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Net Microbial Activity, Vegetation Dynamics, and Ecosystem Function in Created and Natural Palustrine Forested Wetlands in Southeastern Virginia, USA

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Net Microbial Activity, Vegetation Dynamics, and Ecosystem Function in Created and Natural Palustrine Forested Wetlands in Southeastern Virginia, USA

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ABSTRACT

Ecological function is defined as a balance between nutrient uptake and retention, accumulation of above- and below-ground biomass, and the ability of a system to maintain stability despite environmental stress. More recent research has refined this definition to include the accumulation of organic matter into soils and high levels of community primary productivity as important functions of wetland systems. The primary objective of this study was to use these concepts in order to evaluate the performance of a palustrine, forested wetland constructed based on current recommendations, and an adjacent natural wetland. Our data showed that soil moisture, soil organic matter, total carbon, and total nitrogen were all lower in the created wetland than in the natural wetland, while bulk density was higher in the created wetland. Moreover, soil respiration, nitrogen mineralization, and denitrification were all lower in the created wetland, while nitrogen fixation was greater in the created wetland, and methane emissions were negligible from both sites. These analyses indicate that, even when constructed based on current recommendations, environmental conditions in created wetlands produce a challenging environment for the establishment of vegetation. Analysis of plant communities supported this finding. Above- and below-ground biomass production and nutrient uptake were significantly lower in the created wetland, while the created wetland was also less diverse in richness, evenness, and the Shannon index. However, the created wetland featured higher levels of primary production and sequestered more carbon both during the growing season and over the course of the year long sampling period.

Net Microbial Activity, Vegetation Dynamics, and Ecosystem Function in Created and Natural Palustrine Forested Wetlands in Southeastern Virginia, USA

INTRODUCTION:

Wetland Mitigation and Ecosystem Function in Palustrine Forested Wetlands

Introduction

Palustrine wetlands include all non-tidal wetlands dominated by trees, shrubs, persistent emergent plants, or emergent mosses or lichens, as well as small, shallow open water ponds or potholes (Cowardin, 1979). By definition, palustrine wetlands also lack flowing water and contain ocean-derived salts in concentrations less than 0.05% (Cowardin, 1979). Wetlands within this category include swamps, marshes, potholes, bogs, or fens.

Palustrine forested wetlands, as well as other wetland types, are an intricate component of the environment and provide important services to both natural and anthropogenic systems. In natural systems, wetlands are areas of high primary productivity and provide habitat and breeding grounds for aquatic insect larvae, freshwater fish, migrating waterfowl, and a variety of other flora and fauna (Brinson and Rheinhardt, 1996; Mitsch and Wilson, 1996). Wetlands also play an important role in the hydrological cycle, providing both short- and long-term surface water storage, groundwater recharge and discharge, and removal of sediment, nutrients, and pollutants from surface water runoff entering larger bodies of water (Mitsch and Wilson, 1996). In the nutrient cycle wetlands often serve as net-carbon and nitrogen sinks (Bowden, 1987). In addition, many of the functions found in natural wetlands have corresponding functions in anthropogenic systems. For example, in the built-environment, existing natural wetlands provide storage for storm water runoff and serve as sediment and nutrient traps to ensure the health of ground water stores needed for human consumption (Brinson and Rheinhardt, 1995; Brooks et al., 2005).

Despite playing an important role in sediment and nutrient retention, water quality improvement, and atmospheric gas cycling, human interests have often taken precedence over the preservation of naturally functioning, wetland ecosystems within the United States. However, due to the value of these services, their staggering replacement cost,

and the continued, rapid loss of wetlands due to land-use transformation, the United States Congress acted to protect natural wetlands in the 1972 Clean Water Act (Copeland, 1999; Whigham, 1999). The 1977 update to the CWA included a provision that mandated 'no net loss' of existing wetlands, and granted further regulatory power to the US Army Corps of Engineers (USACE) and the Environmental Protection Agency (EPA) (Copeland, 1999). This protection strategy served as a way to curb the loss of wetlands and was enacted with the goal not only of no net loss, but of net gain to both freshwater wetland area and function (Copeland, 1999).

In effect, the CWA, and 1989 administration policy did not halt development on existing wetlands. Rather the development of wetlands continued, but permits issued by the Army Corps and EPA had to be accompanied by plans to create or restore a wetland of equal, or larger, size to the area being developed. This aspect of federal wetland protection policy has led to the creation of the fields of wetland delineation and mitigation (Tiner, 1993; Silverstein, 1994; Whigham, 1999). Delineation refers to classifying an area as a 'wetland' suitable for protection under the outlined policies based on *in situ* hydrological conditions, soil types, and vegetation. Conversely, wetland mitigation attempts to avoid ecosystem destruction, minimize impacts of development, and then, if needed, use engineering and construction practices to either restore function to an existing, yet compromised wetland, or create a wetland where one did not previously exist (Silverstein, 1994; Brinson and Rheinhardt, 1995). In order to be listed as a success, these restored and created wetlands must be of equal or larger size to the wetland area being impacted, and just as importantly, provide equal function (USEPA, 1989).

The concept of ecosystem function in palustrine forested wetlands is difficult to quantify and measure. In a general sense, ecological function is defined as a balance of nutrient uptake and retention, accumulation of above- and below-ground biomass, and the ability of a system to maintain stability despite environmental stress (Odum, 1969). More recent research has refined this definition to include the accumulation of organic matter into

soils and high levels of community primary productivity as important functions of natural wetlands (Balcombe et al., 2005).

Based on these concepts, past research indicates that created wetlands, while of equal or greater area, have often fallen short of natural wetlands in terms of function (Brooks et al., 2005). The primary function wetland mitigation projects fail to recreate properly is a naturally functioning plant community (Mitsch and Wilson, 1996). Mitigation projects essentially re-create early successional stages in natural environments, introducing plants to excavated substrates within an engineered hydrological system (van der Valk, 1981). Many of the difficulties associated with creating wetlands are therefore related to: 1) inadequate hydrology, 2) poor soil development, 3) high mortality of planted individual seedlings, 4) lower levels of individual seedling success in terms of growth, and 5) the inability of plant communities to proceed through natural succession and achieve the full function of a stable community (Mitsch and Wilson, 1996). These individual problems combine to create systems that often fall short of naturally functioning wetlands in terms of nutrient cycling and retention, and ecosystem exchange of atmospheric gasses.

Research has shown changes in microtopography, plant growing techniques, and soil aeration all have the potential to improve both individual, and community, growth and function in created wetlands (Armstrong et al., 1967; Donovan et al., 1988; Shaw et al., 2002; Moser et al., 2007). Other proposed methods of improving success of wetland mitigation plant communities include the addition of organic material to mitigation projects and the use of organic soil amendments (Lin and Mendelssohn, 1997; Bailey et al., 2007). However, little research has been done to address the role microbial communities play in soil development in created wetlands, or the impacts of existing site conditions on vegetation dynamics and ecosystem function.

Objectives

This study focused on addressing some of these questions. We had two specific objectives for this study:

- ***Quantify differences between hydrology, soil characteristics, and microbial activity in a palustrine, forested wetland constructed based on current recommendations, and in an adjacent natural wetland.***
- ***Quantify differences between vegetation dynamics and ecosystem function in a palustrine, forested wetland constructed based on current recommendations, and in an adjacent natural wetland.***

In this experiment, hydrology was approximated as depth of the water table and soil moisture, while measured soil characteristics included organic matter, bulk density, total carbon (C), total nitrogen (N), and soil carbon to nitrogen (C:N) ratios. Net microbial activity was quantified as soil microbial respiration, methanogenesis, nitrogen fixation, nitrogen mineralization, and denitrification in both wetlands. Microbial respiration and methanogenesis were measured *in situ* as net ecosystem exchange of carbon dioxide (CO_2) and methane (CH_4), while nitrogen fixation, nitrogen mineralization, and denitrification were measured using the acetylene reduction technique, anaerobic incubation procedure, and acetylene block method respectively in soil cores collected from both sites. Vegetation dynamics and ecosystem function were defined as the accumulation of above- and below-ground biomass in planted *Betula nigra* seedlings, carbon to nitrogen (C:N) ratios in the foliage of the seedlings, community composition, and community net ecosystem exchange of carbon dioxide.

Although past studies have documented both microbial activity and vegetation dynamics in natural systems, little work has been done to determine whether significant differences exist between natural wetlands and wetlands created for mitigation purposes. By exploring potential differences in microbial activity and plant communities in these sites, and relating these differences to ecosystem function, it was hoped that research would better explain the underlying causes for the differing success rates observed in natural and created systems.

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CHAPTER ONE:

Review of Construction Techniques, Soil Development and Microbial Processes, and Vegetational Dynamics in Palustrine Forested Wetlands

Created Wetland Construction

Introduction

Current federal restoration initiatives include projects that, when taken together achieve a net increase of 100,000 acres of wetlands each year (EPA, 2000). The increase in wetland area outlined in these initiatives are based on section 404 of the 1977 Clean Water Act, which requires the restoration or creation of wetland habitat to compensate for the loss of natural wetlands, and their associated functions, caused by development. Of these two options, 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, while many of the individual projects contained within these overall restoration initiatives are unsuccessful, the failure of created wetlands, i.e. not achieving equal function of natural wetlands, is especially common (Mitsch and Wilson, 1996; Zedler, 1997; Whigham, 1999; Spieles, 2005). One specific, documented problem in these systems is the poor survival, and slow growth rates, of planted woody vegetation, two measures that serve as a proxy for failed function (Spieles, 2005).

In order to minimize project failure and improve created wetland function, federal and state agencies, non-profits, and research universities have taken steps to provide guidelines and acceptable practices for vegetation planting in created wetlands (EPA, 2000). These range from very general management principles to more specific strategies for species selection and planting. Following is an overview of these recommended practices and the biological, physical, and chemical conditions present in both natural and mitigated wetlands that make them necessary.

Wetland Construction Techniques

The US Environmental Protection Agency's (EPA) Watershed Ecology Team provides a list of general guiding principles for the design and construction of wetland mitigation projects (EPA, 2000). While the principles focus on scientific and technical issues, they also include management techniques. The 16 recommended principles of restoration are:

1. Preserve and protect aquatic resources
2. Restore ecological integrity
3. Restore natural function
4. Restore natural structure
5. Work within the watershed/landscape context
6. Understand the potential of the watershed
7. Develop clear, achievable and measurable goals
8. Focus on feasibility
9. Use reference sites
10. Anticipate future changes
11. Involve a multi-disciplinary team
12. Design for self-sustainability
13. Use passive restoration, when appropriate
14. Restore native species, avoid non-native species
15. Use natural fixes and bioengineering
16. Monitor and adapt where changes are necessary

Principles apply to both the physical and biological landscape, and can be used to guide restoration of ecosystem function, defined as natural nutrient cycling regimes, accumulation of biomass, and high levels of productivity (D'Avanzo, 1987; Larson, 1987; Kusler and Kentula, 1989; Confer and Niering, 1992), and structure, defined as natural successional plant communities (Odum, 1969; Brinson and Rheinhardt, 1996; Brooks et al., 2005).

Applicable EPA principles, supported by external research include incorporating historical site conditions into vegetation plans (Maltby, 1987; FICWD, 1989; Kentula et al., 1992) and using reference sites comparable in structure and function to project sites (van der Valk, 1981; Brinson and Rheinhardt, 1995; Mitsch and Wilson, 1996; Balcombe, 2005).

Site Preparation: Following initial excavation to create natural hydrologic conditions, proper site preparation is the first step in any wetland creation project. Research shows site excavation creates high soil compaction and exposes clay subsoils, both of which create additional, difficult growing conditions for vegetation in wetland mitigation projects (Brinson and Rheinhardt, 1995; Whittecar and Daniels, 1999; Bruland and Richardson, 2004; Atkinson et al., 2005). During site preparation, wide track, low ground pressure equipment is therefore preferred because heavy equipment results in soil compaction (Garbisch, 2002). If sites have undergone significant excavation, and have experienced heavy traffic from construction equipment, they should be disked or chisel plowed a minimum of two times to reduce compaction prior to planting. Research shows disking improves microtopography defined as tortuosity, limiting slope, limiting elevation difference, and depth to the water table making conditions more favorable for vegetation community development (Moser et al., 2007). Research also suggests that improving soil aeration via disking improves survival and growth of trees grown in wetland environments (Armstrong and Boatman, 1967).

Official recommendations suggest tillage should be to a depth of 8-15 inches, and to a bulk density of less than 85lbs. per cubic foot for loam, and less than 107 lbs. per cubic foot for fine textured soils. Tillage should occur when the soil is drained, yet moist (Munro, 1991; Sauer, 1998; Schweitzer, 1998). If the soil is approaching saturation, tillage will create either a hard-packed pan immediately below the tillage or a slurry of organic material prone to significant water loss through evaporation (Sauer, 1998). Recommendations ensure proper reconstruction of soil-geologic densities and profiles so that they are similar in both texture and permeability to natural wetland soils, thereby

facilitating groundwater interchange and the rooting of woody species (Haering et al., 1992; Daniels et al., 2005).

After site tillage, research suggests covering at least 1–2% of the overall site acreage with coarse woody debris greater than 2" in diameter (Garbisch, 2002). A mix of woody debris, including logs and stumps, provides wildlife cover and a source of slowly decomposing organic material, and inoculates the site with some plant propagules, forest ectomycorrhizae, and invertebrates (Garbisch, 2002; USACE New England District Regulatory Branch, 2002).

Organic Amendments: Research indicates low amounts of soil organic matter are often found in created wetland sites along the eastern seaboard (Stolt et al., 2000; Bruland and Richardson, 2004; Bergshneider, 2005; Daniels et al., 2005; Bailey et al., 2007). This is primarily due to traditional mitigation practices such as top soil scraping used to create microtopography. While creating favorable physical structure, site grading often removes accumulated organic material in the surface A-horizon.

The best way to combat low levels of soil organic matter in wetland mitigation projects is to conserve and return the natural organic matter, woody debris, and topsoil removed from the site during construction (US EPA, 2000; NRCS, 2002). Economically and practically it is significantly more efficient to conserve on site rather than to import foreign organic material (Eggers, 1992). In order to do this, when A- and B-horizons are excavated the topsoil A-horizon should be separated from the subsoil, stockpiled, and protected on site for future use (Haering et al., 1992; Daniels and Whittecar, 2003).

However, returning excavated organic matter to project sites is not always possible. This has led many researches to examine the impacts of amending soils in wetland mitigation projects with organic material (Brinson et al., 1995; Brinson and Rheinhardt, 1996; Stauffer and Brooks, 1997; Whittecar and Daniels, 1999; McKinstry and Anderson, 2003;

Anderson and Cowell, 2004; Bruland and Richardson, 2004; Bergshneider, 2005; Bailey et al., 2007).

Research examining the effect of organic matter loading rate on vegetation composition, standing crop biomass, woody vegetation development, and ecosystem gas exchange showed that 56 – 112 Mg ha^{-1} of organic mulch improved soil conditions and created an environment comparable to natural wetland sites (Bailey et al., 2007). A related study by Bergshneider (2005) examining organic amendment based on: 1) soil properties reflective of hydric soil development, 2) the formation of redoximorphic features, and 3) the growth and vigor of hydrophytic vegetation showed with increasing organic compost loading up to 112Mg ha^{-1}, redox potential decreased, redoximorphic feature formation increased, and total biomass of *Betula nigra* and *Quercus palustris* peaked. The same study also showed that with loads increasing to 224 Mg ha^{-1} subsurface bulk density remained constant, surface bulk density decreased, and soil moisture peaked (Bergshneider et al., 2005). Similarly, research in the Mid-Atlantic Region of the United States by Daniels et al. (2005) showed that approximately 100 Mg/ha of organic amendment was the optimal loading rate for reconstructing hydric soil conditions.

If a mitigation project is to be amended with organic material, research suggests the site should be over-excavated 6-12 inches depending on site hydrology and groundwater inputs (Cummings, 2000; Daniels and Whittecar, 2003). After the site has been graded to the subsoil B- or C-horizons, organic matter should be incorporated into the existing subsoil until soil organic matter content is at least 5% (Haering et al., 1992). A minimum of 5% organic content provides both a substrate for vegetation rooting and the source of organic material needed to support microbial activity. The 5% level is intended as a target, following soil development, and is very difficult to achieve via one-time organic matter loadings during site construction.

Research suggests when selecting a proper soil amendment, prior to site excavation a standard agricultural analysis of existing soil present on site should be compared to an

analysis of the proposed organic amendment (USACE New England District Regulatory Branch, 2002). Organic matter amendments can consist of topsoil, compost, organic muck, leaf mold, and donor soils from other sites (Cummings, 2000; Daniels and Whittecar, 2003). When a donor soil is being used, seedbank studies should be conducted to ensure invasive species are not introduced (DeBerry and Perry, 2000).

Organic amendments can be difficult to mix into excavated soils (Haering et al., 1992; Whittecar and Daniels, 1999; Daniels and Whittecar, 2003). Once the amendment has been added, the surface should be tilled with a chisel-plow or heavy disk to loosen the soil to a bulk density of less than 1.4 Mg/m^3 (Whittecar and Daniels, 1999). Disking or plowing to a depth of 6-8 inches reduces soil compaction caused by excavation and mixes organic material into exposed soil horizons (Haering et al., 1992; Whittecar and Daniels, 1999; Daniels and Whittecar, 2003). Research shows clay soils benefit more from disking rather than chisel plowing. After organic matter has been mixed into the soil, research shows 1-3 inches of wood chips or leaf mulch should be added to the soil surface to help moderate soil temperatures and retain soil moisture during times of high evaporation (Haering et al., 1992).

Vegetation Establishment: Research shows that tree species being introduced to created wetland sites must have adaptations that: 1) allow for rapid growth, 2) create the ability to colonize during dry conditions, and 3) and encourage adaptation to prolonged inundation and survival in poorly oxygenated or anaerobic soil environments (Cronk and Fennessey, 2001) because construction methods create conditions ranging from dry to increasingly saturated (Campbell et al., 2002; Bruland and Richardson, 2004; DeBerry and Perry, 2004).

Much attention has been paid to the establishment of canopy tree species in wetland mitigation projects, however the establishment of the understory is often overlooked (Garbisch, 2002). According to the USDA Natural Resources Conservation Service (2000) understory diversity can be increased through:

1) Transplanting trees and shrubs from areas that will be filled or cleared;
2) Use of nursery raised plant materials (on average, 10 species per acre)
3) Addition of topsoil from a donor site where invasive or undesirable species are not a concern;
4) Transplanting blocks of topsoil from areas that will be impacted to the mitigation site.

Further research suggests the development of a forest understory can be enhanced by establishing hydrophytic vegetation typical for the wetland type being established (Galatowitsch and van der Valk, 1994; National Research Council, 2001; NRCS, 2002). Research also suggests a mix of seeds, seedlings, and mature plants often jump starts community development (Clewell and Lea, 1990).

In addition to trees and shrubs, Federal Conservation Practice 612 suggests introducing plants that are aggressive ground-level colonizers to stabilize sediment, retain soil moisture, improve structure, delay the establishment of weedy competition, and provide habitat (NRCS, 2002). When planted with other vegetation, annual cover crops like buckwheat, rye, wheat, or millet or specially prepared wetland seed mixes accomplish all of these goals (HQ USACE, 2002).

Proposed plant lists and planting plans are dependent upon project goals. These goals may include improving local habitat, reducing erosion, or decreasing estuary nutrient loading (USDA Soil Conservation Service, 1992; HQ USACE, 2002). The size and configuration of the mitigation site also affect plant selection and introduction. Small, narrow sites can often be revegetated through a combination of natural introduction and regeneration while larger sites are more dependent on anthropogenic plant introduction. Other factors influencing plant selection include site hydrological conditions and the availability of source plant material (USDA Soil Conservation Service, 1992; HQ USACE, 2002).

Tree species often used wetland mitigation projects in the Piedmont region of Virginia include: *Betula nigra, Liquidamber styraciflua, Platanus occidentalis, Quercus bicolor, Quercus palustris, Quercus phellos*, and *Salix nigra.*

Planting Stock: There are a variety of different planting stock types available for introduction to wetland mitigation sites: seeds, bare-root seedlings, cuttings, tubelings, container or potted stock, and ball and burlap plantings (Clewell and Lea, 1990; Sauer, 1998).

Direct seeding is often used to establish both heavy seeded species such as *Quercus* and *Carya*, as well as light seeded species such as *Acer*, *Liquidamber*, *Fagus*, *Fraxinus*, and *Ulmus* (Clewell and Lea, 1990; Munro, 1991; Sauer, 1998). Direct seeding is often required because traditional creation practices such as top soil scraping remove not only the existent vegetation, but also any accumulated organic material in the surface O- and A-horizons including seed banks. Research shows *Quercus* and *Carya* individuals introduced by direct seeding have especially high survival rates in seasonally saturated soils throughout the southern United States (McKevlin, 1992).

Research suggests seeds gathered on-site or from adjacent and nearby properties have higher survival rates and are therefore better suited for use in mitigation (Dunne and Samanns, 1998). When foreign plant material is introduced, seeds should include indigenous species present in local reference wetlands, with source material available within a 200-mile radius (Sauer, 1998; HQ USACE, 2002). Specifically to the State of Virginia, introduction of seeds must comply with Virginia Seed Law (Sections 3.1-262 Code of Virginia) and Virginia Seed Regulations (2 VAC 5-290-10 et seq).

When planning anthropogenic seed introduction HQ USACE (2002) recommends considering: 1) the quantities of live seed per acre required for the project, 2) dates for seeding based on germination rates, and 3) methods, either hand or mechanical seeding, required to attain desired seed coverage.

However, all seeds need not be introduced anthropogenically. In naturally regenerating forests, introduced tree seeds often range between thousands and tens of thousands per acre, numbers impossible to match by anthropogenic introduction (Munro, 1991; Dunne and Samanns, 1998). Recruitment of light-seeded, pioneer species such as *Acer rubrum*, *Liquidamber styraciflua*, *Salix nigra*, and *Platanus occidentailis* from adjacent wetland sites should therefore be considered an integral component of some planting plans (Sauer, 1998).

Research shows the following factors create favorable conditions for natural introduction of light-seeded species:

1) Narrow sites less than 100 yards in width with adjacent wetland, seed-bearing trees.
2) Sites exposed to overbank, seed bearing flood waters.
3) Sites with soil and hydrological conditions not significantly impacted by construction.
4) Viable seedbanks dominated by non-invasive species in close proximity to the planting zone.

While direct seeding is one method of tree establishment, the majority of vegetated tree plantings in forested wetlands rely on three types of planting stock: 1) bare-root seedlings, 2) tublings, and 3) potted trees (Perry and Atkinson, 2008). Bare-root seedlings are saplings up to one year in age, transplanted without an established root ball and the subsequent associated soil microbial community. Tubelings are similar to bare-root seedlings except they are slightly older with a more well-developed root system. Tublings are still transplanted without a root ball. Tubelings are often used to establish fast growing early successional species like willows, cottonwood, and alder (Virginia Department of Forestry, 1993). Potted trees are transplanted with a well-formed root ball, and the associated microfauna, and are categorized by the size of the container in which they are grown. Containers typically range in size from one to three gallons, but can also include in-ground grow bags up to 25 gallons in size. Trees grown in containers

also range in age and size. Trees can be from one to several years in age. One benefit to using potted trees and container stock is that both can be planted later in the season than bare-root seedlings and tubelings (Virginia Department of Forestry, 1993).

Research also shows that container stock has higher survival rates in many types of soil than bare-root seedlings and tubelings (Clewell and Lea, 1990). Specifically, container stock is more tolerant of saturation and inundation than both bare-root seedlings and tubelings. Lower mortality rates for container stock results in fewer individuals needed per acre to establish woody species than when bare-root seedlings are used (Virginia Department of Forestry, 1993). However, potted trees are often more than five or ten times more expensive than seedlings or tublings and also take significantly more effort to transplant. It has yet to be determined whether the increased cost of potted trees results in a more rapid development of the wetland ecosystem to which they are introduced, thereby justifying the increased cost added to the project (Perry and Atkinson, 2008).

Root Production Method: One proposed method of further increasing the survival and growth of individual trees in wetland mitigation projects is the Root Production Method (RPM) (Grossman et al., 2003; Dey et al., 2004). RPM trees are produced in nurseries by air pruning roots and growing trees in well aerated soils to encourage the growth of a dense, fibrous root system (Shaw, 2004; Zemp, 2004).

Specifically, Root Production Methods collect seed propagules within 161km of the planned planting site, grade for quality, and then start selected seeds in 4cm of composted rice hull, pine bark and sand. The compost is prepared in a 4:4:2 ratio to create 35-40% porosity, and amended with slow release fertilizers (Grossman, et al. 2003). This combination promotes air pruning of the tap root, and increased production of lateral roots (Dey et al., 2004). One to two months after emergence, seedlings are transplanted into individual, bottomless containers 10cm deep to continue air root pruning of the tap root. Seedlings are then graded based on height, stem caliper, and root development, with only individuals in the top 50% selected (Grossman, 2003). As seedlings continue

to develop, they undergo a series of transplanting to larger containers and are acclimated to outdoor environments. Containers continue to be shallow and bottomless to encourage air pruning of the tap root.

Research shows after two growing seasons in the nursery, RPM produced *Quercus alba* seedlings have basal stem diameters greater than 2.0cm and heights exceeding 1.5m, a significant improvement over conventionally produced seedlings (Dey et al., 2004). Root dry weights and root volumes for the same RPM seedlings averaged 101-117g and 222-252ml, compared to 18g in weight and 26-33ml in volume for conventionally grown bare-root seedlings, also indicating a significant improvement in growth (Dey et al., 2004). Research shows these trends are typical of RPM grown trees (Grossman et al. 2003, Shaw et al. 2004). Because of the potential to improve sapling development, naturally slow growing species, such as *Quercus palustris*, *Quercus bicolor*, *Quercus macrocarpa*, are often the subject of RPM treatments (Dey et al., 2004).

Research comparing the field growth of RPM and conventionally produced *Quercus alba* seedlings indicate that early rapid shoot growth and acorn mass are significantly greater and occur earlier in RPM produced trees (Grossman et al., 2003). Research also shows that in forest restoration projects, first-year survival was significantly higher and basal diameter was significantly greater in RPM produced seedlings than in bare-root seedlings (Shaw et al., 2004). Tree height was also greater in RPM grown trees, however data analysis showed this difference to not be significant (Shaw et al., 2004). Finally, we have found no research investigating the effect using genetically selected plants has on the overall diversity of a site.

Planting Practices: Because of the variable nature of site hydrology, and the effects construction has on these characteristics, Federal Conservation Practice 652 recommends not finalizing planting plans until after the wetland has been constructed in order to confirm site-specific soil and hydrological conditions (NRCS, 2002).

When direct seeding is being used as a method of species introduction, studies suggest heavy seeds, such as acorns, be planted at a depth of 1-6 inches from late fall until late spring. Heavy seeds can either be planted by hand or by using a modified one or two row planter (Munro, 1991; McKevlin, 1992). When either of these methods are used, conservative germination rates of 35% can be expected (Schweitzer, 1998). At that rate, planting 1000-1500 seeds per acre would result in 300-500 seedlings per acre (Schweitzer, 1998). If weedy competition is expected to be heavy during the growing season, it may be necessary to mow or disk the seeded area repeatedly. While expensive, this upkeep may be crucial to seedling survival if competition is severe.

When natural introduction of light-seeded species from nearby sites contribute to the establishment of species, bare-root seedlings of upper-canopy species such as *Quercus*, *Carya*, *Liquidamber*, *Platanus*, *Acer*, *Fraxinus*, and *Ulmus* should be planted at a density of 110-300 per acre to improve future horizontal and vertical structural complexity (Clewell and Lea, 1990; HQ USACE, 2002).

Federal Conservation Practice 652 suggests when natural introduction of seeds is not a viable alternative, planting a mix of early successional, nurse species via direct seeding as a cover crop, and mixing in shade tolerant, late primary successional bare-root seedlings at densities up to 300 stems per acre creates a similar level of ecosystem complexity (Clewell and Lea, 1990; McKevlin, 1992; NRCS, 2002). When mixed with low stem density plantings of bare-root seedlings, anthropogenically introduced cover crops of early successional, nurse species: 1) contribute organic matter to recently disturbed soils, 2) decrease soil compaction in the rooting zone, and 3) provide shade to minimize the establishment of invasive weed species (McKevlin, 1992; HQ USACE, 2002). Research suggests that these steps encourage the colonization of the entire wetland area within three years of planting (NRCS, 2002).

Higher stem densities (300-500) can also be used and will promote the growth of large stems with small crowns (Clewell and Lea, 1990). Research also shows higher stem

density plantings should be utilized when poor competitors such as *Chamaecyparis thyoides* are used in revegetation (Clewell and Lea, 1990; Sauer, 1998). High density stands work to minimize external competition from other introduced species. Conversely, low stem density plantings may be more appropriate for the introduction of strong competitors of certain species such as *Salix nigra* and *Acer rubrum* (Sauer, 1998).

Seedlings, tubelings, and container stock slated for use in wetland mitigation projects should not be raised in saturated environments. Along with increasing production costs, being grown in an oxygen-poor environment creates individuals with significantly less developed root structures which gives them at a competitive disadvantage after site introduction (USDA Soil Conservation Service, 1992). However, prior to planting, seedling stock should be acclimated to site conditions. If container stock comes with an associated root ball, research shows loosening or breaking up the ball prior to planting increases survival rates (USDA Soil Conservation Service, 1992).

During planting, research suggests small changes in location and elevation significantly alter success and growth rates of trees in wetland mitigation sites (Bailey et al., 2007; Moser et al., 2007). *Taxodium distichum* tubelings grown in a created tidal freshwater swamp showed increased above- and below-ground biomass when planted on a 15cm ridge when compared to those planted at soil surface level or in 15cm ditches (Dickenson, 2007). Separate studies show accumulation of biomass, measured as stem height, diameter, and root structure, in *Nyssa aquatic* and *Salix nigra* was significantly impacted by elevation with higher rates found in individuals grown at mid-levels or ambient water level (Donovan et al., 1988).

Monitoring Project Success

Success of vegetation plantings in wetland mitigation projects is hinged on careful monitoring and reinforcement for up to two years after initial construction (EPA, 2000; Brinson and Rheinhardt, 1995). Monitoring and long-term management responsibilities should be identified in the plan. Methods should be identified for measuring success

criteria in terms of plant survival, ecosystem function, and the presence or absence of invasive species.

Early survival of introduced trees is strongly influenced by herbivory (McKevlin, 1992). For example, one study suggested rodents girdled and killed between 25% and 65% of five-year-old planted Atlantic white cedar in a created wetland in southeastern Virginia (USDA Natural Resources Conservation Service, 2000). Tree tubes or shelters, site fencing, trapping and/or hunting, chemical deterrents, attracting predators, and removing herbivore cover have all been used in forest reestablishment projects with mixed levels of success (HQ USACE, 2002). Research shows tree shelters extending 2 inches into the ground, and removed two to three years after planting significantly reduce predation, especially by rodents (USDA Soil Conservation Service, 1992). No measure is 100% effective in curbing predation, however an assumption of 10% mortality in tree seedlings is often enough to offset the combined loss of individuals due to the effects of predation and environmental stress (USDA Soil Conservation Service, 1992).

Research shows ecosystem function, defined as biomass accumulation and productivity, can be estimated via non-destructive measures of vegetation in wetland projects (Brinson, 1993; Perry and Hershner, 1999). Research shows individual canopy cover, bole basal diameter, and maximum height were the best predictors of individual sapling growth in a created forested wetland in Virginia (Bailey et al., 2007).

Research also shows the introduction of invasive species can be controlled by selective mowing, the use of weed barriers or tree mats, selective site disking, and the use of herbicides (USDA Natural Resources Conservation Service, 2000). Supporting studies suggest invasive species control via these methods increases the growth of hardwood tree species 10-20% over a five year period (Sauer, 1998; Garbisch, 2002). Site plans should specify threshold percentages of invasive species that would trigger remedial action (Dunne and Samanns,1998). In Virginia, undesirable, invasive plant species are listed on the Virginia Department of Conservation and Recreation's Invasive Alien Plant List

(USDA Natural Resources Conservation Service, 2000). Additional information on invasive plant species can be found in the USDA Plants Database.

Wetland soil development and microbial processes

Introduction

Wetlands cover approximately 3% of the Earth's land surface, store up to 30% of the global pool of soil carbon, and improve water quality by serving as efficient transformers of agricultural, industrial and domestic point- and non-point discharges (Prather et al., 1995; Cao et al., 1996). Their ability to serve as sources, sinks, and transformers of important ecosystem and atmospheric compounds such as nitrogen and carbon makes wetlands critical in mitigating the adverse effects of eutrophication, greenhouse gas emissions, and toxic substances in soils, surface water, and ground water (Chanton and Dacey, 1991). Many of these transformations occur in wetland soils and are directly mediated by diverse groups of aerobic and anaerobic microorganisms (Drew and Lynch, 1980).

