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Wetland Vegetation Dynamics and Ecosystem Gas Exchange in Response to Organic Matter Loading Rates

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Wetland Vegetation Dynamics and Ecosystem Gas Exchange in Response to Organic Matter Loading Rates

A Thesis
Presented to

The Faculty of the School of Marine Science
College of William and Mary

In Partial Fulfillment
of the Requirements for the Degree of
Masters of Science

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David E. Bailey
2006
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The requirements for the degree of

Master of Science

Approved, August 15, 2006

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Wetland Vegetation Dynamics and Ecosystem Gas Exchange in Response to Organic Matter Loading Rates
ABSTRACT

Created wetlands are often limited in soil organic matter, usually a long-term product of ecosystem succession. Although many studies have tested the effect of adding organic material to these systems, few if any, have quantified the effect of various loadings of organic matter in created wetlands. The goal of this study was to determine how vegetation composition, standing crop biomass, woody vegetation development, and ecosystem gas exchange varied in a created freshwater wetland along a gradient of soil organic carbon (0 to 336 Mg ha\(^{-1}\) loading rates). Plot surface elevation varied positively with OM loadings, suggesting that inundation/aeration may modify OM effects. Soil nutrients (C, N, C:N, and P) also positively correlated with loading rate. Vegetation measurements suggested an overall similarity of plant assemblage composition and biomass regardless of loading rate, and a slight increase in tree size with loading rate. Gross primary production and net ecosystem exchange were weakly positively and negatively correlated with loading rate, respectively. Respiration was strongly positively correlated with loading rate, and was likely the controlling factor of CO\(_2\) gas flux among treatments. Soil nutrient values and vegetation composition, as well as ecosystem gas flux balance appear to be the best parameters upon which to base an organic matter loading rate decision. In this study, adding an organic matter amendment between LR 2 (56 Mg ha\(^{-1}\)) and 3 (112 Mg ha\(^{-1}\)) seems most appropriate, and may provide a “jumpstart” for the created non-tidal wetlands while also minimizing changes in surface elevation due to the added bulk material.
Chapter 1

A review of non-tidal wetland ecology and created wetlands

Introduction

Freshwater wetlands are important and unique features of the global landscape. These environments often provide favorable circumstances to facilitate physical and biogeochemical functions that are critical to the maintenance of healthy environmental conditions in waterways (Mitsch and Gosselink 2000). Some of these purported hydrologic and water quality functions include short- and long-term surface water storage, maintenance of a high water table, groundwater discharge or recharge points, transformation and cycling of elements, retention and removal of dissolved elements, and accumulation of inorganic sediments (NRC 1995, Cole 2002). Other ecosystem functions performed by wetlands include fish and wildlife habitat, primary production, waste assimilation, and global carbon and nutrient cycling (Odum 1978). It is important to note that the ability of wetlands to perform certain ecosystem functions depends on their extent and their location within the larger landscape. For example, the functionality of a forested wetland will vary; if it lies along a river, it has a greater functional role in maintaining water quality than if it were isolated from the stream (Mitsch and Gosselink 2000).

Recognition of the importance of wetland functions to our own well-being (i.e. ecosystem services), and the acknowledged loss of 53% of the natural wetland area in the contiguous United Stated (USEPA 1989, Dahl 1990), convinced the United States Congress to insure the maintenance of adequate wetland area under the 1977 Clean Water Act (CWA). Section 404 of the CWA is the primary guidance concerning wetland protection, administered under the regulatory power of the US Army Corps of Engineers (USACE) overseen by the Environmental
Protection Agency (EPA). In 1990, President George Bush, Sr. recommended a policy of ‘No Net Loss’ of wetlands as a national goal (Mitsch and Gosselink 2000). As such, many permits issued under section 404 of the CWA authorizing the filling or draining of wetlands are accompanied by directives to create or restore a similarly sized or larger wetland area (i.e. compensation). Importantly, however, compensatory wetland restoration and creation are “last resort” regulatory options (Whigham 1999) following avoidance and minimization of wetland disturbance. Wetland creation involves using engineering and construction practices to establish conditions conducive to the development of wetland hydrology, soils, and vegetation in areas where wetlands did not previously or historically exist. Creating a new wetland as a mitigation practice is more difficult than wetland restoration due to the complexity of establishing the necessary hydrologic regime (Stolt et al. 2000).

Succession in ecosystems, according to the framework of E.P. Odum (1969), involves the transfer and flow of energy through time that shows perceivable directionality, is affected by both the physical environment and community itself, and leads to a theoretical stabilized system where energy efficiency for both biomass and symbiotic organism interaction are maximized. As ecosystems mature, and energy usage shifts over time from high biomass production to ecosystem maintenance, changes in the overall ecosystem energy balance should reflect a progression from a net autotrophic regime (photosynthesis (P) / respiration (R) ratio >1) to a net heterotrophic regime (P/R = 1). Such successional concepts, especially early successional processes (primary succession) are rarely studied in wetlands (van der Valk 1981), and are only now beginning to be applied to created wetlands (Noon 1996, DeBerry and Perry 2004).

Organic matter is important in the primary succession conceptual model because it accumulates in developing ecosystems over time, mitigating perturbations caused by the physical
environment (Odum 1969). Organic matter accumulation is an important and characteristic feature of most wetland systems. However, created wetlands usually contain very low soil organic carbon relative to natural wetlands; traditional creation practices such as topsoil scraping remove not only the existent vegetation, but also any accumulated organic material in the surface A horizon including seed banks. Many studies have advocated amending wetland creation sites with organic material either in the form of salvaged natural wetland soils or mulches (Stauffer and Brooks 1997, Whittecar and Daniels 1999, McKinstry and Anderson 2003, Anderson and Cowell 2004, Bruland and Richardson 2004).

Monitoring organically amended created wetlands not only allows the comparison of created wetland success between amended and unamended sites, it also facilitates the characterization of primary succession processes at different organic mater loading rates by simulating one aspect of the chronosequence of seral stages in created wetland development. The purpose of this study was to evaluate the primary succession processes occurring in a created forested wetland, in terms of both vegetative characteristics and ecosystem energy exchange, along a gradient of soil organic carbon input that mimics the conceptual idea of increasing organic matter with increasing ecosystem maturity. The parameters of interest in this study were herbaceous vegetation community composition and primary productivity, woody vegetation development, and the relative rates of photosynthesis and respiration (carbon flux) of the vegetation-soil continuum. The goal of this study was threefold: 1) to determine how various parameters (plant community composition, standing crop, woody vegetation growth, and Net Ecosystem Exchange) in created freshwater wetlands vary with respect to different loadings of organic matter amendments in wetland soils, 2) to monitor ecosystem function within a primary successional system with respect to an added soil organic carbon gradient, and 3) to use the
measured parameters to elucidate the effect of adding various loadings of an organic matter amendment on created wetland success.

**Literature Review**

Successional processes in wetlands are the result of physical, chemical, and biological factors that influence such ecosystem characteristics as plant community composition, primary productivity, and energy exchange in the form of CO$_2$ flux into and emitted from the wetland. In created wetland systems, these factors act to control or influence pedogenesis and chemical and nutrient cycling, which then shape the attendant vegetative community. The following sections will explore the fundamental and relevant literature that describe the soil and chemical processes affecting wetland primary succession, and will then focus on the historic and current research concerning created wetland systems and primary succession within these systems.

**Wetland Soils and Pedogenesis**

*Soil Development*

Soil development tends to be more advanced on older sites – that is, soil horizonation and strength of expression increases with time since the last major disturbance or since the start of uninterrupted soil creation (Odum 1969, Marks and Bormann 1972, Odum 1985, Chadwick and Graham 2000). In wetlands, this is due to the fact that soil-forming processes such as weathering, incorporation of organics, and soil oxidation/reduction (redox) processes are time-dependent (Jenny 1941, Stevens and Walker 1970, Mausbach and Richardson 1994) and are inevitably linked to the colonizing vegetation (Craft 2001) and to the depletion of free oxygen in the soil (Mitsch and Gosselink 2000, Megonigal et al. 2004). Thus, soil development in non-
tidal, freshwater wetlands is defined by in situ physical, chemical, and biological attributes present at a given point in time.

Wetlands are identified in part by the hydric characteristics of their underlying soil substrate. Hydric soils are defined as soils that “formed under conditions of saturation, flooding, or ponding long enough during the growing season to develop anaerobic conditions in the upper part” (NTCHS 1985). Hence, anaerobiosis – the process of oxygen depletion in the soil via biogeochemical pathways – is a critical defining factor in wetland soils.

Because molecular oxygen diffuses 10,000 times more slowly through water than air, microbial metabolism in a saturated soil preferentially utilizes O$_2$ faster than it can be replaced by diffusion, and the system becomes anaerobic (Megonigal et al. 2004). Under inundated conditions, oxygen may be present in shallow surface water due to the rapid transport of atmospheric O$_2$ across the air-water interface, low populations of oxygen-consuming organisms in the water column, photosynthetic O$_2$ production from algae, and surface water mixing (Gambrell and Patrick 1978). As such, a continuous supply of dissolved oxygen is usually present at the soil-water interface, resulting in a thin oxidized layer at the soil surface as O$_2$ follows a concentration gradient downward (Ponnamperuma 1972).

As organic substrates are oxidized during microbial metabolism, free oxygen is preferentially utilized as a terminal electron acceptor at a redox potential of 400 to 600 mV. As a result, the O$_2$ concentration below the thin oxidized layer drops abruptly in waterlogged soils, and may be completely depleted within a matter of millimeters. At this point, a hierarchy of potential terminal electron acceptors may be available to microorganisms that can respire under increasingly anaerobic conditions, and these organisms preferentially reduce inorganic chemical substrates based on the redox potential of the respective transformations (Table 1; Mitsch and
Gosselink 2000, Wang and Patrick 2000). Chemical reactions that take place under anaerobic conditions, or reduction-oxidation (redox) reactions, are a primary component in both the creation of hydric soil redoximorphic features and the driving force behind nutrient cycles responsible for selecting for and against certain plant species inherent in primary succession (Mitsch and Gosselink 2000). These redox reactions are discussed below in the Chemical and Nutrient Cycling section.

<table>
<thead>
<tr>
<th>Element</th>
<th>Oxidized Form</th>
<th>Reduced Form</th>
<th>Redox Potential (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>NO$_3^-$ (nitrate)</td>
<td>N₂O, N₂, NH$_4^+$ (nitrous oxide, nitrogen gas, and ammonium)</td>
<td>250</td>
</tr>
<tr>
<td>Manganese</td>
<td>Mn$^{4+}$ (manganic)</td>
<td>Mn$^{2+}$ (manganous)</td>
<td>225</td>
</tr>
<tr>
<td>Iron</td>
<td>Fe$^{3+}$ (ferric)</td>
<td>Fe$^{2+}$ (ferrous)</td>
<td>120</td>
</tr>
<tr>
<td>Sulfur</td>
<td>SO$_4^{2-}$ (sulfate)</td>
<td>S$^{2-}$ (sulfide)</td>
<td>-75 to -150</td>
</tr>
<tr>
<td>Carbon</td>
<td>CO$_2$ (carbon dioxide)</td>
<td>CH$_4$ (methane)</td>
<td>-250 to -350</td>
</tr>
</tbody>
</table>

Soil Organic Matter

The progressive increase in soil organic matter with site age is accompanied by increases in denitrification capacity (Johns et al. 2004), C:N ratios (Nair et al. 2001), and plant species diversity (Reinhartz and Warne 1993), and decreases in bulk density, pH, and soil chroma (Bischel-Machung et al. 1996, Nair et al. 2001, Campbell et al. 2002). Soil organic matter provides the materials and energy necessary to sustain metabolism within wetland ecosystems, including providing plant nutrients through its decomposition and mineralization (Neue 1985),
and provides the reducing power for microbially mediated chemical transformations under anaerobic conditions (Vepraskas and Faulkner 2001).

Soil organic matter and organic carbon are coupled inexorably with nutrient cycling, and regulate the conservation and recycling of nutrients between microbial, plant, and soil communities (Collins and Kuehl 2001). As microorganisms oxidize organic compounds, organically bound plant nutrients such as nitrogen and phosphorus are mineralized into their inorganic forms. Concomitantly, microorganisms use carbon and other nutrients from plant residues for their own growth, thereby temporarily immobilizing these compounds into microbial biomass (Qualls and Richardson 2000). As microbes die and decompose, assimilated carbon and nutrients are again mineralized and become available for further decomposition or successive uptake. These processes occur in both aerobic and anaerobic environments; however, different physiological groups of microorganisms are active in the two states, and the oxidized forms of plant nutrients are reduced in anaerobic environments due to the loss of oxygen as an electron acceptor (Collins and Kuehl 2001). Bulk organic matter decomposition by anaerobic microbes is less efficient and much slower than in upland systems; therefore, wetland soils accumulate organic matter at much higher rates than do uplands.

Chemical and Nutrient Cycling

Redox Reactions

Nitrogen

The first reaction following the onset of anaerobiosis is the reduction of nitrate (NO₃⁻) to nitrite (NO₂⁻), and eventually to nitrogen gas (N₂), which represents a potential loss of nitrogen from the system (Phung and Fiskell 1973, Poe et al. 2003, Megonigal et al. 2004). This process
is termed denitrification, and can reduce the bioavailability of nitrogen for plants in freshwater wetland systems (Brinson et al. 1984, Ambus and Lowrance 1991, Groffman 1994). Several other nitrogen transformations may take place, including nitrogen fixation, ammonification, nitrification, and microbial immobilization (DeLaune et al. 1996, Cirmo 1998). The thin, oxidized layer of soil at the soil-water interface is an important component of the nitrogen cycle in freshwater wetlands, because the concentration gradients set up across this interface allow diffusion of intermediary nitrogen forms upward or downward to the aerobic (e.g., nitrification) and/or anaerobic (e.g., denitrification) sites of the major transformations (Gambrell and Patrick 1978, Mitsch and Gosselink 2000).

**Manganese and Iron**

After most of the nitrate-N in the system has been reduced, manganese and iron reduction occur. Both elements behave in a similar manner in wetland substrates, with two valence states resulting in either dissolved (reduced) or precipitated mineral (oxidized) forms (Gambrell et al. 1989, Nealson and Saffarini 1994, Vepraskas and Faulkner 2001). Importantly, however, manganese reduces before iron and at higher redox potentials in most anaerobic environments. Oxidized (manganic) manganese is black in color, and often forms hard nodules in the profile where free oxygen is reintroduced into the soil matrix (e.g., fluctuating water tables, or artificially drained hydric soils) (Ponnamperuma 1972). Oxidized (ferric) iron is red in color, and zones of iron oxide precipitates may be found in fluctuating water table conditions similar to manganese (Vepraskas 1994). These redoximorphic features, called “mottles” in earlier literature and the 1987 COE Manual (Environmental Laboratory 1987), indicate reintroduction of atmospheric oxygen into pore spaces within the reduced soil medium into which dissolved,
reduced (ferrous) iron follows a concentration gradient to bind with the available oxygen. A similar condition may occur in reduced soils when wetland plants oxidize the rhizosphere surrounding the roots (Mendelssohn et al. 1995, Weiss et al. 2003,). Certain wetland plants have the ability to transport atmospheric oxygen from the aerial organs (shoot) through airspace tissue (aerenchyma) down to the roots, where the exuded oxygen forms iron oxide precipitates within the adjacent soil around the root. These “oxidized rhizospheres” (also, oxidized root channels) are of biological importance, because the presence of iron oxide can immobilize important nutrients such as phosphorus, and can form plaques around the root which serve as barriers to nutrients (Gambrell and Patrick 1978).

