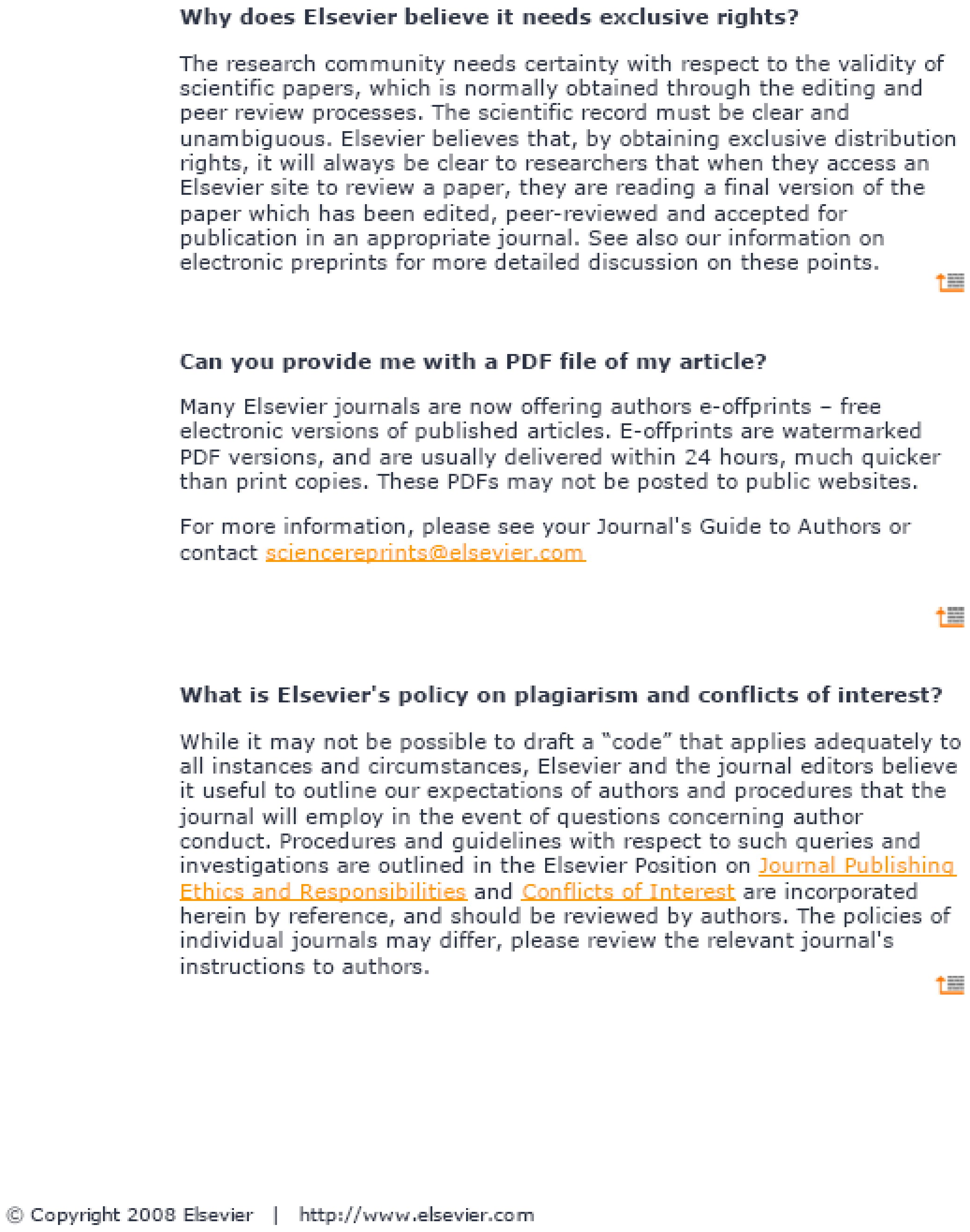

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VITA

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