Wetland Soils

Geochemically, wetlands contain hydric soils "formed under conditions of saturation, flooding, or ponding long enough during the growing season to develop anaerobic conditions in the upper part" (NTCHS, 1985). Therefore, varying levels of soil oxygen depletion, and the presence of anaerobic biogeochemical pathways, are universal characteristics of wetland soils.

Wetland soils range from mineral to organic, eutrophic to oligotrophic, and saline to fresh (Zehnder and Stumm, 1988). Wetland soils also differ from most upland and aquatic sediments because many undergo intermittent flooding and draining. Variability in the water table influences oxygen availability and creates a fluctuating aerobic-anaerobic system which supports microbial communities able to exploit a wide range of electron acceptors during respiration (Matthews and Fung, 1987).

Soil Development: Soil development in created wetland sites increases with increasing site age (Odum, 1969; Marks and Bormann, 1972; Odum, 1985; Chadwick and Graham, 2000) as physical and chemical weathering, the incorporation of organic material into the soil substrate, and soil oxidation and reduction (redox) processes increase with respect to time (Stevens and Walker, 1970; Mausbach and Richardson, 1994). Constructed, i.e. young, wetlands are also characterized by a progressive increase in soil organic matter with increasing site and ecosystem age (Odum, 1969).

Soil organic matter serves as a nutrient source (Neue, 1985), and provides metabolic energy that drives reduction in microbial mediated biogeochemical pathways under anaerobic conditions (Vepraskas and Faulkner, 2001), and, along with organic carbon, regulates the conservation and recycling of nutrients between microbial, plant, and soil communities (Collins and Kuehl, 2001). As soil organic matter increases, C:N ratios increase while bulk density, pH, soil chroma, and coarse mineral fractions all decrease with increasing soil organic matter (Bischel-Machung et al., 1996; Nair et al., 2001; Campbell et al., 2002). In wetlands, the accumulation of organic matter is primarily dictated by changes in the water table, which influence aerobic conditions and organic matter decomposition.

While creating favorable physical structure, site grading often removes accumulated organic material in the surface A horizon. Studies indicate low amounts of soil organic matter are observed in wetland mitigation sites in Florida, Pennsylvania, and Virginia. Values typically are 10-18% higher in natural wetlands than in created wetlands (Table 1.1). Additional research has confirmed this relationship for the Piedmont Region of Virginia and found that soil organic matter concentrations in mitigated wetlands are significantly lower than those found in native forested hydric soils.

Microbial Activity: Microbial activities also influence soil organic matter content, nutrient availability, redox potential, pH, and trace element solubility, precipitation, sorption and mobility (Drew and Lynch, 1980). Microbial activities vary depending on

soil characteristics and other site-specific properties such as hydrology and anthropogenic nutrient inputs (Drew and Lynch, 1980). These variations make it difficult to predict nutrient and carbon cycling across wetlands, or even within a specific wetland type or region, without prior, extensive experimentation on a site-to-site basis.

In natural environments microbes play a crucial role in the soil matrix; microbes: 1) break down soil bulk density increasing porosity and encouraging oxygen diffusion, 2) serve as the dominant denitrifying agent thereby increasing nutrient availability for plants, and 3) make different compounds available for plants to use as a terminal electron acceptor in anaerobic environments (Drew and Lynch, 1980; Garbeva et al., 2004). Wetland microbial communities specifically differ from those found in upland and aquatic soils because intermittent flooding creates a broad spectrum of aerobic and anaerobic conditions. Due to the loss of oxygen as an electron acceptor in these variable anaerobic conditions, in order to inhabit this environment, physiological groups of microbial organisms must able to exploit a wide range of electron acceptors during respiration (Collins and Kuehl, 2001; D'Angelo and Reddy, 1998). Potential electron acceptors include: oxygen (O_2), nitrate (NO_3), iron (Fe(III)), sulfate (SO_4), and carbon dioxide (CO_2) (Sorensen et al., 1979; Lovley and Klug, 1986).

As microbes break down decomposing material through the oxidation of organic compounds, nutrients such as nitrogen and phosphorus are mineralized into their inorganic forms and released into the local environment for plant uptake. However, some of these nutrients are accumulated into microbial biomass and immobilized, only to be re-released with successive microbial decomposition (Qualls and Richardson, 2000).

Research shows wetland microbial respiration rates positively influence nutrient availability (McKinley and Vestal, 1992; Amador and Jones, 1993; Aerts and Toet, 1997), pH (Bergman et al., 1998), temperature (Westermann and Ahring, 1987 Bridgham and Richardson, 1992; Prieme, 1994) and a variety of other soil conditions necessary for

ecosystem development (Burford and Bremner, 1975; Yavitt and Lang, 1990; Jorgensen and Richter, 1992; Crozier et al., 1995).

Microbial activity levels differ by several orders of magnitude on a site-by-site basis depending on hydroperiod and soil content (Zink and Allen, 1998). Research shows microbial activity can be measured *in situ* by quantifying rates of aerobic respiration, denitrification, sulfate reduction, and methanogenesis (D'Angelo and Reddy, 1998; Garbeva et al., 2004).

Soil Respiration

Fixation of carbon dioxide (CO_2) through photosynthesis is the primary source of carbon to a wetland system and natural freshwater wetlands often serve as net carbon sinks through the accumulation of atmospheric carbon dioxide (Armentano and Menges, 1986; Schlesinger and Andrews, 1999). However, decomposition of plant material through leaching, fermentation, and respiration somewhat balances carbon dioxide uptake by converting carbon stored as plant biomass to plant litter, detritus, microbial biomass, and dissolved organic carbon (DOC) (Minderman and Vulto, 1973; Dickinson, 1974). These forms of detritus-based carbon can either be highly refractory and incorporated into the soil matrix through bioturbation and burial into existing sediments, or highly labile and converted through further microbial decomposition and mineralization to inorganic carbon dioxide and methane via aerobic and anaerobic respiration and methanogenesis (Anderson, 1973; Coleman, 1973; Cheng et al., 1993). This carbon dioxide and methane is often then lost from the system through diffusion into the atmosphere (Curry, 1969) (Figure 1.1).

Along with hydrology, soil carbon availability largely controls oxygen supply in wetland soils, either directly by fueling aerobic heterotrophic respiration, or indirectly by supporting the anaerobic production of reductants, such as Fe(II), that subsequently react with O_2, both of which act to reduce soil oxygen concentrations (Alexander, 1961; Kelting et al., 1998). In fact, research shows even small amounts of organic carbon can

produce anaerobic conditions in soils that are either partially or completely saturated (De Jong and Schappert, 1972; Dickinson, 1974). Organic carbon is most abundant at the surface of soils and sediments where detritus is deposited, most of which is derived from aerobic photosynthesis (Dickinson, 1974). The availability of oxygen determined by both soil water and carbon content then influences additional rates of soil organic matter decomposition by dictating whether decomposition occurs through aerobic or anaerobic metabolic pathways (Elkan and Moore, 1960; Cheng et al., 1993).

Aerobic Respiration: In saturated wetland environments molecular oxygen diffuses significantly slower than in well drained upland soils; characteristically O_2 is therefore used at a much higher rate in biological and chemical processes than it can be replaced by atmospheric transport (Elkan and Moore, 1960; Froment, 1972; Hendrickson and Robinson, 1984; Bowden et al., 1993). Oxygen is therefore the limiting reagent in most biological and chemical pathways creating anaerobic soil conditions (Megonigal et al., 2004). However, due to higher rates of air-water gas exchange, oxygen is often present at shallow soil depths creating microenvironments at the soil surface suitable for low levels of oxygen-consuming processes (Ponnamperuma 1972; Gambrell and Patrick, 1978). Oxygen availability decreases rapidly below the oxidized upper layer of the soil profile and is often completely depleted within a few millimeters. Moreover, pulsing wetland hydroperiods further complicate soil aerobic/anerobic conditions by creating fluctuating anoxic site conditions (Atkinson et al., 1993; Daniels et al., 2005). The importance of oxygen throughout the soil profile is due to oxygen's preferential use as the terminal electron acceptor in many biogeochemical redox processes such as aerobic respiration.

Microbial mediated aerobic respiration is often difficult to quantify given the intricacies of separating it from total soil respiration (Minderman and Vulto, 1973; Silvola et al., 1996; Thierron and Laudelout, 1996). Total soil respiration in wetlands is a measure of the combined rates of microbial mediated respiration of soil organic matter, and the metabolic respiration of plants which is occurring in vegetative root structures. Of this

total, aerobic microbial respiration accounts for 10-20% (Trumbore, 2000; Miller et al., 2001).

Aerobic microbial respiration is itself, also directly influenced by vegetation. Wetland plants increase rates of aerobic respiration by serving as conduits of O_2 infusion deep into the soil profile through both root exudation, and diffusion down root channels (Minderman, and Vulto, 1973; Kelting et al., 1998). In the absence of these processes, the depth of O_2 penetration into saturated soils and sediments is only a few millimeters (Hendrickson and Robinson, 1984).

When considering aerobic respiration, it is the lability of soil organic matter (SOM), site hydrology, and temperature that most strongly influence rates of carbon dioxide release (Wickland et al., 2001; Chimner, 2004). In wetland environments, aerobic conditions and an ample, though variable input of freshwater facilitate the highest microbial mediated decomposition rates of SOM, while continuous anaerobic conditions are the least favorable (Brinson et al., 1981). During periods where the water table is low, aerobic conditions allow greater oxygen diffusion into unsaturated soils, thereby stimulating microbial activity, increasing decomposition of SOM, and facilitating high rates of aerobic respiration (Bubier et al., 1998; Chimner, 2004). However, during periods of saturation or inundation, anaerobiosis sets in and decomposition due to microbial respiration decreases significantly.

Microbial respiration varies seasonally, due to both temperature and the amount of detritus entering the soil matrix. Optimal temperatures for microbial respiration range from between 25° C and 30° C (Craft, 2001). Therefore, high rates or microbial respiration are often observed during the late summer and early autumn when temperatures remain high and litterfall increases (Howes et al., 1985; Wickland et al., 2001). Research also shows high soil organic matter content stimulates microbial respiration and increases carbon mineralization rates (Gloser and Tesarova, 1978).

The majority of organic matter deposited in wetland habitats is composed of a complex mixture of biopolymers. These compounds consist partly of highly labile SOM such as easily degradable proteins, carbohydrates, and lipids (Cheng et al., 1993; Collins and Kuehl, 2001). However, organic matter biopolymers also contain structural components of plants consisting of highly recalcitrant lignans and cellulose with high C:N ratios (Collins and Kuehl, 2001; Megonigal et al., 2004). Organic matter biopolymers are degraded in a multistep process. Microorganisms first degrade polymers to monomers such as amino acids, fatty acids, and monosaccharides. These monomers are then further mineralized to carbon dioxide through aerobic respiration, or to a combination of carbon dioxide and methane through anaerobic processes (Collins and Kuehl, 2001).

Anaerobic Respiration: Molecular oxygen diffuses approximately 104 times slower through water than air, meaning that oxygen (O_2) demand for aerobic respiration greatly exceeds supply in saturated wetland environments (Mitsch and Gosselink, 2000). As oxygen becomes depleted in anaerobic environments, heterotrophic microbes are forced to use other terminal electron acceptors. These acceptors are selected based on hierarchical redox potentials (Table 1.2) (Mitsch and Gosselink, 2000; Wang and Patrick, 2000; Megonigal et al., 2004).

Megonigal et al. (2004) showed that variations in the presence and abundance of these electron acceptors explains both large and small scale patterns of anaerobic metabolism in wetland environments. For example, iron reduction dominates anaerobic ecosystems featuring mineral dominated soils or sediments while methanogenesis plays an important role in freshwater wetlands that exist on predominantly organic soils (Elkan and Moore, 1960; Coleman, 1976; Mitsch and Gosselink, 2000).

Patterns of anaerobic metabolism are also explained by depth of location within the soil matrix. The aerobic, upper layers of soil are the source of most terminal electron acceptors, some of which, such as oxygen and sulfate (SO_4), diffuse into the anaerobic zone, while others are regenerated at the aerobic–anaerobic interface due to oxidation of

compounds such as ammonium (NH_4), iron (Fe(II)), manganese (Mn(II)), and hydrogen sulfide (H_2S) (Mitsch and Gosselink, 2000, Megonigal et al., 2004) (Figure 1.2). Regeneration at the aerobic–anaerobic interface can supply a large fraction of the terminal electron acceptors consumed in anaerobic metabolism.

While the aerobic zone is often found only close to the soil-atmosphere interface, bioturbation often transports oxygen deeper into the soil horizon where it is either consumed in aerobic respiration, or serves to re-oxidize compounds utilized in anaerobic metabolism such as iron (Fe(III)) and manganese (Mn(IV)) (Mayer et al., 1995; Vepraskas and Faulkner, 2001). Wetland plants also promote regeneration by serving as conduits of oxygen infusion deep into the soil profile both directly through root exudation, and passively through diffusion down root channels. (Vepraskas and Faulkner, 2001) In the absence of physical mixing, burrowing, or vegetative oxygen transport, the depth of oxygen penetration into saturated soils and sediments is a few millimeters. The influence of plants and animals on anaerobic metabolism is great in that both effectively increase the aerobic–anaerobic surface area (Mayer et al., 1995; Vepraskas and Faulkner, 2001).

Unlike aerobic conditions, where the conversion of monomers to mineralized products is rather simple because O_2 respiring bacteria can degrade monomers completely to carbon dioxide, under anaerobic conditions this process requires a consortium of bacteria that degrade monomers in a series of steps (Mitsch and Gosselink, 2000, Megonigal et al., 2004). The first step is primary fermentation to low molecular weight products such as alcohols and volatile fatty acids. Next, primary fermentation products are either mineralized to carbon dioxide and methane, or they undergo secondary fermentation to smaller volatile fatty acids (Mitsch and Gosselink, 2000, Megonigal et al., 2004). Finally, the secondary fermentation products are mineralized by respiratory organisms using inorganic terminal electron acceptors, a process that yields carbon dioxide, or a combination of carbon dioxide and methane (Vepraskas and Faulkner, 2001; Megonigal et al., 2004).

Methanogenesis

Wetlands also play an important role in the global carbon cycle and are responsible for the majority of methane emissions from natural sources (Bartlett and Harris 1993). Research suggests wetland methane emissions account for between 100 million tons (Tg) and 231 Tg of methane annually, which accounts for 15% to 45% of all global methane emissions (Prather et al., 1995; Cao et al., 1996).

This staggering contribution is primarily due to the ability of wetlands to provide habitat for methane-producing, methanogenic archaea. The variable hydrologic nature of most wetland soils creates a range of conditions conducive to microbial production of methane (Chapman et al., 1996). Microbial communities produce methane through the decomposition of organic matter in systems replete of oxygen that contain an abundant source of easily degradable organic matter (Dise et al., 1993).

Methane fluxes in wetland soils result from the interaction of several biological and physical processes which influence production and consumption (Hogan, 1993). Methane production is predominantly controlled by the absence of oxygen and the presence of easily degradable organic carbon (Dolfing, 1988; Fukuzaki and Nagai, 1990). Conversely, methane consumption is controlled by methane concentrations in the soil matrix, and oxygen availability (Jakobsen, 1983; Gerard and Chanton, 1993).

Methane fluxes are also strongly influenced by gas transport to and from wetland soils (Glenn et al., 1993). Gas transport is affected by soil aeration and porosity, and vegetative mediated transport (Kelley et al., 1995). In the first case gas transport is controlled by soil water, however in the second case it is sometimes influenced by climate and weather, temperature, and rates of vegetative evapotranspiration (Kelly and Chynoweth, 1980).

Methanogens and Methane Oxidizers: Methanogens are microorganisms that produce methane as a metabolic byproduct in anoxic conditions (Svensson and Sundh, 1992; Kotsyurbenko et al., 1993). They are classified as archaea, and thrive in environments where other electron acceptors such as oxygen, nitrate, sulfate, and iron have been depleted (Sadowsky and Schortemeyer, 1997). Research suggests that most biogenic methane result from either carbon dioxide reduction, or acetate (CH_3COO^-) fermentation (Zehnder and Stumm, 1988).

Aerobic and anaerobic methane oxidizers are closely related to methanogens (Megonigal, 1996). Aerobic methane oxidizers utilize methane as a substrate in the presence of oxygen while anaerobic methane oxidizers take advantage of high methane concentrations to reduce compounds such as sulfate and nitrate (Oremland, 1988).

Methane Production in Wetland Soils: Methane production in wetland soils occurs when organic matter is degraded anaerobically. Methanogenesis requires a fully saturated environment that excludes atmospheric O_2, a labile organic carbon substrate, and the absence of other free energy electron acceptors such as NO_3^- and SO_4^{2} (Oremland, 1988).

Several microbial communities that degrade organic material are needed to begin methanogenesis. Fermentive bacteria start this process by reducing the complex organic structures of carbohydrates, proteins, and lipids present in the soil matrix to simpler molecules such as acetate (CH_3COOH), fatty acids, carbon dioxide and hydrogen (H_2) gas (Oremland, 1988). In the next phase of the methane production pathway, acetogenic bacteria use fatty acid products to produce acetate, with carbon dioxide and H_2 by-products (Oremland, 1988). Finally, the products of these reactions, i.e. acetate, carbon dioxide, and hydrogen, support methanogens in their production of methane gas.

While research suggests other substrates can be responsible for up to 5% of total methane production, in freshwater wetland systems this production primarily occurs through one

of two pathways (Dolfing, 1988; Wang et al., 1996). Either acetotrophic archaea use acetate as a substrate in the production of methane via acetate fermentation, or separate methanogenic microbial communities use available hydrogen gas to reduce carbon dioxide via the carbon dioxide reduction pathway (Oremland, 1988). Reaction pathways, and the relative proportions of reaction products, are controlled by the structure of the initial organic substrate, as well as environmental factors such as pH, P_{H2} (the partial pressure of hydrogen gas in the atmosphere) and P_{CO2} (Zehnder and Stumm, 1988). Each of these pathways yields methane gas with distinctive carbon, and hydrogen, stable isotope signatures (Dacey et al., 1994). While data suggest that methane produced in wetland soils often occurs via a simultaneous combination of these two pathways, a clear understanding of how acetate fermentation and CO_2 reduction are balanced is noticeably absent in the primary literature.

However, research does suggest that there is often a strong correlation between organic matter quality and methane production, independent of pathway (Denier van der Gon and Neue, 1995). For example: 1) data show linkages between recent aerobic carbon dioxide production and anaerobic methane production in dried and fresh, undisturbed wetland soil cores; 2) data show positive correlations between new methane production and labile organic matter content, and 3) data indicate positive correlations exist between rates of methanogenesis and soil carbohydrate content (Denier van der Gon and Neue, 1995). These studies suggest soil organic composition is a major determinant of wetland methane production (Denier van der Gon and Neue, 1995).

Methane Oxidation: Methane oxidation is the complimentary process to methane production and is essential for understanding wetland methane emissions because it mitigates methane release from soils (Saarino et al., 1988; Roslev and Henriksen, 1997). Methane oxidation is carried out by methanotrophs and can either be an aerobic or anaerobic process. Anaerobic methane oxidation obviously uses O_2 as an electron acceptor, while in saturated wetland soils the principal electron acceptor in anaerobic methane oxidation is SO_4^{2-} (Matthews and Fung, 1987).

In wetland soils, aerobic oxidation primarily occurs at the soil-water or soil-atmosphere interface, in areas of high soil porosity which allows for the downward diffusion of atmospheric gases, or along root channels where vegetative transport of O_2 into the soil matrix occurs (Holzapfel Pschorn and Seiler, 1986; Fechner and Hemond, 1992; Gerard and Chanton, 1993). Conversely, anaerobic methane oxidation utilizing sulphate can occur at any point in the soil matrix where sulphate and methane are both abundant.

Research suggests that based on variable, *in situ* conditions, between 60% and 90% of methane produced in wetlands is oxidized within the soil (Epp and Chanton, 1993). Of this quantity, the majority of methane is oxidized either in the oxic top layer at the soil-water interface or in the oxic rhizosphere (Calhoun and King, 1997). Research also suggests that the highest rates of methane oxidation are observed in wetland soils where methanogenesis has been occurring for an extended period of time, and where methane concentrations are higher in the soil matrix than in the atmosphere (Calhoun and King, 1997).

Limiting Factors in the Production of Methane: Methane production in wetland soils is limited by microbial biomass, organic substrate composition, and environmental conditions (Roslev and King, 1994; Roslev and King, 1995). Limitation by biomass typically occurs due to restraints in methanogenic microbes, and not fermenting communities. This is because methanogenic archaea feature growth rates lower than those of fermenters, and because substrates for fermentation rarely, if ever, exceed microbial processing capabilities (Pavlosthatis and Giraldo-Gomez, 1991; Schimel et al., 1993). However, substrates for methanogens have frequently been observed in excess of community uptake (Shannon and White, 1996).

Damage to methanogenic populations often results from aerobiosis; either directly by poisoning, or indirectly by carbon starvation due to competition for substrates with other aerobic microorganisms (Pavlosthatis and Giraldo-Gomez, 1991; Magnusson, 1993;

Roslev and King, 1995). In wetland soils, methanogenic archaea often require significant recovery time following periods of aerobiosis, even after the onset of anaerobic conditions. This slow response is primarily due to low individual and community growth rates (Zehnder and Stumm, 1988). In fact, research has specifically found a correlation between post-dry year periods in freshwater, forested wetlands and reduced methane emissions (Amaral and Knowles, 1994).

During prolonged periods of anaerobiosis, methanogenic microbial biomass ceases to be the rate limiting factor and organic substrate content becomes the major obstacle to methane production (Bartlett and Harris, 1993; Denier van der Gon and Neue, 1995). In fact, research shows that in anaerobic conditions, the addition of direct methanogenic substrates, such as hydrogen and acetate, and indirect substrates, such glucose and photosynthates, enhance methane production (Ball, 1997). Moreover, additional research found a positive correlation between methane emission and organic matter input in wetland soils (Williams and Crawford, 1984; Bender and Conrad, 1994).

Methane production is also limited by environmental conditions such as the presence of alternative electron acceptors (Wolin et al., 1969; Bender and Conrad, 1994). Alternative electron acceptors affect methanogenesis either through the creation of toxic conditions resulting from enzyme and cell poisoning at elevated redox potentials, or via direct inhibition when alternate microbial groups out compete methanogens for available electron donors (Cappenberg, 1975; Bridgham Richardson, 1992; Lovley et al., 1996; Calhoun and King, 1997). This occurs due to differences in thermodynamic energy yields and reaction kinetics; alternative electron acceptors such as NO_3, Fe_3^+, Mn_4^+, SO_2^- ^{4}, and possibly humic acids suppress methane production because reduction of alternative electron acceptors supplies more energy than methanogenesis (Williams and Crawford, 1984; Conrad, 1989). Two distinct mechanisms could be responsible for this effect: 1) reduction of electron acceptors could reduce substrate concentrations to a value too low for methanogenesis; or 2) the presence of electron acceptors could result in a redox potential which is too high for methanogenesis (Drew and Lynch, 1980).

Wetland Methane Emissions: Methane emissions from wetland soils result from the balance of methane produced in saturated, anaerobic zones, and the methane lost through oxidization in aerobic regions of the soil matrix (Aerts and Caluwe, 1999). In wetland soils, transfer of methane from soils to the atmosphere occurs mostly through the aerenchyma of wetland aquatic plants; however some methane is also lost through the upward diffusion of escaping methane bubbles (Wang et al., 1996).

Prolonged submersion and the addition of organic matter are two additional factors responsible for increasing methane emissions from wetland soils (Valentine et al., 1994; Waddington et al., 1996). Conversely, both intermittent drainage and the use of nitrogen fertilizers reduce methane emissions (Waddington et al., 1996). Research has also shown a strong correlation between carbon dioxide fixation and methane emissions in forested wetlands, though this could be an indirect consequence of observed increases in vegetational transport capacity which result from increases in biomass (Rusch and Rennenberg, 1998).

Methane Emissions from Created and Natural Wetland Systems: Little research has been done to examine how created wetlands differ from natural wetlands in total methane emissions. This is an especially potent area of research given that current federal restoration initiatives include projects that achieve a net increase of 100,000 acres of wetlands each year, and establish two million miles of riparian conservation buffers annually (EPA, 2000). Given the documented transition of natural wetlands to mitigated wetlands on the landscape level, and the fact that natural wetlands are one of the largest contributors to the atmospheric methane budget, a study of methane production in created and natural wetlands is extremely relevant. Methane is an important greenhouse gas with a global warming potential 21 times greater than carbon dioxide. If natural systems are being replaced with created wetlands at an ever increasing rate, and created wetlands differ in their production or retention of methane in the face of increasing atmospheric

carbon dioxide concentrations, then this conversion has the potential to influence global climate change on a large scale in the near future.

Nitrogen Cycling

Nitrogen is a limiting nutrient in most wetland environments and strongly influences plant primary productivity and biomass accumulation, particularly in freshwater wetland ecosystems (Valiela, 1983l; Megonigal et al., 2004). Nitrogen limitations exist because eukaryotes are unable to assimilate the N_2 nitrogen that exists in the atmosphere. In terrestrial environments, nitrogen can only be utilized by plants as ammonium (NH_4^+), nitrate (NO_3^-), or organic-N (Vitousek and Howarth, 1991). These are the most labile, biologically available forms of nitrogen since they have single or double covalent bonds that require far less energy to break than the triple bonds of N_2 (Megonigal et al., 2004). Sources of labile nitrogen include biological nitrogen fixation, precipitation, streamflow, and groundwater (Figure 1.3) (Valiela, 1983, Vitousek et al., 1997). Wetlands however present an even more unique case of nitrogen limitation as low oxygen levels strongly limit the availability of nitrate making ammonium the most commonly utilized nutrient. This condition results from the rapid transformation of labile nitrogen to N_2 through denitrification, one of the most prevalent forms of anaerobic microbial metabolism in soils (Vitousek and Howarth, 1991; Megonigal et al., 2004).

Denitrification occurs in anaerobic environments because physiologically versatile nitrogen oxides can replace O_2 as terminal electron acceptors with only a small loss of energy yield (Payne, 1981; Tiedje, 1988; Megonigal et all., 1993). Anaerobic metabolism, like aerobic metabolism, is therefore influenced by O_2 availability; most organisms perform best under aerobic conditions, and only resort to anaerobic metabolism as a result of localized oxygen depletions (Firestone, 1982; Seitzinger, 1988). As a result, the thin, oxidized layers of soil at the soil-water interface play a crucial role in the freshwater wetland nitrogen cycle. Over time, concentration gradients form across this interface and encourage diffusion of nitrogen upward or downward. When this form of diffusive transport moves soluble nitrogen to aerobic environments, nitrification

occurs and increases availability of biological labile nitrogen (Gambrell and Patrick 1978, Williams et al., 1992; Mitsch and Gosselink 2000). However, diffusive transport to anaerobic environments ultimately leads to denitrification, or the reduction of nitrate (NO_3^-), to nitrite (NO_2), and then to nitrogen gas (N_2 and N_20) (Drury et al., 1992). This process counterbalances nitrification and acts to reduce the availability of reactive nitrogen in freshwater wetlands (Brinson et al., 1984; Ambus and Lowrance, 1991; Groffman, 1994; Poe et al., 2003; Megonigal et al., 2004).

Nitrogen Fixation: Nitrogen fixation is the process through which atmospheric nitrogen gas (N_2) is converted to ammonia (NH_3), nitrate (NO_3^-), and nitrogen oxides, and is always a microbial mediated process (Bebout et al., 1987; Galloway et al., 1995; Currin et al., 1997; Cronk and Fennessy, 2001). In wetlands, nitrogen fixation occurs continuously regardless of water level and oxygen availability, achieving peak rates during period of highest temperatures and light (Bebout et al., 1987; Cronk and Fennessy, 2001). In many cases, soil microbes develop a symbiotic relationship with plants, and inhabit root nodules where they derive energy from the plant, while providing additional nitrogen for plant functions. Ammonia (NH_3) is the most common product of these processes; however in the soil matrix, ammonia is quickly converted to ammonium (NH_4^+) (Cronk and Fennessy, 2001).

The majority of biological N_2 fixation occurs anaerobically since the nitrogenase enzyme is strongly inhibited by O_2 (Megonigal et al., 2004). As a result, many aerobic N_2 fixing microorganisms such as diazotrophs have specialized structures such as heterocysts, which keep localized sites of N_2 fixation free of oxygen (Megonigal et al., 2004). In the absence of such structures, N_2 fixation varies with O_2 concentrations, often peaking at night when oxygenic photosynthesis is absent (Bebout et al., 1987; Currin et al., 1996).

Nitrogen Mineralization: In wetlands, ammonium (NH_4^+) is primarily produced through either: 1) ammonification, which is the mineralization of organic nitrogen; or 2) dissimilatory nitrate reduction to ammonia (DNRA). Ammonium is then often further

mineralized to nitrate (NO_3) (Stevens et al., 1997; Megonigal et al., 2004). Nitrogen mineralization is the combination of these three proceses.

Ammonification is a microbially-mediated process where particulate organic nitrogen (PON) is mineralized to NH_4^+ (Robertson and Kuenen, 1991; Stevens et al., 1997). Because mineralization is dependent on labile organic matter, NH_4^+ concentrations are highest in the upper levels of the soil where labile PON is highest (Kemp et al., 1990; Tobias et al., 2001). These concentrations then decrease with increasing depth in the soil profile. Ammonification peaks in the fall when decomposing litter fall increases PON concentrations (Anderson et al., 1997). Ammonification is an important process in wetlands because plant uptake only occurs during the growing season, ammonium however is produced throughout the year, immobilized in microbial biomass, and then released as highly labile PON when ammonium is required for uptake (Anderson et al., 1997; Stevens et al., 1997).

Due to a high demand for nitrogen by all organisms, the NO_3 pool in upland ecosystems is relatively small (Vitousek and Howarth, 1991). In wetland and aquatic ecosystems, NO_3 availability may be limited further by anaerobic conditions and low nitrification rates (Megonigal et al., 2004). Microbial metabolism and anthropogenic activity are the primary sources of NO_3 in wetland ecosystems. Nitrate is a waste product of chemoautotrophic nitrification, a series of two dissimilatory oxidation reactions, each performed by a distinct group of bacteria (Tiedje, 1988). In the first step, NH_4 is oxidized to NO_2 by *Nitrosomonas europaea* and other "Nitroso-" genera including *Nitrosococcus* and *Nitrosospira* (Megonigal et al., 2004). In the second step, NO_2 is oxidized to NO_3 by nitrite-oxidizing bacteria belonging to "Nitro-" genera such as *Nitrobacter* and *Nitrospira* (Megonigal et al., 2004). The two genera constitute the *Nitrobacteriaceae* (Megonigal et al., 2004).

Nitrate reduction studies have focused overwhelmingly on denitrification at the expense of other NO_3 sinks such as dissimulatory NO_3 reduction to NH_4 (Currin et al., 1996)

Dissimilatory nitrate reduction to ammonia (DNRA) is the microbially mediated process in which nitrate (NO_3^-) is reduced to ammonium (NH_4^+) (Megonigal et al., 2004). DNRA rates are influenced by: microbially activities, the amount of nitrate entering the system, and the ability of dissolved organic carbon (DOC) in the soil substrate (Tobias et al., 2001). In wetlands with low nitrate inputs, this process is not as important as ammonification (Megonigal et al., 2004). The ecological implications of reducing NO_3 to NH_4, instead of N_2 are significant because NH_4 is readily retained in the ecosystem through plant assimilation, while N_2 is often lost to the atmosphere (Megonigal et al., 2004). Thus, DNRA contributes to eutrophication by reducing the quantity of fixed nitrogen that is returned to the atmosphere as N_2 (Megonigal et al., 2004).

Denitrification: In wetlands, nitrogen loss is primarily due to denitrification where nitrogen gas (N_2) produced through microbial activity, diffuses into the atmosphere (Payne, 1981; Tiedje et al., 1982; Drury et al., 1982). In the denitrification process, organic carbon acts as an electron donor, and nitrate (NO_3^-) is the terminal electron acceptor reduced by anaerobic bacteria to nitrite (NO_2^-), then to nitrous oxide (N_2O) and finally to nitrogen gas (Ye et al., 1994). Denitrification is the most common form of anaerobic respiration based on nitrogen and respiratory denitrification is more energetically favorable than Fe(III) reduction, SO_4 reduction or methanogenesis, and tends to be the dominant form of anaerobic carbon metabolism when NO_3 or NO_2 are available (Megonigal et al., 1993; Tiedje et al., 1994).

The nitrous oxide (N_2O) and nitric oxide (NO) produced through denitrification are very potent gases (Drury et al., 1992). Nitrous oxide in particular is a greenhouse gas that is 300 times more effective at radiative forcing than carbon dioxide on a molar basis. (Megonigal et al., 2004) Due to this effective strength, studies estimate that N_2O has accounted for approximately 6% of the radiative forcing since 1750 (Williams et al., 1992; Ramaswamy et al., 2001). Additionally, N_2O promotes O_3 destruction in the stratosphere (Ramaswamy et al., 2001).