Sulfur

The reduction of sulfate typically follows iron and manganese in the redox hierarchy (Table 1). Although sulfate reduction occurs in non-tidal, freshwater wetlands, it is more characteristic of marine and estuarine wetland systems due to the prevalence of sulfate in seawater (Megenigal et al. 2004). In freshwater systems, sulfate reduction is limited by the comparatively low concentrations of sulfate (Craft 2001), and methanogenesis (see below) is the predominant respiratory pathway at low redox states. However, in some wetland systems, sulfur inputs from atmospheric deposition can elevate sulfate reduction rates in freshwater wetlands, resulting in a concomitant reduction in methane production (Wieder et al. 1990, Gauci et al. 2004). Sulfate reduction is strongly linked to organic carbon oxidation, and usually requires a refined, highly labile (easily broken down), short-chain carbon source (Megenigal et al. 2004).

Methanogenesis and Fermentation
At the bottom of the redox hierarchy, carbon itself is reduced during microbial respiration. Carbon reduction is a very low energy-yielding metabolic reaction (Table 1), and typically occurs only when all other terminal electron acceptors have been exhausted within the system. The two major anaerobic carbon transformations in wetland soils are methanogenesis and fermentation. The former uses CO$_2$ as a terminal electron acceptor, and the latter uses simple, low molecular-weight organic compounds (Mitsch and Gosselink 2000). Methanogenesis is the final step in organic carbon degradation within anaerobic environments, resulting in the release of methane (CH$_4$) gas as a waste product by methanogenic microorganisms. Thus, for microbes to successfully utilize the methanogenic pathway, a source of labile organic carbon must be readily available (Megonigal and Schlesinger 1997, Megonigal et al. 2004). In freshwater wetlands, this source is typically the standing vegetation (although phytoplankton also play a role in surface water systems). Plants supply organic compounds to the substrate via decomposition of detritus and/or through the exudation of intracellular constituents (Gambrell and Patrick 1978, Brinson et al. 1981, Moran et al. 1989). This link between methane emissions and primary productivity has been demonstrated in natural and anthropogenic freshwater wetland systems (Whiting and Chanton 1993, Megonigal et al. 2004).

**Phosphorus**

The phosphorus cycle is of interest in freshwater wetlands because it is often a limiting nutrient in these systems (Aerts et al. 1992, Chapin et al. 2004), and because wetlands represent a potential removal mechanism for elevated nonpoint source phosphorus levels in urbanized and agricultural landscapes (Richardson 1985). The phosphorus cycle is unique among the other major biogeochemical cycles in that it has no gaseous phase – phosphorus occurs as soluble or
insoluble organic and inorganic complexes adsorbed to soil particles (Qualls and Richardson 1995, Mitsch and Gosselink 2000). Although redox potential does not directly affect phosphorus transformations, an indirect effect may occur in the presence of other elemental redox forms such as ferric iron, which immobilizes otherwise bioavailable phosphate by precipitation (Ponnamperuma 1972, Mohanty and Dash 1982). Other removal mechanisms include precipitation with calcium and aluminum, adsorption onto clay or organic particles and iron or aluminum oxides (Gambrell and Patrick 1978), and incorporation in living biomass via direct uptake primarily by microbes and to a lesser extent by plants. However, as anoxia proceeds in saturated soils, iron-bound phosphorus may be released as bioavailable phosphate when ferric iron is reduced to ferrous iron by anaerobic microbial respiration (Mitsch and Gosselink 2000). Thus, freshwater wetlands can function as sources and/or sinks for phosphorus (Richardson and Marshall 1986), depending on the biogeochemical setting and other factors such as pH (Verhoeven et al. 1990).

The Carbon Cycle

In the biosphere’s carbon cycle, many wetlands, such as organic soil bogs and fens of northern latitudes and pocosins of the southeastern US, function naturally as net carbon sinks by accumulating carbon dioxide from the atmosphere (Armentano and Menges 1986). In fact, northern bogs can have peat accumulation rates as high as 0.2 cm yr$^{-1}$ (Collins and Kuehl 2001) and carbon turnover times exceeding 2000 years (Oades 1988). However, wetlands are both sources and sinks of organic material, and are affected by hydrologic characteristics such as proximity to freshwater input, geomorphic orientation of marsh drainage system, and meteorological phenomena (de la Cruz 1978). A diagram of the carbon cycle (DeBusk 1996 in
Collins and Kuehl 2001) in wetlands is given in Figure 1, and is presented as a series of compartments of carbon storage.

In the general cycle, inorganic carbon, in the form of CO$_2$, is fixed through the photosynthesis of plants and algae and stored as biomass. Litterfall and subsequent decomposition of plant material through leaching, fermentation, and respiratory processes transfers plant-bound carbon into storage as litter or detritus, microbial biomass, or dissolved organic carbon (DOC). Incorporation of carbon substances into the soil matrix occurs through the activity of invertebrates (i.e. bioturbation) or burial under deposited sediment or additional litter. Soil organic matter can then be immobilized into microbial biomass or undergo further decomposition, eventually combining with leached products from plant roots and exogenous
sources to be finally converted and released as CO₂ or CH₄ by anaerobic respiration and methanogenesis. The ecological value of carbon cycle processes in wetlands is based on movement of detritus into neighboring bodies of water and into aquatic food webs, to be consumed as secondary production by heterotrophic organisms, further broken down by mechanical or biological processes, or mineralized by bacteria (de la Cruz 1978).

**Plant Community Responses to Environmental Conditions**

Soil properties can be affected by plant composition, species diversity, and successional development of the standing vegetation (Marks and Bormann 1972, Parsons and Ware 1982, Hooper and Vitousek 1997). As we have seen, accumulation of detritus in wetland systems is a controlling factor in the development of wetland soils (Vepraskas 1994, Megonigal et al. 2004). In this respect, vegetation provides a feedback mechanism for the development of substrates that typically characterize natural wetland communities by providing organic matter in the form of detritus to initiate microbially mediated reduction (Stauffer and Brooks 1997). Therefore, the structure, age, composition, and density of the standing vegetation represent potential mediating factors in the development of wetland soils.

**Plant Succession**

Few topics in ecology are as widely known and widely debated as plant succession. The concept of succession – namely, the unidirectional, sequential replacement of species within a community over time (Smith 1990) – has been criticized as a useful model for describing plant communities (Bazzazz 1996). However, the fact that species shifts through time can be observed and quantified in natural systems renders the concept useful in discussing ecosystem processes.
dependent on vegetative dynamics, particularly in wetlands (van der Valk 1981, Neiring 1987, King and Allen 1996, Noon 1996, Mitsch and Gosselink 2000, Spencer et al. 2001). This is perhaps because wetlands have been considered intermediary steps in a “hydrarch succession” sequence (Wilson 1935, Mitsch and Gosselink 2000) that follows the development of vegetation from an open water system (lake) to a terrestrial system.

The hydrarch sequence is a concept of autogenic succession, which presupposes that changes in the community are brought about by the plants themselves (Smith 1990, Barbour et al. 1999). In this context, plant-soil interactions are perceived as site-specific phenomena – plants colonize a wetland substrate, contribute organic matter to the substrate, and the attendant biogeochemical responses in the system influence successional changes to the community over time. However, the recent conception of wetlands as pulsed systems (Niering 1987, Odum et al. 1995) limits the usefulness of traditional concepts of autogenic succession and the climax community, in that the development of wetland vegetation is now viewed in response to environmental conditions (i.e., allogenic succession) predicated by the hydrologic regime and other geomorphic controls. Therefore, a discussion of vegetation dynamics and plant-soil interactions in wetlands should consider the autogenic effects of initial floristic composition (Niering 1987, Noon 1996) and site-specific organic matter inputs from the developing vegetation community (Campbell et al. 2002), as well as allogenic processes related to other environmental variables (Niering 1987, Craft 1997, Mitsch and Gosselink 2000).

*Autogenic and Allogenic Relationships*

Wetland plants retain a diversity of adaptations that allow establishment, growth, and persistence in anaerobic soil conditions (Cronk and Fennessey 2001). Thus, the types of plants...
that may colonize a saturated or inundated soil must have adaptations that allow for rapid growth and survival in a poorly oxygenated soil environment. The most extensive literature source on early recruitment and colonization of recently disturbed non-tidal, freshwater wetland substrates comes from studies in wetland creation and restoration sites, which implicate a diversity of hydrophytes that can become established in such environments (Reinhartz and Warne 1993, Noon 1996, Wilson and Mitsch 1996, Campbell et al. 2002, Whigham et al. 2002, DeBerry and Perry 2004). Under these conditions, aboveground biomass equivalency with adjacent natural wetlands can be achieved even in the early stages of plant development (Whigham et al. 2002, DeBerry and Perry 2004), indicating that early colonizers allocate a significant proportion of growth to aerial plant components. This is presumably facilitated by enhanced photosynthetic capacity due to solar radiation exposure in the emergent macrophyte community (Brinson et al. 1981), and by the plants themselves – the colonizing species are generally annuals, or facultative annuals, with the capacity to persist under potentially stressful, low-nutrient conditions (van der Valk 1981, DeBerry and Perry 2004). As the vegetative community develops, biomass turnover contributes organic matter to the soil, and the complex suite of biogeochemical transformations described in previous sections are initiated.

Energy flow in non-tidal, freshwater wetland systems is detritus-based (Day 1984, Mitsch and Gosselink 2000). As Odum (1969, 1985) points out, young sites typically exhibit open mineral cycles with low structural complexity. As sites mature, biogeochemical energetics shift toward closed mineral cycles with high structural complexity. This shift is facilitated by the incorporation of biogenic organic products into the soil profile, which also functions to increase water-holding capacity in the system, thereby influencing soil redox state, mineral cycling, and microbial community development (D’Angelo et al. 2005). Therefore, as the vegetative
community in non-tidal, freshwater wetland sites proceeds along a successional trajectory from emergent to forested cover types, the potential sources of organic carbon will be augmented with a parallel increase in structural complexity in the community. Forested systems support a diversity of growth forms, including trees, shrubs, and understory herbaceous plants, and the quality of the detritus improves accordingly (i.e., more protein- and nutrient-rich organic products from leaves, fruits, flowers, tubers, etc.). In addition, plant community development results in the production of a deep root system, which supports a diversity of soil microbiota and further influences the redox state of soil via gas transport through the vascular tissues down to the profile (Ehrenfeld and Toth 1997, Craft 2001,).

As the biogeochemical environment “improves” with respect to bioavailable nutrient and organic carbon sources, a concomitant response in redox processes mediated by organic matter inputs further influences the availability of growth-limiting nutrients such as N and P (Armstrong and Boatman 1967, Gambrell and Patrick 1978, Koerselman et al. 1990, Aerts et al. 1992). The structural complexity of the system increases, and nutrient availability gradients may become established across the wetland substrate in response to hydrologic regime and other factors (e.g., pH, variable nutrient inputs, etc.) (Aerts et al. 1992, Bridgham et al. 1995, Bragazza and Gerdol 2002).

Such gradients are also influenced by allogenic processes such as nutrient inputs from exogenous sources in open wetland systems (Craft and Richardson 1993a, Craft et al. 1995, Craft and Richardson 1997, Cirmo 1998, Chiang et al. 2000). These inputs are regulated by physical controls such as hydrologic regime and geomorphic setting (Megenigal and Day 1988, Mausbach and Richardson 1994, Richardson et al. 2001), by the condition of the contributing upgradient watershed (Brinson et al. 1984, Craft and Richardson 1993b, Newman et al. 2001, 23
Qualls et al. 2001), and by the source-sink and redox functional status of the wetland for nutrient subsidies (Bridgham and Richardson 1993, Cirmo 1998). Thus, the distribution and abundance of plants may change in accordance with resource limitations established by such gradients (Burke et al. 2003), and a feedback mechanism is established whereby organic carbon inputs from the plant community moderate the soil biogeochemical setting, and the resultant biogeochemical setting moderates the distribution and abundance of plant species over the successional stages of vegetation community development.

*Photosynthesis and Respiration*

An understanding of rate determinants of photosynthesis and respiration is essential when considering successional changes and ecosystem maturity based on energy flows in the form of net carbon exchange in wetlands. Emergent wetlands are among the most productive plant communities on Earth, owing mostly to near constant water supply and high nutrient concentrations. Most emergent macrophytes exhibit sun plant characteristics and are capable of utilizing full sun with minimal photoinhibition at high irradiance levels (Wetzel 2001). Variations in the photosynthetic efficiency and overall photosynthetic rates of wetland plants are associated with light (photosynthetically active radiation, PAR), temperature, and soil moisture, though these variations are also affected by other allogenic and autogenic factors.

Gross photosynthetic production is controlled primarily by latitude and modified by topography, both of which correspond to control solar radiation, mean annual temperature, precipitation, and evaporation (Oades 1988). Mann and Wetzel (1999) showed that photosynthetic efficiency in a common emergent hydrophyte, *Juncus effuses*, was highest (4.5%) at the lowest irradiance levels and decreased to about 0.5% at full sunlight without any apparent
photoinhibition. Temperature optima for maximum average net photosynthesis ranged from 35 to 40°C for _J. effuses_, but photosynthetic efficiency decreased markedly at higher temperatures. Similar light and temperature trends have been found for other emergent and floating plant species, such as _Typha latifolia_ (Wetzel 2001). Although water stress reduces photosynthesis, growth, and total plant respiration (Ryan 2001), adequate soil moisture is necessary for large photosynthetic uptake of CO₂ (Schreder et al. 1998), as plants constantly lose water through their stomata while they are photosynthesizing (Knorr 2000). In fact, the stomata of aquatic macrophytes generally do not close to nearly the extent observed in terrestrial flora during lighted hours, and thus higher transpiration rates occur in aquatic macrophytes than in land plants (Wetzel 2001). Photosynthetic water loss rates are determined by vegetation temperature, as evidenced by the strong temperature dependence of saturated vapor pressure observed in stomatal cavities (Knorr 2000). Vegetation temperature differs from air temperature due to sensible heat flux between the vegetation canopy and the free air, controlled largely by the net radiation of the vegetation canopy and the opening and closing of the stomata (Knorr 2000). There is also considerable evidence that photosynthetic capacity in both C₃ and C₄ plants is closely correlated with leaf nitrogen content (Jones 1988). Indirect determinants of photosynthesis and, consequently, primary productivity include water flow, and hence geomorphic position (i.e. “open” or “closed” system), which act to replenish the nutrient and oxygen supply to roots (Brinson et al. 1981). The more “open” a wetland, the greater the Net Primary Productivity (NPP), as predictable periodic inundation brings in O₂ and nutrient-rich sediment, and flushes toxins (Craft 2001). “Closed” systems (i.e. bogs, pocosins) receive water and nutrients only from precipitation, and thus have low NPP compared to “open” systems (Craft 2001).
Ecosystem respiration is the net result of the microbially mediated respiration of soil organic matter fractions and the metabolic respiration of plants (Trumbore 2000). Respiration rate determinants in soils include organic matter quality (labile vs. refractory), hydrologic conditions, and temperature (Chimner 2004), with temperature being the single most important factor in soil organic matter loss (Brinson et al. 1981). Substrate quality influences carbon mineralization rates, as greater amounts of labile and refractory material increase and decrease mineralization rates, respectively. The labile fraction contains easily degradable polysaccharides such as proteins, carbohydrates, and lipids, whereas refractory or recalcitrant compounds are resistant to decomposition due to large fractions of high molecular-weight, high C:N ratio structural components such as lignocellulose, hemicellulose, and complex polysaccharides like chitin and waxes (Collins and Kuehl 2001, Megonigal et al. 2004). Many studies have found conflicting results regarding the effect of moisture on litter decomposition (Brinson et al. 1981, Bayley et al. 1985). Theoretically, the most rapid decomposition rates should occur with aerobic conditions under a regime of wetting and drying, while continuously anaerobic conditions are least favorable for decomposition (Brinson et al. 1981). Overall, lower water table depths have been found to increase soil respiration rates in wetlands due to greater oxygen diffusion into unsaturated soils leading to more efficient aerobic respiration and increased diffusion of CO$_2$ (Bubier et al. 1998, Chimner 2004). Lastly, although soils formed in submerged conditions do not follow climate patterns as closely as aerobic soils, as temperature is less influential over a wider range (Neue 1985), warmer temperatures stimulate microbial activity, resulting in greater CO$_2$ production from microbially-mediated organic carbon mineralization (Chimner 2004). Studies have shown that microbial activity and decomposition generally increase with increasing
temperature up to ~35-40°C (Craft 2001), while temperatures < 5°C inhibit microbially mediated redox reactions (Megenigal et al. 1996).