Denitrifying bacteria are aerobes that substitute NO_3 or NO_2 for O_2 as a terminal electron acceptor in the absence of oxygen (Payne, 1981; Firestone, 1982). Denitrification is therefore dependent on the availability of O_2, as well as the presence of organic carbon and nitrate produced through ammonia and nitrite oxidation (Cronk and Fennessy, 2001). As a result, denitrification is regulated by factors such as nitrification rate as well as factors like soil water content which influence the transfer of substrates to sites where denitrification occurs.

Given ample oxygen sources, aerobic respiration is still favored over denitrification metabolically because it yields more free energy than NO_3 respiration (Megonigal et al., 2004). The presence of O_2 therefore inhibits many denitrification enzymes, and while some organisms can denitrify at O_2 concentrations up to 80% of air saturation, more rapid denitrification rates are observed in strictly anaerobic environments (Robertson and Kuenen, 1991). The presence of water is therefore essentially a prerequisite for denitrification because it slows O_2 diffusion by a factor of 104 when compared to air (Robertson and Kuenen, 1991). In saturated wetland soils, water reduces the O_2 supply by blocking a fraction of soil pores, either partially or completely blocking air-filled pathways to the atmosphere (Megonigal et al., 1993). As a result of this dynamic, denitrification rates in soils are often positively related to water content (Drury et al., 1992; Groffman and Tiedje, 1991).

Soil texture is another good indicator of denitrification rates scale because it relates water content and soil porosity with respect to gas and solute diffusion (Groffman, 1991). For example, at a given soil water content, the small pores found in clay soils are more likely to be completely blocked than the relatively large pores found in loam and sand soils (Kemp et al., 1990). Soil texture also influences temporal variability in soil water content because for the most part it establishes the water infiltration rate and water holding capacity of a given soil (de Klein and van Logtestijn, 1996; Sexstone et al., 1985). When examining rates of denitrification, a useful expression for soil water content is percent waterfilled porosity, or the ratio of volumetric water content to total soil porosity

(Williams et al., 1992). Based on this metric, research suggests that denitrification increases dramatically above 65% water-filled porosity on average, with higher values for sandy soils (74– 83%) than clayey soils (50–74%) (Barton et al., 1999).

Vegetational Dynamics

Introduction

Wetland creation attempts are often initiated by the removal of upland surface soil materials exposing mineral subsoil strata (Atkinson et al., 1993). The extent to which the revegetation sequence may be viewed as primary succession is dependent upon the amount of soil removal and the viability of the pre-existing seed bank (Mitsch and Gosselink, 2000). The presence of wetland species in the seed bank following construction will often be minimal or lacking based on the antecedent upland condition of the site (Kusler and Kentula, 1989). Species present during the first few years of vegetation establishment that were not planted or seeded are assumed to be volunteers from offsite sources (Reinhartz and Warne, 1993), and therefore represent the primary seral stage of vegetation succession. For the purposes of this review, we define ''created wetland'' in the context of wetland regulation in the United States as the ''establishment of a wetland . . . where one did not formerly exist'' (Federal Register Vol. 60, No. 228, p. 58613).

Soil Conditions and Vegetational Communities

Soil conditions can lead to poor growth and survival of planted and colonizing vegetation. Research shows that the low levels of soil organic matter often found at created wetland sites contributes significantly to the poor survival rates of planted trees (Stolt et al., 2000; Bruland and Richardson, 2004; Bergshneider, 2005; Daniels et al., 2005; Bailey et al., 2007). Reinhartz and Warne (1993) found that as soil organic matter increased plant species diversity increased.

Vegetative Influences on Soil Development: Soil properties are not constant and are strongly influenced by plant composition, species diversity, and successional

development of the vegetative community (Marks and Bormann, 1972; Hooper and Vitousek, 1997). Through the accumulation of detritus and organic matter, vegetation provides a feedback mechanism for the development of substrates in both natural and mitigation wetland environments, and helps initiate nutrient cycling (Vepraskas, 1994; Stauffer and Brooks, 1997; Megonigal et al., 2004).

Research shows energy flow in non-tidal, freshwater wetland systems is detritus-based, with younger sites exhibiting low structural complexity (Odum, 1969; Day, 1984; Odum, 1985; Mitsch and Gosselink, 2000). Biogeochemical cycles become increasingly complex in structure as sites age, above-ground plant material decays, and organic material is incorporated into the soil profile. Incorporation of organics into the soil begins a feedback loop by altering hydrological characteristics, redox states, nutrient cycling, and microbial communities (D'Angelo et al., 2005). Increasingly complex nutrient cycles then allow for the development of increasingly complex above- and below-ground vegetated ecosystems, which continue to act in the feedback cycle. This connective loop can be described in a general sense by organic carbon inputs from the plant community moderating the soil biogeochemical setting, and the resultant biogeochemical setting in turn, moderating the distribution and abundance of plant species over the successional stages of vegetation community development.

On a more complex level, as above-ground ecosystems age and become more complex, detritus becomes more protein and nutrient rich, which further encourages growth of below-ground root structures and the development of soil microbiota, both of which influence soil redox potentials by transporting atmospheric gases into the soil profile and increasing the availability of growth-limiting nutrients such as N and P (Armstrong and Boatman, 1967; Gambrell and Patrick, 1978; Aerts et al., 1992; Ehrenfeld and Toth, 1997; Craft, 2001). The structure, age, composition, and density of the standing vegetation therefore represent potential influencing factors in the development of wetland soils.

Vegetation Establishment

Research conducted by Noon (1996) found that wetlands are colonized by different types of species at different site ages. He suggested that succession in wetlands can be categorized as: the Arrival and Establishment Phase, from year zero to year three, and the Autogenic Dominance Stage, classified as beyond year three. Noon (1996) concluded that the Arrival and Establishment Phase is largely controlled by which species are randomly introduced, and in what order, while the Autogenic Dominance State is governed by interactions between established species (Noon, 1996). This was confirmed by DeBerry and Perry (2004) for a southeastern Virginia created wetland.

Studies also showed that early successional species such as *Salix nigra* and *Acer negundo* colonize forested wetlands first in wetlands in the mid-Atlantic region of the United States (Spencer et al., 2001; DeBerry and Perry, 2004). Conversely, slow-growing species such as *Quercus alba* and *Carya cordiformus* appear later in the forest successional process (Whittaker, 1978; Spencer et al., 2001).

Additional research supports these findings and suggests that disturbed forested wetlands become revegetated through both primary and secondary successional processes (Campbell et al., 2002; Balcombe et al., 2005; DeBerry and Perry, 2004). Spencer et al. (2001) specifically found that post-disturbance, natural wetlands are colonized via the establishment of nurse species and through coppicing.

In natural wetlands, early colonizing species are typically annual hydrophytes, or facultative annuals, and have the ability to survive despite stressful, high saturation, low-nutrient conditions (van der Valk, 1981; DeBerry and Perry, 2004). Research suggests that because the introduction of species is controlled anthropogenically, wetland mitigation projects should focus on planting these types of early successional and nurse species that can restore community function through natural ecosystem interactions as time progresses (US EPA, 2000; DeBerry, 2006).

Primary successional nurse species are aggressive colonizers that feature high growth rates and include *Acer negundo*, *Nyssca sylvatica*, and *Salix nigra* (DeBerry and Perry, 2004). Primary successional nurse species also allocate the majority of their growth to aerial plant components due to ample solar exposure and subsequent high levels of photosynthesis. The establishment of nurse species creates favorable conditions for later establishment of hardwood species, such as *Quercus spp.* And *Carya spp.*, that appear in later successional stages (Whittaker, 1978; Clewell and Lea, 1990; Sauer, 1998). Research shows nurse species shade competing vegetation, add organic matter to the soil, fix atmospheric nitrogen, and increase vertical structural complexity (Clewell and Lea, 1990; Dunne and Samanns, 1998).

Succession: Succession is defined as the unidirectional, sequential replacement of species within a community over time (Smith, 1990). Succession in ecosystems also involves the directional transfer of energy as the system approaches equilibrium (Odum, 1969). Equilibrium in this case is defined as a balance of energy between biomass accumulation and organismal demands. As ecosystems mature, energy usage shifts from high biomass production to ecosystem maintenance, reflected in a progression from a net autotrophic regime to a net heterotrophic regime. Net autotrophic regimes are defined as a having a P:R ratio greater than one where P represents photosynthesis and R represents respiration. Conversely, net heterotrophic regimes feature a P:R ratio equal to, or less than one (van der Valk, 1981).

While this has often been criticized as an anthropogenic construct (Bazzazz, 1996), species shifts over time are observable, quantifiable characteristics of natural systems. In freshwater wetlands, autogenic succession, where plants influence change in their local environment over time, has therefore historically been proposed as a useful method for describing ecosystem processes (van der Valk, 1981; Neiring, 1987; Noon, 1996; King and Allen, 1996; Mitsch and Gosselink, 2000; Spencer et al., 2001). In this type of theoretical succession, plants are introduced and colonize a site, contribute detritus and

organic matter to the soil substrate, and change the site over time through their influence on biogeochemical cycles.

However, some work suggests wetlands are pulsed systems which, rather than following traditional climax theories, are highly variable successional community dependent on changing site conditions (Odum, 1985; Niering, 1987). This relationship is described as allogenic succession where the hydrology and geomorphic conditions influence plant establishment as much as vegetation influences site conditions. Recent research also suggests that successional processes in wetlands affect, and are affected via feedback loops by the physical, chemical, and biological environment. For example, plant community composition, primary productivity, and energy exchange in the form of carbon dioxide flux influence pedogenesis and chemical and nutrient cycling, which in turn exert further influence on the evolving vegetative community (Bailey et al., 2007). Descriptions of successional process in freshwater wetlands therefore need to consider both autogenic and allogenic processes in vegetation establishment and community development (Niering, 1987; Noon, 1996; Craft, 1997; Campbell et al., 2002).

Nutrient Uptake and Retention

Fixation of carbon dioxide through photosynthesis is the primary source of carbon into a wetland system; as such natural freshwater wetlands often serve as net carbon sinks through the accumulation of atmospheric carbon dioxide (Armentano and Menges, 1986). This ability to accumulate organic and inorganic carbon is strongly influenced by site hydrology; i.e. freshwater input, geomorphic orientation of the drainage system, and meteorological phenomena (de la Cruz, 1978).

In a general sense, inorganic carbon (CO_2) is stored as organic biomass through photosynthesis. Decomposition of plant material through leaching, fermentation, and respiration then converts carbon stored as plant biomass to plant litter, detritus, microbial biomass, and dissolved organic carbon (DOC). This carbon is either highly refractory and incorporated into the soil matrix through bioturbation and burial into existing

sediments, or highly labile and converted through further microbial decomposition and mineralization to inorganic carbon dioxide and methane via aerobic and anaerobic respiration and methanogenesis. This carbon dioxide and methane is often then lost from the system through diffusion into the atmosphere (see previous section on Soil Development and Microbial Processes).

These processes are important in freshwater wetlands because they provide organic detritus for terrestrial and aquatic food webs which is then consumed in secondary production by heterotrophic organisms, further broken down by mechanical or biological processes, and mineralized by bacteria (Figure 2.2.1) (de la Cruz, 1978).

Photosynthesis: On a large scale, gross photosynthetic production is controlled by latitude and modified by topography and season. These combined aspects affect solar radiation, local temperatures, precipitation, and evapotranspiration (Oades, 1988). On a smaller scale, in wetland plants, rates of photosynthesis vary significantly with light abundance, measured as photosynthetically active radiation (PAR); ambient air temperature; and soil moisture.

Research in freshwater systems also suggests that photosynthetic efficiency in most wetland plants is highest at low irradiance levels and then decreases as conditions approach full sunlight without any photoinhibition. Photosynthetic efficiency also reaches a max at optimal temperatures which range between 35° C and 40° C, and then decreases at both lower and higher temperatures (Mann and Wetzel, 1999; Wetzel, 2001).

In a seasonal sense, net photosynthesis increases with the beginning of the growing season, peaks in mid summer, and then declines through the fall (Drake and Read, 1981; Mann and Wetzel, 1999; Miller et al., 2001). Photosynthesis is also strongly affected by soil redox potential (Eh) and rates of carbon dioxide fixation decline with low values of Eh (Pezeshki and DeLaune, 1992). Increased soil aeration and higher local elevations

both raise Eh, and therefore increase rates of carbon dioxide fixation (Pezeshki et al., 1992).

While water stress caused by inundation negatively affects rates of photosynthesis and respiration, soil moisture is required for large photosynthetic carbon dioxide uptake (Schreader et al., 1998; Ryan, 2001). Research also suggests that rates of photosynthesis in wetlands are affected by hydrology and nutrient flow. Wetlands with an open hydrological system, and many freshwater inputs, have a steady supply of oxygen and other nutrients required for high levels of Net Primary Productivity (NPP); conversely, a closed system with fewer inputs has inconsistent oxygen and nutrient supplies leading to lower NPP (Brinson et al., 1981; Craft, 2001).

Vegetative Respiration: Ecosystem respiration in wetlands is a measure of the combined rates of microbially mediated respiration of soil organic matter, and the metabolic respiration of plants. Microbial mediated respiration accounts for 10-20% of total community respiration (Trumbore, 2000; Miller et al., 2001). Ecosystem respiration rates are therefore strongly influenced by the lability of soil organic matter (SOM), site hydrology, and temperature since these factors all influence microbial respiration (Wickland et al., 2001; Chimner, 2004). Community respiration highest in mid-summer, and conversely, significantly lower in both the spring and fall (Drake and Read, 1981; Wickland et al., 2001).

Respiration is significant because it occurs constantly, while photosynthesis only occurs during periods of ample sunlight. This relationship results in losses of up to 34% of the carbon sequestered during the day (Drake and Read, 1981). Plant mediated respiration releases 50% of the carbon sequestered through photosynthesis, while the other 50% is stored as structural components, nutrients, or lost as litter (Ryan, 1991). Rates of plant respiration increase with temperature and tissue nitrogen content (Ryan, 1991; Reich et al., 1998).

carbon dioxide as a by-product of plant respiration is released: 1) directly into the atmosphere from above-ground biomass, 2) into the substrate from root structures and rhizomes, and/or 3) into the atmosphere through plant lacunae (Howes et al., 1985). Respiration by root structures and rhizomes decreases significantly with age as active tissue is replaced with starch, with the exception coming during the spring when older rhizomes feature higher respiration rates as stored energy in the rhizomes becomes utilized by above-ground growth (Bouchard and Lefeuvre, 2000).

Nitrogen Uptake: Human activity has roughly doubled the rate of terrestrial N_2 fixation through the production of fertilizer and the cultivation of legumes (Vitousek et al., 1997). While these activities have enhanced plant productivity in agricultural systems, run-off of excess labile nitrogen in surface and groundwater has also influenced productivity in aquatic systems (Galloway et al., 1995). A great amount of research has been conducted examining the influence of excessive nitrogen on aquatic eutrophication and production in algal communities (Meyer-Reil and Ko¨ster, 2000). However, to our knowledge, little work has been done investigating nitrogen uptake by either herbaceous or woody vegetation in freshwater wetlands. Since nitrate removal is a key function of natural wetlands that many created wetlands attempt to recreate, studies of these processes is especially pertinent.

Plant-Microbial Coupling in the Methane Cycle: Roots affect methane production in wetland systems both positively and negatively (Chanton and Dacey, 1991; Pezeshki, 1991; King, 1994). Vegetational oxygen transport into saturated soils through below-ground structures suppresses methane production by creating localized aerobic conditions (Paterson et al., 1997). However root decay and the exudation of photosynthates promote methane production by providing organic substrates for reduction (Megonigal et al., 1999). Most studies fail to separate these two processes; therefore the term 'rhizodeposition' is used to refer to the sum of both rapid root turnover and photosynthate exudation (Grosse et al., 1992). Additionally, aerenchyma tissue found in wetland plants improves methane ventilation to the atmosphere, which also acts to increase wetland

methane emissions (Dacey et al., 1994). Aerenchyma is a type of tissue found in the roots and stems of many wetland plants which is an evolutionary response to long term inundation (Chanton and Dacey, 1991). Aerenchyma tissue encourages the exchange of gases between roots and shoots through air-filled cavities. These cavities provide low resistance, internal pathways for the passive diffusion of gases such as oxygen and methane between wetlands soils and the ambient atmosphere (Chanton and Dacey, 1991).

Chanton and Dacey (1991) suggested that the relative contributions of root-associated methane production to total methane emissions through the latter two pathways could be significant in wetland soils. Other studies showed contributions of root-associated methane production can vary between 4% and 52% of total methane emissions (Gerard and Chanton, 1993; Mindota and Kimura, 1994; Minoda et al., 1996). Because microorganisms are generally carbon limited, other studies suggest an increase in soil carbon through rhizodeposition often stimulates microbial activity (Denier van der Gon and Neue, 1995). Additional research demonstrated that removing above ground vegetation decreases methane emissions by more than 10%, indicating the significant impact of vegetation on methane ventilation (Grosse et al., 1992). Finally, another study showed that approximately 90% of the methane released to the atmosphere travels through aerenchyma tissue in herbaceous and woody wetland species, again highlighting the importance of root-associated activity on methane emissions (Paterson et al., 1997).

Paterson et al. (1997) also suggested a tight coupling between photosynthesis and methane production in wetland soils; in fact, they found a positive correlation between net ecosystem exchange, gross primary production, and methane emissions exists for wetlands distributed from the subarctic to the subtropics. However, other studies could not determine whether carbon isotopes originating in the leaf were incorporated into methane through acetate fermentation or carbon dioxide reduction (Grosse et al., 1992; Mindota and Kimura, 1994). While increased methane production through acetate fermentation would indicate an energetic link between vegetative carbon fixation and microbes, use of the carbon dioxide reduction pathway does not necessarily indicate a

tight relationship between microbial methane production and above ground vegetation, and observed increases in methane emissions could be due to ventilation through aerenchyma tissue (Chanton and Dacey, 1991; Grosse et al., 1992).

Work by Calhoun and King (1997) demonstrated that changes in water table depth strongly impacted the relationship between photosynthesis and methane production in wetland soils. This relationship was based on species composition due to differences in root depth and distribution within the soil matrix (Calhoun and King, 1997). In species with shallow root systems, in sites that are only periodically inundated, rhizodeposition typically occurs within the aerobic zone at the soil-atmosphere interface which cannot accommodate methanogenesis because of the presence of O_2 (Rusch and Rennenberg, 1998). However, species with deeper root systems, grown in the same environment, release photosynthate exudates in both surface aerobic zones, and deeper zones in the soil where anaerobic conditions are prevalent and methane production can occur (King, 1994; Megonigal and Schlesinger, 1997).

Elevated Carbon Dioxide Concentrations and Methane Emissions: Past research has examined the effects of elevated carbon dioxide on methane emissions in soil cores and mesocosms representing peat lands, salt marshes, rice paddies, and freshwater forested wetlands. These studies found that methane emissions increase substantially as a direct result of elevated carbon dioxide, particularly in temperate and tropical zones (Hogan, 1993; Dacey et al., 1994; Megonigal and Schlesinger, 1997).

Research has also examined couplings between photosynthetic rates and methane emissions in the face of elevated atmospheric carbon dioxide. The relationship between increased rates of photosynthesis and elevated methane emissions remained constant in a high carbon dioxide environment, thus providing additional evidence for a direct link between net ecosystem exchange and methanogenesis in most wetland ecosystems (Megonigal et al., 1999). The proposed mechanisms for this effect are the same mechanisms used to explain the impacts of root-associated activity on methane emissions

at ambient carbon dioxide concentrations: 1) increased rates of root turnover and photosynthate exudation provide additional organic substrate for acetate fermentation by methanogens; and 2) additional aerenchyma tissue found in wetland plants improves methane ventilation from soils to the atmosphere (Megonigal et al., 1999). Research shows the latter pathways is observed because elevated carbon dioxide often increases plant biomass, which should directly increase the quantity of methane ventilated to the atmosphere through plants thereby producing a correlation between elevated carbon dioxide conditions, diffusive methane flux, and biomass (Paterson et al., 1997).

In most cases, a combination of these two processes is observed; in fact, one study reports individual *O. aquaticum* species exhibited a 36% increase in total biomass and a 28% increase in methane emissions in response to elevated carbon dioxide, suggesting higher emissions were due to both increases in substrate through exudation of photosynthates, and improvements in methane ventilation (Paterson al., 1997). However, some studies show elevated atmospheric carbon dioxide concentrations do not always increase plant biomass or aerenchyma tissue, though in the same studies increased methane emissions were still observed (Megonigal et al., 1999). For example, in three of seven wetland studies examining the effects of elevated carbon dioxide, no increase in biomass was reported while methane emissions increased by 62% to 250% (Paterson al., 1997). This suggests that at least in some cases, increased methane emissions are most likely due to increases in rhizodeposition.

In freshwater wetlands, standing vegetation supplies the organic compounds needed through decomposition; a strong connection therefore exists between methane emissions and primary productivity in both natural and mitigated wetlands (Whiting and Chanton, 1993; Megonigal et al., 2004).

Due to observed disparities in the pathway through which increased carbon dioxide concentrations stimulate methane production, further research is needed to determine whether elevated carbon dioxide enhances methane emissions by exciting

methanogenesis, by increasing ventilation through aerenchyma tissue, or by some combination of these two mechanisms. This is an important issue since the first pathway represents an increase in gross methane production, while the second only represents a change in emissions pathway from passive diffusion through the soil-water interface to vegetative transport. Changing the emission pathway, as opposed to altering actual methane production, represents a far less significant change to wetland carbon cycling because it does not necessarily constitute a significant increase in new methane added to the atmosphere.

More research is also needed to help explain additional discrepancies that have been found to exist between the impacts of herbaceous and woody vegetation on methane emissions in high carbon dioxide environments. In fact, research suggests previous work examining herbaceous plant-soil wetland systems, at elevated atmospheric carbon dioxide concentrations, may not be applicable to wetlands dominated by woody vegetation (Moore and Knowles, 1990). This is because while wetland woody vegetation develop root and stem aerenchyma tissues similar to those found in herbaceous species, data suggest gas transport in woody species is significantly less effective than in herbaceous species (Megonigal et al., 1999). Additional research shows that the roots of woody vegetation also have more lignin and suberin than the roots of herbaceous plants, which further decreases methane emissions by directly diminishing photosynthate exudation, and indirectly reducing acetate fermentation (Paterson et al., 1997). This is because carbon allocation to recalcitrant, woody structures reduces the quantity of carbon allocated to relatively labile pools such as fine roots and root exudates (Paterson et al., 1997). Moreover, high concentrations of lignin and suberin limits decomposition of roots and other woody material that also serve to decrease the availability of acetate and other organic substrates used in methanogenesis (Chanton and Dacey, 1991). Given that forested wetlands account for approximately 60% of the total wetland area globally, understanding these differences is crucial to predicting the impacts of future carbon dioxide concentrations on wetland methane emissions to the atmosphere (Matthews and Fung, 1987; Megonigal, 1996).

While many lab-based studies have examined these relationships, more field studies are needed in order to address which specific mechanisms are responsible for increased methane emissions in an elevated carbon dioxide environment, how forested wetlands differ from herbaceous wetlands in their response to these conditions, and how both ecosystems react *in situ*.

Net Ecosystem Exchange of Carbon Dioxide

Net ecosystem exchange (NEE) is often used to measure fluxes of carbon dioxide gas 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). Net ecosystem exchange is measured *in situ* in closed chambers and is used to estimate net primary productivity (Net Primary Productivity (NPP) = Gross Primary Productivity (GPP) – Respiration (R)), compare productivity between two wetlands, and measure factors influencing productivity (Streever et al., 1998).

NEE measurements are also used to in wetlands to determine successional state (Roggero, 2003). Early successional systems are defined by a net autotrophic, high above-ground biomass productivity system where the ratio of photosynthesis (P) to respiration I ratio is greater than one. Conversely, older successional systems are defined as net heterotrophic regimes where P:R is equal to, or less than one (Odum, 1969).

Research often shows soil temperature, solar radiation, trophic status, and water tables are all positively correlated with NEE (Bubier et al., 1998; Frolking et al., 1998; Schreader et al., 1998). Research also shows in most systems NEE measures of carbon flux indicate wetlands serve as a carbon sinks when GPP exceeds R. However this is not always the case; in fact, in some instances, natural wetlands serve as atmospheric carbon sources as oxidation of sediments releases stored carbon (Shreader et al., 1998; Wickland et al., 2001). In these instances, often variations in local climate, which create

exceedingly hot and dry conditions or increase rates of microbial respiration, are the driving factor behind changing a net carbon sink to a net source.

In a general sense, nutrient availability gradients become established across wetland substrates in response to hydrologic regime, geomorphic setting, and other factors such as pH and variable nutrient inputs from exogenous sources (Megonigal and Day, 1988; Aerts et al., 1992; Mausbach and Richardson, 1994; Bridgham et al., 1995; Richardson et al., 2001; Bragazza and Gerdol, 2002).

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Figure Captions

Figure 1.1: Wetland carbon cycle showing the transformations that take place in the production and accumulation of soil organic matter (DeBusk 1996).

Figure 1.2: Aerobic and anaerobic metabolic pathways observed in wetland soils. Aerobic metabolism occurs at the soil surface, indicated at the highest point of the graph. Moving down the graph corresponds with an increase in depth in the soil horizon.

Figure 1.3: Wetland nitrogen cycle showing the transformations that take place in the production and accumulation of soil organic matter (Roggero 2003).

Table Captions

Figures

Figure 1.1

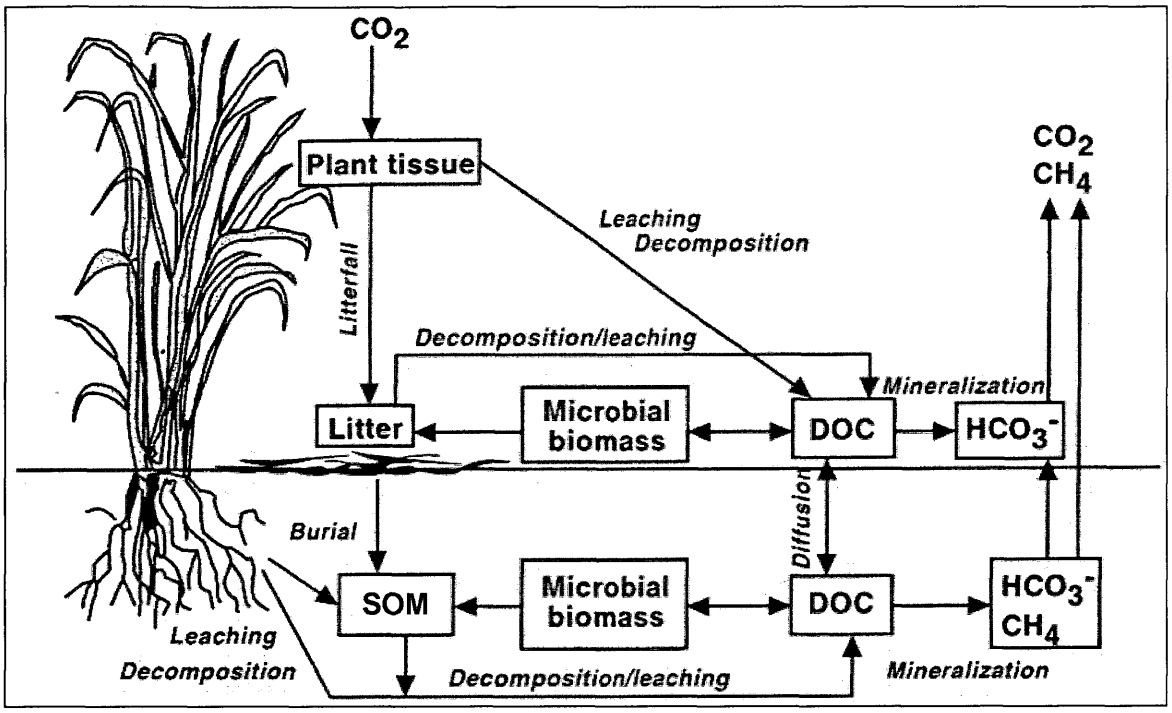


Figure 1.2

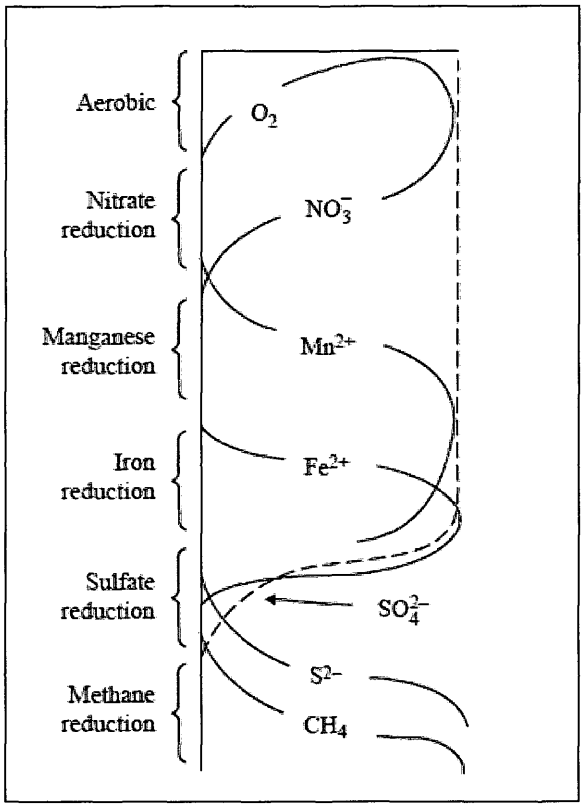
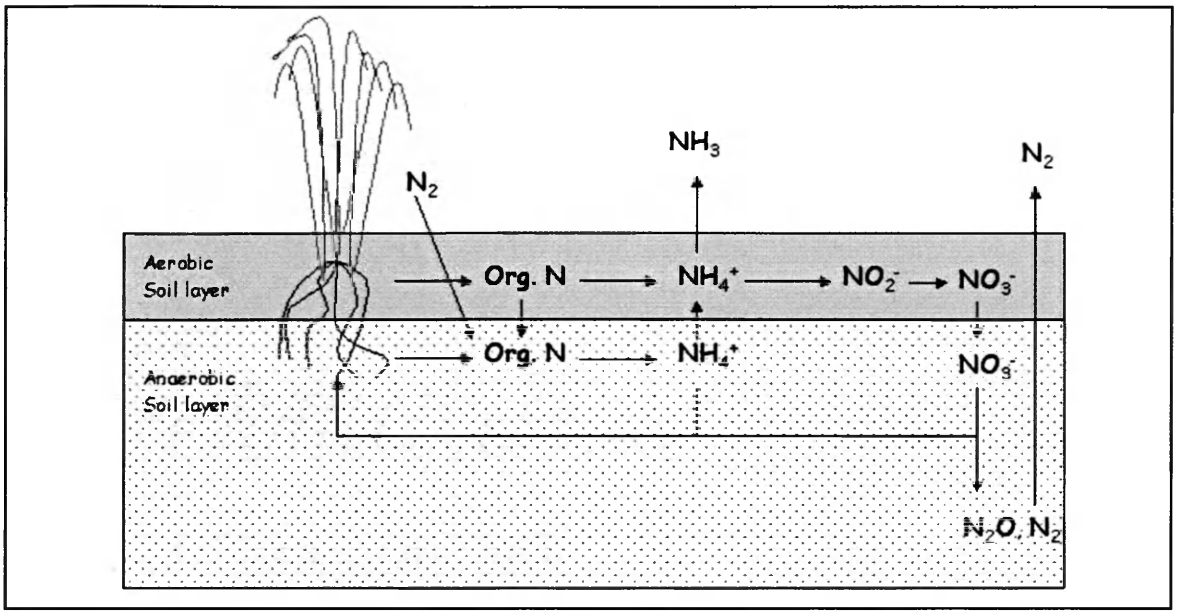

Aerobic
Nitrate reduction
Manganese reduction
Iron reduction
Sulfate reduction
Methane reduction
O_2
NO_3^-
Mn^{2+}
Fe^{2+}
SO_4^{2-}
S^{2-}
CH_4

Figure 1.3


NH3
N2
N2
Aerobic
Soil layer
Org. N
NH4+
NO2-
NO3-
Org. N
NH4+
NO3-
Anaerobic
Soil layer
N2O, N2

Tables

Table 1.1

State	Mitigated Wetland	Natural Wetland
Florida	2.6 ± 0.3 (Anderson and Cowell 2004)	13.8 (Anderson and Cowell 2004)
Pennsylvania	0.5-0.9 (Stauffer and Brooks 1997)	21.7 ± 20.4 (Bischel-Machung et al. 1996)
Pennsylvania	2.3-6.5 (Cole et al. 2001)	
Pennsylvania	4.0 (Brooks et al. 2005)	
Virginia	0.9-1.9 (Whittecar and Daniels 1999)	2.4-11 (Stolt et al. 2000)
Virginia	3.5-7.2 (Bruland and Richardson 2004)	

Table 1.2

Element	Oxidized Form	Reduced Form	Redox Potential (mV)
Nitrogen	NO_3^- (nitrate)	N_2O, N_2, NH_4^+ (nitrous oxide, nitrogen, ammonium)	250
Manganese	Mn^{+4} (manganic)	Mn^{+2} (manganous)	225
Iron	Fe^{+3} (ferric)	Fe^{+2} (ferrous)	120
Sulfur	SO_4^{-2} (sulfate)	S^{-2} (sulfide)	-75 to -150
Carbon	CO_2 (carbon dioxide)	CH_4 (methane)	-250 to -350

CHAPTER TWO:

Hydrology, Soil Characteristics, and Net Microbial Activity in Created and Natural Palustrine Forested Wetlands

Abstract

Eugene Odum (1969) defined ecological function as a balance between nutrient uptake and retention, accumulation of above- and below-ground biomass, and the ability of a system to maintain stability despite environmental stress. More recent research has refined this definition to include the accumulation of organic matter into soils and high levels of community primary productivity as important functions of wetland systems (Balcombe et al., 2005). The primary objective of this study was to use these concepts in order to evaluate the performance of a palustrine, forested wetland constructed based on current recommendations, and an adjacent natural wetland. Our research investigated how hydrology, soil characteristics, and microbial activity varied between these sites. Data indicated that soil moisture, soil organic matter, total carbon, and total nitrogen were all lower in the created wetland than in the natural wetland, while bulk density and soil carbon to nitrogen ratios was higher in the created wetland. Additionally, respiration, nitrogen mineralization, and denitrification were all lower in the created wetland. Nitrogen fixation was greater in the created wetland while methane emissions were negligible from both wetlands. Our research therefore suggests that even when constructed based on current recommendations, ecosystem function, based on Odum's definition, is lower in created wetlands than in natural reference sites. Our research also indicates that conditions potentially combine in created wetlands to produce a challenging environment for the establishment of vegetation.

Introduction

Microbial mediated biogeochemical processes are important in wetland ecosystems because they affect soil development, nutrient cycling and retention, and the successional processes governing plant communities (Langis et al., 1991). Microbial activity is especially important in created wetlands because it has a strong influence on soil development and nutrient retention in systems often featuring poor soil conditions such as high bulk density, poorly developed, or entirely absent, litter layers, and low soil organic matter (SOM) content (Bishel Machung et al., 1996; Stauffer and Brooks, 1997; Stolt et al., 2000; Bruland and Richardson, 2004). Although organic matter accumulation is a characteristic feature of most natural wetlands, traditional creation practices such as top soil scraping often remove up to one meter of organic rich material in the surface soil A-horizon, leaving created wetlands often depleted of organic matter in the surface layers

(Whittecar and Daniels, 1999; Bergschneider, 2005). This is a well documented problem in many created wetlands; in fact, low organic matter content in wetlands has been reported for created sites in Florida (Anderson and Cowell, 2004), Pennsylvania (Stauffer and Brooks, 1997), and Virginia (Whittecar and Daniels, 1999).

Past research has shown poor soil conditions, including low levels of soil organic matter, strongly contribute to created wetland failure (Stauffer and Brooks, 1997). Because of these *in situ* conditions, many studies have advocated amending created wetlands with organic material either in the form of salvaged natural wetland topsoils or imported mulches generated elsewhere (Stauffer and Brooks, 1997; Anderson and Cowell, 2004; Whittecar and Daniels, 1999). The reasoning behind organic amendment is that while organic matter accumulation occurs naturally over decadal time spans in wetlands, adding organic amendments to created wetland soils will accelerate this process and more rapidly achieve functional equivalency in created wetland soils. A great amount of research on this topic has concluded that, at least in some instances, amending soils in wetland creation projects with organic material has the potential to improve local soil conditions (Brinson et al., 1995; Brinson and Rheinhardt, 1996; Stauffer and Brooks, 1997; Whittecar and Daniels, 1999; McKinstry and Anderson, 2003; Anderson and Cowell, 2004; Bruland and Richardson, 2004; Bergshneider, 2005; Bailey et al., 2007).

Because microbes play such a key role in nutrient cycling processes, the lack of an abundant, well-developed microbial community may also strongly impact nutrient transformation and retention functions in created wetlands (Bruland and Richardson, 2004). Increasing organic matter content through soil amendment will likely enhance microbial activity. Specifically, following amendment, microbial biomass and denitrification are expected to increase, as organic matter provides an energy source for heterotrophic microbes, helps retain soil moisture, and contributes to decreases in redox potential (Duncan and Groffman, 1994). However, little work has been done to identify or quantify differences between microbial activity in created and natural wetlands. This is a significant gap in the knowledge base of wetlands science. If significant differences

between activities exist, it might indicate that microbial communities play an important role in wetland creation projects, and have the potential to impact not only nutrient uptake and retention, but also soil organic carbon stores, the lability of other important nutrients, and other general soil conditions needed for the successful establishment of vegetation in created wetlands.

Differences in microbial activities also have the potential to affect global processes. Research shows wetlands are one of the largest contributors to the atmospheric carbon and nitrogen budgets (Mitsch and Gosselink, 2000). These contributions are predominantly due to microbial communities, which are important transformers of detritus and whose cumulative activity produces carbon dioxide (CO_2), methane (CH_4), and nitrous oxide (N_2O), all important greenhouse gases (Duncan and Groffman, 1994; Bruland and Richardson, 2004). If natural and created wetlands differ in their impacts on these biogeochemical cycles, large scale landscape conversion has the potential to drastically impact future climate. Because of this, and the documented transition of natural wetlands to created wetlands on a regional and national level, a study of how microbial activities differ is not only extremely relevant to improving created wetland performance on a site-to-site basis, but also on mitigating potential climate change.

Our study focused on addressing some of these concerns. The specific objective of this study was to quantify differences between hydrology, soil characteristics, and microbial activity in a palustrine, forested wetland constructed based on current recommendations, and an adjacent natural wetland. In this study, net microbial activity was quantified by measuring soil microbial respiration, methanogenesis, nitrogen fixation, nitrogen mineralization, and denitrification in both a natural and a created wetland.

Materials and Methods

Adjacent created and natural wetlands were identified in January 2009, and studied from May 2009 through April 2010 to determine: 1) if differences exist in the microbial activities found in natural and created wetland soils and if these differences influence the

establishment of vegetation; and 2) if these differences impact the production of pertinent greenhouse gases that could potentially affect future climate.

Experimental Design: The experiment consisted of two treatment groups: 1) a created palustrine forested wetland, and 2) a similar adjacent natural wetland. In each treatment, a grid consisting of fifty plots was created. Plots were numbered created one through fifty (C_1-C_{50}), and natural one through fifty (N_1-N_{50}). Data were collected over a twelve month period from May 2009 through April 2010.

Wetland Field Sites: The created-natural wetland complex used in this experiment was part of a wetland bank located in southeastern Virginia. The selected site contained a matrix of both created and natural wetlands. Physical construction on the created wetland utilized in this experiment began in September 2008 and concluded in January 2009. During construction, grading removed all topsoil. However, the topsoil was stockpiled and re-incorporated into the remaining substrate at the completion of initial site excavation. In February 2009, the created wetland was planted with a mix of multiple wetland tree species. A native seed mix was also introduced in February 2009 to stabilize the soil and add organic material following the first growing season.

Sampling: Sampling began five months after the final stages of site construction ($T_{5\ months}$) and then occurred again every month ($T_{6\ months}$, $T_{7\ months}$,...$T_{16\ months}$) for a 12 month sampling period. The five months elapsed time between the final stages of site construction and sampling was planned in order to give the system time to equilibrate.

Each month, hydrologic conditions were measured in three ground water wells in the created wetland, and in three ground water wells in the natural wetland. Three plots from both wetlands were also randomly selected each month for sampling. In each selected plot, soil cores were removed and returned to the laboratory to be analyzed for: soil moisture, soil bulk density, soil organic matter, total carbon, and total nitrogen. Microbial activity was also quantified in each plot by measuring soil microbial respiration, methanogenesis, nitrogen fixation, nitrogen mineralization, and

denitrification. Microbial respiration and methanogenesis were measured *in situ* as net ecosystem exchange of carbon dioxide (CO_2) and methane (CH_4), while nitrogen fixation, nitrogen mineralization, and denitrification were measured using the acetylene reduction technique, anaerobic incubation procedure, and acetylene block method in the laboratory in three additional soil cores collected from both wetlands.

This design yielded three replicate data points for both treatment groups each month for all measures of hydrologic parameters, general soil conditions, and microbial activity. Methods regarding the measurement of specific parameters are outlined in the following sections.

Hydrology: Hydrologic conditions were measured in ground water observation wells installed in each treatment. Elevation of the water table was calculated monthly by measuring the distance from the top of the installations to the surface of the water in the wells. This value was related to sea level by subtracting the vertical distance to water level from the elevation of the top of the installations. Elevation data of both the installations, and the surface of each plot, was calculated using ArcView GIS.

Soil moisture was also calculated monthly in samples collected from the upper 15 cm of the soil in selected plots from both wetlands. Soil moisture was calculated gravimetrically by weighing each soil sample, drying the samples for 48 hours at 60°C, and then re-weighing the samples. Soil moisture was then calculated as the percent difference between these two weights, relative to the samples initial wet weight using the following equation:

$$\text{Soil Moisture} = [(\text{wet weight} - \text{dry weight}) \div \text{dry weight}] \times 100$$

Soil Characteristics: Each month, three soil additional samples were collected from the top 15 cm of the soil profile at each plot using a hand spade. Each sample was thoroughly mixed. One sample was analyzed for soil organic matter content, one was

analyzed for soil bulk density, and one was analyzed for total carbon (C) and nitrogen (N) content, which were then used to calculate soil carbon to nitrogen (C:N) ratios. Organic matter content was determined using loss of weight on ignition (LOI) at 450 ◦C (Storer, 1984). The difference between the dry weight and the ashed weight of each sample represented the organic matter mass in the sample, which was expressed as a percentage of the total sediment dry weight. Soil bulk density (g cm^{-3}) was determined by weight using oven drying of intact soil cores (Blake and Hartge, 1986). Bulk density was calculated as dry weight (g) divided by the volume of soil analyzed (cm^3). Finally, total carbon and total nitrogen contents were measured using a NC2100 controlled combustion elemental analyzer (ThermoQuest Italia S.p.A.) (Nelson and Sommers, 1996).

Microbial Respiration: Soil microbial respiration was quantified by measuring net ecosystem exchange of CO_2 at the soil surface in plots cleared of vegetation. To use net ecosystem exchange of CO_2 as a proxy for soil microbial respiration, a soil collar 60 cm in length and 15 cm in diameter was hammered into the soil matrix directly adjacent to the seedlings randomly selected for sampling. The soil collar was capped with an air tight, transparent, heat-permeable Tefzel® (DuPont®) plastic cover. Approximately 15 cm of headspace area existed between the soil interface and the Tefzel® cap. Vegetation was clipped inside the soil collar at the soil surface one month prior to sampling. One month of elapsed time was required so that all below-ground vegetative contributions to soil respiration would have ceased and the observed CO_2 flux would solely be a product of soil microbial communities. A 15 cm diameter soil collar was used to keep the scale of vegetative clipping to a minimum and preserve shading from adjacent plants in order to keep soil temperatures close to ambient values (Megonigal, per. Comm.).

Net ecosystem exchange of CO_2 in this setup was measured in situ using either a LI-6200 (LI-COR® Biosciences), or a TPS-2 (PP Systems®) portable photosynthesis/respiration system. Both the LI-6200 and the TPS-2 consisted of a portable computer attached to an InfraRed Gas Analyzer (IRGA). For the first 8 months of the sampling period, the LI-COR system was used; however this system was replaced with the newer TPS-2 model

prior to measurements being taken in January 2010. Both the LI-6200 and the TPS-2 systems pumped air out of the headspace area of the soil collar into the IRGA through a tube running from the soil collar to the machine. The IRGA then measured CO_2 concentration (ppm), and returned the air to the soil collar through a second tube (Figure 2.1). Both systems were programmed to record one measurement approximately every 30 s over a 5 min period. All measurements were made during the hours of highest light (approximately 11am to 3pm). In addition to net ecosystem gas exchange measurements, ambient air temperature was also measured using a portable temperature probe.

The set of CO_2 concentration measurements, generated using the afore mentioned techniques, was then used to calculate CO_2 flux as the change in CO_2 per square meter per second (ΔCO_2 ppm m^{-2} s^{-1}). To calculate CO_2 flux, CO_2 concentrations were plotted versus time in seconds, with time serving as the independent x-variable. The slope of this scatter plot was CO_2 flux in parts per million, which was later converted to µmol C m^{-2} h^{-1} using the ideal gas law, PV=nRT, where P equals atmospheric pressure, V is volume of chamber, R is the universal gas constant, T is temperature in Kelvin (K), and n is the number moles of gas in the sample container. Because all of the other variables are known, this equation can be rearranged to solve for the number of moles of gas in the chamber used for measurements of CO_2 concentration. To convert parts per million to µmoles, the slope of the concentration scatter plot is then multiplied by moles of air (n) in the chamber. In this calculation, parts per million of CO_2 equates to one mole of CO_2 per 106 moles of air, or a single µmole (10^{-6} moles) of CO_2 per mole of air. Thus, slope equals µmol CO_2 m^{-2} mol air^{-1} $second^{-1}$ and by multiplying moles of air (n) by slope, you effectively convert ppm to µmoles per unit time. The final step in converting to change in CO_2 per square meter per hour (ΔCO_2 µmol m^{-2} hr^{-1}) is to divide calculated µmoles per unit time by the area of the base of the chamber.

CO_2 fluxes were then modeled to determine both monthly and annual rates of soil respiration (R). In the model, CO_2 flux rates in the soil matrix were controlled by changes in temperature. To model respiration (R), an Arrhenius plot of the natural log of

CO_2 flux (ΔCO_2 m^{-2} hr^{-1}) versus the inverse of temperature in degrees K, with temperature as the independent x-variable, was created. A curve was fit to this plot using the equation:

$$R = y_0 + ax$$

where y_0 was the slope of the Arrhenius plot, a was an empirically-derived constant, and x was the average hourly air temperature in degrees K. Hourly temperature values for each day, of each month in southeastern Virginia were then run through the equation to generate hourly R rates (ΔCO_2 m^{-2}). The hourly temperature values used in this model was measured at the Virginia Institute of Marine Science (VIMS). Finally, hourly R rates were summed for month and year.

Methanogenesis: To quantify methane emissions, gas samples (CH_4) were drawn manually in 10 mL capped syringes from the above chamber's septum, which was located directly adjacent to the tubes running from the LI-6200 re-entered the chamber. Gas samples were analyzed using a Hewlett-Packard® 5890 series II Gas Chromatograph (GC), equipped with a 2.6 mL sampling loop and Flame Ignition Detector (FID) with Molecular Sieve 13x. Samples were drawn at one minute intervals over a 5 minute sampling period. For each sample, the 10 mL syringe was first purged with air drawn from the chamber via its septum. The second drawn sample was preserved using a gas tight stopcock to prevent leakage and stored at 4°C, in the dark, until analysis. CH_4 standards were collected from both 100 ppm and 10 ppm Scotty® 221 L cylinders (Scott Specialty Gases, Inc.) at the beginning of each sampling day. Standards allowed the calculation of correction curves to be created and were used to convert measurements from the GC into CH_4 concentrations of field air samples.

CH_4 measurements were taken at 0% ambient light in the bare-ground patches in order to shade out light and reduce temperature fluctuations during measurements. Instantaneous

CH_4 fluxes were calculated by regressing CH_4 concentrations against time to calculate a slope, i.e. the change in CH_4 concentration per minute.

Nitrogen Fixation: Each month one soil core (7.5 cm in diameter and 15 cm in length) was removed from selected plots using a plastic sampling tube that was sharpened at one end. The cores were immediately removed from the sampling device, put in individual sealed plastic bags, placed on ice and returned to the laboratory. Once at the lab, samples were stored at 4°C until being analyzed.

Soil samples were analyzed using the acetylene reduction technique to estimate nitrogen fixation (Dilworth, 1966; Hardy et al., 1968). Using this technique, 10g of soil was placed in a 125 ml jar, to which 2 ml of distilled water was added. Air (22.5 ml) was then withdrawn from the jar with a 25 ml gastight syringe fitted with a 19 cm, 20 ga needle and replaced with 22.5 ml of acetylene to create an gaseous environment consisting of 10% acetylene. 10 mL of headspace gas was then removed immediately ($T_{initial}$) and stored for later analysis on a gas chromatograph. Samples were then incubated over a 3 hour period after which another 10 mL of headspace gas was removed (T_{final}) and stored for later analysis on a gas chromatograph. Finally, gas samples were analyzed for ethylene concentration with flame ionization detection on a Shimadzu GC-2010 gas chromatograph (Shimadzu Instruments, Inc.). This allowed for the calculation of acetylene reduction to ethylene over time as a function of soil mass. In order to facilitate comparisons with the literature, ethylene flux was converted to N flux assuming a theoretical ratio of 3 moles ethylene produced per 1 moles of N_2 fixed, and expressed on an area basis using soil bulk density data from both wetlands to a depth of 15 cm.

Nitrogen Mineralization: Rates of nitrogen mineralization were calculated in intact soil cores using the anaerobic incubation procedure developed by Waring and Bremner (1964) and then later modified by Keneey (1982). Soil cores were collected from each wetland using the technique previously described. In the lab, soil samples were sub-sampled to produce two 10 g samples. One of the 10 g sub-samples was then analyzed

for ammonium-N (NH_4) concentration. The second sub-sample was added to test tubes with as little head space remaining as possible. 12 ml of distilled water was added to the test tubes to create waterlogged conditions. The test tubes were then incubated at 40°C for 7 days. Following this incubation period, ammonium-N concentrations were again measured and compared to the initial data. The difference between the two measured ammonium-N values over time was used to approximate net N mineralization as a function of soil mass. Ammonium-N flux was then expressed on an area basis using soil bulk density data from both wetlands to a depth of 15 cm.

Denitrification: Rates of denitrification were calculated in intact soil cores using the acetylene block method (Hefting et al., 2004). Soil cores were collected from each wetland using the technique previously described. In the lab, intact soil cores were placed in 250 ml jars. Each jar then received five, 25 ml injections of acetone-free acetylene (C_2H_2), 125 ml total, and were left to equilibrate for 2 hours. Headspace gas samples of 10 ml each were then drawn from each jar ($T_{initial}$). After 3 hours, another 10ml sample was drawn (T_{final}). Both samples were then stored 4°C before being analyzed for nitrous oxide (N_2O) concentration on a Shimadzu GC-2010 gas chromatograph. Final results were used to calculate denitrification rate as the change in N_2O over time as a function of soil mass. Rates of denitrification were then expressed on an area basis using soil bulk density data from both wetlands to a depth of 15 cm.

Statistical Analyses: Simple regression was used to explore relationships among the various parameters constituting vegetative dynamics, general soil conditions, and hydrology measured in both the created and natural wetland over the course of the year long sampling period. Data were further analyzed using two-way analysis of variance (ANOVA) to determine if significant differences existed between the two wetlands in net microbial activity. Analysis of variance (ANOVA) was used to compare time (month) and treatment (created versus natural) as main effects and interactions between time and treatment. Variables used in separate ANOVA were rates of: soil respiration, methanogenesis, nitrogen fixation, nitrogen mineralization, denitrification; and measures of: soil bulk density, soil organic matter, total soil carbon and nitrogen, depth of the water

table, and percent soil moisture. Statistical analyses were performed using R software (GNU Operating Systems, Inc.).

Results

Both sites featured seasonal fluctuations in the measured parameters, likely due to changing patterns in temperature and precipitation. In addition, microbial nutrient transformations never completely ceased, even during the coldest parts of the year. More specific trends in the data are outlined in the following sections. Results of ANOVA are reported in Table 2.1.

Hydrology: Significant differences existed in hydrology between treatment groups (Figure 2.2). Specifically, the depth of the water table, averaged over the entire course of the experiment, was greater in the created site ($p \leq 0.001$). The depth of the water table also varied significantly by month ($p \leq 0.001$). However, there was a significant interaction between these two factors ($p \leq 0.001$). A Tukey post-test indicated that the water table in the created site was higher than the water table in the natural site during the months of May, June, and July, 2009 and January, 2010. However, none of these differences were significant ($\alpha = 0.05$). The only months in which the water table differed significantly between the two wetlands were August, September, October, and December, 2009 ($p = 0.002$, $p \leq 0.001$, $p = 0.002$, $p = 0.009$ respectively). In each of these months the water table was higher in the natural wetland. This relationship was also observed in November, 2009 and February, March, and April, 2010, though not significantly.

There was a significant correlation between the monthly depth of the water table and soil moisture in both sites (created: $R^2 = 0.6605$, $p \leq 0.001$; natural: $R^2 = 0.7781$, $p \leq 0.001$) (Figure 2.3). Similarly to water table, soil moisture also varied significantly between wetlands ($p < 0.001$), reaching a minimum of 4.11% ($\pm$ 0.51) in the created wetland compared to a minimum of 12.51% ($\pm$ 1.06) in the natural wetland.

Soil Characteristics: Analysis showed a significant difference between soil organic matter (SOM) both between sites ($p \leq 0.001$) and across months ($p = 0.042$) (Figure 2.4). However, no clear pattern existed in the monthly variation and there was no significant interaction between the two factors. Soil organic matter ranged from 3.5% (± 0.92) to 9.78% (± 1.09) in the created wetland, and from 9.94% (± 1.39) to 13.72% (± 1.38) in the natural wetland. Approximately 50% of soil organic matter consisted of carbon. Bulk density also varied significantly both between wetlands ($p \leq 0.001$) and across months ($p = 0.002$) (Figure 2.5). However, again, no clear pattern existed in the monthly variation. There was also no significant interaction between these factors. In all months, bulk density in the created wetland was significantly greater than bulk density in the natural wetland. In the created wetland, bulk density ranged from 0.98 g m^{-3} (± 0.05) to 1.18 g m^{-3} (± 0.14); in the natural wetland bulk density ranged from 0.42 g m^{-3} (± 0.02) to 0.72 g m^{-3} (± 0.13). On average bulk density in the created wetland was approximately two times greater than that of the natural wetland (1.11 g m^{-2} (± 0.10) to 0.59 g m^{-2} (± 0.11)). Soil total carbon to total nitrogen ratios also varied significantly by site ($p \leq 0.001$), though not by month ($p = 0.4870$) (Figure 2.6). However, when soil C:N was examined in just the created site, regression analysis showed that C:N increased gradually over the course of the experiment ($R^2 = 0.7781$, $p = 0.009$) (Figure 2.7). No significant interaction existed between the two factors; each month soil C:N was higher in the created wetland than in the natural wetland. C:N ratios ranged from 12.38 (±3.63) to 32.02 (±2.15) in the created wetland, and from 9.11 (±0.91) to 11.28 (±1.31) in the natural wetland. Additional analysis showed a lack of carbonates in samples, total carbon was therefore approximately equal to organic carbon in all soil samples analyzed.

Regression analysis indicated a significant correlation between soil organic matter and soil bulk density (created: $R^2 = 0.6611$, $p \leq 0.001$; natural: $R^2 = 0.3782$, $p \leq 0.001$) (Figure 2.8), soil organic matter and total carbon (created: $R^2 = 0.4075$, $p \leq 0.001$; natural: $R^2 = 0.4704$, $p \leq 0.001$) (Figure 2.9), and soil organic matter and total nitrogen (created: $R^2 = 0.5184$, $p \leq 0.001$; natural: $R^2 = 0.6967$, $p \leq 0.001$) (Figure 2.10) in both the created and natural wetlands. In both wetlands, as a soil organic matter increased,

soil bulk density decreased, and total carbon and nitrogen increased. There was also a significant correlation between soil organic matter and soil C:N in the created wetland ($R^2 = 0.2699$, $p \leq 0.001$) (Figure 2.11), and soil C:N and total nitrogen in both wetlands (created: $R^2 = 0.5505$, $p \leq 0.001$; natural: $R^2 = 0.1527$, $p = 0.015$) (Figure 2.12). As SOM increased in the natural wetland, soil C:N ratios decreased significantly. This general trend was also apparent in the created wetland, though the relationship was not significant. In both wetlands however, as C:N ratios increased total nitrogen decreased significantly.

Microbial Respiration: Modeled monthly microbial respiration varied significantly both by site ($p \leq 0.001$) and month ($p \leq 0.001$) (Figure 2.13). However, a significant interaction existed between these factors ($p \leq 0.001$). From May through December, 2009, microbial respiration was significantly greater in the natural wetland than the created wetland ($p \leq 0.001$ for each month during this time period). However in January, February, and April, 2010 there was no significant difference between respiration in the two wetlands, while in March, 2010 respiration in the created wetland was greater. During the growing season, rates of monthly respiration reached a peak of 191.70 g-C m^{-2} ($\pm$ 8.56) in the created wetland, compared to a peak of 254.65 g-C m^{-2} ($\pm$ 25.78). Conversely, during the winter months, respiration reached a minimum of 30.47 g-C m^{-2} ($\pm$ 15.56) in the created wetland, and 19.01 g-C m^{-2} ($\pm$ 5.96). Modeled annual respiration also differed significantly between the created and natural wetland ($p \leq 0.001$). Annual respiration in the created wetland was estimated to be 1518.34 g-C m^{-2} ($\pm$ 63.08), while annual respiration in the natural wetland was calculated as 1957.65 g-C m^{-2} ($\pm$ 22.74).

The regression of monthly microbial respiration with soil moisture was significant in both wetlands (created: $R^2 = 0.2351$, $p = 0.012$; natural: $R^2 = 0.2741$, $p \leq 0.001$) (Figure 2.14). However there was no significant relationship ($\alpha = 0.05$) with either soil organic matter (created: $R^2 = 0.0012$, $p = 0.6651$; natural: $R^2 = 0.0195$, $p = 0.4249$), or soil C:N ratios (created: $R^2 = 0.0559$, $p = 0.2523$; natural: $R^2 = 0.0035$, $p = 0.5604$), though slightly

increasing rates of respiration with increasing SOM and slightly decreasing rates with increasing C:N ratios were observed.

Methanogenesis: Methane fluxes were determined to be insignificant over the course of the experiment. Instantaneous rates measured *in situ* ranged from 0.00 to 0.04 mg-C m^{-2} min and were therefore determined to be negligible. Therefore methane data was not included in the analysis of microbial activity in either the created or natural wetland.

Nitrogen Fixation: Nitrogen fixation (N-fix) varied significantly between the two sites ($p \leq 0.001$) but not across months ($p = 0.5635$) (Figure 2.15). Additionally there was no significant interaction factor between site and month; for each month of measurements nitrogen fixation was higher in the created site than in the natural site. Over the course of the year, in the created wetland, rates of nitrogen fixation ranged from 0.28 mg-N m^{-2} hr^{-1} ($\pm$ 0.03) to 0.36 mg-N m^{-2} hr^{-1} ($\pm$ 0.10); in the natural wetland, rates ranged from 0.21 mg-N m^{-2} hr^{-1} ($\pm$ 0.03) to 0.27 mg-N m^{-2} hr^{-1} ($\pm$ 0.01).

Regression analysis indicated a significant relationship between nitrogen fixation and soil moisture in the natural wetland ($R^2 = 0.2432$, $p = 0.003$), though this relationship was not significant ($\alpha = 0.05$) in the created wetland ($R^2 = 0.0119$, $p = 0.8314$) (Figure 2.16). In the natural wetland as soil moisture increased, N-fix significantly decreased. There was also a significant relationship between soil C:N and rates of N-fix in the created wetland ($R^2 = 0.5078$, $p \leq 0.001$), though again this relationship didn't hold for both sites as it was not significant ($\alpha = 0.05$) in the natural wetland ($R^2 = 0.0037$, $p = 0.851$) (Figure 2.17). In the created wetland, analysis showed that as C:N ratios increased, rates of nitrogen fixation increased significantly.

Nitrogen Mineralization: Nitrogen mineralization varied significantly by site ($p \leq 0.001$), though not by month ($p = 0.166$) (Figure 2.18). There was also no significant interaction factor between these two parameters; each month nitrogen mineralization was lower in the created wetland. Rates of nitrogen mineralization ranged from 0.41 mg-N m

^{2} hr^{-1} (± 0.30) to 1.41 mg-N m^{-2} hr^{-1} (± 0.31) in the created wetland, and 1.11 mg-N m^{-2} hr^{-1} (± 0.58) to 1.85 mg-N m^{-2} hr^{-1} (± 0.17). in the natural wetland.

Regression analysis indicated a significant, though weak, relationship between soil organic matter and nitrogen mineralization in the created wetland ($R^2 = 0.0027$, $p = 0.851$). However, this relationship was not observed in the natural wetland ($R^2 = 0.0941$, $p = 0.069$) (Figure 2.19). In the created wetland, as SOM increased, nitrogen mineralization increased. A significant relationship did exist between soil C:N and mineralization in both wetlands (created: $R^2 = 0.1512$, $p = 0.015$; natural: $R^2 = 0.1046$, $p = 0.046$). Analysis showed that in both the created and natural wetland, as C:N ratios increased, nitrogen mineralization decreased significantly.

Denitrification: Denitrification varied significantly by both site ($p \leq 0.001$) and month ($p \leq 0.001$) though there was no clear pattern in the monthly fluctuations (Figure 2.20). However, there was a significant interaction factor between these parameters ($p \leq 0.001$). From May through November, 2009, and in January and February, 2010 denitrification was significantly higher in the natural wetland than in the created wetland ($p \leq 0.001$). However, no significant differences in denitrification were observed between the two wetlands in December, 2009, or in March or April, 2010. Over the year long sampling period, denitrification ranged from 0.71 mg-N m^{-2} hr^{-1} (± 0.01) to 1.30 mg-N m^{-2} hr^{-1} (± 0.20) in the created wetland, and from 1.18 mg-N m^{-2} hr^{-1} (± 0.02) to 1.36 mg-N m^{-2} hr^{-1} (± 0.04) in the natural wetland.

Rates of denitrification were significantly affected by soil moisture in both wetlands (created: $R^2 = 0.6438$, $p \leq 0.001$; natural: $R^2 = 0.2721$, $p = 0.002$), though no relationship existed between denitrification and soil organic matter, or soil C:N in either wetland ($\alpha = 0.05$).

Discussion

Optimal microbial activity occurs near "field capacity", which is equivalent to 50% water-filled pore space (Linn and Doran, 1984). Extended periods of saturation above this value may, however, result in poor soil aeration and a reduction of soil oxygen concentrations which leads to a reduction of aerobic mineralization rates as microbial organisms become inactive (Trettin et al., 1996). We therefore hypothesized that net microbial activity would fluctuate seasonally with precipitation, regardless of wetland type. Our data, as well, as past studies (Entry et al., 1995; Megonigal et al. 1996) confirmed this initial hypothesis, and showed that microbial processing rates vary with season as a direct result of changes in soil moisture, and oxygen, as well as temperature. However, these seasonal fluctuations were not observed in processes measured in the laboratory. This is likely a result of controlled, artificial lab conditions, such as temperature, which differed significantly from field conditions.

Microbial processes are also strongly correlated with soil organic matter (Gambrell and Patrick 1978; Brinson et al. 1981; Moran et al. 1989; Whiting and Chanton 1993; Bubier et al. 1998; Chimner 2004, Megonigal et al. 2004). Since other research has suggested that created wetlands often feature lower levels of organics than natural wetlands (Stolt et al., 2000; Bruland and Richardson, 2004; Bergshneider, 2005; Daniels et al., 2005; Bailey et al., 2007), we also hypothesized that many corresponding microbial processes in the created wetland would be lower than in the natural wetland. Additionally, since past research indicated that twelve months was not enough time to add significant amounts of organic material to the substrate via natural methods (Campbell et al., 2002), we hypothesized that observed differences between microbial activity in the created and natural wetlands would not be reduced over the trial period.

A more detailed analysis of the physical and biological characteristics observed in the two wetlands is outlined in the following sections.

Hydrology: Past studies have found that as wetland soils lose moisture, soil redox potential increases from around -290 mV to approximately +210 mV as the soil matrix

shifts from a reducing environment to an oxidizing environment (Patrick and Mahapatra, 1968; Mitsch and Gosselink, 2000). Other studies have shown that when a wetland becomes saturated beyond field capacity, oxygen diffusion becomes limited, soils experience a rapid depletion of O_2, and reducing conditions are established as oxygen (O_2) reduction and then nitrate (NO_3) reduction to ammonium (NH_4), nitrous oxide (N_2O), or dinitrogen (N_2) takes place (Mitsch and Gosselink, 2000). As a result, soil moisture levels and conditions of flooding are often used as a proxy to determine *in situ* redox conditions and microbial activities such denitrification potential (DeMars and Wassen, 1999; Gold et al., 2001; Seybold et al., 2002; Mitchell and Branfireun, 2005; Fiedler et al., 2007; Niedermeier and Robinson, 2007). Differences in site hydrology in our study therefore potentially explain many of the differences in microbial activity observed between the created and natural wetlands.