Plants respire about 50% of the carbon derived through photosynthesis after photorespiration, with the remaining 50% used for growth, propagation, nutrient acquisition, and litter production (Ryan 1991). Plant dark respiration represents the process of glycolysis and the oxidative pentose phosphate pathway, the Krebs cycle, and electron transport to oxidative phosphorylation, with concomitant uptake of O₂, and generation of CO₂ (Ryan 1991). Photorespiration is the oxidation of ribulose bisphosphate carboxylase in the presence of oxygen (Ryan 1991). Rates of respiration, like all enzymatic chemical reactions, increase with temperature, and show Q₁₀ values (an empirical parameter that relates a 10°C change in temperature to a change in activity) of ~2 for herbaceous plants and ~2.3 for woody plants (Ryan 1991). Plant respiration, like photosynthesis is also strongly correlated to tissue nitrogen content, as most of the organic nitrogen in plants is in protein and ~60% of maintenance respiration supports protein repair and replacement (Ryan 1991, Reich et al. 1998). Further, atmospheric CO₂ concentrations can either increase (Amthor 1989) or decrease (Bunce 1990) plant respiration rates, though there are confounding gaps in current knowledge that hamper the development of adequate models of the response of plant respiration to atmospheric CO₂ (Ryan 1991).

**Created Forested Wetlands**

Wetland creation and restoration in the United States, motivated in part by the recognition of their many ecological and societal functions, but owing mostly to the policy of 'No Net Loss', has commonly occurred since the late 1980's and early 1990's. Though some
created and restored wetlands have met mandated permit guidelines of functionality, failure of these systems is common due mostly to a lack of proper hydrology (Mitsch and Gosselink 2000). Other problems include improper geomorphic setting, chemical toxicity such as acid mine drainage, and improper soil conditions including extreme compaction, high bulk density, and low soil organic matter. The need to improve or boost created wetland functionality has led to the contemporary research of created wetland systems and various suggested techniques to improve the success of wetland creation projects. Herein, some aspects of created wetland research will be explored including needs for further study.

The primary goal of compensatory wetland mitigation, whether creation or restoration, is to achieve functional equivalency with natural wetlands. It follows, therefore, that the most common approach to created wetland research is to compare these sites with similar and often adjacent natural reference wetlands (Kentula et al. 1992, Bishel-Machung et al. 1996, Brinson and Rheinhardt 1996, Cole and Brooks 2000, Stolt et al. 2000, DeBerry and Perry 2004, Brooks et al. 2005). Advantages of using a created-reference wetland approach include: 1) specifying mitigation goals through identifying reference standards from data that typify regional conditions, 2) providing templates to which mitigation wetlands can be designed, and 3) establishing a framework whereby a decline or recovery in functions following disturbance can be estimated for single projects and over large areas through time (Brinson and Rheinhardt 1996). Various parameters have been sampled and compared between created and reference wetlands, including soil parameters such as organic matter content, matrix chroma, temperature, and redox potential (Bishel-Machung 1996, Stolt et al. 2000), hydrologic characteristics (Cole and Brooks 2000), animal communities (Snodgrass et al. 2000, Snell-Rood and Cristol 2003,

However, few studies of created wetlands have attempted to apply a concept of primary succession. Noon (1996) created a model of primary succession in for created wetlands under the hypothesis that newly exposed hydric soils are colonized primarily by annual species in the first growing season, perennial species in the second growing season, and vegetative perennials in sites older than 5 growing seasons. Noon (1996) suggested that there are two phases in early wetland primary succession: the Arrival and Establishment Phase in the first 3 years after wetland creation, characterized by successional dependence on chance elements and physical forces (allogenic), and the Autogenic Dominance Stage after the first 3 years, where succession depends on plant community related processes. DeBerry and Perry (2004) observed a strong presence of perennial emergents in a 2-year-old southeastern Virginia created wetland, and found that the vegetative community did not fit the typical model of primary succession (van der Valk 1981), though it did seem to correspond to Noon’s (1996) model. Further studies are needed on primary successional trends in created wetlands, as these systems are an example of one of the only situations where early succession truly occurs, due to the removal of substrate including seed banks.

Still fewer studies utilize the concept of net ecosystem CO$_2$ exchange (Net Ecosystem Exchange, NEE) as a tool to characterize the successional maturity of created wetland systems. A variety of researchers have used NEE techniques to observe CO$_2$ fluxes between wetlands and the atmosphere (Bubier et al. 1998, Frolking et al. 1998, Schreader et al. 1998, Streever et al. 1998, Clark et al. 1999, Wickland et al. 2001). Most studies of this kind have been carried out in boreal or subarctic peatland of North America (Bubier et al. 1998, Frolking et al. 1998,
Schreader et al. 1998), and generally show that soil temperatures, incident solar radiation, trophic status, and water table were closely coupled to NEE. Whereas intuitively carbon flux should flow in the direction of wetland storage (Bubier et al. 1998), Shreader et al. (1998) showed that a subarctic sedge fen was a net source of CO₂ to the atmosphere during an unusually hot, dry summer, suggesting that climate warming would need to be accompanied by a large rainfall increase to maintain an average annual condition of net carbon gain. Similarly, Wickland et al. (2001) measured CO₂ and CH₄ exchange between the atmosphere and a Rocky Mountain subalpine wetland, and indicated that the wetland was a net source of carbon gas to the atmosphere over the 3-year sampling period, despite the long-term carbon accumulation of ~0.7 mol m⁻² yr⁻¹ determined from ¹⁴C analysis. Clark et al. (1999) compared NEE between an evergreen pine upland and deciduous cypress wetland ecosystem in Florida, and observed a relatively low rate of annual carbon accumulation in the cypress wetland relative to the pine ecosystem. Streever et al. (1998) described an in situ closed chamber technique to measure CO₂ flux in marshes, for use in estimating marsh net productivity, comparing the productivity of two or more marshes, or assessing the factors that influence productivity. Perhaps an underutilized purpose for NEE measurements in wetlands is to determine the successional state of these systems, where high biomass production in early successional stages should reflect a progression from a net autotrophic regime (photosynthesis (P) / respiration (R) ratio >1) to a net heterotrophic regime (P/R = 1) (Odum 1969). Such uses of NEE measurements have only recently been applied to wetlands (Roggero 2003), but are absent from studies of created non-tidal wetland systems.
Objectives and Hypothesis

The objectives of this study were to measure and compare the effects of various loading rates of an organic matter amendment in relation to 1) community composition and standing crop of herbaceous vegetation, 2) development of planted woody vegetation, and 3) ecosystem carbon flux measured as Net Ecosystem Exchange (NEE) of CO\textsubscript{2}, within a created freshwater wetland.

The preceding parameters were used to quantify the effects of incorporation of an organic matter soil amendment on several metrics of vegetation growth and community structure as well as carbon (CO\textsubscript{2}) flux in the created wetland. Comparing the vegetation and gas exchange components among the various organic matter loading rates, relative soil nutrient concentrations, and other parameters such as soil surface and water table elevation provided comparisons of ecosystem function among the created wetland plots on a local scale, and provided insight into carbon sequestration/release processes on a global scale. Lastly, this information was related to ecological and management perspectives to discern how adding organic matter to created wetlands influences created wetland success.

Null Hypothesis: Herbaceous plant community composition and biomass, planted woody vegetation, and Ecosystem Gas Exchange will not vary with respect to organic matter loading rate.

Alternate Hypothesis: Herbaceous plant community composition and biomass, planted woody vegetation, and Ecosystem Gas Exchange will vary with respect to organic matter loading rate.
Vegetation community composition, biomass, and tree size are addressed in Chapter 2, while ecosystem gas flux is addressed in Chapter 3. Chapter 4 presents a brief summary of the combined work.
Literature Cited


Chapter 2

Wetland vegetation dynamics in response to organic matter loading rates

INTRODUCTION

Permits to drain or fill wetlands granted under section 404 of the 1977 Clean Water Act are often accompanied by compensatory mitigation requirements designed to replace the functions [i.e. ecosystem services (sensu Odum 1978)] once performed by the disturbed area. Thus, the primary goal of such final mitigation alternatives is to achieve functional equivalency with natural wetlands. The failure of created wetlands to achieve this goal is due mostly to a lack of proper hydrology (Mitsch and Gosselink 2000), but can also be attributed to improper geomorphic setting, lack of microtopography, extreme soil compaction (i.e. high bulk density), improper soil chemical conditions, and low soil organic matter (Whittecar and Daniels 1999).

Variable but low amounts of soil organic matter have been reported for created wetlands in Pennsylvania [0.5-0.9% (Stauffer and Brooks 1997), 2.3-6.5% (Cole et al. 2001), 4% (Brooks et al. 2005)], Florida [2.6 ± 0.3% (Anderson and Cowell 2004)], and Virginia [0.9-1.9% (Whittecar and Daniels 1999), 3.5-7.2% (Bruland and Richardson 2004)]. These values can be compared to organic matter amounts in natural reference wetlands of 21.7 ± 20.4% in Pennsylvania (Bischel-Machung et al. 1996), 13.8% in Florida (Brown 1991), and 2.4-11% in Virginia (Stolt et al. 2000).

Organic matter generally accumulates to a maximum in ecosystems as succession proceeds (Odum 1969), leading many studies to advocate amending wetland creation sites with organic material, either in the form of salvaged natural wetland soils or mulches, to help
mitigation wetlands achieve functional equivalency sooner (Stauffer and Brooks 1997, Whittecar and Daniels 1999, McKinstry and Anderson 2003, Anderson and Cowell 2004, Bruland and Richardson 2004). Indeed, studies comparing mulched and non-mulched created wetlands are common (Bischel-Machung et al. 1996, Brinson and Rheinhardt 1996, Cole and Brooks 2000, Stolt et al. 2000, Brooks et al. 2005). Also, many parameters have been sampled and compared between created and reference wetlands, including soil parameters such as organic matter content, matrix chroma, temperature, and redox potential (Bischel-Machung et al. 1996, Stolt et al. 2000), hydrologic characteristics (Cole and Brooks 2000), animal communities (Snodgrass et al. 2000, Snell-Rood and Cristol 2003, Stanczak and Keiper 2004), and a combination of vegetative and environmental characteristics (Zampella and Laidig 2003, DeBerry and Perry 2004, Balcombe et al. 2005, Brooks et al. 2005). However, few studies (Bergschneider 2005) have explored the differences in created wetland ecosystem functions with several different loadings of an organic soil amendment (e.g. an organic carbon gradient). Early successional processes (i.e. primary succession) are also rarely studied in wetlands (van der Valk 1981), and are only now beginning to be investigated in created wetlands (Noon 1996, DeBerry and Perry 2004).

The purpose of this study was to examine the primary successional processes occurring in a created forested wetland, in terms of vegetative characteristics, along a gradient of soil organic carbon. Our goal was to determine how vegetation composition, standing crop biomass, and woody vegetation development varied in a created freshwater wetland with respect to different loadings of a soil organic matter amendment. Our hypothesis was that the vegetative parameters would vary with respect to the soil organic carbon-loading gradient. Using the results of this
study, we were able to develop recommendations for organic matter amendments in created wetland systems.

SITE DESCRIPTION

The Charles City Wetland Mitigation Site (CCW) is a 21 ha constructed mitigation wetland owned by the Virginia Department of Transportation (VDOT) in Charles City County, VA (76°55'33" W, 37°20'37" N) (Figure 1a). The site can be classified as palustrine emergent headwater wetlands (Cowardin et al. 1979, DeBerry and Perry 2004), with 18 ha designated as forested wetlands. In 1996, the upper soil profile was excavated to an initial depth of -0.5 to -0.6 m (Bergschneider 2005). However, during summer and fall 2003 much of the site was regraded to a lower elevation, and some areas were ‘ripped’ to an approximate depth of 0.25-0.5 m to decrease the effects of compaction and create more favorable conditions for vegetation (Schmidt 2002). Although construction specifications called for the replacement of topsoil, there is no evidence that such replacement occurred. CCW is characterized by surface exposure of a plastic E or argillic horizon (Btg) that typically exceeds 1 m in depth (Bergschneider 2005). Precipitation is the dominant hydrologic factor in CCW, and fall and winter months are generally accompanied by up to 0.6 m of standing water (Schmidt 2002). In the summer and fall, surface water appears to be perched over the restrictive Btg, particularly following heavy rain events. Following initial grading, the site was stabilized with a seed mix of annual ryegrass (Lolium perenne ssp. multiflorum) and switchgrass (Panicum virgatum) (DeBerry and Perry 2004).

The study site consisted of a 680 m² area located along the northern edge of the CCW (Figure 1b) that contained twenty 4.57 x 3.05 m (15 x 10 ft) plots separated by 3.05 m (10 ft)
alleyways. In June 2002 each plot received one of five organic matter loading rates, ranging from 0 to 336 Mg ha$^{-1}$ (Table 1) intended to 1) bracket the currently utilized and recommended rates employed by the wetland design and construction industry, and 2) coincide with rates reported for other disturbed land re-vegetation studies in Virginia (Bergschneider 2005). The plots were arranged in a randomized complete block design (Figure 2), creating four treatment plot replicates per loading rate. Bergschneider (2005) tested for and found no block x treatment effects within the experimental site. The organic amendment material consisted of dry, mixed wood and yard waste compost processed by Grind-All LLC of Richmond, Virginia. This product was chosen due to its relative stability, history of use, moderate degree of decomposition, and relatively low total nitrogen content (Bergschneider 2005). Chemical composition analysis of the amendment material is listed in Appendix 1. Amendments were incorporated into the plots in June 2002 via disking and roto-tilling by tractor. Plots in loading rates (LRs) 4 and 5 supported a layer of unincorporated organic amendment (compost) atop the mineral soil surface, with a thicker layer in LR 5 than 4. Bergschneider’s (2005) study confirmed an organic matter mounding effect in 2003 by showing that redox potential ($E_h$) readings in LRs 4 and 5 were consistently higher than those in LRs 2 and 3, presumably due to gains in surface elevation and subsequent increases in oxygen diffusion rates.

Between December 1 and 15, 2002, five Betula nigra and Quercus palustris saplings were planted on 1.2 m (4 ft) centers in each experimental plot. Each sapling was fertilized with two (2) 16-8-12 controlled release fertilizer tablets buried within the planting pit near the tree roots. (Note: In late September 2005, the authors noted that 3 of the assumed B. nigra saplings [all in plot 20] were actually B. alleghaniensis). Further details regarding the preparation of the experimental plots can be found in Bergschneider (2005).
METHODS

Elevation

Relative elevations of each plot were measured on July 18, 2005 using a Topcon® AT-G7 Autolevel and a standard stadia rod. Elevation measurements were collected from the center point of each plot and from the base of each *B. nigra* sapling (up to 6 total points per plot). The relative measures were then compared to a known surveyed elevation benchmark and converted to elevations above sea level. The average study site elevation was 10.4 m above sea level, although significant variability existed among plots.