Our data showed hydrologic conditions varied significantly between the two wetlands, but only for a few select months. During the spring, early summer, and winter, conditions were roughly equivalent in the created and natural wetlands. However, during the late summer and fall, while the natural wetland remained relatively moist, the created wetland became quite dry. As soils become increasingly dry as the water table drops, they switch from a reducing to an oxidizing environment (DeMars and Wassen, 1999; Gold et al., 2001; Seybold et al., 2002; Mitchell and Branfireun, 2005; Fiedler et al., 2007; Niedermeier and Robinson, 2007); therefore during August, September, October, and December, 2009 when the created wetland was significantly drier than the natural wetland, aerobic conditions likely persisted.

We believe that aerobic conditions in the created wetland initially stimulated higher rates of microbial respiration, mineralization, and subsequent uptake of organic matter in the created wetland; however after all available organic matter had been decomposed, respiration rates rapidly declined. This theory explains both the low SOM found in the created wetland, as well as the low mean monthly rates of respiration observed. In the natural wetland however, where soil moisture values remained high, we think that

degradation of organic matter entering the wetland as litterfall was slow, but persistent, as the soil microenvironment remained anaerobic for much of the year. Other studies have made similar conclusions and have found that SOM significantly increased as soil moisture increased (Entry et al., 1995; Trettin et al., 1996; Hunt et al., 1999).

Spatial and temporal changes in aerobic and anaerobic conditions in wetland soils also impact rates of ammonification, nitrification and denitrification (Reddy et al., 1980; Patrick, 1982; Reddy et al., 1989; Hill, 1996; Clement et al., 2002; Bruland and Richardson 2004). As such, we also attribute many of the differences observed in the nitrogen cycle to *in situ* hydrological conditions.

Soil Characteristics: Differing soil characteristics and nutrient levels were expected to be major drivers of the microbial parameters observed at the two wtelands (Kicklighter et al., 1994; Trettin et al., 1996; Davidson et al., 1998). As expected, soil organic matter, soil carbon content, and soil nitrogen content were generally higher in the natural wetland, while bulk density and soil C:N were generally higher in the created wetland.

The range of organic matter values we found in the created wetland (3.95-9.78%) were similar to those reported for surface horizons of southern forested reference wetlands on mineral soils (2.8-18%) (Lockaby and Walbridge, 1998), though they did trend towards the lower end of the spectrum and were significantly less than the range found in the natural wetland (9.95-13.72%). Moreover, while the range of soil carbon values in the created wetland (1.43-3.64%) was at times higher than the concentration generally viewed as indicative of nutritional limitation for many deciduous floodplain species (2%), it was significantly lower than the range of values found in the natural wetland (3.71-5.78%) and low enough to indicate some nutrient limitations over the course of the year long study (Baker and Broadfoot, 1979).

Nitrogen levels in the created wetland ranged from 0.10-0.31% which were on par with values reported for reference wetlands in the Virginia Coastal Plain (0.2-0.4%), and

consistent with nitrogen levels observed in unammended created wetlands in Virginia (0.03-0.2%) (Stolt et al., 2000). The latter was unexpected since the created wetland was amended with organic material following construction. Soil nitrogen in the created wetland was also significantly lower than in the adjacent natural wetland (0.46-0.58%). The increasing soil C:N ratios observed in the created wetland indicated that over time, these nutrient limitations were not being ameliorated but rather were becoming more pronounced.

Aside from the increasing soil C:N observed in the created site, there were few significant changes during the one year period in the soil characteristics of either the created or natural wetland. The slight variations that were observed were most likely a result of normal seasonal fluctuations in ambient *in situ* conditions, which have been documented in other studies (Raich et al., 1990; Crill, 1991; Davidson et al., 1998). The low levels of soil carbon and nitrogen in the created wetland confirm the general nutrient deficiency reported for other created wetlands (Bishel-Machung et al., 1996; Whittecar and Daniels, 1999; Stolt et al., 2000). Low nutrient conditions in the created wetland most likely resulted from excavation which removed most nutrient rich topsoil, even though some was replaced.

While it may appear intuitive that over time, as vegetation dies and is incorporated into the soil matrix, soil organic matter, carbon, and nitrogen will all increase, while soil bulk density will decrease, others have shown that this is often not the case (Campbell et al., 2002). Campbell et al. (2002) specifically found that soil bulk density in created wetlands does not necessarily decrease over time, and that, if it does, it only decreases at the soil surface as litterfall is not incorporated into subsurface horizons compacted during construction.

In addition to providing nutrients that fuel soil microorganisms, organic matter binds soil particles into aggregates and improves rates of water infiltration, which in turn improves groundwater recharge, a key function created wetlands are attempting to recreate (Hunt et

al., 1999). Past studies also show SOM significantly impacts the water holding capacity of soil (Entry et al., 1995). In fact, studies have shown that certain types of soil organic matter can hold up to 20 times their weight in water and that for each one percent increase in SOM, the available water holding capacity of the soil can increase by as much as 3.7 percent (Mitsch and Gosselink, 2000). Low levels of organic matter in the created wetland could therefore be acting in a negative feedback loop by contributing to decreases in soil moisture during the fall months, and enhancing the aerobic degradation of SOM, which in turn would decrease organic matter levels even further and decrease the water holding capacity of soils (Kowalenko et al., 1978; Rustad et al., 2001). This cycle would remain unchanged over time, unless wetland hydrology changed significantly, and provides a possible explanation for created wetland failure as peat will not accumulate under aerobic conditions.

While Eugene Odum (1969) noted that increases in soil organic matter are characteristic of developing ecosystems approaching ecological 'maturity;' a more recent study by Bishel-Machung et al. (1996) found that this may not be characteristic of created wetlands as increases in the organic fraction are often not observed with increasing site age, even in sites as old as 10 years. Our results seem to support this finding, as SOM did not increase significantly over the course of the year. Past studies have also found that bulk densities remain high even in the oldest created wetlands, indicating that soil compaction is not lessening significantly over time, which our research also supported (Campbell et al., 2002). The failure to develop organic, low density soils in turn inhibits the development of *in situ* vegetational communities. Stauffer and Brooks (1997) showed that soil organic matter is critical for plant community establishment and is therefore an important parameter to use in the assessment of created wetlands.

Organic matter consists of a variety of components. These include, in varying proportions and many intermediate stages, an active organic fraction including microorganisms (10–40 percent), and a resistant or stable fraction (40–60 percent), referred to as humus (Updegraff et al., 1995). Often C:N ratios are used to indicate what fractions of labile

and recalcitrant material are contained within the soil (Updegraff et al., 1995). The high C:N ratio found in the created wetland indicated a more resilient substrate was present, while the low C:N ratio in the natural wetland indicated an easily decomposable base, which further highlighted the different conditions in each wetland (Singh and Gupta, 1977; Gambrell and Patrick, 1978).

Our results were similar to those of previous studies that examined soil development in created and natural wetlands. These studies showed that few physical characteristics of created wetlands, such as soil texture and organic matter content, were similar to levels found in natural reference wetlands, even in sites as old as ten years (Bishel-Machung et al., 1996; Whittecar and Daniels, 1999; Stolt et al., 2000; Campbell et al., 2002). This uncertainty further emphasizes the problem of short or nonexistent monitoring periods characteristic of most mitigation projects and further highlights the need for long-term monitoring to accurately document the success or failure of created wetlands (Mitsch and Wilson, 1996; Cole, 1999).

Microbial Respiration: We found that microbial respiration differed significantly between the created and natural wetland, and varied seasonally in both wetlands based on temperature and soil moisture. Generally, higher rates of respiration were observed in the natural wetland. This is likely a result of higher concentrations of labile SOM. While our results don't indicate this, past studies have shown higher SOM is strongly correlated with higher rates of microbial respiration (Trettin et al., 1996; Kasimir-Klemedtsson et al., 1997). The two months that the created wetland had higher microbial respiration rates (February and March, 2010) may have been due to a lower water table combined with equivalent levels of soil organic matter. Past studies support this hypothesis and have found that prolonged anaerobiosis in wetland environments leads to a significant reduction of microbial respiration despite high levels of easily degradable soil organic matter (Kowalenko et al., 1978; Oechel et al., 1998).

Past studies showed the rate of microbial respiration is influenced by the quality of soil organic matter (Insam and Domsch, 1988; Duncan and Groffman, 1994; Trettin et al., 1996; Schlesinger and Andrews, 1999). While our results weren't significant, we observed slightly increasing rates of respiration with increasing SOM, and slightly decreasing rates with increasing soil C:N ratios, which confirmed the results of these earlier studies.

In respiration, different bi-products are released: carbon dioxide, energy, water, plant nutrients, and resynthesized organic carbon compounds. Successive respiration of these organic carbon compounds results in the formation of a more complex organic matter called humus through a process called 'humification' (Allison et al., 1949; Green et al., 1993; Van Delft et al., 1999; Prescott et al., 2000). Humus has a significant affect on soil properties. As it decomposes, humus increases soil aggregation and aggregate stability, improves the ability of soil to attract and retain nutrients, and contributes to soil carbon, nitrogen, and phosphorous reserves available for plant uptake (Allison et al., 1949; Green et al., 1993; Van Delft et al., 1999; Prescott et al., 2000). As a result, microbial respiration both directly and indirectly influences nutrient cycling processes (Trettin et al., 1996), and lower rates of respiration in the created wetland likely made conditions less tenable for the establishment of vegetation.

Humus is also resistant to rapid decomposition, and increases the capacity of wetland soils to sequester and store carbon from the atmosphere (Armentano and Menges, 1986; Kicklighter et al., 1994). Therefore, even though higher rates of microbial respiration in the natural wetland may have initially released more carbon dioxide, over an extended period of time (more than one year) higher rates of respiration should stimulate above- and below-ground biomass production, and the accumulation of stable humified organic matter in the soil itself, both of which combine to increase the natural wetland's ability to act as a carbon sink (Armentano and Menges, 1986).

Methanogenesis: Methane emissions were negligible from both the created and natural wetland over the course of the experiment. The reduction of organic matter, and the production of methane, is the most energetically inefficient means of energy production (Westermarm and Ahring, 1987; Bartlett and Harris; 1993). As such, methanogenesis typically occurs only under complete saturation when all other available electron acceptors have been utilized (Westermarm and Ahring, 1987; Bartlett and Harris; 1993). Therefore, we propose that our results could likely be due to the fact that soils in both wetlands did not remain saturated long enough for available nitrate, manganese, iron, and sulfate stores to be reduced.

Nitrogen Fixation: Our results show rates of nitrogen fixation were generally higher in the created wetland than in the natural wetland. We propose that this relationship exists because total nitrogen was significantly higher in the natural wetland, and soil C:N ratios were significantly lower, both of which indicate a lack of nitrogen limation which lowers the need for soil microbiological communities to fix atmospheric nitrogen in order to augment soil nitrogen stores. This follows previous work that has shown that the presence of fixed nitrogen suppresses nitrogen fixation in wetland sites (Dierberg and Brezonik, 1983; Ogan, 1982; Ogan, 1983). In addition to soil nitrogen content, soil temperature also has a significant influence on rates of nitrogen fixation according to the Arrhenius equation. However, since both wetlands experienced similar temperature regimes, soil temperatures were not expected to influence differences between the two wetlands in terms of the rate of nitrogen fixation (Maag and Vinther, 1996).

Rates of nitrogen fixation in both wetlands were on par with observed rates in wetland soils. Previous studies have shown these values can range from a few hundredths of a g $N\ m^{-2}\ yr^{-1}$ to about 10 g $N\ m^{-2}\ yr^{-1}$ with most values around 1 g $N\ m^{-2}\ yr^{-1}$ (Bowden, 1987) When summed over the course of the study, results indicate nitrogen fixation in the created wetland were approximately 2.84 g $N\ m^{-2}\ yr^{-1}$ (± 0.54) while in the natural wetland rates were approximately equal to 2.15 g $N\ m^{-2}\ yr^{-1}$ (± 0.13). It is important to note however, that past work has suggested that the acetylene reduction technique used in

this experiment significantly overestimates rates of nitrogen fixation due to variable C_2H_2:N_2 reduction ratios, differential solubility and diffusion of C_2H_2 and N2 and de novo ethylene production in soil (Hardy et al., 1968; Rice and Paul, 1971; Smith, 1980).

Nitrogen Mineralization: Past studies have shown that higher levels of SOM are strongly correlated with higher rates of mineralization, and that mineralization rates are often low where disturbance has reduced soil organic matter (Pastor et al., 1987; Zak and Grigal, 1991). Our results confirmed these findings. We propose that rates of nitrogen mineralization were significantly lower in the created wetland as a result of lower soil organic matter content.

Lower rates of mineralization in the created wetland were also likely a result of hydrology. While ammonification of organic nitrogen occurs under both aerobic and anaerobic conditions, nitrification, which counteracts the ammonium mineralization process by converting soil ammonium to nitrate, requires the presence of oxygen, and as a result only occurs in aerated soils or sediments such as those found in the created wetland (Cassman and Munns; 1980; Pastor et al. 1987; Reddy et al., 1989; Zak and Grigal, 1991). Our findings are also supported by past research, which found that as soil water content falls below the optimum (less than 10 cm from the soil surface), which was the case in the created wetland, nitrogen mineralization declines (Cassman and Munns, 1980; Bowden, 1987).

However, the laboratory incubations we used may have significantly underestimated nitrogen mineralization in the field because they were conducted under temperature and moisture conditions different than those observed *in situ* (Bowden, 1984; Gianello and Bremner, 1986). Laboratory methods may also have underestimated mineralization due to the short incubation period (7 days) and because different substrates within the soil are utilized under anaerobic conditions than under aerobic conditions, which likely occurred *in situ* (Gianello and Bremner, 1986).

Denitrification: Denitrification is the respiratory reduction of nitrate (NO_3) by microbes to gaseous oxides of nitrogen (N_20) and ultimately to dinitrogen gas (N_2) (Bartlett et al., 1979; Groffman et al., 1999). As such, it is an effective means of removing fixed nitrogen from groundwater (Gersberg et al., 1984; Groffman and Hanson, 1997; Hunter and Faulkner, 2001), one specific ecosystem service natural wetlands provide that created wetlands attempt to recreate.

Our results showed rates of denitrification varied significantly between the created and natural wetland and were generally higher in the natural site. This is likely a result of hydrology. Under the saturated conditions characteristic of the natural wetland, oxygen tensions are usually low, which favors denitrification (Davidson and Swank, 1986). Conversely, since the created wetland remained dry for much of the year, denitrification likely only occurred when short-term events generated partial anaerobiosis. This relationship is reflected in the strong correlation observed between soil moisture and denitrification in both wetlands. Our results are therefore consistent with earlier work which found that when water table levels were between 10 and 30 cm of the soil surface the highest rates of denitrification were observed, while when water table levels dropped below 30 cm, denitrification reached a minimum (McClain et al. 2003). The relationship between soil saturation and denitrification in soils has also been observed in other studies (Groffman and Tiedje, 1989; Parsons et al., 1991; Clement et al., 2002; Dhont et al., 2004).

Most denitrification rates reported in literature, including ours, are for measurements of potential denitrification resulting from lab incubations, rather than direct field measurements (Muller et al., 1980). While laboratory methods are often necessitated because the large background of atmospheric nitrogen (N_2) obscures emissions from denitrifiers in the field, it's important to note some of their limitations. Tiedje et al. (1982) showed that the acetylene block technique we used, overestimates *in situ* rates of denitrification, often by as much as 40 to 1000 times (Tiedje et al., 1982). As such, our

results must be viewed as having questionable relevance to what is actually occurring in either wetland.

Summary and Recommendations

The specific objective of this study was to quantify differences between hydrology, soil characteristics, and microbial activity in a palustrine, forested wetland constructed based on current recommendations and in an adjacent natural wetland. Our research indicated that hydrology and soil conditions varied greatly between the two sites. Results also showed that microbial communities process detritus and contribute to nutrient cycles very differently in the two wetlands. Most of these differences can be explained by either observed differences in hydrology and soils, such as low soil moisture and organic matter, and high soil C:N ratios.

Observed differences between the created and natural wetland are perplexing given that the created wetland was amended with stockpiled topsoil during construction. Theoretically, re-application of topsoil should have alleviated some of the limiting soil conditions typical of created wetlands (Brinson et al., 1995; Brinson and Rheinhardt, 1996; Stauffer and Brooks, 1997; Whittecar and Daniels, 1999; McKinstry and Anderson, 2003; Anderson and Cowell, 2004; Bruland and Richardson, 2004; Bergshneider, 2005; Bailey et al., 2007). However, given the large scale of the construction project, and the difficulty in re-incorporating topsoil into reaming substrates after site excavation, perhaps these conditions are not uncommon.

Also, contrary to our initial thoughts, research showed that perhaps natural systems produce more greenhouse gases than their created counterparts, as microbial respiration and nitrous oxide production were both higher in the natural wetland, and methane emissions were negligible from both sites. These differences were largely due to the prevalent role of water table dynamics in wetland carbon and nitrogen cycling, as increased variability in soil moisture often results in large fluctuations in soil redox

conditions, which in turn stimulate carbon and nitrogen outgassing (Maag et al., 1997; Rustad et al., 2001).

We believe differences observed in hydrology and soil conditions will combine to limit the establishment of vegetation, impact the success of the created wetland, and through feedback loops ensure that successional processes in soils do not occur as the created wetland ages. As such our primary recommendation is to ensure that hydrology and soil conditions in created sites more closely match those of natural reference wetlands. However, it's important to note that our measurements occurred on such a small scale, and in some instances took place in laboratory settings, that to draw definitive conclusions from the data might be erroneous.

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Figure Captions

Figure 2.1: Diagram of the soil respiration sampling equipment. Air is pulled from the chamber directly above the soil surface into the IRGA, which measures CO_2 content. The air is then returned to the chamber. A PAR meter, air temperature probe, and soil temperature probe also gather information.

Figure 2.2: Graphical representation of the water table in the created and natural wetlands observed in this study. For the majority of the year, the water table behaved similarly in both sites, however during the late summer and fall months, depth of the water table increased significantly in the created wetland.

Figure 2.3: Significant correlations existed between depth of the water table and soil moisture measured in samples collected from both the created natural wetland. A positive relationship was observed in both sites, suggesting that at higher water table elevations, conditions of saturation increased significantly.

Figure 2.4: Soil organic matter (SOM) in both sites over the course of the experiment. Significant seasonal differences were observed in both sites, with SOM increasing significantly in the autumn as litterfall inputs increased.

Figure 2.5: Soil bulk density differed significantly between the created and natural wetland, with higher values being observed in the created site. These conditions most likely resulted from site excavation which occurred during construction. Data indicate these differences were not ameliorated over the course of the experiment.

Figure 2.6: Soil C:N ratios varied significantly in the created wetland over the course of the year long sampling period, though they appear to be more constant in the natural site. Higher C:N ratios in the created wetland indicate soil nitrogen limitations which could inhibit plant growth.

Figure 2.7: Analysis of soil C:N in the created wetland, independent of data generated in the natural site, indicate ratios increased significantly over time. These results indicate that nutrient limitations became more pronounced over the course of the experiment and highlight the need for additional organic matter inputs to enhance the establishment of vegetation in created wetland systems.

Figure 2.8: Significant correlations exist between soil organic matter (SOM) and bulk density in both sites. This relationship has been well documented in other studies and indicates that increasing SOM has the potential to alleviate conditions created by high soil bulk density.

Figure 2.9: A positive relationship existed between soil organic matter (SOM) and total carbon at both sites. As SOM increased, there was an observed, significant increase in total soil carbon.

Figure 2.10: Data analysis indicates that as soil organic matter increased, there was a significant increase in total soil nitrogen in both the created and natural wetlands.

Figure 2.11: The relationship between soil organic matter (SOM) and soil C:N varied between the two sites investigated in this study. In the created wetland, a negative relationship existed where As SOM increased, there was a significant decrease in C:N. However, this relationship did not exist in the natural wetland.

Figure 2.12: A significant correlation existed between soil C:N and total nitrogen in both study sites. As C:N increased in both the created and natural wetland, soil total nitrogen rapidly decreased.

Figure 2.13: Contributions of soil microbial respiration to the production of CO_2 in both the created and natural wetlands. In both sites, significant seasonality was observed, with respiration declining in the autumn and winter with decreasing temperatures, and then increasing in the spring, again based on changes in temperature.

Figure 2.14: Along with seasonality, a significant relationship between microbial respiration and soil moisture was observed. As soil moisture increased, rates of respiration decreased in both wetland sites as soil conditions shifted from aerobic to anaerobic.

Figure 2.15: Rates of nitrogen fixation measured in both the created and natural wetland. While there was a large amount of variation in the data, significant differences existed between the rates of nitrogen fixation observed in the created and natural wetlands, with higher rates of fixation being observed in the created site.

Figure 2.16: Data indicated a negative relationship between soil moisture and nitrogen fixation existed in the natural wetland where as soil moisture increased, rates of fixation decreased. However this relationship was not significant in the created site.

Figure 2.17: In the created wetland, soil C:N was positively correlated with nitrogen fixation. However this relationship was not observed in the natural site most likely because there was not enough variation in C:N conditions to influence fixation.

Figure 2.18: Nitrogen mineralization in both sites. Significant spread around the data made drawing conclusions difficult.

Figure 2.19: A positive relationship existed between soil organic matter and nitrogen mineralization in both sites. Though this general trend was observed in the natural site, it was only significant in the created wetland.

Figure 2.20: Data indicated a negative relationship between soil C:N and nitrogen mineralization in both wetland ecosystems. Less available soil nitrogen clearly would result in lower rates of mineralization.

Figure 2.21: Rates of denitrification varied significantly between the created and natural wetland with strong seasonality being observed in soils collected from the created site. Rates of denitrification increased significantly in the created wetland following increased litterfall inputs at the end of the growing season.

Figure 2.22: Significant relationships existed between soil moisture and denitrification in both the created and natural wetlands. This was expected as denitrification only occurs in anaerobic environments.

Figure 2.23: As soil organic matter increased in the natural wetland, rates of denitrification increased significantly, however this relationship was not observed in the created site.

Table Captions

Table 2.1: Results of ANOVA for *in situ* soil conditions and microbial measures, as well as for laboratory incubations in soils collected from both the created and natural wetland.

Figures

Figure 2.1

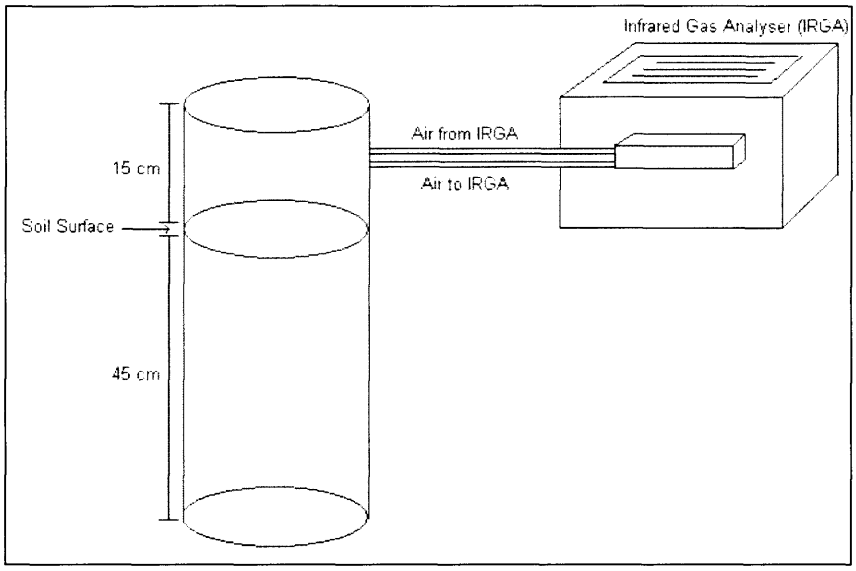


Figure 2.2

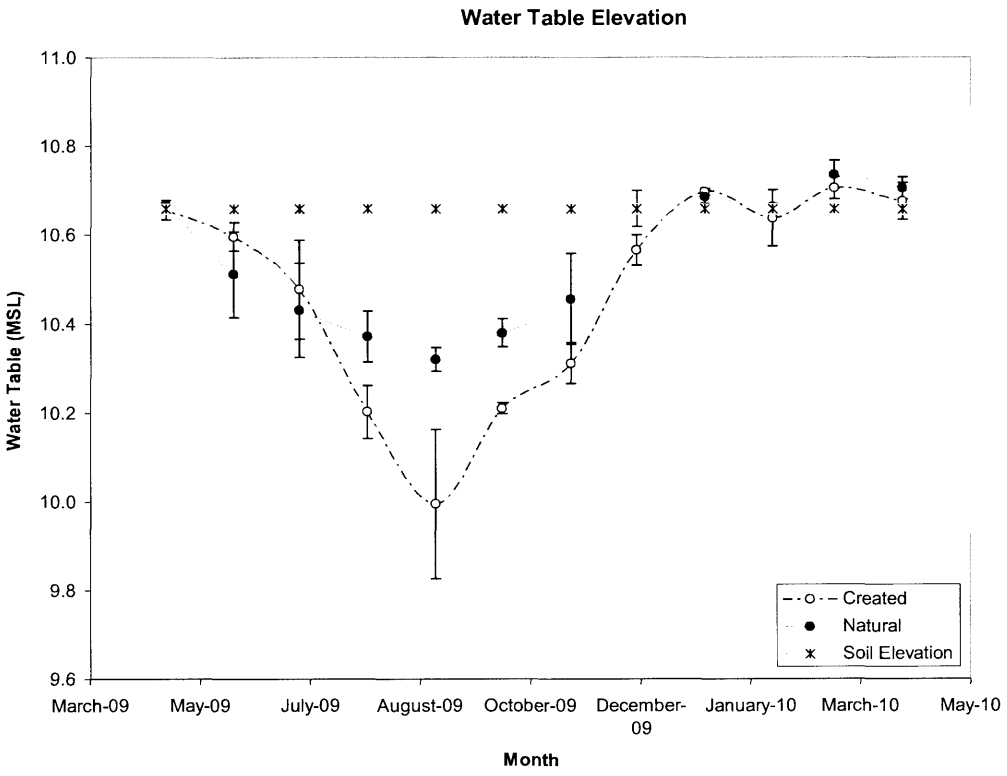

Water Table Elevation
11.0
10.8
10.6
10.4
10.2
10.0
9.8
9.6
Water Table (MSL)
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural
Soil Elevation

Figure 2.3

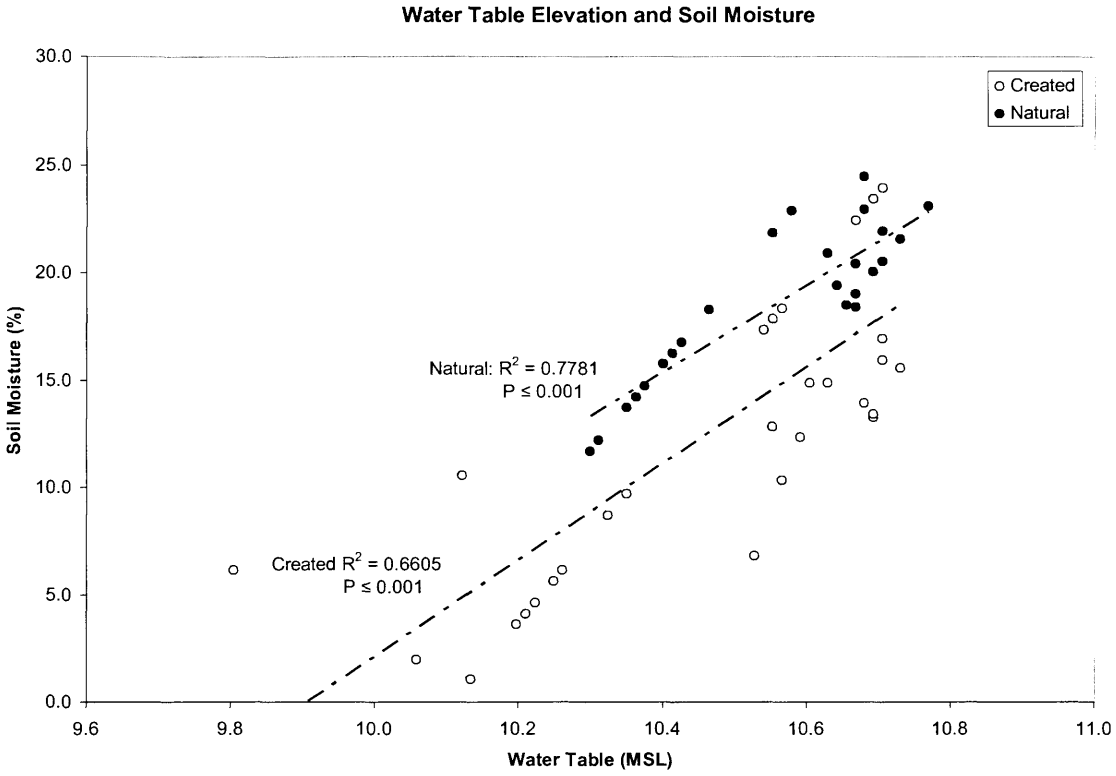

Water Table Elevation and Soil Moisture
Created
Natural
Natural: R2 = 0.7781
P ≤ 0.001
Created R2 = 0.6605
P ≤ 0.001
Soil Moisture (%)
Water Table (MSL)
30.0
25.0
20.0
15.0
10.0
5.0
0.0
9.6
9.8
10.0
10.2
10.4
10.6
10.8
11.0

Figure 2.4

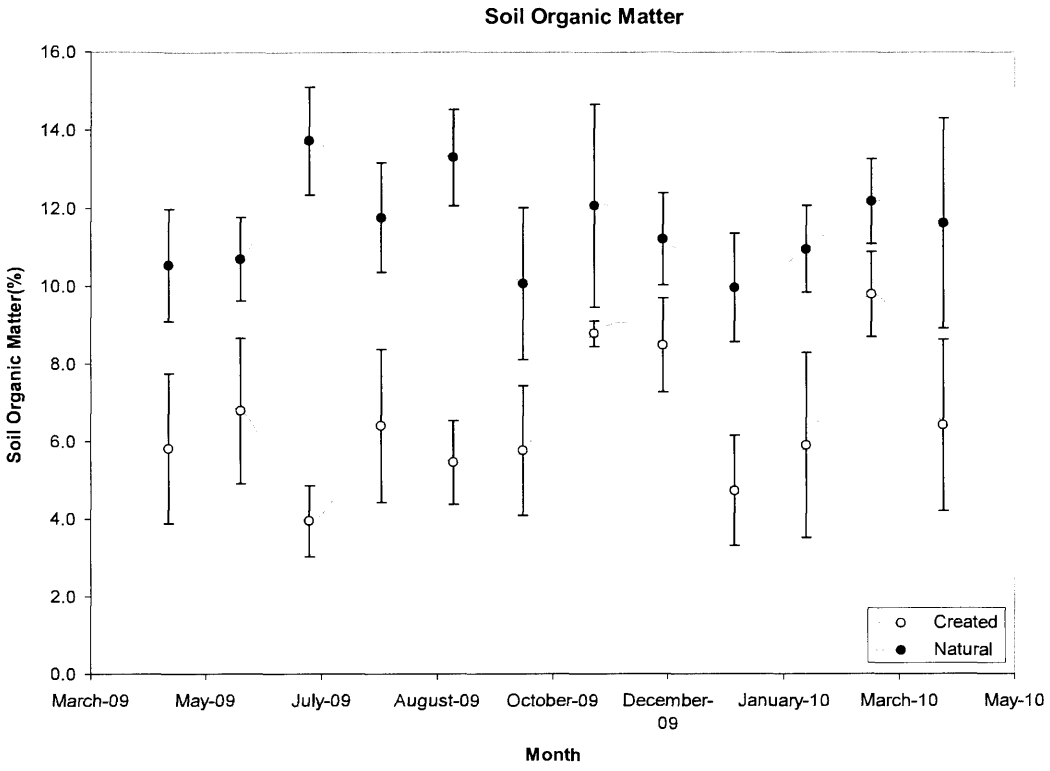

Soil Organic Matter
16.0
14.0
12.0
10.0
8.0
6.0
4.0
2.0
0.0
Soil Organic Matter(%)
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 2.5

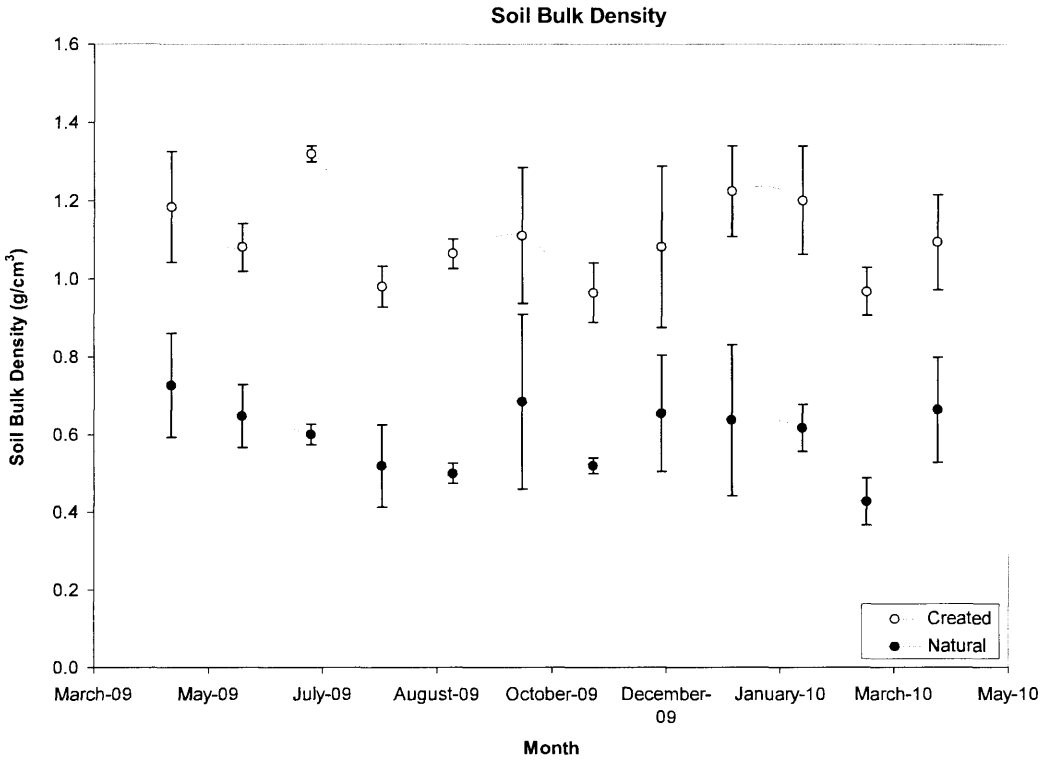

Soil Bulk Density
Soil Bulk Density (g/cm3)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 2.6

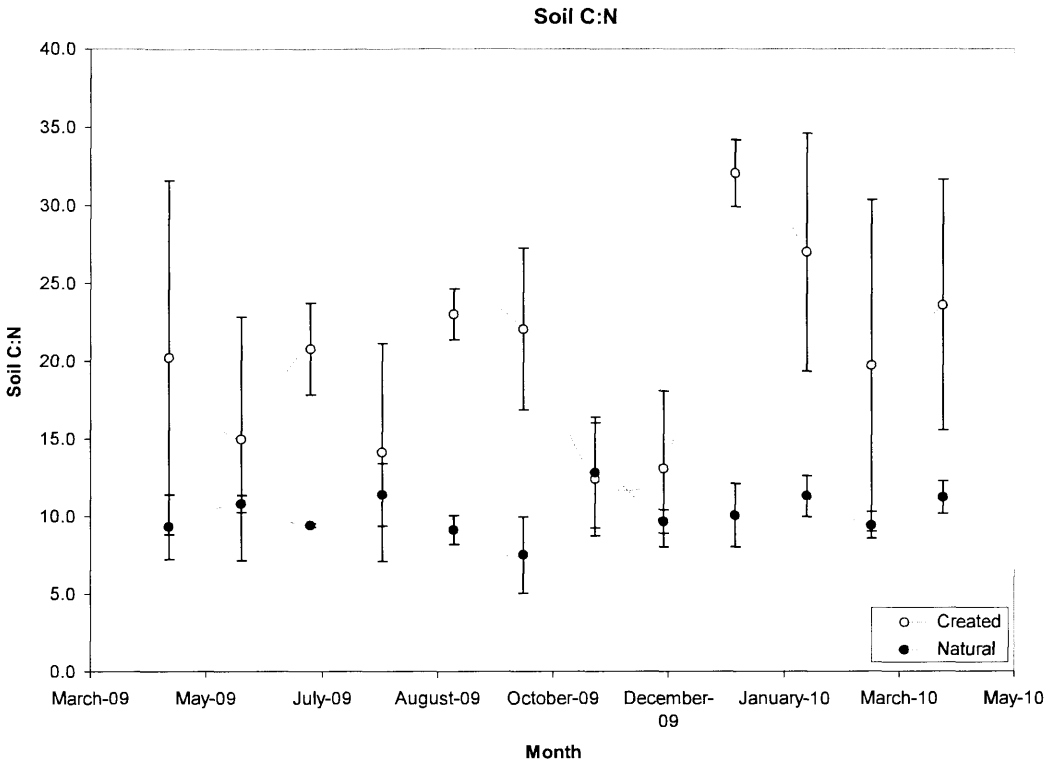

Soil C:N
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Soil C:N
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 2.7

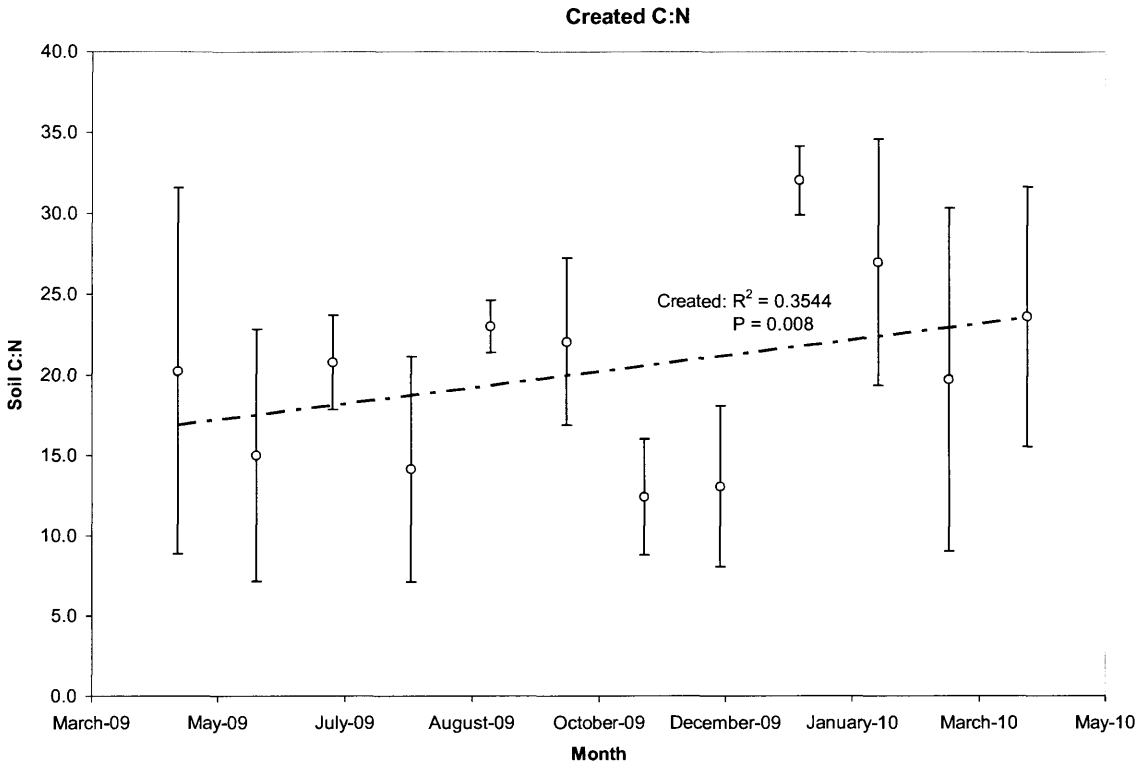

Created C:N
40.0
35.0
30.0
25.0
20.0
15.0
10.0
5.0
0.0
Soil C:N
Created: R2 = 0.3544
P = 0.008
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 2.8

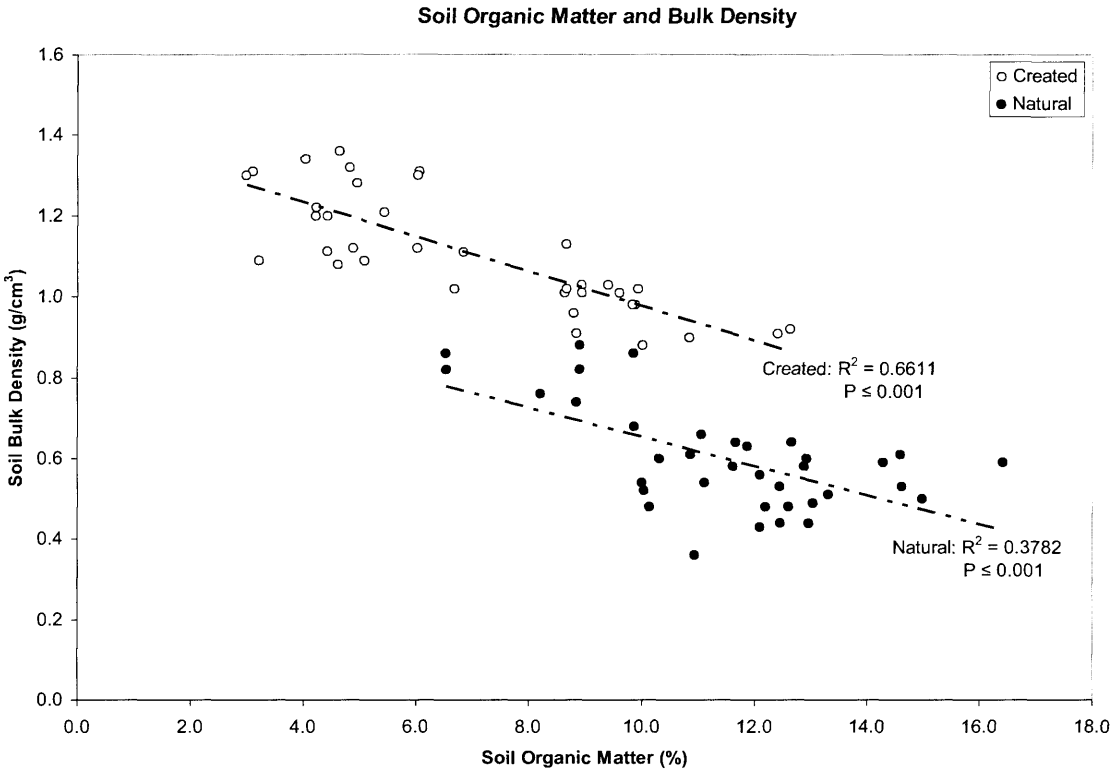

Soil Organic Matter and Bulk Density
Soil Bulk Density (g/cm3)
Soil Organic Matter (%)
Created
Natural
Created: R2 = 0.6611
P ≤ 0.001
Natural: R2 = 0.3782
P ≤ 0.001
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0

Figure 2.9

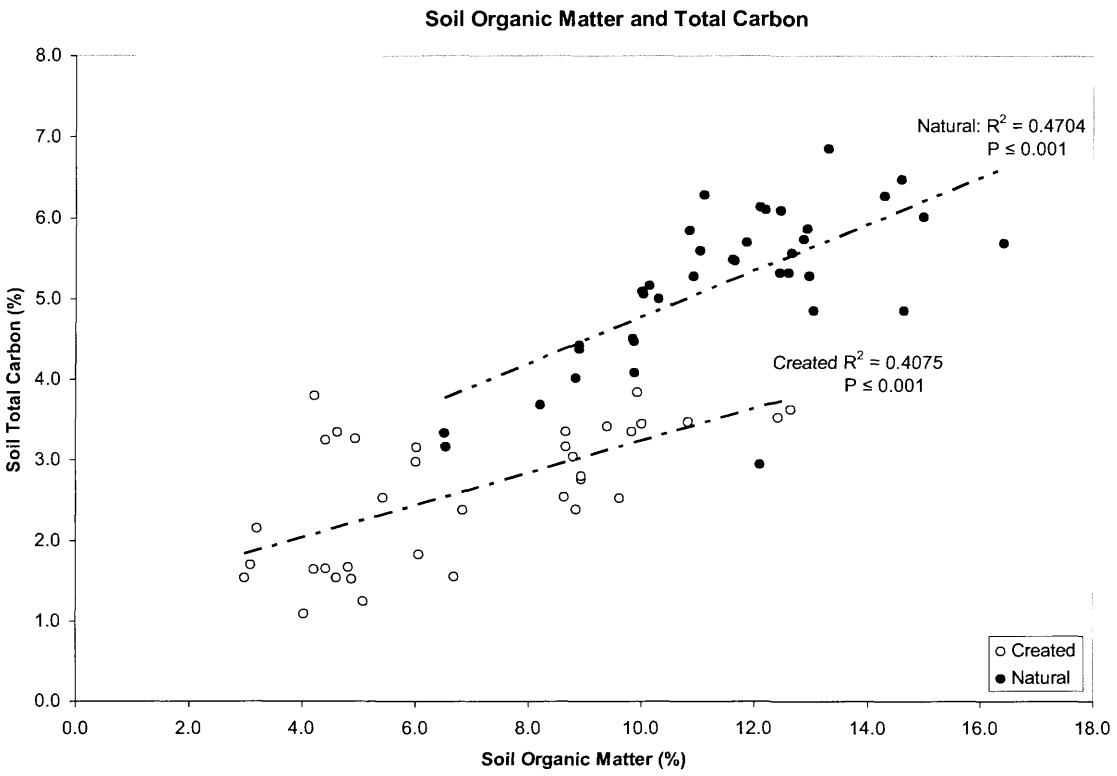

Soil Organic Matter and Total Carbon
Natural: R^2 = 0.4704
P ≤ 0.001
Created R^2 = 0.4075
P ≤ 0.001
Created
Natural
Soil Total Carbon (%)
Soil Organic Matter (%)
0.0
1.0
2.0
3.0
4.0
5.0
6.0
7.0
8.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0

Figure 2.10

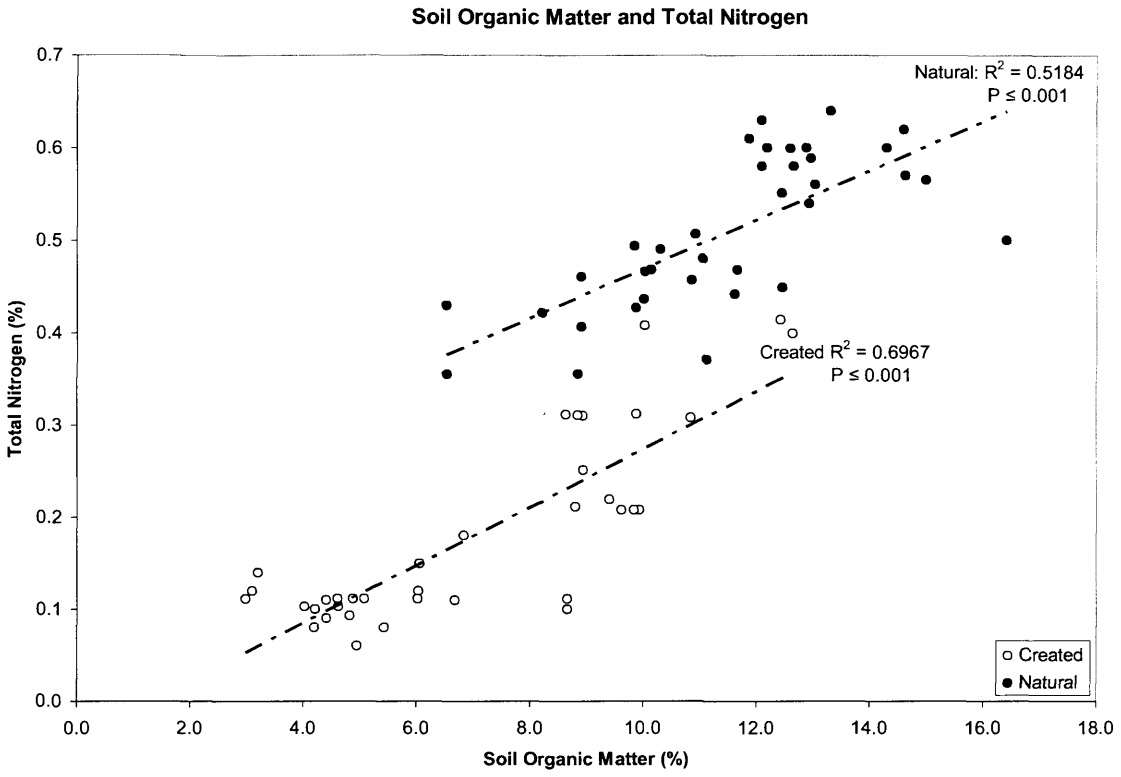

Soil Organic Matter and Total Nitrogen
Natural: R2 = 0.5184
P ≤ 0.001
Created R2 = 0.6967
P ≤ 0.001
Created
Natural
Total Nitrogen (%)
Soil Organic Matter (%)
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0

Figure 2.11

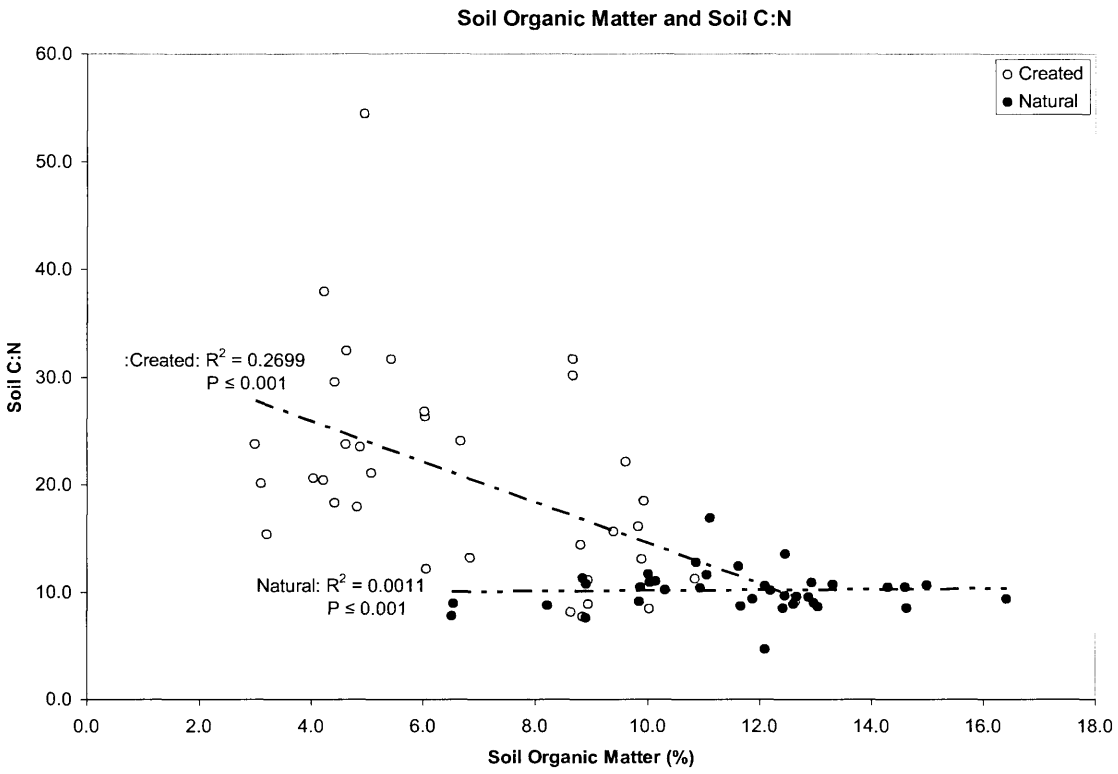

Soil Organic Matter and Soil C:N
Created
Natural
:Created: R2 = 0.2699
P ≤ 0.001
Natural: R2 = 0.0011
P ≤ 0.001
Soil C:N
Soil Organic Matter (%)
60.0
50.0
40.0
30.0
20.0
10.0
0.0
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0

Figure 2.12

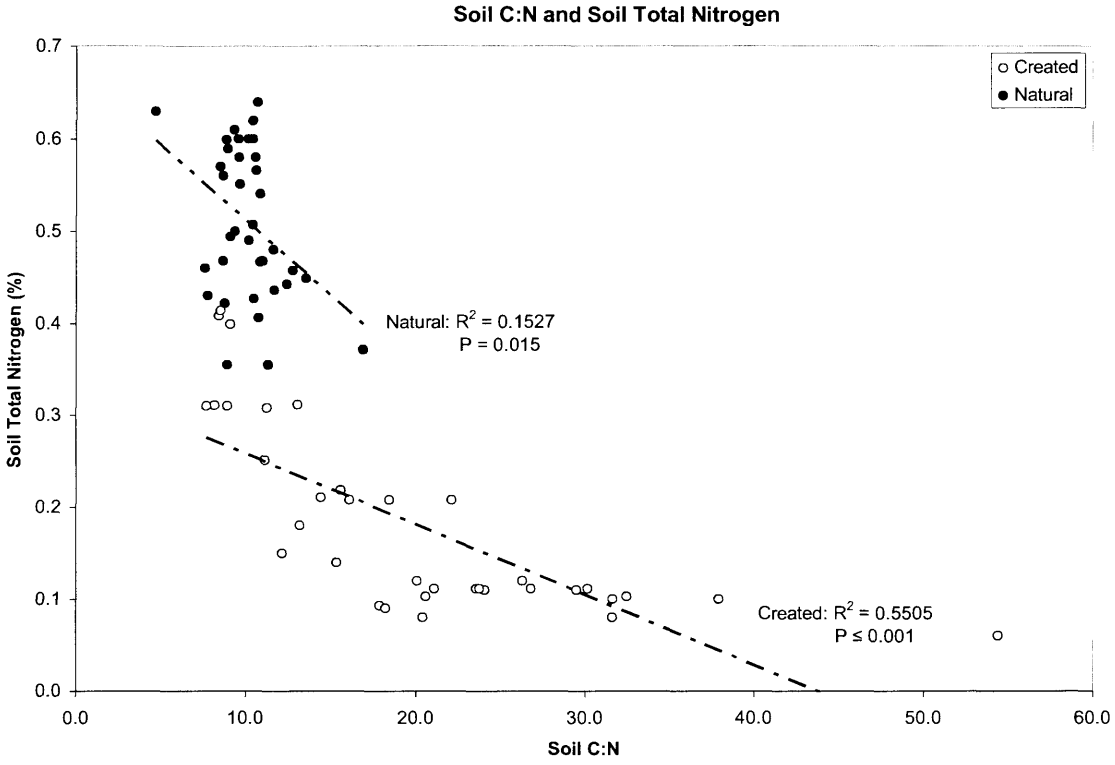

Soil C:N and Soil Total Nitrogen
Created
Natural
Natural: R^2 = 0.1527
P = 0.015
Created: R^2 = 0.5505
P ≤ 0.001
Soil Total Nitrogen (%)
Soil C:N
0.0
0.1
0.2
0.3
0.4
0.5
0.6
0.7
0.0
10.0
20.0
30.0
40.0
50.0
60.0

Figure 2.13

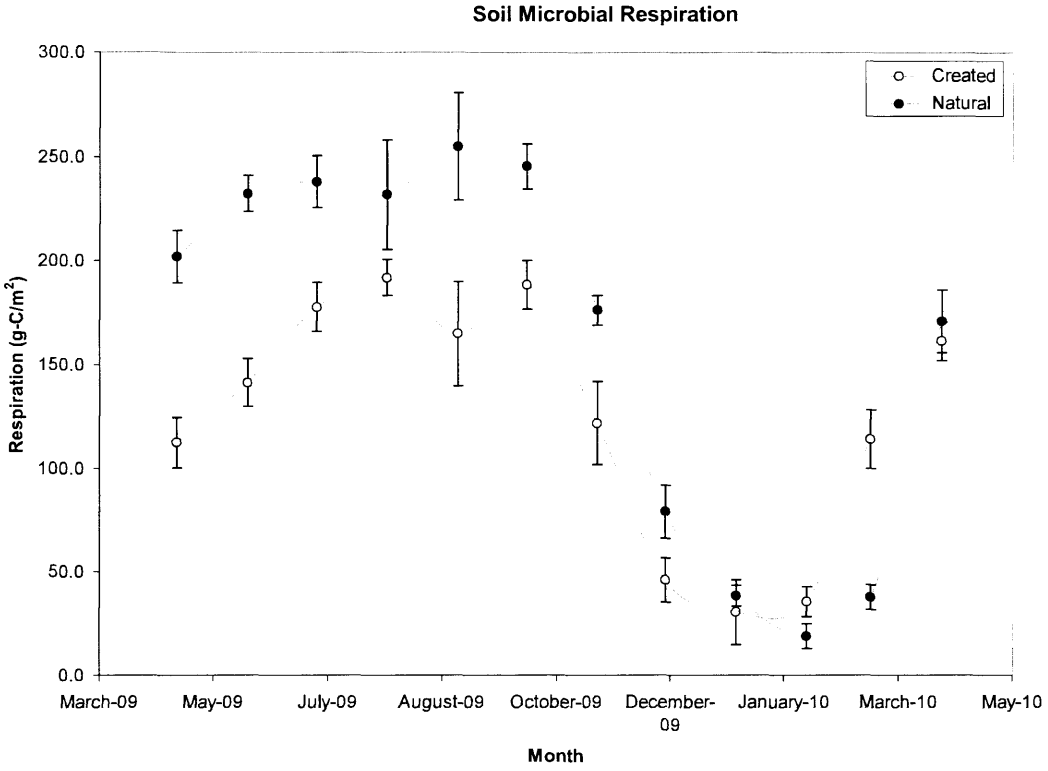

Soil Microbial Respiration
Created
Natural
Respiration (g-C/m2)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 2.14

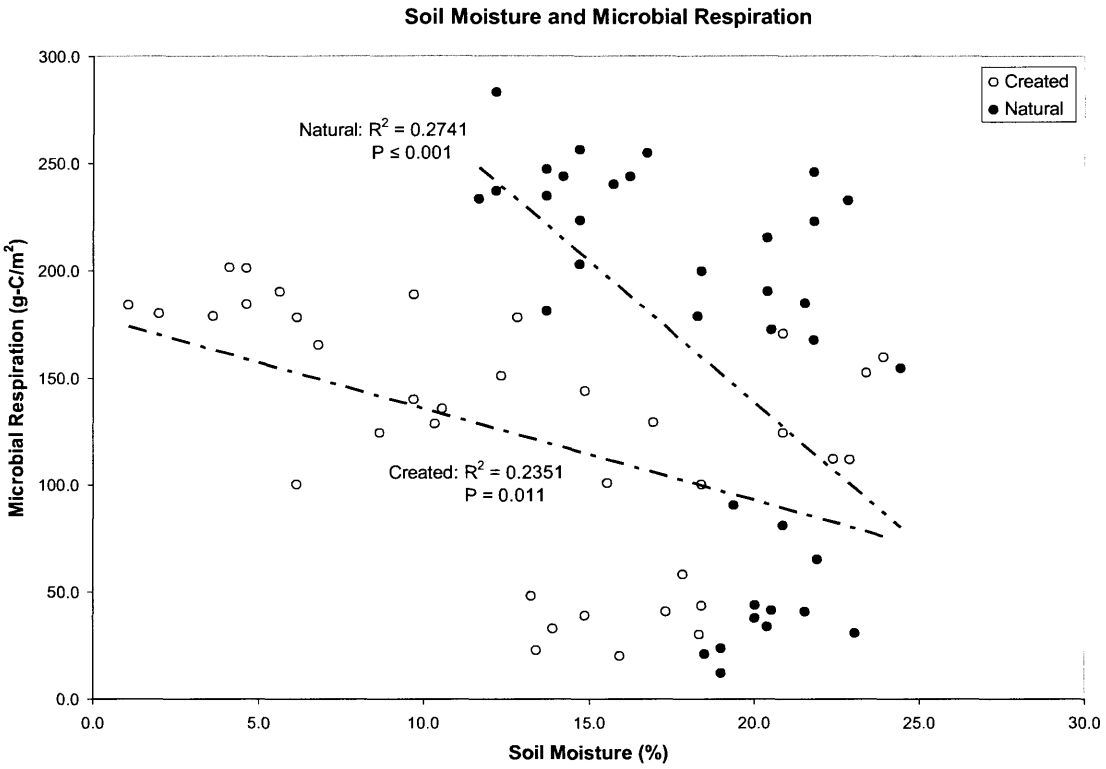

Soil Moisture and Microbial Respiration
Created
Natural
Natural: R2 = 0.2741
P ≤ 0.001
Created: R2 = 0.2351
P = 0.011
Microbial Respiration (g-C/m2)
Soil Moisture (%)
300.0
250.0
200.0
150.0
100.0
50.0
0.0
0.0
5.0
10.0
15.0
20.0
25.0
30.0

Figure 2.15

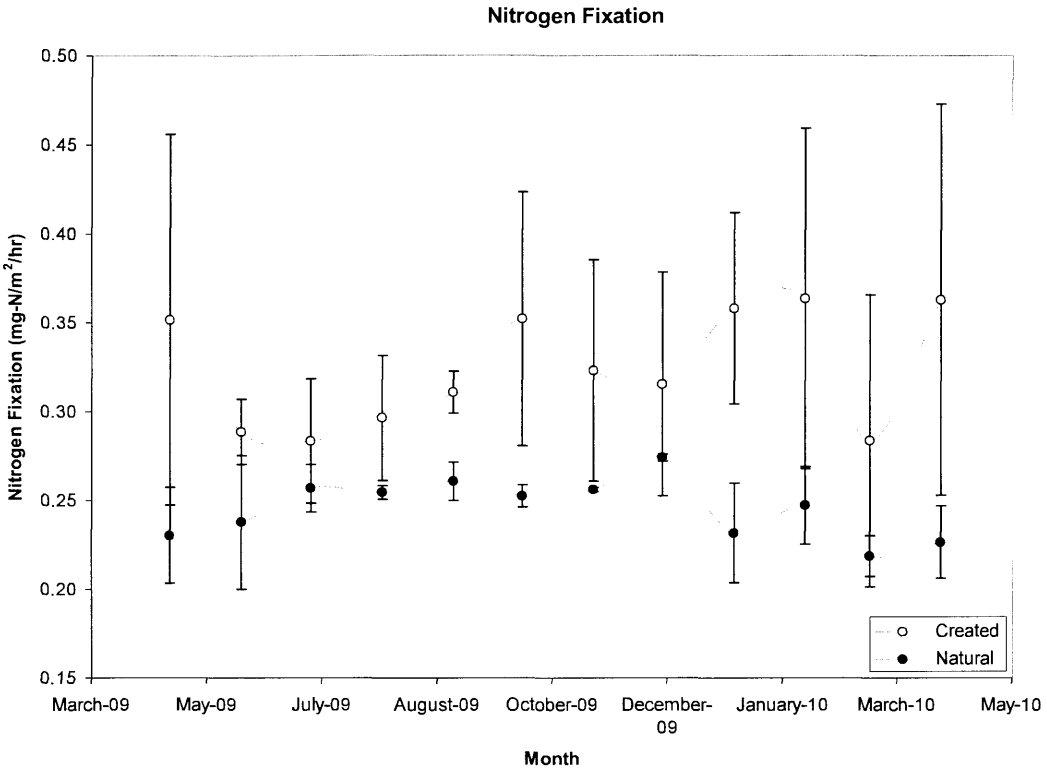

Nitrogen Fixation
Nitrogen Fixation (mg-N/m2/hr)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 2.16

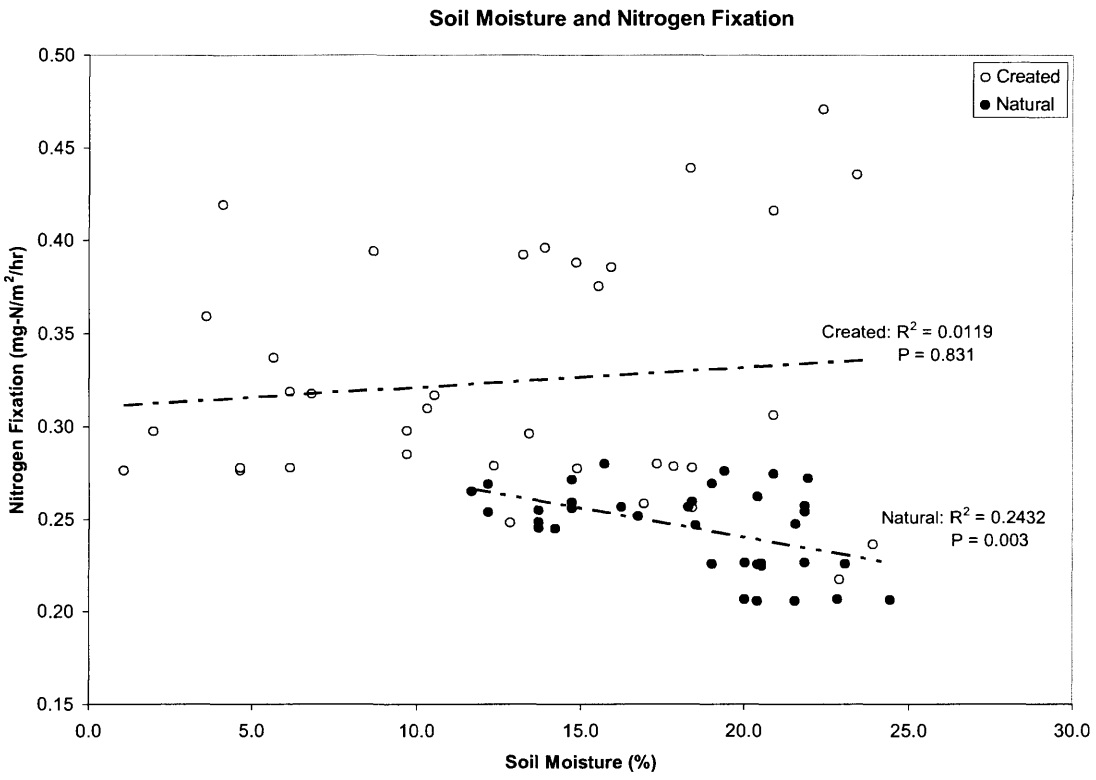

Soil Moisture and Nitrogen Fixation
Nitrogen Fixation (mg-N/m²/hr)
Soil Moisture (%)
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.0
5.0
10.0
15.0
20.0
25.0
30.0
Created
Natural
Created: R² = 0.0119
P = 0.831
Natural: R² = 0.2432
P = 0.003