Soil Nutrients

One sample of the top 10 cm of the soil profile (from the soil surface) was collected in each plot on August 22, 2005 using a posthole digger. Each sample was thoroughly mixed and analyzed for total carbon (C) and nitrogen (N) content via a controlled combustion Elemental Analyzer (Nelson and Sommers 1996). Due to the assumed lack of carbonates present in the soil at CCW (Bergschneider 2005), total C was presumed to approximately equal organic carbon. Dilute double-acid extractable phosphorus (P), along with other micronutrients (K, Ca, Mg, Zn, Mn, Cu, Fe, and B) (Appendix 2), was determined by Inductively Coupled Plasma Spectroscopy (Donohue and Heckendorn 1996). All soil nutrient analyses were performed by the Virginia Tech Soil Testing Laboratories.

Plant Assemblage
Herbaceous vegetation was sampled monthly (April-October 2005) in each of the 20 plots for community composition. Measurements of vegetative cover were collected from two randomly placed 1 m x 1 m PVC quadrats in each plot. Percent cover per species was visually estimated directly in the field as a value of 1 to 100% or trace (<1%) using a modified Braun-Blanquet cover scale (Daubenmire 1959, DeBerry and Perry 2004) where: <1% = trace, 1 to 5% = 3%, 5 to 25% = 15%, 25 to 50% = 37.5%, 50 to 75% = 62.5%, 75 to 95% = 85%, and 95 to 100% = 97.5%. Standing dead plant material and bare ground were treated as unique species. Plant taxonomy and nomenclature followed Gleason and Cronquist (1991) or the Natural Resources Conservation Service (2006) Plants Database.

Estimates of cover and frequency were converted to relative measures, and Importance Values (IV) were calculated as the sum of relative cover and relative frequency for each species (Atkinson et al. 1993). The IV of each treatment replicate were averaged over the growing season to calculate IV at each loading rate. Dominant species for each treatment were selected by ranking in order of decreasing IV, with dominants comprising the first 50% of the total and any additional species greater than 20% (50:20 rule). Species Richness (SR) was determined both as the total number of species for each loading rate during the 2005 growing season as a whole and as a per quadrat (m²) average. Evenness (J') and the Shannon Diversity Index (H') values (Zar 1984) were calculated for each loading rate using IV and SR data. These values were calculated with standing dead and bare ground removed from the calculations, but including planted tree species Betula nigra, B. alleghaniensis, and Quercus palustris; removing these species from SR, J', and H' calculations would inaccurately depict the current plant community.
The Ellenberg Community Coefficient Similarity Index (SIₑ) (Mueller-Dombois and Ellenberg 1974) was used as a measure of plant community similarity among loading rates. This calculation weights species presence and absence between two communities by IV, and is summarized in the following equation:

\[
SIₐ = \frac{M_c/2}{M_a + M_b + (M_c/2)}
\]

where \( M_c \) is the sum of IV common to both loading rates, \( M_a \) is the sum of IV unique to loading rate \( a \), and \( M_b \) is the sum of IV unique to loading rate \( b \).

Weighted Averages (WA) were calculated per species as the product of the sum of per replicate IV and the indicator index of that species (Region 2, Reed 1988). Indicator values ranged from 1 (OBL) to 5 (UPL), with intermediate indicators assigned in between (FACW+ = 1.67, FACW = 2, FACW- = 2.33, FAC+ = 2.67, FAC = 3, FAC- = 3.33, FACU+ = 3.67, FACU = 4, FACU- = 4.33). These replicate values were then averaged over the growing season for each loading rate. This calculation is summarized in the following equation:

\[
WA = \frac{\sum_{i=1}^{m} y_i u_i}{\sum_{i=1}^{m} y_i}
\]

where \( y_1, y_2, \ldots, y_m \) are the relative IV values for each species in a loading rate, and \( u_1, u_2, \ldots, u_m \) are the indicator values of each species. WA was calculated including the planted tree species.

Standing Crop Biomass

Aboveground standing crop measurements were obtained by clipping a sub-sample of all living and standing dead plant material at the soil surface from two randomly placed 0.25 m² (0.5 x 0.5 m) PVC quadrats in each plot. These samples were collected on August 22, 2005, as late summer is thought to represent peak seasonal biomass in southeastern Virginia wetlands (Perry
and Atkinson 1997, Perry and Hershner 1999, DeBerry and Perry 2004). Harvested material was separated by species and dried at 40°C until constant mass was achieved.

Woody Vegetation Development

Sampling of woody vegetation development characteristics was performed once on June 21, 2005 for all Betula nigra saplings within the 20 plots. Quercus palustris saplings were omitted from these measurements due to high mortality. Morphometric characteristics measured included total height, crown diameter, main stem diameter, and number of stems. Total height was sampled using standard meter tape, while crown diameter and main stem diameter were quantified using macro-calipers (Haglof, Inc. “Mantax Precision” Calipers) and micro-calipers (SPI 6”/1 mm Poly Dial Calipers), respectively. Crown diameter was measured at three different angles at the visual diameter maximum and averaged to determine the final value. Main stem diameter was measured once at the base of the main stem (trunk) or just above the visual top of stem base swelling (hypertrophy) often accompanying trees growing in flooded conditions (Cronk and Fennessey 2001).

Morphometrics were analyzed using Principal Components Analysis (PCA), which enabled the data to be analyzed simultaneously, providing a visualization of data structure not available using simple regression techniques. PCA was used to produce an index that represented woody vegetation development, which could then be analyzed using statistical techniques described in the following section. Development of Betula nigra saplings was quantified using an index represented by the scores on the first Principal Component in the PCA. Multivariate statistics were performed on MATLAB® software (The MathWorks 2005).
Data Analysis

Data that did not meet conditions of normality were either \( \log_{10} \) transformed or analyzed using non-parametric statistics. Due to the gradient of organic matter loadings, simple regression was the primary technique used to explore relationships between various parameters and loading rate, plot elevation, and/or soil nutrients. In cases where data did not fit a linear/curvilinear model, one-way Analysis of Variance (ANOVA) or the non-parametric Kruskal-Wallis test was used to test for contrasts among treatments. Tukey’s family error rate was used for pair-wise comparisons of elevation and tree development vs. loading rate, while the non-parametric Wilcoxon Rank Sum Test was used for non-normal data including soil C and N. Tests for normality, significance, and pair-wise comparisons were performed using Minitab®, release 14 (Minitab 2005), while various regressions were performed using SigmaPlot, v. 9.0 (Systat 2004). Unless otherwise indicated, data are reported as means ± the Standard Error (SE).

RESULTS

Elevation

Linear regression revealed a positive relationship between loading rate and surface elevation \((p \leq 0.001, R^2 = 0.58)\) (Figure 3) with a maximum elevation increase of approximately 11 cm. Pair-wise comparison showed that plots in LRs 1-3 were not significantly different in elevation, while LRs 4 and 5 were significantly higher than LRs 1-3, and LR 5 was higher than 4. Differences in plot elevation among loading rates were the result of incomplete incorporation of the organic material as noted in Bergschneider (2005), particularly in the higher LRs (4 and 5).
where conventional tillage (disking and roto-tilling) simply could not mix the large volumes applied.

Soil Nutrients

Linear regression was positive between soil C (p \leq 0.001, R^2 = 0.60), N (p \leq 0.001, R^2 = 0.66), P (p \leq 0.001, R^2 = 0.66), and C:N (p \leq 0.001, R^2 = 0.49) and loading rate (Figure 4), indicating that soil chemical parameters were affected by and increased with increasing organic matter loadings. The C, N, P, and C:N relationship with loading rate appeared positive from 0 to 224 Mg ha\(^{-1}\). However, there was an obvious decrease or stasis in these properties from 224 to 336 Mg ha\(^{-1}\), likely related to the small sample size (n = 4) and disproportionate effect of one of the LR 5 soil samples (plot 20). This data was not removed due to an inability to justify its exclusion (i.e. no obvious reasons for differences between this replicate and others in LR 5). Increasing C:N indicated that organic matter in the system may become more refractive with increasing loading rate.

Plant Assemblage

Sixty-four (64) vascular plant species representing twenty-seven (27) families were collected from the experimental block during the study (Table 2). Forty-five (45) were perennial, fifteen (15) annual, six (6) perennial/annual, and one (1) biennial. \textit{Scirpus cyperinus} (FACW+) was the dominant or co-dominant species in each plot (IV range from 18.2 to 33.3), with standing dead vegetation co-dominant (IV range from 13.8 to 19.5) (Table 3). Other co-dominants included open unvegetated ground (bare ground) in LRs 1-4 (IV range from 7.8 to 12.8) and \textit{Eleocharis obtusa} (OBL) in LRs 1 and 2 (IV range from 9.8 to 10.8). Among LRs 1-3,
common subdominant species included *Typha latifolia* (IV range from 9.8 to 10.8), *Juncus acuminatus* (IV range from 2.5 to 4.7), and *Polygonum hydropiperoides* (IV range from 1.9 to 4.2). Common subdominant species in LRs 4 and 5 included *Juncus effusus* (IV range from 4.6 to 7.6), *Andropogon virginicus* (IV range from 3.3 to 4.5), and *Acalypha rhomboidea* (IV range from 2.0 to 3.8).

**Diversity Measurements.** Species richness (SR) values ranged from 7.8 ± 0.4 species m⁻² in LR 2 to 5.3 ± 0.3 species m⁻² in LR 5 (Table 4). A weak ($R^2 = 0.05$) but significant ($p \leq 0.001$) decrease in SR with increasing loading rate was detected. Evenness ($J'$) values ranged from 0.92 ± 0.01 in LR 2 to 0.86 ± 0.01 in LR 5 (Table 4), with linear regression suggesting a weak ($R^2 = 0.02$) yet significant ($p = 0.013$) negative relationship with loading rate. Shannon index ($H'$) values showed a high of 1.8 ± 0.1 in LR 2 and a low of 1.4 ± 0.1 in LR 5 (Table 4). Linear regression suggested a weak ($R^2 = 0.05$) yet significant ($p \leq 0.001$) negative relationship between $H'$ and loading rate.

**Community Similarity.** The Ellenberg Community Coefficient Similarity Indices ($S_{IE}$) were high (> 0.5) among all loading rate comparisons (Table 5). These indices, combined with the similar ranges and weak correlations of SR, $J'$, and $H'$ vs. loading rate, suggested that species composition of the plant communities of all loading rates were very similar to each other.

**Weighted Averages (WA).** The WA values ranged from 1.6 ± 0.03 in LR 2 to 2.1 ± 0.1 in LR 5 (Table 4) and showed a positive linear relationship with both loading rate ($p \leq 0.001, R^2 = 0.27$) and surface elevation ($p \leq 0.001, R^2 = 0.35$) (Figure 5). The range in WA was narrow, and generally contained a majority of wetland (OBL through FACW) plant species in all plots (Figure 6). However, there was also an apparent increase in the abundance (i.e. IV) of upland...
species (FAC through UPL) versus wetland species (FAC+ through OBL), as evidenced by the change in the ratio of wetland vs. upland species abundance from LR 1 (4.9) to LR 5 (1.2).

Standing Crop Biomass

Peak season biomass estimates ranged from 580 ± 97 in LR 2 to 790 ± 60 in LR 5 (Table 4), but showed neither a significant correlation with loading rate (p = 0.283) nor significant differences among loading rates (Kruskal-Wallis Test, p = 0.205). However, a quadratic regression model revealed a significant relationship (p = 0.007, $R^2 = 0.23$) between biomass and surface elevation (Figure 7a). These results indicate that peak biomass occurs at an optimum plot elevation, with values decreasing at both higher and lower elevations. However, linear regressions were not significant between biomass and either soil N (p = 0.547), P (p = 0.539), or C:N (p = 0.845).

Closer inspection of biomass values showed that plot 14 (LR 1) biomass values (1360 and 1444 g m$^{-2}$) were both much higher than the rest of the LR 1 replicates (120-560 g m$^{-2}$). Also, plot 17 (LR 4) replicate samples were very different from one another, with one sample showing very high biomass (1912 g m$^{-2}$) and the other showing very low values (608 g m$^{-2}$). Because the biomass values for these plots were the highest seen in all of the experimental block, and are higher than what is usually reported in these systems, these data points were removed and the analysis was rerun without these outliers. Under these conditions, biomass estimates showed a significant linear relationship with both loading rate (p = 0.020, $R^2 = 0.15$) and plot elevation (p = 0.030, $R^2 = 0.13$) (Figure 7b). Even with outliers removed, linear regressions were not significant between biomass and either soil N (p = 0.203), P (p = 0.092), or C:N (p = 0.462). However, there were no known factors regarding the site preparation or ambient
environmental characteristics of plots 14 and 17 that would explain such unusual biomass values. Therefore, the discussion of standing crop biomass in the following sections will be based on data including the outliers, with a supplemental section that discusses the modified data set.

Woody Vegetation Development

Principal Components Analysis revealed similar loadings on the first principal component for total height, crown diameter, and main stem diameter (Figure 8). Due to the similar magnitude of these loadings, as well as the common metric of size (length or width) between these morphometrics, the scores on the first principal component were used as an index of tree “size”. Pair-wise comparisons showed significant increases in tree size between LRs 1-3 and 4-5 (Figure 9). Linear regression showed a significant positive relationship of tree size versus loading rate (p ≤ 0.001, $R^2 = 0.21$) (Figure 9), surface elevation (p ≤ 0.001, $R^2 = 0.28$) (Figure 10), and soil P (p ≤ 0.001, $R^2 = 0.15$) (Figure 11).

DISCUSSION

Loading Rate Effects on Elevation

Since elevation varied positively with loading rate (Figure 3), it became an important environmental gradient to consider in our study. Differences in elevation among loading rates reflected the incomplete incorporation of the organic amendment material, particularly in the higher-level amendments (LRs 4 and 5), into each plot during site preparation (W.L. Daniels pers. com.). This elevation effect was noted in an earlier study of the same experimental block.
by Bergschneider (2005), suggesting that organic matter amendments in LRs 4 and 5 have not settled to a significant degree between studies.

Elevation can be used as a proxy for a soil moisture/water saturation gradient, driven by inundation frequency and duration. This assertion is justified by 1) the relatively small aerial extent (680 m²) of the experimental block, 2) the dominance of precipitation-driven hydrology, and 3) the determination of CCW as a groundwater recharge site (Despres 2004). These three factors lead us to assume that the CCW experimental block as a whole experienced a uniform hydrologic input, and, as such, generally experienced hydrologic inundation more frequently and for longer duration (i.e. hydroperiod) in lower elevation plots than in higher elevation plots. Changes in physical and chemical conditions along hydrologic gradients are well known, and can affect soil O₂ (oxygen) availability (Gambrell and W. H. Patrick 1978, Nedwell 1984, Craft 2001), soil redox potential (Eh) (Ponnamperuma 1972, Vepraskas and Faulkner 2001), and nutrient availability (Bayley et al. 1985, Richardson 1985, Bedford et al. 1999). The potential importance of elevation, and therefore hydrological differences among plots and loading rates, in this study are discussed further within the following “Plant Assemblage”, “Standing Crop Biomass”, and “Woody Vegetation Development” sections.

Soil Nutrients

Changes in soil chemical concentrations were expected to be a major driver of the potential differences in the measured parameters among loading rates, especially in relation to standing crop biomass and woody vegetation development. As expected, soil C content, as well as N and P (Figure 4), generally increased with increasing loading rate. Increases in these parameters logically reflected the addition of increasing volumes of coarse organic matter
amendments, which also contained increasing volumes of N and P in their organic forms (Bergschneider 2005).