Figure 2.17

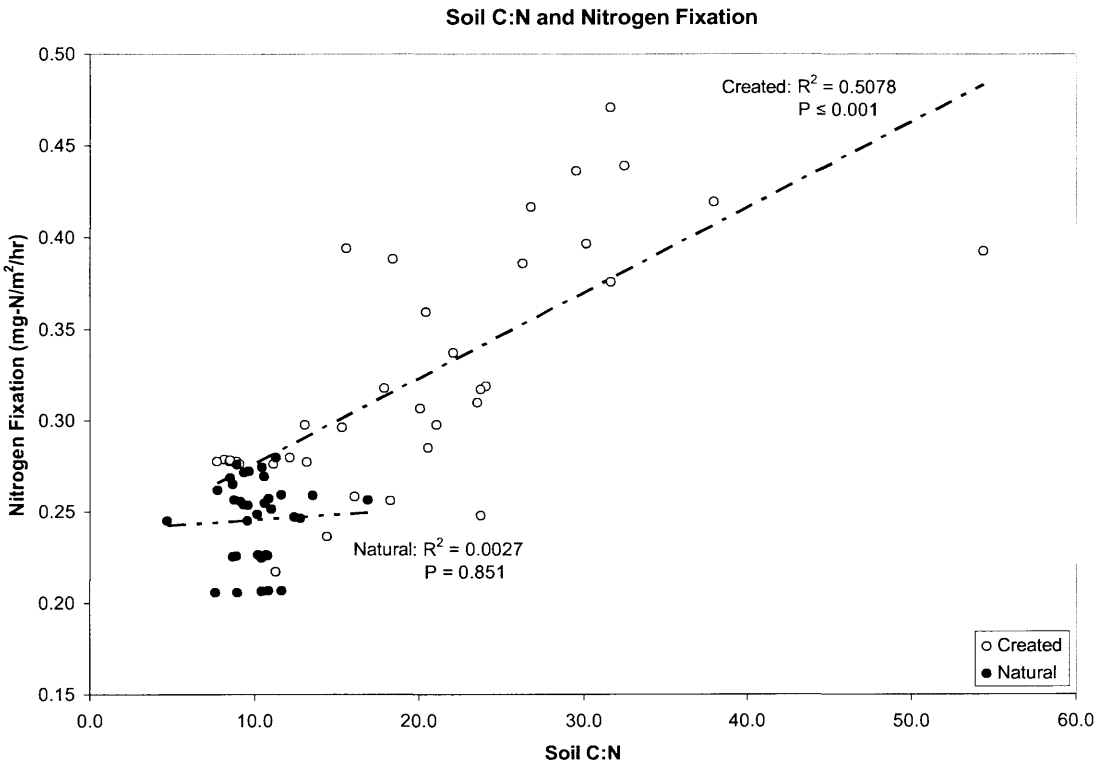

Soil C:N and Nitrogen Fixation
Created: R^2 = 0.5078
P ≤ 0.001
Natural: R^2 = 0.0027
P = 0.851
Created
Natural
Nitrogen Fixation (mg-N/m^2/hr)
Soil C:N
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.0
10.0
20.0
30.0
40.0
50.0
60.0

Figure 2.18

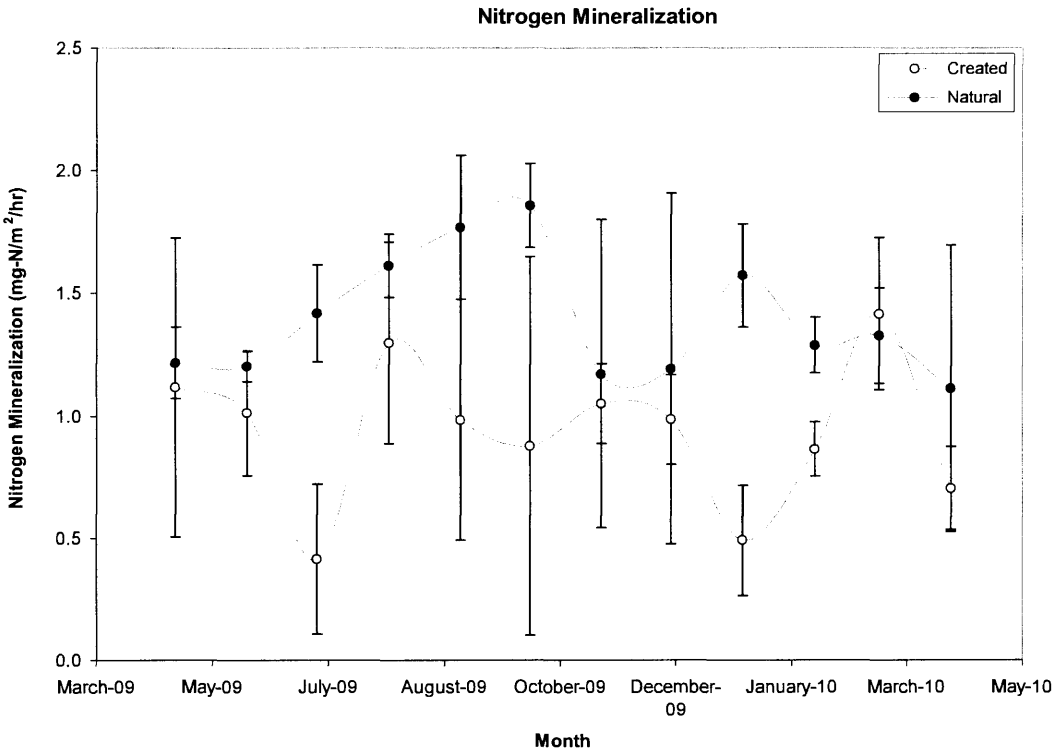

Nitrogen Mineralization
Created
Natural
Nitrogen Mineralization (mg-N/m 2/hr)
2.5
2.0
1.5
1.0
0.5
0.0
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 2.19

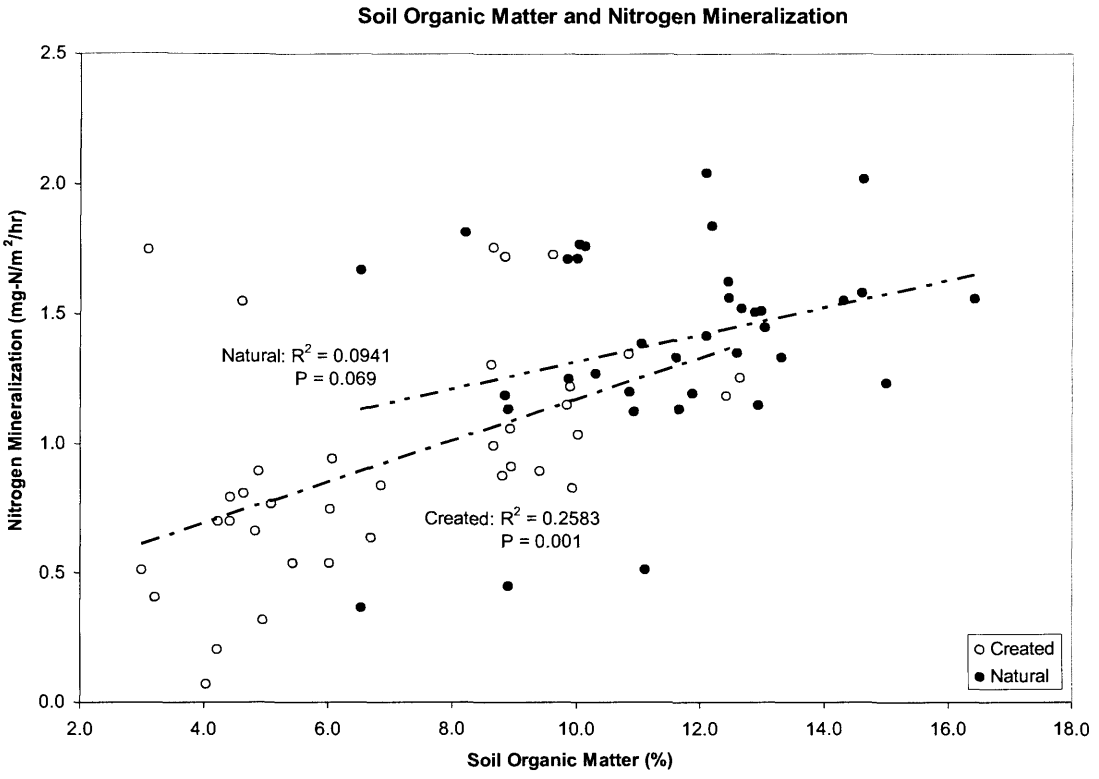

Soil Organic Matter and Nitrogen Mineralization
Nitrogen Mineralization (mg-N/m2/hr)
Soil Organic Matter (%)
Natural: R2 = 0.0941
P = 0.069
Created: R2 = 0.2583
P = 0.001
Created
Natural
2.5
2.0
1.5
1.0
0.5
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0

Figure 2.20

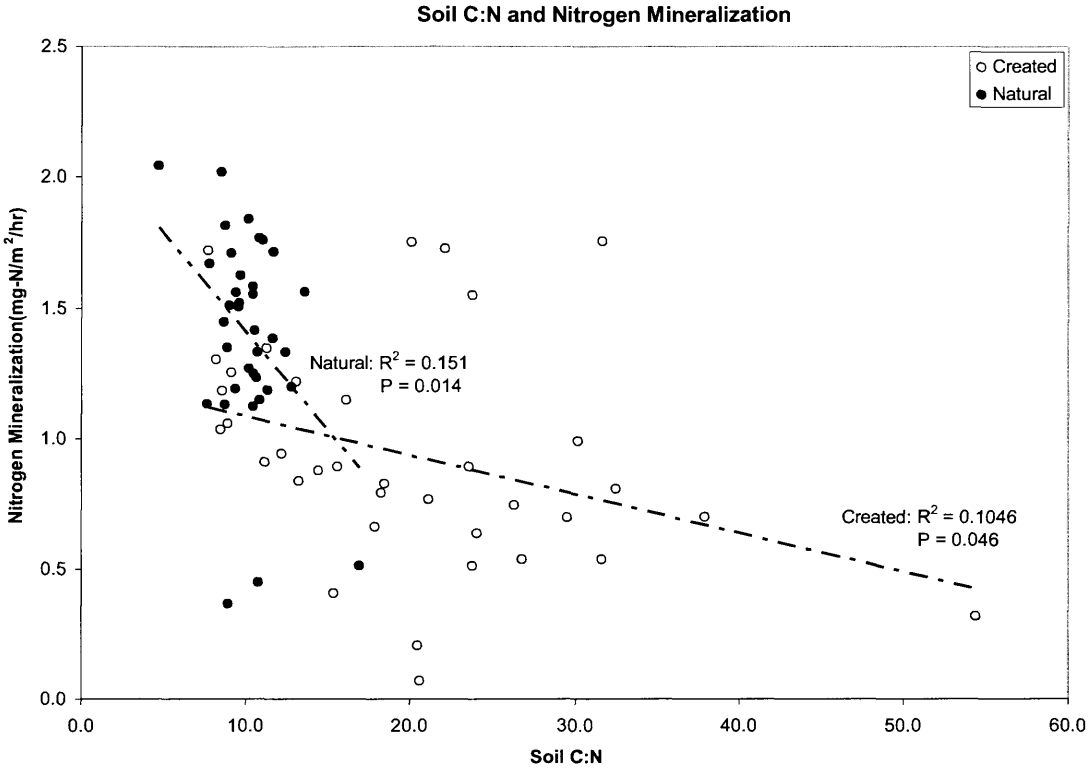

Soil C:N and Nitrogen Mineralization
Created
Natural
Natural: R2 = 0.151
P = 0.014
Created: R2 = 0.1046
P = 0.046
Nitrogen Mineralization(mg-N/m 2/hr)
Soil C:N
2.5
2.0
1.5
1.0
0.5
0.0
0.0
10.0
20.0
30.0
40.0
50.0
60.0

Figure 2.21

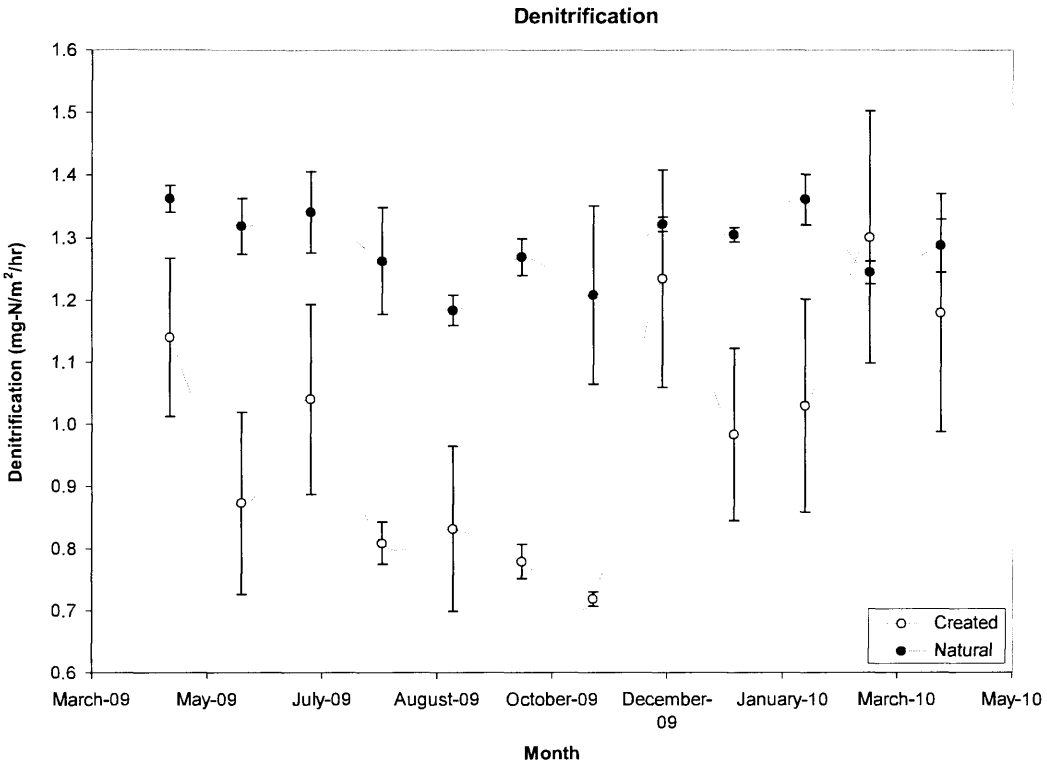

Denitrification
Denitrification (mg-N/m^2/hr)
1.6
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 2.22

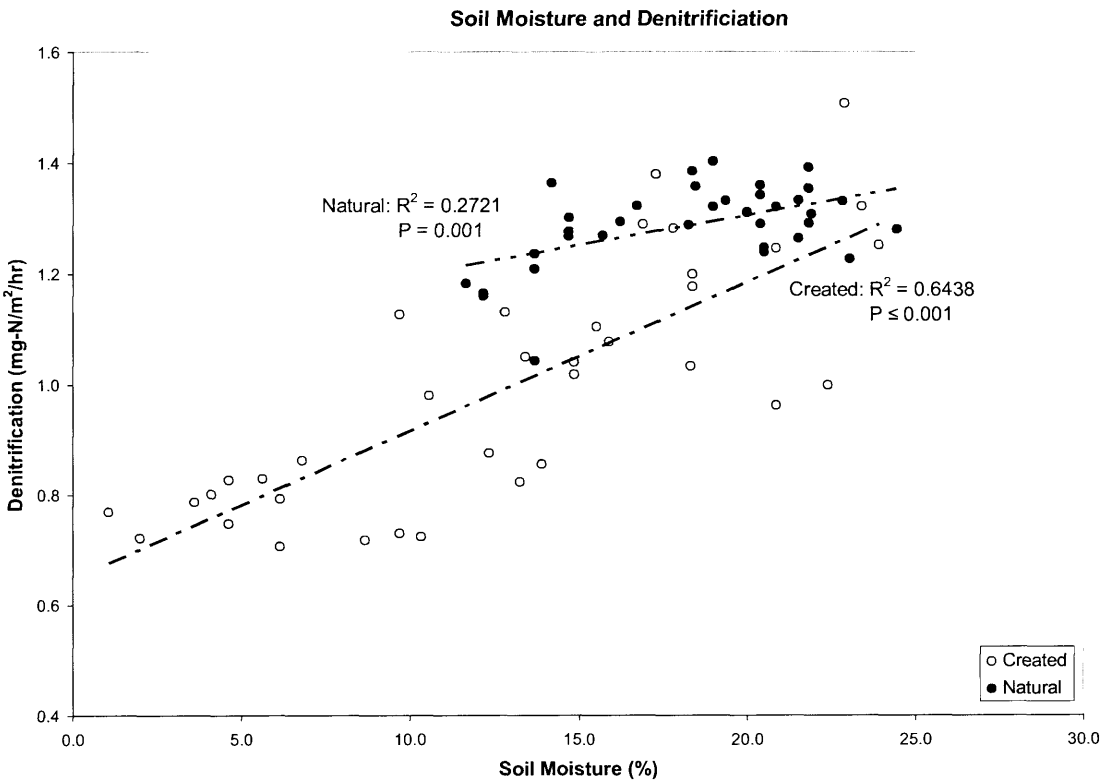
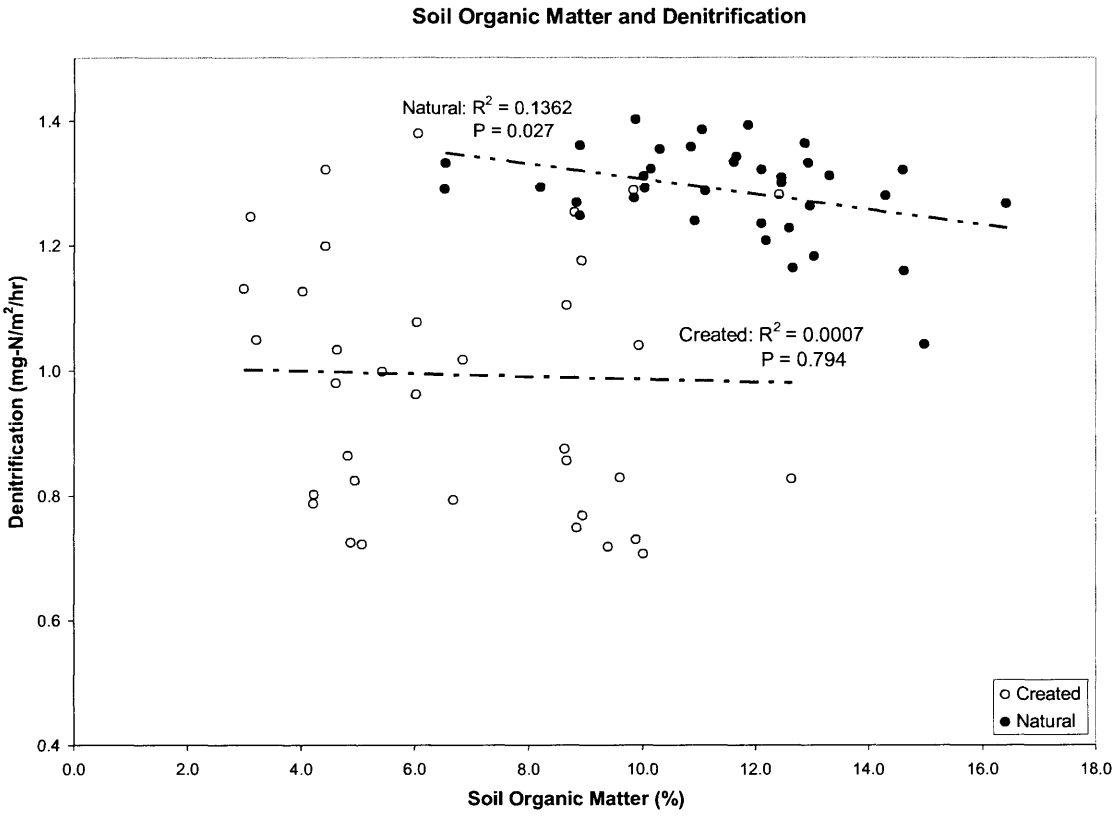

Soil Moisture and Denitrificiation
Natural: R^2 = 0.2721
P = 0.001
Created: R^2 = 0.6438
P ≤ 0.001
Created
Natural
Denitrification (mg-N/m^2/hr)
Soil Moisture (%)
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.0
5.0
10.0
15.0
20.0
25.0
30.0

Figure 2.23


Soil Organic Matter and Denitrification
Natural: R2 = 0.1362
P = 0.027
Created: R2 = 0.0007
P = 0.794
Denitrification (mg-N/m2/hr)
Soil Organic Matter (%)
Created
Natural
0.4
0.6
0.8
1.0
1.2
1.4
0.0
2.0
4.0
6.0
8.0
10.0
12.0
14.0
16.0
18.0

Tables

Table 2.1

Parameter	*Factor*	*df*	*F*	*P*
Water Table	Site	1	23.131	< 0.001
	Month	11	55.781	< 0.001
	Site × month	11	4.973	< 0.001
Soil Moisture	Site	1	92.548	< 0.001
	Month	11	21.640	< 0.001
	Site × month	11	4.113	< 0.001
Bulk Density	Site	1	333.021	< 0.001
	Month	11	3.401	0.00154
	Site × month	11	0.936	0.51512
Organic Matter	Site	1	75.800	< 0.001
	Month	11	2.065	0.0419
	Site × month	11	1.937	0.0575
C:N	Site	1	36.497	< 0.001
	Month	11	0.968	0.487
	Site × month	11	1.252	0.280
Respiration	Site	1	121.939	< 0.001
	Month	11	158.569	< 0.001
	Site × month	11	18.617	< 0.001
Annual Respiration	Site	1	128.741	< 0.001
Nitrogen Fixation	Site	1	43.139	0.790
	Month	11	0.633	< 0.001
	Site × month	11	0.882	0.563
N Mineralization	Site	1	27.593	< 0.001
	Month	11	1.490	0.166
	Site × month	11	1.739	0.092
Denitrification	Site	1	135.893	< 0.001
	Month	11	6.537	< 0.001
	Site × month	11	3.968	< 0.001

CHAPTER THREE:

Vegetation Dynamics and Ecosystem Function in Created and Natural Palustrine, Forested Wetlands

Abstract

Palustrine, forested wetlands are frequently degraded or destroyed as a consequence of development. Under federal legislation, wetlands are often created to mitigate the effects of these actions. However, past studies have shown that created wetlands may not adequately replace all ecosystem functions lost. The objective of this study was therefore to build on earlier research and quantify specific differences between vegetation dynamics and ecosystem function in a palustrine, forested wetland constructed based on current recommendations, and in an adjacent natural wetland. Vegetation dynamics were defined as the accumulation of above- and below-ground biomass, nutrient uptake, community composition, and net community ecosystem exchange of carbon dioxide. Results indicate above- and below-ground biomass production and nutrient uptake were significantly lower in the created wetland, likely as a result of environmental conditions. Additionally, results show the created wetland was less diverse in terms of richness, evenness, and the Shannon index. However, being an early successional system, the created wetland featured higher levels of primary productivity and sequestered more carbon during the growing season, and over the course of the year long sampling period than the natural wetland. However these results are far from conclusive as measurements only occurred during the first year following construction. Our data therefore highlight the need for more exhaustive investigations of these processes in created wetlands as they age.

Introduction

Ecosystem function in wetland plant communities is characterized as the balance between nutrient uptake and retention, size of the standing biomass crop, and the ability of a system to maintain stability despite environmental stress (Odum, 1969). More recent research has refined this definition to include high levels of diversity and community primary productivity as other important functions of wetland plant communities (Balcombe et al., 2005).

Ecosystem function is typically lower in created wetlands than in natural systems. This dynamic exists because in most cases, wetland construction practices remove all existing vegetation, soils, and seed banks, rendering most created wetlands primary successional ecosystems replete of organic matter (van der Valk, 1981; Brinson and Rheinhardt, 1995; Whittecar and Daniels, 1999; Bruland and Richardson, 2004). These observed low levels

of organics often pose significant obstacles to both the establishment of vegetation, and to the development of a fully functioning plant community (Atkinson et al., 2005). In natural wetlands, the accumulation of organic matter in the soil matrix acts to mitigate perturbations caused by the physical environment, thereby contributing to successional processes which lead to the development of a healthy ecosystem (Stolt et al., 2000; Bruland and Richardson, 2004). In created systems, however, the opposite trend is observed, as low levels of soil organic matter often significantly hinder vegetational function (Bergshneider, 2005; Daniels et al., 2005). Likewise, the lack of a viable seed bank in created systems poses a significant impediment to the establishment of a fully functioning community. The removal of all *in situ* seed banks makes created sites dependent upon natural or artificial introduction of species from external sources (Dunne and Samanns, 1998). These factors often combine to negatively influence vegetational function in wetlands created for mitigation purposes (DeBerry and Perry, 2004).

In the past, ecosystem function in plant communities has been compared along an assortment of varying environmental gradients, including created and natural systems, and ecosystems at different successional stages using traditional practices such as the measurement of plant cover, density, standing crop biomass, and nutrient uptake by plants (Atkinson et al., 1993; Hooper and Vitousek, 1997; McKenna, 2003). In wetland mitigation projects, the use of reference sites during the initial monitoring phases following site construction is particularly useful as a means to compare ecosystem processes in less disturbed systems with those in sites targeted for restoration (Kentula et al., 1992; Brinson and Rheinhardt, 1996; Fule´ et al., 1997; Palik et al., 2000). Reference sites also provide an important metric of natural function which can be used to evaluate the success of created systems (Balcombe et al., 2005).

Because the successful establishment of plant communities plays such a crucial role in developing ecosystem function in created wetlands, understanding the variables driving the structure of plant communities in these systems is necessary to understand the differences in function often observed between created and natural wetlands (DeBerry

and Perry, 2004; Bailey et al., 2007). Many factors influence which plant species are found in a particular wetland. In created wetlands, the species pool is composed of species present in the seed bank, species able to disperse into the wetland via natural methods, and species anthropogenically introduced (van der Valk et al., 1978; Jurik et al., 1994; Zobel et al., 1998; Casanova and Brock, 2000; Mahaney, 2001). After initial introduction, abiotic and biotic environmental factors then act to influence individual performance and species abundance in the plant community (van der Valk, 1981; Zobel et al., 1998; Mahaney, 2001). Environmental factors therefore often act as filters which select what species constitute a wetland plant community (van der Valk, 1981; Keddy, 1992). Using this principle, past studies have created a set of predictive 'assembly rules' that define this relationship based on life history traits (Gaudet and Keddy, 1988; Weiher and Keddy, 1995; Grime, 1998). These life history traits help indicate how a specific species, or group of species, will respond to variations in hydrology and soil conditions (van der Valk, 1981). This technique represents a fundamentally different approach than methods that attempt to predict community structure based on immigration success, and is perhaps more relevant to studies of created wetlands given that most plant communities in these systems are anthropogenically introduced and not limited by dispersal. In wetland creation, where immigration rates are of less importance, it is the therefore the ability of propagules and introduced plants to tolerate the specific environmental factors of a site that determines the success of an individual plant, the presence or absence of a species, overall community composition, and ecosystem function (Keddy and Constabel, 1986; Leck et al., 1989; Britton and Brock, 1994). For example, in wetland creation, a large number of propagules may be introduced, but only a few may be able to tolerate the conditions at the site (Jurik et al., 1994; Dittmar and Neely, 1999). A strong emphasis in created wetland studies therefore needs to be placed on the way in which site characteristics such as hydrology and nutrient availability filter out individuals and small subsets of species, thereby impacting community assembly.

One environmental parameter that acts as a major determinant of plant community development in created wetlands is hydrology. Site hydrology can be described by its

depth, duration, frequency, rate of filling and drying, and timing and predictability of flooded and dry phases (Bunn et al. 1997). Alternating wet and dry periods affect plant establishment by stimulating or inhibiting germination, by modifying oxygen availability in the rhizosphere, by influencing concentrations of nutrients and toxic substances in the soil, and by desiccating aquatic plants or inundating terrestrial plants (Mitchell and Rogers, 1985; Brock and Britton, 1995; Bornette and Amoros, 1996). These characteristics can affect species richness by creating establishment opportunities for species and preventing competitive exclusion consistent with the Intermediate Disturbance Hypothesis (Connell, 1978; Galatowitsch and van der Valk, 1994; van den Brink et al., 1995; Bomette and Amoros, 1996; Seabloom et al., 1998). Research has also shown that decreases in the groundwater table may cause an increase in nutrient availability by accelerating mineralization rates in the soil, which could stimulate both above- and below-ground production (Black, 1968; Etherington, 1975).

The success of planted species in created wetlands influences: 1) the timing, rates and pathways of resource use, 2) vegetation effects on the physical environment, and 3) interactions with other species (Connor and Simberloff, 1979; Parrish and Bazzaz, 1982; Drake, 1991). Thus, varying levels of success in introduced species may alter ecosystem processes such as nutrient cycling and retention, productivity, decomposition, community composition, and ultimately ecosystem function (Vitousek 1986; Chapin et al. 1997; Vitousek 1997; Tilman et al. 1997). Developing a better understanding of how introduced species respond to *in situ* conditions in created wetlands is therefore essential to predicting how created systems differ from natural wetlands with regards to success in achieving ecosystem function. Traditional practices such as the measurement of standing crop biomass, nutrient uptake, and diversity, along with more recent techniques which measure carbon dioxide (CO_2) gas fluxes can be used to accomplish this (Hooper and Vitousek et al., 1997; Bubier et al., 1998; Wickland et al., 2001; Balcombe et al., 2005). These techniques evaluate ecosystem function in created and natural wetlands along a gradient of differing environmental parameters in order to determine how and why function differs in the two ecosystems.

Carbon flux gas measurements are particularly useful and measure: carbon uptake (community production, gross primary production, or photosynthesis); carbon emissions (total plant and soil respiration); and their net difference (net ecosystem exchange, NEE) (Roggero, 2003; Bailey et al., 2007). Unlike simple biomass harvesting or community metrics, these techniques incorporate the activities of both plants and soil to measure ecosystem gas exchange for the community, so that both above- and below-ground production and respiration are captured (Streever et al., 1998; Roggero, 2003; Bailey et al., 2007). One purpose for NEE measurements in wetlands is to quantify the successional state of these systems. Past research has shown that as ecosystems mature, energy use shifts from a high biomass production, net autotrophic regime (photosynthesis (P) / respiration (R) ratio >1), to a more balanced state where the majority of resources are devoted to ecosystem maintenance (P/R = 1) (Odum 1969). Based on this concept of bioenergetics, one could theoretically use the P/R ratio as a functional index of the relative maturity of ecosystems, as well as a metric for quantifying ecosystem function in plant communities; however, very few studies have actually utilized this model in ecosystems research, especially in wetlands ecology (Roggero, 2003; Bailey et al., 2007). The few studies that have utilized carbon flux techniques in wetland environments either compared the flux rates of different wetland ecosystem types, or carbon flux in one wetland system along gradients of vegetation, soil moisture, trophic status, or managed changes in water levels (Bridgham and Richardson, 1992; Bubier et al., 1998; Clark et al., 1999). Additionally, while some studies have occurred in both freshwater tidal systems, and created salt marshes, the use of NEE measurements as an indicator of successional state and ecosystem function remain notably absent from studies pertaining to non-tidal freshwater wetlands created for mitigation purposes (Roggero, 2003).

The primary objective of this study was to use traditional measures of plant communities, in conjunction with more recently developed carbon flux techniques, in order to quantify vegetation dynamics in a palustrine, forested wetland constructed based on current recommendations, and in an adjacent natural wetland. Vegetation dynamics were defined

as the accumulation of above- and below-ground biomass, nutrient uptake, community composition, and net community ecosystem exchange of carbon dioxide.

Materials and Methods

Adjacent created and natural wetlands were identified in January 2009, and studied from May 2009 through April 2010 to determine: 1) if differences exist in ecosystem function in natural and created wetlands, and 2) what environmental parameters influence vegetation dynamics in the two sites.