The range of C (i.e. organic matter) values found in this study (1.7-17%) was similar to those reported for surface horizons of southern forested wetlands on mineral soils (2.8-18%) (Lockaby and Walbridge 1998). However, the average C value of LR 1 (1.7%) was below the soil surface organic matter concentration of 2%, reported by Baker and Broadfoot (1979) as indicative of nutritional limitation for many deciduous floodplain species. N levels ranged from 0.2-0.7% for LRs 2-5 in our study, values encompassing and extending higher than those reported for reference wetlands in the Virginia Coastal Plain (0.2-0.4%) (Stolt et al. 2000). However, N content of LR 1 (0.1%) was lower than these natural wetland values, and was consistent with levels in Virginia unamended created wetlands (0.03-0.2%) (Stolt et al. 2000).

Available P levels measured in this study (2.8-18 mg kg⁻¹) were within ranges reported from numerous southeastern US floodplain forest soils (2-18 mg kg⁻¹) (Lockaby and Walbridge 1998). However, LRs 1 (2.8 mg kg⁻¹) and 2 (6.3 mg kg⁻¹) were below the “crude deficiency level” for available soil P (7 mg kg⁻¹) reported in Lockaby and Walbridge (1998) for hardwood floodplain species.

The low levels of N and P in LR 1 confirm the general nutrient deficiency reported for other created wetlands (Bischel-Machung et al. 1996, Whittecar and Daniels 1999, Stolt et al. 2000). Considering the increases in nutrient levels with increasing loading rates, our results reinforce the importance of incorporating organic matter into created wetland soils in terms of establishing a nutrient environment more similar to that of natural or reference wetland systems. However, it is important to note that addressing potentially complicating factors such as
hydrological changes (i.e. elevation) at higher loading rates should be of prominent concern when designing such experiments. This is discussed further in the following section.

Plant Assemblage

Vegetation composition in the CCW experimental block (Table 2) was similar to that of other created wetlands in Virginia (Atkinson et al. 1993, Stolt et al. 2000, DeBerry and Perry 2004, Atkinson et al. 2005). We had hypothesized that vegetation composition would vary noticeably among different loadings of organic matter; however, we did not detect such differences. The overall similarity in dominant vegetation among all loading rates was likely attributable to the prevalent communities of *Scirpus cyperinus* bordering the experimental block (DeBerry 2006). However, the increase in dominance of *S. cyperinus* and decrease of *Eleocharis obtusa* with increasing loading rate suggested a potential successional trend with organic amendment amount.

*Scirpus cyperinus*, an abundant native in many wetland types ranging from eastern portions of Canada south to Texas and Louisiana (Godfrey and Wooten 1979), dominated the CCW experimental block as a whole, and generally increased in dominance with increasing loading rate (Table 3). The overall dominance of this species was consistent with several studies of wetlands in Virginia (Jones et al. 1993, Yu et al. 1998, Atkinson and Cairns 2001, Atkinson et al. 2005). This species is a biennial, and can produce hundreds of thousands of small ($\leq 1$ mm), lightweight ($1 \times 10^{-5}$ g) seeds (Shipley and Parent 1991), which can be transported via wind, water, or animals to suitable habitat patches (Larson 1999). In addition, it can tolerate a wide range of environmental conditions (Kadlec 1958, 1961, Wilcox et al. 1985, Larson 1999). For example, *S. cyperinus* is known to colonize dry sites in wetlands, such as during seasonal or
managed draw downs, and also has the ability to tolerate prolonged inundation (Kadlec 1958, 1961). This pattern was consistent with the *S. cyperinus* dominance at CCW during the 2005 study; the experimental plots were inundated in all but the highest loading rates throughout the winter and early spring 2005 (December-April), dried out completely at the surface in early summer (June), and remained dry until late fall (November).

*Eleocharis obtusa* is a common, cespitose, obligate wetland annual that occurs throughout the eastern U.S. in “muddy places” (Strausbaugh and Core 1977). This species co-dominated in LRs 1 and 2, but decreased in abundance in LRs 3-5 (Table 2). Although little ecological information is available for *E. obtusa*, it is possible that relatively large areas (high IV) of bare ground (Table 3) could have encouraged its establishment as a dominant in LRs 1 and 2. The amount of bare ground decreased with increasing loading rate (IV range from 12.8 to 7.8). This result is consistent with Stauffer and Brooks (1997), who reported higher vegetative coverage in organically amended plots than in unamended plots.

Larger areas of bare ground could potentially lead to higher and more diurnally fluctuating soil temperatures in the upper profile (Stolt et al. 2000) due to the reduced amount of plant shading in these areas (Aust and Lea 1991). Higher soil temperatures generally encourage higher microbial activity (Zak et al. 1999), leading to faster organic matter decomposition rates (Reth et al. 2005) and perhaps a more stressful plant environment in terms of rooting stress due to higher respiration rates (Londo et al. 1999). Such temperature responses could potentially initiate a positive feedback loop in low organic matter sites: open ground leads to higher soil temperatures, increasing organic matter respiration rates, leading to decreased soil organic matter, causing lower plant production, thereby leading to low plant coverage and more bare ground. However, evaluation of several growing seasons would be required to test this
hypothesis. As such, evidence from this study suggests that *E. obtusa* opportunistically colonizes unutilized habitats, as represented in this study by bare ground.

DeBerry and Perry (2004), in a similar study of an unamended portion of the CCW site and an adjacent reference wetland, found that *S. cyperinus* was a dominant plant in an adjacent emergent reference wetland (IV = 29.6), while *E. obtusa* was dominant in the created wetland (IV = 33.4). Therefore, the results of this study suggested that plots with higher loadings of incorporated organic matter (i.e. LRs 3-5) more closely resembled adjacent reference wetlands, as compared to plots with little or no organic matter amendments. In terms of similar IV, LR 3 most closely resembled DeBerry and Perry’s (2004) reference wetland, especially when considering *S. cyperinus* [26.89 (this study), 26.7 (DeBerry and Perry 2004)] and *Juncus effusus* [6.12 (this study), 5.1 (DeBerry and Perry 2004)].

Standing dead vegetation was prevalent in the experimental block, and visual observation showed that much of it was senesced *S. cyperinus* material. Thus, the co-dominance of standing dead vegetation in each loading rate (Table 3), and its positive relationship with loading rate, most likely reflected the dominant production of *S. cyperinus* in previous growing seasons as well as its relatively low decomposition rate (Kittle et al. 1995). The large volume of standing dead vegetation in all loading rates suggested an abundance of refractive carbon within this created wetland that could serve as a future autogenic soil carbon source. However, the portion of this material that will be incorporated into the soil as organic matter is unknown. Further, plant root material, not measured in this study, can be the major source of organic matter to the soil in some ecosystems (Megonigal and Day 1988, Collins and Kuehl 2001).

We hypothesized that plant community diversity would vary with organic matter loading rate, and to some extent this occurred; weak but significant negative relationships were seen
among species richness (SR), evenness (J'), and diversity (Shannon Index, H') (Table 4). These trends agree with Anderson and Cowell (2004), who reported significantly lower SR and H’ values in mulched wetlands relative to non-mulched wetlands in Florida. Our results suggest a potential relationship between nutrient availability to a few of cosmopolitan species and substrate availability to several specialized niche species (sensu Moore et al. 1989). However, the narrow ranges of diversity values may still indicate a similarity of diversity over all loading rates, as was corroborated by direct comparisons of plant community similarity (SIe) (Table 5). This high degree of similarity may have been an artifact of the overall dominance of S. cyperinus and co-dominance of standing dead vegetation. Whether S. cyperinus is a species that facilitates plant diversity (nurse species) or suppresses plant diversity (invasive species) over time warrants further study.

Weighted Average (WA) values likely reflected changes in elevation among plots rather than differences in loading rate. As would be expected, WA increased significantly with increasing elevation (Figure 5), suggesting a negative relationship between the relative abundance of hydrophytic species and elevation. This relationship was seen (Figure 6), as the cumulative IV of upland species (FAC through UPL) increased with increasing LR (and elevation), while the cumulative IV of wetland species (FAC+ through OBL) stayed relatively constant. Indeed, many studies have reported vegetation composition changes along hydrologic gradients (Beatty 1984, Messina and Conner 1998, Wall and Darwin 1999, Waddington et al. 2001, Burke et al. 2003, Nicol et al. 2003, Fraser and Karnezis 2005). More specifically, Vivian-smith (1997) found that microtopographical differences, even as minor as 1-3 cm, produced significant plant community structure differences in experimental wetland communities. Therefore, the 11 cm difference in the average elevations among plots in loading rates 1 (10.36
m) and 5 (10.47 m) could potentially have acted as a selective pressure on plant species colonization and survival. Even so, our data indicated that all plots supported a majority of hydrophytic plants (FAC+ through OBL) (55-83%). Thus, loadings of organic matter do not solely determine or encourage the establishment of hydrophytes in created wetland systems, and other factors such as elevation (i.e. hydroperiod/oxygen availability) and seed dispersal may be more important determinants.

Standing Crop Biomass

Biomass ranges (including outliers) for this study (580-790 g m\(^{-2}\)) fell within those for natural inland freshwater marshes (500-5500 g m\(^{-2}\)) (Mitsch and Gosselink 2000), were on the low side of the range reported for several studies of created wetlands in central Pennsylvania (520-1697 g m\(^{-2}\)) (Cole et al. 2001), and were generally higher than those reported in an earlier study from our created wetland site (146-896 g m\(^{-2}\)) (DeBerry and Perry 2004) and mulched wetland creation areas in west-central Florida (349 g m\(^{-2}\)) (Anderson and Cowell 2004).

We hypothesized that aboveground standing crop biomass would vary positively with organic matter loadings; however, this was not the case. Our results were consistent with others (Cole et al. 2001, Anderson and Cowell 2004) who did not detect total biomass differences in mulched and non mulched wetlands. In a 2003 study of the same experimental plots, Bergschneider (2005) also failed to detect loading rate-based differences in biomass. These results suggest that aboveground plant biomass may not be directly dependant on soil organic matter, at least when considering early successional communities.

Elevation was a better predictor of biomass than loading rate (Figure 7), and the relationship between the two suggested that at some elevation (~10.45 m) the predominant
environmental conditions (i.e. nutrient availability, plant available water, redox potential, etc.) were optimal for plant (aboveground) primary production. Plant biomass and growth differences along hydrologic gradients have been well documented (Mitsch and Ewel 1979, Brinson et al. 1981, Craft 2001, Craft et al. 2002, Fraser and Karnezis 2005). Bayley et al. (1985) inferred that sequences of drought and flooding provided a substantial nutrient source to vegetation that was not available in continuously flooded marshes, leading to higher plant production. This finding potentially explains results from the current study, in that plots with elevations corresponding with the highest biomass values were such that occasional flooding during the growing season (presumably by summer rains) may have made limiting nutrients (especially P) temporarily available for vegetation, and that subsequent drying may have allowed for optimal aerobic, yet moist, growing conditions.

Removing outlying points from the biomass dataset suggested linear relationships with both loading rate and elevation. Because of the significance of each curve, and the similar amount of data explained by each curve (i.e. $R^2 = 0.14$ and 0.13), it is difficult to derive which factor or suite of factors affected biomass production more. More data, perhaps over several growing seasons, may be needed to explain why the outlying points were so much higher than the rest of the dataset. It is possible that the linear relationships seen were artifacts of the modified data, and, therefore, do not warrant further discussion.

We also anticipated that plant biomass would vary positively with soil nutrients, particularly N and P, as macronutrient availability represents a potential constraint on vegetative productivity (Lockaby and Walbridge 1998). However, we did not detect a correlation between biomass and soil nutrients (N, P, C:N) in either the original or modified (outlier-removed) dataset. It is possible that plant available nutrients were utilized by vegetation before the
sampling period (August 2005). Also, levels of N and P in the soil (representing both plant available and unavailable chemical species) may not have been indicative of the plant available nutrient resource pool, perhaps due to immobilization within coarse-organic amendments (e.g. ground-woody debris larger than a few centimeters in diameter). Further, perhaps the amount of nutrients provided by the organic matter additions, especially the higher LRs (4 and 5), was in excess of concentrations required by existing vegetation, and that some other physical or chemical factor or suite of factors was limiting to growth. It is also possible that available soil nutrients were allocated to belowground biomass by the plot vegetation (Day and Megonigal 1993), and therefore were not reflected in our biomass analysis.

Although the incorporation of organic matter into created wetland soils certainly has the potential to improve the plant rooting environment, either through lowering soil bulk density, muting soil temperature fluctuations, or providing a nutrient source, this early successional created wetland appears to have responded more directly to soil surface elevation and presumably saturation and inundation. Further investigation should consider sampling belowground biomass production, analyzing soils for plant available macronutrient compounds such as nitrate (NO$_3^-$), ammonium (NH$_4^+$), and phosphate (PO$_4^{3-}$), and perhaps analyzing plant tissue nutrient ratios to determine limiting nutrient status in these systems.

Woody Vegetation Development

As with biomass, we expected tree sizes to vary positively with organic matter amendment loadings. Although loading rate was a significant predictor ($R^2 = 0.21$), our results suggested that elevation differences among plots ($R^2 = 0.28$) better explained the differences in tree size. This finding was corroborated by the pair-wise analysis of tree size vs. loading rate,
which showed a clear and significant separation between LRs 1-3 and 4-5 [an elevation difference of 8 cm (10.37 - 10.45 m)] (Table 1), but similar tree sizes within the two groups. Bergschneider (2005) found a similar increase in tree size between LRs 2 and 3. *Betula nigra* is a flood-tolerant species, and can withstand soil inundation for one to three months during the growing season (Norby and Kozlowski 1983). However, Norby and Kozlowski (1983) showed that dry weights of roots, stems, and leaves of flooded *B. nigra* individuals were reduced to 24, 76, and 73% of those of unflooded individuals after 5 weeks. Further, woody wetland species are known to show slower growth rates in flooded bottomland hardwood forests vs. similar wetlands with a lower hydroperiod (Malecki et al. 1983, Megonigal et al. 1997).

The relationship of tree size with elevation (Figure 10) was linear, and differed from that of herbaceous plant biomass, which showed a quadratic relationship with elevation. The difference in elevation relationship between the two structural forms may be explained by the differences in rooting depth between the herbaceous and woody species. For example, in LR 5, the set of plots with the highest average elevation (10.47 m), *B. nigra* individuals may be able to send roots through the unincorporated organic amendment layer into the mineral portion of the soil profile where available P presumably exists (Richardson 1985, Craft 2001), perhaps in higher concentrations resulting from nutrient (P) leaching from decomposition of the highly loaded organic material. Therefore, tree saplings in the higher loading rates may have been able to exploit nutrient (P) sources in both the organic amendment layer and the mineral soil that may have been unavailable to the more shallow-rooted herbaceous vegetation. This explanation is plausible as tree size did show a significant positive relationship with P (Figure 11, \( p \leq 0.001, R^2 = 0.15 \)). These findings suggest that early sapling development does not respond to soil organic matter alone. Instead, it appears as though the combination of elevation-related hydrology and
organic amendment-related nutrient gradients may better explain the linear tree size relationship seen in this study. As succession proceeds within the experimental plots, and as the organic matter amendments in the higher loading rates continue to settle due to physical breakdown and microbially-mediated OM oxidation, hydrology could become less of a differentiating factor among the loading rates. It is possible, then, that *B. nigra* sizes would more closely track organic matter loading rate given more time for such processes to occur. Future studies would be required to test this hypothesis.

**Plant Production and Soil Phosphorus**

The removal of the modified (i.e. outlier-removed) biomass data, and a closer inspection of the tree size data, revealed a potential relationship between aboveground plant production of both herbaceous and woody species and soil P, up to a saturation point. As shown in Figure 12, herbaceous biomass appears to increase linearly with soil P up to a concentration of 10 mg kg$^{-1}$ ($p = 0.017$, $R^2 = 0.25$), after which biomass was variable but relatively constant. Further, tree size linearly increased with soil P ($p \leq 0.001$, $R^2 = 0.30$), up to a concentration of 13-15 mg kg$^{-1}$ (Figure 13), after which tree size was fairly constant. The Virginia Department of Conservation and Recreation (2005) lists soil P concentrations of 11-15 mg kg$^{-1}$ as indicative of "medium fertility" for cultivated crops such as corn. However, it is thought that native plant species are actually more efficient at P uptake and can tolerate much lower available P levels than crops (W.L. Daniels, pers. com.). Thus, it is possible that the increases in aboveground production up to an intermediate soil P concentration seen in the study reflects P limitation in the lower loading rates, with increasing availability until plants could no longer take advantage of the superfluous resource (i.e. saturation). This saturation effect could potentially explain the weak relationship
between biomass and tree size with loading rate. That is, as increasing loadings of the amendment material increased soil P, these factors did not respond linearly in the higher loading rates. The potential relationship between plant production and soil P could use further study, in part to determine a P fertility threshold for native wetland vegetation.

RECOMMENDATIONS

The lack of correlation between biomass and organic matter loading rate indicates that aboveground plant biomass should not be used as a functional indicator of created wetland success. This is corroborated by other studies that reported either no relationship between soil organic matter and biomass (Cole et al. 2001, Anderson and Cowell 2004) or that created wetland aboveground biomass equivalency with adjacent natural wetlands can be achieved even in the early stages of plant development (Whigham et al. 2002, DeBerry and Perry 2004).

Further, adding high loadings of organic matter into created wetlands (i.e. LRs 4 and 5) could inadvertently raise soil surface elevations and decrease the frequency of inundation/anaerobic conditions. This is illustrated by the positive relationship of Weighted Averages (WA) and loading rates. For example, Wentworth et al. (1988) suggested and Atkinson et al. (1993) confirmed that WA ≤ 2.0 was an acceptable value on which to designate wetlands on the basis of vegetation alone. Using this criterion, LRs 1-3 would be considered “wetlands” and LR 4 and 5 would not (Table 4). As such, elevation changes due to organic matter mounding in these higher loading rates could have an effect on regulatory wetland status within the CCW experimental block. Therefore, we recommend that organic matter loadings into created wetland soils be applied such that the material is fully incorporated into the soil.
profile, and that the loading rate used be moderate enough to not significantly alter soil surface elevation.

Given that standing crop biomass did not vary with loading rate, and that the tree size-loading rate relationship may be better explained by plot elevation, a recommendation for an organic matter loading in similar created wetland systems should instead be based on soil nutrient values, including the potential aboveground production-soil P relationship, and vegetation composition. Therefore, an organic matter soil amendment similar to LR 3 (112 Mg ha\(^{-1}\)) is appropriate, as average nutrient levels (C = 6.7%, N = 0.3%, P = 8.3 mg kg\(^{-1}\)) were within range of natural systems and vegetation composition was most similar in this loading rate to that of adjacent early-successional reference wetlands (DeBerry and Perry 2004). This organic matter loading seemed to provide a “jumpstart” for the created plots, while also minimizing the change in elevation (+2 cm from LR 1) due to the added bulk material.
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Table 1. Summary of mean (+1 SE) variation over loading rates for surface elevation, soil carbon (C), nitrogen (N), phosphorus (P), and C:N collected on July 18, 2005 (elevation) and August 22, 2005 (soil nutrients) in CCW, Charles City County, VA. LR 1 = 0 Mg ha\(^{-1}\), LR 2 = 56 Mg ha\(^{-1}\), LR 3 = 112 Mg ha\(^{-1}\), LR 4 = 224 Mg ha\(^{-1}\), LR 5 = 336 Mg ha\(^{-1}\).

<table>
<thead>
<tr>
<th>Soil Variable</th>
<th>LR1</th>
<th>LR2</th>
<th>LR3</th>
<th>LR4</th>
<th>LR5</th>
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<td>Loading Rate (Mg ha(^{-1}))</td>
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<td>56</td>
<td>112</td>
<td>224</td>
<td>336</td>
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<td>Surface Elevation (m)</td>
<td>10.36 ± 0.004</td>
<td>10.36 ± 0.01</td>
<td>10.38 ± 0.01</td>
<td>10.43 ± 0.01</td>
<td>10.47 ± 0.01</td>
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<td>C content (%)</td>
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<td>3.37 ± 0.14</td>
<td>6.66 ± 1.08</td>
<td>16.53 ± 3.06</td>
<td>15.08 ± 3.51</td>
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<td>N content (%)</td>
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<td>P content (mg kg(^{-1}))</td>
<td>2.75 ± 0.23</td>
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<td>C:N</td>
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<td>18.29 ± 0.57</td>
<td>20.94 ± 0.76</td>
<td>23.89 ± 1.18</td>
<td>22.58 ± 1.35</td>
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</table>
Table 2. Plant species occurring in collecting plots and relative IV during the 2005 growing season (April-October) in CCW, Charles City County, VA. Species names are accompanied by wetland indicator status (Indicator), plant life strategy (Duration), and are separated by each loading rate (LR) they occurred in. Nomenclature follows NRCS (2006). Planted species are denoted by *. LR 1 = 0 Mg ha⁻¹, LR 2 = 56 Mg ha⁻¹, LR 3 = 112 Mg ha⁻¹, LR 4 = 224 Mg ha⁻¹, LR 5 = 336 Mg ha⁻¹.

<table>
<thead>
<tr>
<th>Species</th>
<th>Indicator</th>
<th>Duration</th>
<th>LR1</th>
<th>LR2</th>
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<th>LR4</th>
<th>LR5</th>
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<td>Acer rubrum L.</td>
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<td>Perennial</td>
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<td>0.94</td>
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<td>Alisma plantago-aquatica L.</td>
<td>OBL</td>
<td>Perennial</td>
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<td>Apocynum cannabinum L.</td>
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<td>Baccharis halimifolia L.</td>
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<td>Perennial</td>
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<td>Bidens aristosa (Michx.) Britt.</td>
<td>FACW⁻</td>
<td>Annual/Biennial</td>
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<td>1.81</td>
<td>2.29</td>
<td>0.52</td>
<td>0.26</td>
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<td>1.12</td>
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<td>Eclipta prostrata (L.) L.</td>
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<td>Annual/Perennial</td>
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<td>0.36</td>
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Table 3. Summary of dominant species in each loading rate based on IV using the 50:20 rule for the 2005 growing season (April-October) in CCW, Charles City County, VA. LR 1 = 0 Mg ha\(^{-1}\), LR 2 = 56 Mg ha\(^{-1}\), LR 3 = 112 Mg ha\(^{-1}\), LR 4 = 224 Mg ha\(^{-1}\), LR 5 = 336 Mg ha\(^{-1}\).

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<td><em>Scirpus cyperinus</em></td>
<td>20.73</td>
<td>18.17</td>
<td>26.89</td>
<td>27.31</td>
<td>33.30</td>
</tr>
<tr>
<td>Standing Dead</td>
<td>14.53</td>
<td>13.75</td>
<td>17.85</td>
<td>19.53</td>
<td>19.25</td>
</tr>
<tr>
<td>Bare Ground</td>
<td>12.75</td>
<td>11.53</td>
<td>10.38</td>
<td>7.80</td>
<td></td>
</tr>
<tr>
<td><em>Eleocharis obtusa</em></td>
<td>9.89</td>
<td>10.79</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Table 4. Summary of mean (±1 SE) variation over loading rates for total species richness (TS), per quadrat species richness (SR), evenness (J'), Shannon Diversity Index (H'), weighted average (WA), and standing crop biomass over the 2005 growing season (April-October) in CCW, Charles City County, VA. Values do not including Bare Ground or Standing Dead categories. LR 1 = 0 Mg ha⁻¹, LR 2 = 56 Mg ha⁻¹, LR 3 = 112 Mg ha⁻¹, LR 4 = 224 Mg ha⁻¹, LR 5 = 336 Mg ha⁻¹.

<table>
<thead>
<tr>
<th>Vegetation Variable</th>
<th>Loading Rates (LR)</th>
<th>n</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>LR1</td>
<td>LR2</td>
</tr>
<tr>
<td>Total Species (TS)</td>
<td>39</td>
<td>35</td>
</tr>
<tr>
<td>Species Richness (SR)</td>
<td>7.39 ± 0.45</td>
<td>7.75 ± 0.37</td>
</tr>
<tr>
<td>Evenness (J')</td>
<td>0.89 ± 0.01</td>
<td>0.92 ± 0.01</td>
</tr>
<tr>
<td>Shannon Index (H')</td>
<td>1.71 ± 0.07</td>
<td>1.82 ± 0.05</td>
</tr>
<tr>
<td>Weighted Averages (WA)</td>
<td>1.63 ± 0.03</td>
<td>1.57 ± 0.03</td>
</tr>
<tr>
<td>Standing Crop Biomass</td>
<td>603.5 ± 179.8</td>
<td>580.0 ± 96.9</td>
</tr>
</tbody>
</table>
Table 5. Matrix of Ellenberg Community Coefficient Similarity Index ($SI_e$) from relative IV of species, calculated for each month of the 2005 Growing Season (April-October) and averaged for each treatment comparison in CCW, Charles City County, VA. LR 1 = 0 Mg ha$^{-1}$, LR 2 = 56 Mg ha$^{-1}$, LR 3 = 112 Mg ha$^{-1}$, LR 4 = 224 Mg ha$^{-1}$, LR 5 = 336 Mg ha$^{-1}$.

<table>
<thead>
<tr>
<th></th>
<th>LR 5</th>
<th>LR 4</th>
<th>LR 3</th>
<th>LR 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>LR 1</td>
<td>0.76</td>
<td>0.82</td>
<td>0.88</td>
<td>0.92</td>
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<tr>
<td>LR 2</td>
<td>0.76</td>
<td>0.85</td>
<td>0.90</td>
<td></td>
</tr>
<tr>
<td>LR 3</td>
<td>0.80</td>
<td>0.84</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LR 4</td>
<td>0.86</td>
<td></td>
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</tr>
</tbody>
</table>
Figure 1. Charles City Mitigation Wetland (CCW) site location, Charles City County, VA (Bergschneider 2005). Part a represents the location of Charles City County in Virginia. Part b shows the location of the experimental block (denoted by the "W") within CCW.
Figure 2. Schematic design of CCW experimental plots in Charles City County, VA.
Figure 3. Mean (±1 SE) elevation vs. Loading Rate in CCW, Charles City County, VA, from April to October 2005. Different letters above the treatment means denote significant differences based on Tukey’s Family Error Rate.
Figure 4. Linear regression of mean (±1 SE) soil Carbon (C), Nitrogen (N), Phosphorus (P) content and C:N vs. Loading Rate in CCW, Charles City County, VA, on August 22, 2005. P-values reported indicate significance of the linear regression at the $\alpha = 0.05$ level. Different letters above the treatment means denote significant differences at the $\alpha = 0.05$ level based on Wilcoxon Rank Sum Test for C and N and Tukey's family Error Rate for P and C:N.
Figure 5. Linear regression of mean (±1 SE) per plot weighted average (WA) values vs. elevation in CCW, Charles City County, VA, from April to October 2005. The p-value reported indicates significance of the linear regression at the $\alpha = 0.05$ level.
Figure 6. Total IV of general wetland indicator categories (Reed 1988) for different loading rates in CCW, Charles City County, VA, April to October 2005. FACW and FACU categories include +/- modifiers, while the FAC category includes the + modifier.
Figure 7a. Biomass values vs. Elevation in CCW, Charles City County, VA, on August 22, 2005. The p-value reported indicates significance of the quadratic regression at the $\alpha = 0.05$ level.
Figure 7b. Linear regression of biomass values (outliers removed) vs. elevation in CCW, Charles City County, VA, on August 22, 2005. The p-value reported indicates significance of the linear regression at the $\alpha = 0.05$ level.
Figure 8. Sorted loadings on first principal component from a Principal Components Analysis calculated using log transformed morphometric data. Total height, crown diameter, and main stem diameter show similar loadings on the first principal component (PC1), and have the common metric of size (length or width), therefore the scores on PC1 were used as an index of tree development.
Figure 9. Linear regression of mean (±1 SE) tree “size” vs. Loading Rate in CCW in Charles City County, VA on June 21, 2005. Different letters above the treatment means denote significant differences based on Tukey’s family error rate. The p-value reported indicates significance of the linear regression at the $\alpha = 0.05$ level.
Figure 10. Linear regression of PC1 Scores (tree size) vs. elevation in CCW, Charles City County, VA, on June 21, 2005. The p-value reported indicates significance of the linear regression at the $\alpha = 0.05$ level.
Figure 11. Linear regression of mean (±1 SE) tree “size” (PC1) values vs. soil phosphorus (P) content in CCW, Charles City County, VA, on August 22, 2005. The p-value reported indicates significance of the linear regression at the α = 0.05 level.
Figure 12. Linear regression of mean (±1 SE) biomass values vs. soil phosphorus (P) content (0 to 12 mg kg\(^{-1}\)) in CCW, Charles City County, VA, on August 22, 2005. Biomass values are modified such that 4 outlying data points have been removed. The p-value reported indicates significance of the linear regression at the \(\alpha = 0.05\) level.
Figure 13. Linear regression of mean (±1 SE) tree “size” (PC1) values vs. soil phosphorus (P) content (0 to 15 mg kg⁻¹) in CCW, Charles City County, VA, on August 22, 2005. The p-value reported indicates significance of the linear regression at the α = 0.05 level.
Chapter 3

Wetland ecosystem gas exchange in response to organic matter loading rates

INTRODUCTION

Mitigation requirements based on section 404 of the 1977 Clean Water Act often require the replacement of specific acreages of wetland habitat to compensate for the loss of natural wetlands and their associated functions. Wetland creation is typically more difficult than restoration due to the complexity of establishing the necessary hydrology, soils, and vegetation in areas where wetlands did not previously or historically exist (Stolt et al. 2000). Thus, failure (i.e. not achieving functional status of natural wetlands) of these systems is common (Zedler 1997, Whigham 1999).

One reason for created wetland failure has been improper soil conditions, including low soil organic matter (Stauffer and Brooks 1997). Although organic matter accumulation is a characteristic feature of most wetland systems, traditional creation practices such as top soil scraping typically remove up to 1 meter of surface soil (W.L. Daniels, pers. com.), including organic-rich material in the A horizon (Whittecar and Daniels 1999, Bergschneider 2005). Indeed, lower organic matter than that of natural wetlands has been reported for created wetlands in Pennsylvania (Stauffer and Brooks 1997, Cole et al. 2001, Brooks et al. 2005), Florida (Anderson and Cowell 2004), and Virginia (Whittecar and Daniels 1999, Bruland and Richardson 2004). As such, many studies have advocated amending wetland creation sites with organic material either in
the form of salvaged natural wetland soils or mulches (Stauffer and Brooks 1997, Whittecar and Daniels 1999, McKinstry and Anderson 2003, Anderson and Cowell 2004, Bruland and Richardson 2004). Because organic matter accumulation occurs over centuries, the hope is that adding organic amendments to created wetland soils will help to "jump start" the maturation process, and achieve functional equivalency (to natural wetlands) sooner.

Since construction practices usually remove vegetation, soils, and existing seed banks, most created wetlands occur as primary successional ecosystems. Organic matter (OM) is important in these ecosystems since its accumulation over time acts to mitigate perturbations caused by the physical environment; OM therefore acts as a stabilizing force, theoretically leading to a "climax" system where energy efficiency for both biomass and symbiotic organism interaction are maximized (Odum 1969). Based on the work of E.P. Odum (1969), as ecosystems mature, and energy use shifts over time from high biomass production to ecosystem maintenance, changes in the overall ecosystem energy balance should reflect a progression from a net autotrophic regime (photosynthesis (P) / respiration (R) ratio >1) to a balanced state (P/R = 1). As such, one could theoretically use the P/R ratio as a functional index of the relative maturity of ecosystems.

Few studies have utilized Odum's concepts as a tool to characterize ecosystem successional maturity. However, several studies have utilized techniques of measuring carbon gas (CO$_2$, CH$_4$) fluxes as C uptake (Community Production, Gross Primary Production, or Photosynthesis), C emission (Respiration), and their net difference (Net Ecosystem Exchange, NEE) to observe energy fluxes between wetlands and the
atmosphere (Bubier et al. 1998, Frolking et al. 1998, Schreader et al. 1998, Streever et al. 1998, Clark et al. 1999, Neubauer et al. 2000, Wickland et al. 2001). These techniques provide an opportunity to use Odum’s (1969) bioenergetics theory to observe the relative successional maturity of wetland ecosystems. Such uses of CO₂ flux measurements have only recently been applied to wetlands (Roggero 2003, Cornell et al. 2006), but are absent from studies of created non-tidal wetland systems.

The purpose of this study was to examine the primary succession processes occurring in a created forested wetland, in terms of ecosystem gas exchange, along a gradient of soil organic carbon. Our goal was to determine how Gross Primary Production, Respiration, and Net Ecosystem Exchange varied in a created freshwater wetland with respect to different loadings of organic matter in the soil. We hypothesized that Ecosystem Gas Exchange parameters would vary with respect to organic carbon loading. Using the results of this study, we were able to compare how various organic matter amendments affect energy flow in a primary successional system, as well as to develop management recommendations for organic matter loading rates in created wetland systems.

SITE DESCRIPTION

The Charles City Wetland Mitigation Site (CCW) is a 21 ha constructed mitigation wetland owned by the Virginia Department of Transportation (VDOT) in Charles City County, VA, USA (76°55'33" W, 37°20'37" N) (Chapter 2, Figure 1a). The site can be classified as palustrine emergent headwater wetlands (Cowardin et al. 1979,
DeBerry and Perry 2004), with 18 ha designated as forested wetlands. CCW was initially constructed in 1996, although sections have been regraded as recently as 2003 (see Bergschneider 2005). This site is characterized by silty clay loam soils typically exceeding 1 m in depth. Precipitation is the dominant hydrologic factor in CCW, and fall and winter months are generally accompanied by up to 0.6 m of standing water (Schmidt 2002).

The study site consisted of a 680 m² area located along the northern edge of the CCW (Chapter 2, Figure 1b) containing twenty 4.57 x 3.05 m (15 x 10 ft) plots separated by 3.05 m (10 ft) alleyways. In June 2002 each plot received one of five organic matter (wood/yard waste compost, Appendix 1) loading rates, ranging from 0 to 336 Mg ha⁻¹ arranged in a randomized complete block design (Chapter 2, Figure 2), creating four plot replicates per treatment. Amendments were incorporated into the plots via disk ing and roto-tilling by tractor. Plots showed a positive correlation between loading rate and surface elevation due in part to incomplete incorporation of the amendment material, especially in Loading Rates 4 and 5 (Chapter 2, Table 1, Figure 3). Further details regarding the preparation of the experimental plots as well as previous studies at CCW can be found in Chapter 2 as well as in Bergschneider (2005).

METHODS

Water Table Depth

Elevations of each plot were determined as described in Chapter 2 (Elevation). The depth of the water table was measured periodically (2-4 times per month) in one of
~30 wells maintained by the Virginia Department of Transportation (VDOT) at the CCW. Well #2 data (VDOT, unpublished data) were chosen as the approximate level of the water table in the experimental plots due to the close proximity of the well to the plots (~10 m north of the experimental block). The water table elevation was estimated using relative water table height data from Well #2 and surface water depth data taken from an adjacent plot (Plot 12) during CO$_2$ flux sampling. Since the plot surface elevation was known, elevation of the water table during flooded periods could be determined by a regression of water table surface (during flooded periods) of Plot 12 (i.e. plot surface elevation + depth of surface water) vs. relative water table depth in Well #2 ($p = 0.004$, $R^2 = 0.96$). Plot elevations were then subtracted from the water table elevation to determine water table depths relative to the surface of each plot.

**CO$_2$ Flux**

Ecosystem exchange of CO$_2$ was measured using one of two aluminum-framed chambers (Figure 1) (e.g. Streever et al. 1998, Roggero 2003, Cornell et al. 2006). Each chamber was covered in airtight, transparent, Tefzel® film (DuPont, Inc., Circleville, OH). The chambers were clamped onto 0.25 m$^2$ aluminum bases (0.5 x 0.5 m), one installed in the center of each plot two to four weeks prior to sampling. The bases were inserted to a point at which approximately 3 cm of the base was above the soil surface. The height of vegetation within the chamber base determined which chamber was used for each plot (1.0 or 1.5 m tall).

The chambers were connected via bev-a-line tubing to a LI-6200 Portable Infrared Gas Analyzer (IRGA) (LI-COR, Inc., Lincoln, NE), which cycled air between
the chamber and the IRGA and periodically measured and recorded the concentration of 
\( \text{CO}_2 \). Prior to measurement, the IRGA was zeroed with ambient air passing sequentially 
through a \( \text{CO}_2 \) scrub (soda lime) and a desiccant (magnesium perchlorate, \( \text{Mg(ClO}_4)_2 \)). 
The IRGA was then calibrated with a gas standard containing 500 ppmv \( \text{CO}_2 \) in \( \text{N}_2 \) (Scott 
Specialty Gases, Inc. Plumsteadville, PA).

In the field, \( \text{CO}_2 \) levels were monitored with the chambers in place, but open to 
the atmosphere, until a steady-state concentration had been reached. After this 
equilibration period (usually 5-10 minutes), the chamber lids were closed, the change in 
\( [\text{CO}_2] \) was allowed to stabilize (usually \( \sim \) 1 minute), and the sampling period was started. 
\( [\text{CO}_2] \) was measured and logged at \( \sim \)30 second intervals during a five minute sampling 
period, and thus 11 measurements were recorded by the IRGA for each light level.

Incident irradiance (Photosynthetically Active Radiation, PAR) was measured 
concurrently with \( \text{CO}_2 \) flux using a LI-190 Quantum Sensor (LI-COR, Inc., Lincoln, NE) 
placed at the top of the inside of each chamber. These measurements were recorded at 1 
minute intervals using a LI-1000 data logger (LI-COR, Inc., Lincoln, NE). Air 
temperature within the chamber was also concurrently recorded at 5 second intervals 
using a HOBO® U10 data logger (Onset, Inc., Bourne, MA).

\( \text{CO}_2 \) fluxes were sampled monthly in each plot from June 2005 through May 
2006. The majority of sampling was performed in full light, between 0900 and 1600 
(Eastern Standard Time), to maximize light intensities. Four sampling treatments were 
performed in each plot each month: one in full (ambient) light, two at different 
intermediate lights levels (using two separate jackets of window screening), and one in 
the dark (using an opaque blanket). Due to low photosynthetic activity, only the dark
measurements were performed in December 2005 and January 2006. Measurements at all light levels for each plot were measured on the same day, and monthly sampling for all 20 plots was completed within two to four days.

Calculations and Modeling

Short-term field measurements of CO$_2$ fluxes were scaled to monthly and annual rates using a CO$_2$ flux model. In the model, CO$_2$ flux rates at various time scales are controlled by changes in PAR and air temperature, measured at the Virginia Institute of Marine Science (VIMS, ~40 km east of CCW) and at a nearby NOAA weather station, Williamsburg, VA (~20 km east of CCW, 76°71'70" W, 37°23'30" N) (NOAA 2006), respectively. CO$_2$ fluxes were estimated for a one year period from June 2005 through May 2006.

Monthly measurements of gross primary production (GPP) were calculated from field measurements at full light and two shaded treatments, plus the respiration measurements (dark fluxes) taken immediately following light measurements. Changes in GPP over various time scales were determined by fitting GPP vs. PAR curves, one per plot, per month. Curves were fit by hyperbolic curves using SigmaPlot®, v. 9.0 (Systat 2004). GPP was modeled as:

$$\text{GPP} = \left( \frac{a \times I}{b + I} \right)$$

where $I$ = average hourly irradiance (PAR), and $a$ and $b$ are empirically-derived constants with the units of μmol C m$^{-2}$ h$^{-1}$ and μE m$^{-2}$ h$^{-1}$, respectively (Neubauer et al. 2000).

Respiration (R) rates were calculated using hourly temperature data as:

$$R = y_0 + ax$$
where $y_0$ is the slope of an Arrhenius plot of the natural log of CO$_2$ flux versus the inverse of temperature in degrees K, $a$ is an empirically-derived constant, and $x$ is average hourly air temperature in degrees K.

Hourly GPP and R rates were summed to obtain monthly and annual rates, and NEE was calculated as:

$$\text{NEE} = \text{GPP} + \text{R}$$

Gas exchange was expressed relative to the ecosystem with GPP as positive values and respiration as negative values.

Additional Carbon Sources

CH$_4$ (methane) fluxes were sampled monthly in each plot from July 2005 to June 2006. Instantaneous CH$_4$ flux rates were determined to be insignificant (monthly average range from -0.05 to 0.09 mg C m$^{-2}$ min$^{-1}$). Therefore, CH$_4$ data were not included in the C flux analysis for CCW. However, monthly instantaneous CH$_4$ fluxes and the methods used are reported in Appendix 5.

Data Analysis

Simple regression was used to explore relationships among various parameters (GPP, R, NEE, and GPP:R) and loading rate. Tests for normality were performed using Minitab®, release 14 (Minitab 2005), while various regressions were performed using SigmaPlot, v. 9.0 (Systat 2004). Unless otherwise indicated, data are reported as means ± the Standard Error.
RESULTS

Annual CO₂ Fluxes

Respiration (R) values ranged from 796 ± 171 to 1912 ± 182 g C m⁻² yr⁻¹ in LRs 1 and 5 (Table 1, Figure 2), respectively, and correlated positively with loading rate (p = <0.001, R² = 0.62) (Figure 3). Gross primary production (GPP) ranged from 938 ± 108 g C m⁻² yr⁻¹ (LR 1) to 1452 ± 96 g C m⁻² yr⁻¹ (LR 5), with values positively correlating with loading rate (p = 0.002, R² = 0.41). Annual NEE ranged from -459 ± 240 to 141 ± 67 g C m⁻² yr⁻¹ in LRs 5 and 1, respectively, displaying a negative correlation with loading rate (p = 0.006, R² = 0.34). The weak regression of NEE and loading rate was possibly due to high variability in GPP and R among treatment replicates, which were used to calculate NEE by difference.

Monthly CO₂ Fluxes

Loading rates 1 and 5 showed the lowest and highest R rates, respectively, regardless of month, with the other loading rates sequentially falling in between (Figure 4). The highest R rates occurred in July with slightly lower rates in August. These high summer rates coincided with high seasonal temperatures, and may also have been influenced by hydrologic variation (i.e. soil moisture). That is, although the highest annual temperatures typically occur in August, it is possible that the relatively lower water table in July (see Figure 4) enhanced aerobic, and therefore more efficient, respiration, creating the highest R rates observed in this study. This hydrologic effect on respiration is consistent with a regression of monthly respiration rates versus water table.
depth (Figure 7), which shows a significant negative correlation between respiration rate and water table height (p < 0.001, \( R^2 = 0.60 \)). The lowest R rates in the study occurred in the winter months (December-February).

Monthly GPP values (Figure 5) showed more variability among replicate plots than R. Generally, LRs 1 and 5 showed the lowest and highest GPP, respectively, in any given month, with LRs 2 - 4 following more-or-less sequentially. However, there were exceptions, such as LR 4 with the highest GPP in June, and LR 1 with the second highest GPP in April. The highest overall GPP rates occurred from July to August, while the lowest measured GPP occurred in February. Because measurements on a subset of plots showed no significant photosynthesis in any loading rate during December and January, we did not measure GPP during these months. However, we assume that the actual lowest GPP (near zero) occurred in December and January.

All loading rates showed generally high NEE (Figure 6) in late spring (May and June) and early fall (September), with lower NEE from late fall to early spring (October to April) and the lowest NEE in mid summer (July). Low NEE (net heterotrophic activity) from late fall to early spring was caused by low GPP due to seasonal senescence of vegetation (data not shown). Further decreases in NEE in early spring (March and April) were likely due to the effect of increases in temperature on respiration rate, while GPP was still relatively low. Increasing NEE in the late spring (May) coincided with full leaf-out of vegetation and the resulting increased GPP. Peak NEE for all loading rates except LR 4 occurred in May; this was likely due to low R controlled by a high water table and moderately low temperatures, as well as increasing GPP due to longer periods of high solar irradiance.
All loading rates had their lowest NEE values during July (range from -38 ± 40 [LR 1] to -102 ± 40 g C m⁻² yr⁻¹ [LR 3]). These low values reflected high respiration brought on by high summer temperatures as well as dry conditions (as shown by water table depth), which would have allowed aerobic respiration of organic matter. Indeed, all loading rates showed higher R in July than any other month (see Figure 4). The positive spike in NEE in September can be attributed to a decrease in R due to an increase in soil moisture, while conditions were still optimal for high GPP (i.e. warm temperatures, long periods of high irradiance).

Most loading rates showed net heterotrophic activity (i.e. NEE < 0) the majority of the year, with LRs 2, 3, 4 and 5 showing net autotrophic activity (i.e. NEE > 0) for only four, three, four, and two, months, respectively. LR 1, on the other hand, was net autotrophic for one half (6 months) of the year. All loading rates were net autotrophic during September, while all except for LR 4 were net autotrophic in May. All plots were net heterotrophic in July and from December through March, while most plots showed NEEs near or below 0 in October, November, and April.

Ratio of Photosynthesis to Respiration (GPP:R)

GPP:R ranged from 0.8 ± 0.1 in LR 5 to 1.3 ± 0.1 in LR 1, and appeared to trend negatively with loading rate (Figure 8). LRs 1 and 2 were above Odum’s (1969) theoretical line of 1:1, while LRs 3 -5 fell below the 1:1 line, indicating that they were annually net autotrophic and net heterotrophic, respectively.
DISCUSSION

CO₂ Fluxes

The range of annual GPP and R rates measured in this study (938-1452 and 796-1912 g C m⁻² yr⁻¹, respectively) were on the high side of annual rates measured in other studies. For example, Hirota et al. (2006) reported GPP at 51 and R at 37 g C m⁻² yr⁻¹ in a marsh on the Qinghai-Tibetan Plateau, Clark et al. (1999) found GPP to be 647 and R to be 562 g C m⁻² yr⁻¹ in a Florida Cypress ecosystem, and Neubauer et al. (2000) measured gross community GPP and R rates of 1062 and 1269 g C m⁻² yr⁻¹, respectively, in a freshwater tidal marsh in Virginia. Mean NEE rates in this study were also highly variable among loading rates (range of 141 to -459 g C m⁻² yr⁻¹), and encompass the range of those of the previous studies listed (84 to -207 g C m⁻² yr⁻¹). One major difference between the current study site and the other studies is age. Hirota et al. (2006), Clark et al. (1999), and Neubauer et al. (2000) all studied relatively mature, natural ecosystems compared to our ecosystem (CCW), which partially explains the near-zero NEEs in those studies. The slightly more negative NEE in Neubauer et al. (2000) was explained in part by tidally-controlled lateral transport of organic debris into the marsh, thereby increasing the respiration component. CCW, on the other hand, represents an early successional system, and would be expected to have NEE values far from equilibrium. The different loading rates appear to modify whether gas flux will trend positively or negatively with maturity; however, all trends would be expected to approach equilibrium.
High variability in annual NEE within treatment replicates may be due to slight
differences in plant community composition and/or plant sizes within chamber bases,
which could then affect photosynthetic and plant respiratory rates. Species richness data
(Chapter 2, Table 4) corroborates this hypothesis, as standard deviations ranged from 36
to 55% of the mean (n = 56) of growing season plant community diversity measurements
among loading rates sampled in the same plots (though not within the chamber bases) as
the current study. Further, standing crop biomass standard deviations (Chapter 2, Table
4) ranged from 22 to 88% of the mean (n = 8) among loading rates. Thus, it is feasible
that within-treatment plant communities could produce highly variable GPP and plant R
due to differences in species composition and biomass. Further, typically diverse non-
tidal freshwater wetlands (e.g. sedge meadows, this study) could reasonably be expected
to have higher spatial CO$_2$ flux variability than would wetlands with lower plant diversity
(e.g. salt marshes).

The significant positive correlation between annual GPP and loading rate
indicates that increasing amounts of organic material encouraged higher plant production,
perhaps due to increasing soil nutrients N and P (see Chapter 2, Figure 4) or inundation
frequency differences driven by plot elevations (see Chapter 2, Figure 3). However,
mean annual GPP only increased by a factor of 1.5 from LR 1 (938 g C m$^{-2}$ yr$^{-1}$) to LR 5
(1452 g C m$^{-2}$ yr$^{-1}$). The narrow ranges in GPP among loading rates agree with the results
of studies comparing standing crop biomass, another measure of annual plant production,
between natural and created wetlands (DeBerry and Perry 2004), amended and
unamended wetlands (Anderson and Cowell 2004), and along gradients of soil organic
matter (Cole et al. 2001, Bergschneider 2005). In fact, standing crop data from this study
showed no significant correlation or significant differences among loading rates (Chapter 2, Table 4). In addition, using similar field methods as the current study, both Cornell et al. (2006) and Roggero (2003) found similar rates of annual GPP between created salt marshes (low soil organic matter) and natural reference marshes (high soil organic matter).

Annual respiration values showed a positive relationship with loading rate, most likely accounted for by microbial respiration of the increasing organic amendments. Given adequate soil moisture and temperature, acceptable organic matter quality, and an available heterotrophic microbial community (Craft 2001), rates of organic matter decomposition would logically increase with increasing organic material availability. In addition, increased soil organic matter usually results in lower soil bulk densities (Collins and Kuehl 2001). In fact, a recent study of the same research plots Bergschneider (2005) measured bulk densities that were significantly different among, and negatively correlated with, loading rate (range from LR 1 = 1.3 g cm$^{-3}$ to LR 5 = 0.5 g cm$^{-3}$). Such decreases in bulk density likely have the effect of increasing the depth of aeration in the soil profile, thereby exposing more of the available organic matter to oxidation (particularly in dry conditions).

Further, as plot elevation and loading rate were positively correlated, it is also possible that some of the increase in respiration with loading rate was related to a decrease in soil inundation. For example, studies have shown that the mean water table position is a good predictor of mean respiration rates (Bubier et al. 1998, Wickland et al. 2001). Other studies suggest that wetland ecosystems can change from net CO$_2$ sinks to

Importantly, however, ecosystem respiration is the result of the metabolic respiration of plants, in addition to the microbially mediated respiration of soil organic matter fractions (Trumbore 2000). Although plant respiration was not directly measured in this study and could not be estimated as a percentage of the total, it is generally accepted that plants respire about 50% of the carbon derived through photosynthesis after photorespiration, with the remaining 50% used for growth, propagation, nutrient acquisition, and litter production (Ryan 1991). Because of this proportionality, the narrow annual GPP range among loading rates, and the fact that mean annual R increased by a factor of 2.5 from LR 1 (796 g C m$^{-2}$ yr$^{-1}$) to LR 5 (1912 g C m$^{-2}$ yr$^{-1}$), it can be inferred that plant-related respiration could not totally explain the differences in total respiration among treatments.

The negative trend in NEE with loading rate seems related mostly to differences in R rather than GPP among loading rates. Thus, respiration was likely the dominant CO$_2$ flux determinant (i.e. controls NEE) in this study.

Successional Maturity

The negative correlation between the photosynthesis to respiration ratio (GPP:R) and loading rate generally followed the predictions of Odum (1969). That is, if one considered high soil organic matter (OM) as a proxy for successional maturity, one would expect the ratio of photosynthesis to respiration to decrease (approach 1) from early to later successional stages. Of course, OM accumulation is only one of many attributes
(i.e. plant diversity and structure, biogeochemical cycling, etc.) expected to change as an ecosystem matures. As such, OM loading rate is not a perfect proxy for successional state, and care must be taken to cautiously interpret ecosystem function when using this parameter.

LRs 1 and 2 showed average GPP:R above 1, and thus displayed characteristics of an early successional system. LR 1 had no OM amendments and very low soil organic carbon content (1.7 ± 0.3%, Chapter 2, Table 1, Figure 4), and thus would be expected to have higher production than respiration. Judging from the low organic content of created wetland soils (Whittecar and Daniels 1999, Stolt et al. 2000) as well as the quickly accumulating plant biomass of southeastern Virginia created wetlands (DeBerry and Perry 2004), the LR 1 ratio is likely representative of many non-tidal created wetlands in this area. The slight decrease in the LR 2 ratio was probably related to the increased soil organic content from LR 1 (see “CO$_2$ Fluxes”), but organic matter is apparently still accumulating in these plots (i.e. ratio > 1). Of course, LR 2 is not in a late successional stage, and obviously lacks the plant structure of mature forested wetlands. However, the energy flow is balanced, and appears to mimic that of a climax community.

LRs 3-5 all show GPP:R ratios below 1. Ratios below 1 are typically produced in cases of organic pollution (Odum 1969). It is difficult to imagine a “naturally” functioning ecosystem that could maintain such a ratio, as the respiration component would be directly proportional to the amount of organic matter produced in the system. Other anthropogenic circumstances can lead to unusually low GPP:R ratios. For example, forest clearing can produce P/R ratios below 1, due to decomposition of dead organic matter immediately following clear cutting (Smith 1996). Just as cleared forests
will eventually reach a balance between production and respiration (if regeneration is allowed), so too should LRs 3-5. It appears that the high loadings of organic matter in these treatments added more organic carbon to the system than can be maintained under the ambient environmental conditions. The response, then, was high respiration rates (relative to production), acting to return the system to a carbon (i.e. energy) equilibrium. The decreasing ratios with increasing loading rate can be explained simply by the successively higher concentrations of organic carbon.

SUMMARY AND RECOMMENDATIONS

Annual fluxes of CO$_2$ at the CCW indicated generally decreasing NEE of carbon with increasing organic amendment loadings. These net fluxes were controlled primarily by changes in respiration. The photosynthesis to respiration ratios (GPP:R) appeared to mimic the theoretical decrease with successional maturity suggested by Odum (1969). However, the higher loading rate ratios fell below the 1:1 relationship indicative of climax systems, and instead were more suggestive of organically polluted environments. The lower loading rates were suggestive of early successional systems, however, and appear to be accumulating carbon as is typical of primary successional systems.

Comparisons of carbon flux components (GPP, R, NEE) as practiced in this study showed sensitivity to many environmental gradients, and provide an opportunity to study successional processes and differences in ecosystem parameters on a quantitative basis. Using these techniques, wetland function can potentially be monitored on theoretical grounds, instead of using only superficial indicators such as standing crop biomass.
Our results indicate that organic amendments do affect CO₂ flux processes in created, early successional wetland systems. However, organic amendment loading rate recommendations must be based on the specific goals of individual mitigation projects. In a general sense, the higher rates (LRs 3-5) would most likely prove unnecessary since the ecosystem energy balance would act to oxidize excess organic material until equilibrium was reached (i.e. P/R = 1). If the primary mitigation goal is to maximize carbon sequestration, not incorporating an amendment (i.e. LR 1) may produce best results. However, opting for this option would ignore many of the positive effects that studies such as Bergschneider (2005) have shown to accompany organic amendments. Thus, a low to moderate amendment such as LR 2 could potentially balance various mitigation goals such as plant diversity and soil fertility and bulk density, along with providing net carbon sequestration or balance.
Literature Review


NOAA. 2006. DS3505 - Surface Data, Hourly Global 72029799999. National Climatic Data Center, NNDC Climate Data Online.


Table 1. Summary of mean (±1 SE) annual variation over loading rates for gross primary production (GPP), respiration (R), net ecosystem exchange (NEE = GPP - R), and the photosynthesis to respiration ratio (GPP:R) from June 2005 to May 2006 in CCW, Charles City County, VA.

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<th>LR4</th>
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<td>GPP:R</td>
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<td>0.92 ± 0.1</td>
<td>0.81 ± 0.1</td>
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Figure 1. Schematic of carbon flux sampling equipment. Air is pulled from the chamber into the IRGA, where CO₂ content is measured. The air is then returned to the chamber. A photosynthetically active radiation (PAR) meter and air temperature sensor also gather information.
Figure 2. Annual gross primary production (GPP), respiration (R) and net ecosystem exchange (NEE) for each organic matter amendment treatment at the Charles City Mitigation site (CCW), Charles City Co. Virginia. Error bars represent ± one Standard Error (n = 4). LR 1 = 0 Mg ha$^{-1}$, LR 2 = 56 Mg ha$^{-1}$, LR 3 = 112 Mg ha$^{-1}$, LR 4 = 224 Mg ha$^{-1}$, LR 5 = 336 Mg ha$^{-1}$. 
Figure 3. Linear regressions of mean (±1 SE) annual gross primary production (GPP), respiration (R) and net ecosystem exchange (NEE) vs. organic matter loading rate at the Charles City Mitigation site (CCW), Charles City Co. Virginia. R is presented as negative C flux, relative to gains and losses from the ecosystem. NEE represents the difference between GPP and R (NEE = GPP – R). All p-values reported represent significance at the α = 0.05 level.
Figure 4. Monthly respiration (R) for each Loading Rate from June 2005 to May 2006 at the Charles City Mitigation site (CCW), Charles City Co. Virginia. The heavy blue line represents water table elevation above sea level in meters (msl).
Figure 5. Monthly gross primary production (GPP) for each Loading Rate from June 2005 to May 2006 at the Charles City Mitigation site (CCW), Charles City Co. Virginia. The heavy blue line represents water table elevation above sea level in meters (msl). GPP data were not collected from December 2005 to January 2006 due to very low photosynthetic rates.
Figure 6. Monthly net ecosystem exchange (NEE) for each Loading Rate from June 2005 to May 2006 at the Charles City Mitigation site (CCW), Charles City Co. Virginia. The heavy horizontal line represents a CO$_2$ flux of 0 g C m$^{-2}$ month$^{-1}$. The heavy blue line represents water table elevation above sea level in meters (msl). Positive NEE (> 0) indicate a net autotrophic system (i.e. carbon uptake) while negative NEE (< 0) indicates a net heterotrophic system (i.e. carbon release).
Figure 7. Linear regression of respiration (R) CO$_2$ flux vs. water table height relative to plot soil surface in CCW, Charles City County, VA, for June-September 2005. Positive water table depths are above the soil surface (0 cm). The p-value reported indicates significance of the linear regression at the $\alpha = 0.05$ level.
Figure 8. Annual photosynthesis (GPP) to respiration (R) ratio vs. Loading Rate in CCW, Charles City County, VA, from June 2005 to May 2006. The dashed line represents a GPP:R of 1:1. Error bars represent ± one Standard Error (n = 4).
Chapter 4
Summary and Conclusions

Chapters 2 and 3, though focusing on different suites of parameters, combine to provide a reasonably complete picture of several created wetland ecosystem functions at the Charles City Wetland Mitigation Site, and how those functions were effected by and varied among organic matter amendment loadings. Plant communities, regardless of loading rate were similar; there were few dominant and co-dominant species relative to the overall site species richness. Standing crop biomass was similar among loading rates, while both Gross Primary Production and tree size both varied positively (although weakly) with loading rate. Respiration data showed a positive correlation with loading rate, as modeled by air temperature. However, water table depth also appeared to play a critical role moderating summer respiration rates. Growing season water table depths (using plot elevation as a proxy) also seemed to explain trends in biomass and tree size, suggesting that hydrology may be an important determinant for these parameters.

The overall similarity of plant community composition and standing crop among loading rates, as well as the narrow range in gross primary production all seem to suggest that soil amendments may not provide the predominant environmental forcing or may exert conflicting forcings on these factors. However, significant trends in respiration and NEE with loading rate support the assertion that organic matter does control ecosystem energy exchange to some extent. That is, adding organic matter to an early successional wetland system can change the carbon balance from one focused on production and carbon sequestering to one dominated by respiration and carbon export. In either case,
ecosystems not in production-respiration balance will approach that balance as succession proceeds.

Both the vegetation and the CO$_2$ flux analyses show that adding higher end (i.e. LRs 4 and 5) loadings of organic matter amendments to created wetland soils is unsubstantiated due to the lack of differences in the vegetation and the negative NEE results. In fact, the negative NEEs indicate that excess organic matter is being oxidized in these treatments because environmental conditions cannot support it. Thus, from a management perspective, adding such high volumes of material may not be an efficient use of resources, and could in fact add needless cost to wetland construction. The combined results from Chapters 2 and 3 thus suggest an amendment loading rate between LR 2 (56 Mg ha$^{-1}$) and 3 (112 Mg ha$^{-1}$), based on the recommendations in Chapter 2 tempered by the apparent carbon balance noted in Chapter 3.
Appendices

--- Yard-waste Compost Samples ---

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Appendix 1. Results of analysis of organic matter amendments performed by A and L Laboratories, Inc. Samples collected on July 9, 2002 (from Bergschneider 2005)
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Appendix 2. Table of chemical parameters for surface soil (top 10 cm) samples collected on August 22, 2005 in CCW, Charles City County, VA. Data are separated by plot (i.e. treatment replicate), with four replicates per organic matter amendment loading rate (LR). Soils were analyzed and data were provided by the Virginia Cooperative Extension Soil Testing Laboratory, Virginia Tech University, Blacksburg, VA.
Appendix 3. Total monthly PAR from June 2005 to May 2006 measured at the Virginia Institute of Marine Science, Gloucester Point, Virginia.
Appendix 4. Mean monthly air temperature from June 2005 to May 2006 measured at the NOAA National Climatic Data Center station, Williamsburg, Virginia (76°71'70" W, 37°23'30" N).
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</table>

*CH$_4$ data were collected in the same static chambers used for CO$_2$ flux measurements. The chamber (either 1 or 1.5 m tall, depending on height of vegetation was secured to the chamber base using clamps and covered with an opaque blanket to shade out light and reduce temperature fluctuations during measurements. For each plot, 10 ml of gas were withdrawn from a septum in the wall of the chamber at 0, 5, 10, 20, and 40 minutes. Samples were collected during the same weeks that CO$_2$ flux measurements were performed (usually the first week of the month), from July 2005 to June 2006. Rubber bands were placed around the plunger and stopcock of each syringe to insure that leakage would not result in a change in CH$_4$ concentration before analysis. Gas samples were analyzed within five days of collection using a Hewlett-Packard model 5890 gas chromatograph, equipped with a 2.6 mL sampling loop and Flame Ignition Detector (FID) with Molecular Sieve 13x. The instrument was calibrated routinely before and during analysis using 1.00, 9.07, and 100.00 ppmv CH$_4$ in N$_2$ standards (Scott Specialty Gasses, Inc.). CH$_4$ concentrations were regressed over the 40 minute sampling period to calculate a slope, i.e. the change in CH$_4$ concentration per minute.

Appendix 5. Table of seasonal instantaneous (i.e. per minute) methane (CH$_4$) fluxes collected from July 2005 to June 2006 in CCW, Charles City County, VA. Data are separated by plot (i.e. treatment replicate), with four replicates per organic matter amendment loading rate (LR). CH$_4$ collection and analysis methods are described below the table.*