Experimental Design: The experiment consisted of two treatment groups: 1) a created palustrine, forested wetland, and 2) a similar adjacent natural wetland. Treatments were first identified and observed in January 2009. In each treatment, a grid consisting of fifty plots was created. Plots were numbered created one through fifty (C_1-C_{50}), and natural one through fifty (N_1-N_{50}). All plots in the two treatments were subjected to natural hydrologic and soil conditions. A single bareroot *Betula nigra* seedling was planted in each plot in March 2009. Seedlings were also numbered created one through fifty (C_1-C_{50}), and natural one through fifty (N_1-N_{50}) corresponding to the plots in which they were planted. Data were collected in each treatment from May 2009 through April 2010.

Wetland Field Sites: The same created-natural wetland complex which was used for earlier research investigating microbial activity in wetlands was also utilized for this experiment. A full description of this site can be found in the methods section of the afore mentioned research on microbial processing rates.

Tree Seedlings: *Betula nigra* was selected for this experiment because of its wetland indicator status (FACW) and common use in wetland mitigation projects (USDA Natural Resources Conservation Service, 2008). Bare-root planting stock was used because it lacks a root ball and the associated microbial microfauna. Any observed differences in plant function and site net microbial activity may therefore be attributed to the

differences in the two environments, and not growth technique, transplanted soil, or imported microbial communities.

Betula nigra (River Birch) is a deciduous tree that reaches 25 m to 30 m in height, with a stem diameter of 50cm to 150cm (USDA Natural Resources Conservation Service, 2008). *Betula nigra* commonly features multiple trunks, with highly variable, dark gray or brown, scaly bark which sometimes exfoliates in papery sheets. Leaves are alternate, serrated, and oval; they range from 4 cm to 8 cm in length and 3 cm to 6 cm in length (Radford et al., 1968; Grimm, 1983). *Betula nigra* is native to the eastern United States and ranges from New Hampshire south to northern Florida and west to southern Minnesota and eastern Texas (USDA Natural Resources Conservation Service, 2008). *Betula nigra* is most often found growing in riparian zones, floodplains, wetlands and other habitats featuring moist alluvial soils in the state of Virginia (Radford et al., 1968; Grimm, 1983).

Sampling: Sampling began five months after the final stages of site creation concluded ($T_{5\ months}$), and three months after all *Betula nigra* seedlings were planted. Sampling then occurred again every month ($T_{6\ months}$, $T_{7\ months}$,...$T_{16\ months}$) for a 12 month sampling period. The five months elapsed time between the final stages of site construction and sampling, and the three months elapsed time between planting and the first sampling point, were planned in order to give the system time to equilibrate, and seedlings time to adjust to their new environment before measurements began.

Each month, hydrologic conditions were measured in three ground water wells installed in the created wetland, and in three ground water wells installed in the natural wetland. Additionally, each month, three randomly selected plots from the created wetland, and three randomly selected plots from the natural wetland were also chosen for soil and vegetation sampling. The seedlings within the plots were measured for above- and below-ground biomass accumulation and nutrient uptake in plant tissues, while community composition was measured in the 0.5 m^2 area around the selected seedlings.

Community net ecosystem exchange of carbon dioxide (CO_2) was also measured in the 0.5 m^2 plot centered around the selected seedlings.

This experimental design yielded three replicates per treatment group at each monthly sampling point for all measures of vegetative function. Methods regarding the measurement of the specific parameters constituting vegetative function are outlined in the following sections.

Hydrology: Hydrologic data generated for earlier research, which investigated microbial activity in the same created and natural wetlands used in this experiment, were utilized for analysis in order to assess the impacts of environmental parameters on vegetation and ecosystem function. A full description of these methods can be found in the afore mentioned research on microbial processing rates.

Soil Characteristics: Soil characteristics measured in the earlier research project looking at microbial activity were also utilized for analysis in this experiment. In the earlier experiment, soil data were generated each month, in three randomly selected plots from the created wetland, and in three randomly selected plots from the natural wetland were chosen for sampling. In this experiment, plots where soil sampling occurred were paired with *Betula nigra* seedlings such that soil characteristics were measured in soils directly adjacent to the selected seedlings each month. This ensured that soil characteristics could be matched with vegetation dynamics and ecosystem functions both spatially and chronologically. Soil characteristics were measured in cores. Soil cores were removed from selected plots, returned to the laboratory, and analyzed for: soil moisture, soil bulk density, soil organic matter, total carbon, and total nitrogen.

Biomass Accumulation: Above-ground biomass was quantified by measuring: 1) total plant height, 2) main stem diameter, and 3) crown diameter. Morphometric characteristics were selected based on work done Bailey et al. (2007) which showed these metrics were the best indicator of growth. Below-ground biomass was quantified by

measuring dry weight of root structures. Total plant height was measured from the soil surface to the top of the plant using a standard meter tape. Main stem diameter was measured at the base of the main stem using micro-calipers. Crown diameter was measured at three different angles at the visual diameter maximum using macro-calipers and then averaged to determine the final crown diameter. Finally, dry weight of below-ground biomass was measured by sacrificing the plant after above-ground vegetation had been sampled, and quantifying the dry weight of below-ground root structures. Dry weight was calculated by drying the roots in an oven for a 48 hour period at 30°C.

Total plant height, main stem diameter, and crown diameter were measured immediately after planting in all individuals to create a baseline against which future growth measurements could be compared. A baseline for below-ground biomass was also created by sacrificing 15 *Betula nigra* seedlings not planted in the field treatments. In these individuals, below-ground biomass was quantified by measuring dry weight of root structures using the method previously described. The average of these 15 values was then used as a baseline against which all future measurements of below-ground biomass were compared.

Monthly measurements of morphometrics were converted to percent change relative to initial values using the following equation:

$$\text{Percent Change} = [(\text{Growth}_{\text{final}} - \text{Growth}_{\text{initial}}) / \text{Growth}_{\text{initial}}] \times 100$$

Morphometrics were analyzed individually, as height, diameter, canopy cover, etc. over month and site, and also converted to relative importance values of total tree growth (RIV_g). Relative importance values were calculated as the average of all measured morphometrics and were also compared to see how total growth differed in seedlings planted in the two wetland types. RIV_g were also converted to mean monthly growth rates by dividing the calculated RIV_g by the total number of months since the seedlings

had been planted. This was done in order to see how growth rates differed over the course of the experiment in the two sites.

Nutrient Uptake: Nutrient uptake was quantified by measuring carbon to nitrogen (C:N) ratios in plant tissues. To measure C:N ratios, stems were destructively harvested after above- and below-ground biomass and net ecosystem exchange of CO_2 had been measured. Stems were analyzed for C:N ratios via dry combustion on a NC2100 controlled combustion elemental analyzer (ThermoQuest Italia S.p.A.), values were then compared over time in both treatment groups. Monthly measurements were also converted to percent change relative to initial values. The initial baseline value for C:N ratios in seedlings was created by sacrificing 15 *Betula nigra* seedlings not planted in the field treatments. In these individuals, C:N ratios were quantified using the method previously described. The average of these 15 values was then used as a baseline against which all future measurements of individual C:N ratios were compared. Finally, percent change values were converted to rates of nutrient uptake by dividing C:N ratios by the total number of months since the seedlings had been planted.

Community Composition: Community composition was measured via the Releve Method using a modified Braum-Blanquet cover scale (Daubenmire, 1959; Atkinson et al., 1993; DeBerry and Perry, 2004). This technique provided an approximate measure of vegetative cover by visually estimating percent cover per species in the field where: 0<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% (DeBerry and Perry 2004). In these measurements, bare ground was treated as a unique species and included as such in all calculations. Plant taxonomy and nomenclature were determined using the Natural Resources Conservation Service Plants Database (USDA Natural Resources Conservation Service, 2008). Vegetative cover estimates were then used to calculate relative species cover and relative species frequency. Relative species cover was calculated as individual species cover divided by total species cover in each plot (Atkinson et al., 1993; DeBerry and Perry, 2004). Relative species frequency was

calculated as of each species relative to the occurrence of all species (Atkinson et al., 1993; DeBerry and Perry, 2004). For example, if *Species a* appeared in the created wetland in May, 2009, and twelve total species were observed that month, the relative frequency *Species a* would be 1 ÷ 12, or 0.083.

Relative importance values were calculated as the average of relative cover and relative frequency for each species, multiplied by one hundred (Atkinson et al., 1993; DeBerry and Perry, 2004). Relative importance values were then averaged monthly and annually to enable comparisons over the duration of the trial period, and ranked in order of decreasing RIV_d to indicate the dominant species in each treatment.

Using *in situ* vegetative cover estimates, species Richness (SR) was calculated as the total number of species in the created and natural wetland on a per quadrat basis (Peet, 1974; Zar, 1984). Species Evenness (J') and the Shannon Diversity Index (H') were then calculated using both RIV_d and SR data. These values were calculated with bare ground removed from the calculations, but including planted *Betula nigra* seedlings. The Shannon Index was calculated using the following equation (Peet, 1974):

$$H' = -\Sigma (p_i \ln p_i)$$

for all species in a plot, where pi is the relative abundance of each species or the proportion of individuals of a given species to the total number of individuals in the community. Species Evenness (J') was calculated using the following equation (Peet, 1974):

$$J' = H' / H'_{max}$$

where H' is the Shannon Index calculated from field data, and H'_{max} is the maximum value of the Shannon Index for a given plot. H'_{max} is calculated as the natural log of Species Richness.

Monthly and annual Weighted Averages (WA) were calculated per plot as the sum, of the product of each species relative importance value and the indicator index of that species (Reed, 1988; Wentworth et al. 1988; Atkinson et al., 1993). This calculation is summarized in the following equation:

$$WA = (y_1u_1 + y_2u_2 + \ldots + y_mu_m) / \Sigma y_i$$

where $y_1, y_{2,\ldots}, y_m$ are the RIV values for each species in a loading rate, and $u_1, u_{2,\ldots}, u_m$ are the indicator values of each species.

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 values were then averaged each month, and over the course of the trial period to create annual weighted averages, for each site. WA were calculated including the planted trees species.

Community composition data was further analyzed using the Ellenberg Community Coefficient Similarity Index (SI_E) (Mueller-Dombois and Ellenberg, 1974). The Ellenberg index is a measure of plant community similarity in the two sites or ecosystems, in this case the created and natural wetlands (Mueller-Dombois and Ellenberg, 1974). This calculation weighted species presence and absence between two communities by RIV_d, summarized in the following equation:

$$SI_E = (M_c/2)/(M_a + M_b + (M_c/2))$$

where M_c was the sum of RIV_d common to both the created and natural wetland, M_a was the sum of RIV_d unique to the created wetland, and M_b was the sum of IV unique to the natural wetland. Using this metric, a value of 1.0 indicates identical communities while a

value of 0.0 indicates two completely dissimilar communities (Mueller-Dombois and Ellenberg, 1974).

Net Ecosystem Exchange of Carbon Dioxide: To measure net ecosystem exchange of $C0_2$ a transparent, enclosed chamber attached to either a LI-6200 (LI-COR® Biosciences), or a TPS-2 (PP Systems®) portable photosynthesis/respiration system was used. The enclosed chamber was made of a lightweight aluminum frame, covered with a transparent, heat-permeable Tefzel® (DuPont®) plastic, and attached to a 0.5 m^2 x 0.15 m aluminum base. Both the LI-6200 and the TPS-2 consisted of a portable computer attached to an InfraRed Gas Analyzer (IRGA). For the first eight months of the sampling period, the LI-COR system was used; however this system was replaced with the newer TPS-2 model prior to measurements being taken in January 2010.

For each plot selected, the 0.5m^2 base was centered around the planted seedling, carefully placed over all existing vegetation, and firmly seated into the soil to ensure no gas escaped where the base came in contact with the soil. After the base had been put in place, the transparent chamber was attached using clamps. CO_2 concentration measurements (parts per million (ppm)) were then taken using the InfraRed Gas Analyzer (IRGA). To do so, both the LI-6200 and TPS-2 pumped air out of the chamber through a tube running from the chamber into the IRGA, which measured CO_2 concentration (ppm) (Figure 3.1). Air was then returned to the chamber through a second tube. Both machines were programmed to record one measurement approximately every 30 seconds over a 5 minute period. All measurements were made during the hours of highest light (approximately 11am to 3pm).

CO_2 measurements were taken under each of the following conditions: 1) full ambient light, 2) 50% ambient light, 3) 25% ambient light, and 4) 0% ambient light. Different light conditions were created by using a combination of shade screens. The first measurement represented net photosynthesis at ambient light; the fourth measurement represented total respiration, or the combination of both vegetative and soil respiration, in

the plot. The second and third measurements showed CO_2 flux which resulted from decreased photosynthetic rates at intermediate light levels. A full set of CO_2 flux was taken at each light regime. In between measurements, the enclosed plastic chamber was opened to allow mixing with ambient atmospheric air and allowed to equilibrate for 15 minutes prior to the next set of measurements being taken.

In addition to measuring CO_2 flux in the existing vegetative community, the following parameters were also measured: 1) photosynthetically active radiation (PAR) using an LI-190 Quantum Sensor (LI-COR® Biosciences), and 2) air temperature using a portable temperature probe.

The set of CO_2 concentration measurements, generated using the afore mentioned techniques, was then used to calculate CO_2 flux as the change in CO_2 per square meter per second (ΔCO_2 ppm m^{-2} s^{-1}). To calculate CO_2 flux, CO_2 concentrations were plotted versus time in seconds, with time serving as the independent x-variable. The slope of this scatter plot was CO_2 flux in parts per million, which was later converted to µmol C m^{-2} h^{-1} using the ideal gas law, PV=nRT, where P equals atmospheric pressure, V is volume of chamber, R is the universal gas constant, T is temperature in Kelvin (K), and n is the number moles of gas in the sample container. Because all of the other variables are known, this equation can be rearranged to solve for the number of moles of gas in the chamber used for measurements of CO_2 concentration. To convert parts per million to µmoles, the slope of the concentration scatter plot is then multiplied by moles of air (n) in the chamber. In this calculation, parts per million of CO_2 equates to one mole of CO_2 per 106 moles of air, or a single µmole (10^{-6} moles) of CO_2 per mole of air. Thus, slope equals µmol CO_2 m^{-2} mol air^{-1} $second^{-1}$ and by multiplying moles of air (n) by slope, you effectively convert ppm to µmoles per unit time. The final step in converting to change in CO_2 per square meter per hour (ΔCO_2 µmol m^{-2} hr^{-1}) is to divide calculated µmoles per unit time by the area of the base of the chamber.

CO_2 fluxes were then modeled to determine both monthly and annual rates of gross primary production (GPP) and total respiration (R). Scaling short-term field measurements of CO_2 flux to monthly and annual rates was done using a CO_2 flux model. In the model, CO_2 flux rates were controlled by changes in PAR and temperature.

Monthly and annual estimates of gross primary production were calculated from field measurements taken in all light conditions (100%, 50%, and 25% ambient light). Gross primary production was quantified by plotting CO_2 flux (ΔCO_2 m^{-2} hr^{-1}) versus PAR, with PAR as the independent x-variable (Neubauer et al., 2000). Hyperbolic curves were fitted to this scatter plot using the equation:

$$GPP = [(a \times I) / (b + I)]$$

where I = average hourly irradiance (PAR), and a and b were empirically-derived constants with the units of μmol C m^{-2} h^{-1} and μE m^{-2} h^{-1} respectively (Neubauer et al., 2000). Hourly irradiance values for each day, of each month in southeastern Virginia were then run through the equation to generate hourly GPP rates (ΔCO_2 m^{-2}). The hourly irradiance values used in this model was measured at the Virginia Institute of Marine Science (VIMS). Finally, hourly GPP rates were summed for month and year.

Respiration (R) rates were calculated in a similar fashion. However, monthly and annual estimates of respiration only utilized field measurements taken at 0% ambient light. In this case, an Arrhenius plot of the natural log of CO_2 flux (ΔCO_2 m^{-2} hr^{-1}) versus the inverse of temperature in degrees K, with temperature as the independent x-variable, was created (Neubauer et al., 2000). A curve was fit to this plot using the equation:

$$R = y_0 + ax$$

where y_0 was the slope of the Arrhenius plot, a was an empirically-derived constant, and x was the average hourly air temperature in degrees K (Neubauer et al., 2000). Hourly

temperature values for each day, of each month in southeastern Virginia were then run through the equation to generate hourly R rates ($\Delta CO_2\ m^{-2}$). The hourly irradiance values used in this model was measured at the Virginia Institute of Marine Science (VIMS). Finally, hourly R rates were summed for month and year.

Monthly and annual rates of GPP and R were then used to calculate NEE as (Neubauer et al., 2000; Roggero, 2003):

$$NEE = GPP + R$$

In this equation GPP is expressed as a positive value, while respiration is represented by negative values. Gas exchange was then expressed relative to the two wetland sites as monthly (NEE_m) and annual (NEE_a) means.

Statistical Analyses: Simple regression was used to explore relationships among the various parameters constituting vegetative dynamics, general soil conditions, and hydrology measured in both the created and natural wetland over the course of the year long sampling period. Data was further analyzed using two-way analysis of variance (ANOVA) to determine if significant differences existed between the two sites in terms of vegetative dynamics. Analysis of variance (ANOVA) was used to compare time (month) and treatment (created versus natural) as main effects and interactions between time and treatment. Variables used in separate ANOVA were measures of above- and below-ground biomass accumulation, nutrient uptake, and net ecosystem exchange of CO_2; and measures of: soil bulk density, soil organic matter, total soil carbon and nitrogen, depth of the water table, and percent soil moisture. Analyses were performed on transformed data. Statistical analyses were performed using R software (GNU Operating Systems, Inc.).

Results

Vegetation dynamics differed significantly between the two wetland sites. Both communities differed greatly in their growth, nutrient uptake capabilities, composition, and influence on atmospheric carbon exchange. Results of ANOVA are reported in Table 3.5.

Hydrology: A detailed analysis of hydrologic data can be found in the previous chapter documenting hydrology, soil developmental processes, and microbial activity.

Soil Characteristics: Measured soil characteristics included: soil moisture, soil bulk density, soil organic matter, total carbon, and total nitrogen. A detailed description of these results can also be found in the previous chapter.

Biomass Accumulation: In both the created and natural wetland, all parameters of growth in planted *Betula nigra* seedlings increased throughout the growing season. Additionally, accumulation of above- and below-ground biomass in seedlings differed significantly in both sites. Height varied significantly by both site ($p \leq 0.001$) and month ($p \leq 0.001$) (Figure 3.2). However a significant interaction factor also existed ($p = 0.013$). A Tukey post-test indicated that height was similar between the two treatment groups for the first three months of the trial period. However, following July, height was significantly greater in the created wetland for the remainder of the experiment. Stem diameter (Figure 3.3), canopy cover (Figure 3.4), and dried weight of below-ground root structures (Figure 3.5) also varied significantly by both site (all p values ≤ 0.001) and across month (all p values ≤ 0.001). However, stem diameter and dried weight of root structures also had a significant interaction factor ($p \leq 0.001$ and $p = 0.035$, respectively). A Tukey post-test indicated that significant overlap existed on a month to month basis with regards to stem diameter, while no clear pattern in the variation was evident. A similar interaction factor existed between weight of below-ground root structures; however, in this case, a more consistent relationship was evident. A Tukey post-test indicated that while an overlap between the monthly mean was present in August and September of 2009, and in February, March, and April of 2010, for the other seven

months the mass of roots was significantly greater in the natural wetland than in the created wetland. Additionally, the mean monthly percent increase in height, stem diameter, canopy cover, and below-ground root structures all varied between the created and natural wetlands all (p-values ≤ 0.001) (Figure 3.6). Relative importance values of total tree growth (RIV_g) also varied significantly by both site ($p \leq 0.001$) and month ($p \leq 0.001$). In this analysis, there was no significant interaction between factors ($p = 0.432$), and tree size was significantly higher in the natural wetland than in the created site over the course of the year long sampling period (Figure 3.7). Finally, growth rates varied by both site ($p \leq 0.001$) and month ($p = 0.001$); however the interaction term in this analysis was also significant ($p \leq 0.001$). A Tukey post-test indicated that rates of growth did not differ significantly during the months of September and December, 2009, and April 2010. For the remainder of the trial period, higher rates of growth were observed in the natural site Figure 3.8).

Regression analysis showed a significant correlation between soil moisture and growth in the natural wetland at $\alpha = 0.05$ ($R^2 = 0.1317$, $p = 0.033$), however this relationship was not significant in the created site ($R^2 = 0.0793$, $p = 0.063$) (Figure 3.9). No correlation existed between soil organic matter and growth in either the created ($R^2 = 0.0030$, $p = 0.742$), or the natural wetland ($R^2 = 0.0226$, $p = 0.386$), and a significant relationship only existed between soil C:N and growth in the created wetland (created: $R^2 = 0.1348$, $p = 0.027$; natural: $R^2 = 0.0001$, $p = 0.950$) (Figure 3.10).

Nutrient Uptake: In the natural wetland, C:N ratios in plant tissue ranged from 25.10 (± 18.59) to 44.74 (± 2.19). However, ratios were much higher in the created site, ranging from 36.78 (± 4.43) to 97.61 (± 16.32).

Results of ANOVA indicated that this difference was significant. Results show that C:N ratios in sampled vegetation varied significantly by site ($p \leq 0.001$), though not by month ($p = 0.056$) (Figure 3.11). Additionally, Individual analysis of C:N ratios in *Betula nigra* seedlings grown in the natural wetland, indicate this increase was significant over time

($R^2 = 0.6346$, $p \leq 0.001$) (Figure 3.12). Similarly, rates of increase in C:N ratios differed by site ($p \leq 0.001$) though not by month ($p = 0.068$); however there was a significant interaction in this analysis ($p = 0.001$). Tukey post-test indicated rates of increase in C:N ratios were not significantly different in May or June, 2009. However, after these first two months C:N ratios in plant tissues increased rapidly in the created site, while values remained more constant in the natural wetland. This resulted in a significant difference between C:N ratios in the created and natural wetlands for the remaining ten months in the trial period (Figure 3.13).

Regression analysis indicated a significant relationship between soil nitrogen and nitrogen content in plant tissues (created: $R^2 = 0.6469$, $p \leq 0.001$; natural: $R^2 = 0.3463$, $p \leq 0.001$) (Figure 3.14). However, no relationship existed between soil moisture and C:N ratios (created: $R^2 = 0.06544$, $p = 0.132$; natural: $R^2 = 0.01736$, $p = 0.4506$), or organic matter and C:N ratios (created: $R^2 = 0.1017$, $p = 0.057$; natural: $R^2 = 0.02211$, $p = 0.3939$) in either site. Finally, no relationship existed between any measured environmental parameters and rates of increase in C:N ratios.

Community Composition: During the experimental period, thirty-seven (37) plant species representing thirteen (13) families were collected from the created wetland (Table 3.1). Twenty-six (26) were perennial, eight (8) annual, and three (3) annual/perennial. In the natural wetland, forty-one (41) species, representing fourteen (14) families were observed (Table 3.1). Twenty-eight (28) were perennial, ten (10) annual, and three (3) annual/perennial. *Juncus effusus* (OBL) was the dominant species in both the created and natural wetland (RIV_d: 22.40 and 18.80, respectively), with unvegetated bareground being the next dominant 'species' in both sites (RIV: 22.56 and 7.2, respectively) (Table 3.2). *Juncus acuminatus*, *Polytrichum juniperinum*, and *Bryoandersonia illecebra* had the next highest importance values, however their RIV were relatively low (~2.00).

In the created wetland, monthly species richness values ranged from 7.66 (± 3.78) to 17.85 (± 5.567.8) species m^{-2}. In the natural wetland, species richness values were much

higher, ranging from 9.33 (± 4.93) to 24.75 (± 2.00) species m^{-2} (Table 3.3). Monthly evenness values ranged from 0.68 (± 0.09) to 0.87 (± 0.08) in the created wetland, and again were higher in the natural site, ranging from 0.88 (± 0.05) to 0.91 (± 0.02). Monthly values of the Shannon index showed a similar response to treatment; Shannon index values were lower in the created wetland, ranging from 1.39 (± 0.37) to 2.70 (± 0.64), compared to 2.68 (± 0.30) and 3.46 (± 0.58) in the natural site.

ANOVA indicated that all of these values (species richness, evenness, Shannon Index) differed significantly between the two sites (all p-values ≤ 0.001). ANOVA also indicated that annual measures of species richness, Shannon Index, and weighted averages all varied significantly by site, thought species evenness did not (Table 3.4). Additionally, linear regression indicated an increase in all measures of diversity in the created wetland over the course of the trial period (richness: $R^2 = 0.3621$, $p = 0.027$; evenness: $R^2 = 0.1833$, $p = 0.047$; Shannon index: $R^2 = 0.5078$, $p = 0.001$) (Figures 3.15, 3.16, and 3.17 respectively). However, this relationship was not observed in the natural site. Except for species richness ($R^2 = 0.4402$, $p = 0.036$), in the natural wetland, the other two measures of diversity remained constant over the course of the experiment (evenness: $R^2 = 0.2306$, $p = 0.102$; Shannon index: $R^2 = 0.2220$, $p = 0.287$). ANOVA also indicated annual values of richness and evenness differed significantly (both p-values ≤ 0.001), though annual measures of the Shannon index did not ($p = 0.124$).

Regression analysis indicated a significant correlation between species richness and soil moisture in the created wetland ($R^2 = 0.3226$, $p = 0.001$) (Figure 3.18). However this relationship was not observed in the natural wetland, and analysis indicated that no significant relationship existed between species evenness and the Shannon index and any of the environmental parameters measured in either site ($\alpha = 0.05$).

The range in weighted averages was narrow, and generally contained the same mix of wetland and upland plant species (OBL through FACU). Weighted Averages ranged from 1.49 (± 0.38) to 2.38 (± 0.35) in the created wetland, and 2.13 (± 0.09) to 3.10 (±

0.62) in the natural wetland. Despite the rather narrow range in values between the two sites, ANOVA indicated a significant difference between weighted averages measured in the created and natural wetland ($p \leq 0.001$). Moreover, linear regression showed that in the created site, these values increased with time ($R^2 = 0.7807$, $p \leq 0.001$) (Figure 3.19). However, this relationship was not observed in the natural wetland ($R^2 = 0.0085$, $p = 0.681$).

Finally, Ellenberg Community Coefficient Similarity Indices (SI_E), both monthly and annually, were high (> 0.7) among treatment groups, and increased over the course of the sampling period (Table 3.4). Regression analysis indicated this increase over time was significant ($R^2 = 0.4673$; $p \leq 0.001$) (Figure 3.20). These indices suggested that despite variations in richness, evenness, and the Shannon index, species composition of the plant communities in both wetland systems were very similar to each other.

Net Ecosystem Exchange of Carbon Dioxide: As expected, both wetland sites showed marked seasonality in gross primary production (GPP), respiration (R), and net ecosystem exchange (NEE), with values decreasing with temperature in winter, and increasing in the spring and late summer (Figures 3.21, 3.22, and 3.23 respectively). Both wetland sites were net heterotrophic in the winter and early spring, before becoming autotrophic in April. In the autumn, heterotrophy was driven by increases in respiration as primary production decreased. In the created wetland, NEE peaked in the mid-summer, however, in the natural site, the peak occurred much earlier, likely due to differences in soil conditions which fueled respiration. A plot of GPP:R further highlighted the differences in carbon dynamics between the two sites (Figure 3.24). A GPP:R ratio less than one indicates net heterotrophy, a ratio greater than one indicates autotrophy (Roggero, 2003; Bailey et al., 2007). Our data show that the created wetland was autrophotrophic for two months more out of the year than the natural wetland, as the natural site became heterotrophic much earlier in the fall.

Two-way ANOVA indicated monthly values of GPP differed significantly by both site ($p \leq 0.001$) and across month ($p \leq 0.001$). However, a significant interaction existed between factors ($p \leq 0.001$). Tukey post-test showed that differences in mean GPP were primarily driven by differences in the months of July, August, and September, 2009 and April, 2010 where primary production was much greater in the created wetland than in the natural wetland. Except for these four months; however, monthly GPP values in the created and natural wetlands were not significantly different. ANOVA also indicated significant differences in respiration across both site ($p \leq 0.001$) and month ($p \leq 0.001$), again with a significant interaction term ($p \leq 0.001$). Post-tests showed respiration was significantly higher in the natural wetland for nine months of the trial period, however significant differences did not exist between the two sites with regard to measured respiration in either January, February, or March, 2010. NEE also varied significantly both between sites ($p \leq 0.001$) and across months ($p \leq 0.001$). Significant interactions between these factors ($p \leq 0.001$), made post-tests necessary which indicated that throughout the trial period, NEE was significantly higher in the created wetland except for the months of January, February, and March 2010, when no significant difference in ecosystem exchange between the created natural site was detected. Finally, annual values of GPP, R, and NEE all differed significantly between sites (all p-values ≤ 0.001) and showed that over the course of the year long trial period, the created wetland was net autrophic while the natural wetland was net heterotrophic. This suggested the created wetland acted as a carbon sink, while the natural wetland served as a carbon source.

Discussion

Species establishment, success, and community composition all vary based on environmental parameters (Bunn et al. 1997; Grime, 1998). Site characteristics therefore act as filters that limit the establishment of species not adapted to *in situ* hydrology and nutrient conditions (Connor and Simberloff, 1979; Drake, 1991; Keddy, 1992; van den Brink et al., 1995). A discussion of the specific environmental parameters observed in this study, and the vegetation dynamics occurring in response to them, is outlined in the following sections.

Hydrology: While *Betula nigra* is a flood-tolerant species able to withstand soil inundation for one to three months during the growing season (Norby and Kozlowski, 1983), past research has shown that water regime significantly impacts biomass accumulation in wetland plants (Bunn et al. 1997; Casanova and Brock, 2000). Past studies have specifically hypothesized that plant productivity in wetlands is highest when site hydrology is characterized by periodic, short duration, flooding (Odum et al., 1979; Conchou and Fustec, 1988; Cronk and Fennessy, 2001). This so called 'subsidy-stress model' theorized that long-lasting floods cause physiological stress to plants, while a complete lack of flooding limits production by minimizing nutrient inputs (Odum et al., 1979). Norby and Kozlowski (1983) confirmed this model for *Betula nigra* and showed that dry weights of roots, stems, and leaves of flooded individuals were reduced by 24%, 76%, and 73% when compared to the same parameters in unflooded individuals after 5 weeks of inundation. Additional research suggested that woody wetland species feature slower growth rates in flooded bottomland hardwood forests when compared to individuals grown in similar wetlands with a lower hydroperiod (Malecki et al., 1983; Megonigal et al., 1997). Based on these studies, it was expected that increasing levels of soil moisture would act as a significant impediment to the growth of planted *Betula nigra* seedlings in both wetlands.

In our experiment, the hydrology of both sites tended to be quite dry during the growing season, indicating that rather than inundation, perhaps nutrient inputs would be limiting. However, there were significant differences in the hydrologic conditions at both sites. During the spring, early summer, and winter, conditions were roughly equivalent in the created and natural wetlands, while in the late summer and fall, the natural site remained relatively moist, though the created wetland became quite dry. This variability explained some of differences in vegetation dynamics observed in the two sites as regression analysis indicated a significant correlation between soil moisture and growth. As percent soil moisture increased, which was representative of increased water table elevation, growth increased significantly. This finding is further supported by earlier research

which found that in dry-lands, conditions of elevated soil moisture often result in greater above- and below- ground biomass production (Keddy and Reznicek, 1986; Duncan and Groffman, 1994; Roberts, 1994). This was the opposite of what was hypothesized. It was expected that increased soil moisture would act as a significant stress and impediment to tree growth. However, because neither site was inundated during the growing season, and the created site in fact became quite dry during the late summer months, data suggest a lack of moisture (drought) was more of a factor in determining growth than an overabundance of water. Based on this finding, it appears logical that the natural site was more conducive to tree growth than the created wetland.

Soil Characteristics: Soil organic matter, soil carbon content, and soil nitrogen content were generally higher in the natural site, while bulk density and soil C:N were generally higher in the created wetland. This result was somewhat surprising. Typically, soil carbon and nitrogen content increase with amendment, while bulk density and soil C:N ratios generally decrease to levels similar to those observed in natural reference wetlands (Stolt et al., 2000; Bruland and Richardson, 2004; Bergshneider, 2005; Daniels et al., 2005; Bailey et al., 2007). Logically, these changes are a result of adding coarse organic matter to the site, which contain high volumes of carbon and nitrogen in their organic forms (Daniels and Whittecar, 2003; Bergschneider, 2005). We expected to see a similar result in measured soil parameters in the created wetland because it had been amended with topsoil removed and stockpiled during early phases of construction. However, this was not the case.

Given the discrepancies between sites in terms of soil characteristics, it was hypothesized that conditions in the natural wetland would result in higher growth rates and higher rates of nutrient uptake in *Betula nigra* seedlings, which data confirmed. This result was expected because earlier research suggested high density, nutrient poor soils, such as those found in the created site, often inhibit the development of *in situ* vegetational communities (Brouwer, 1978; Stolt et al., 2000; Bruland and Richardson, 2004; Bergshneider et al., 2005; Daniels et al., 2005; Bailey et al., 2007). Our result was also

expected because Stauffer and Brooks (1997) found that soil organic matter is critical for plant community establishment in created wetlands, and if SOM is deficient, planted individuals struggle significantly.

Studies have also shown that the poor soil conditions inhibiting plant growth in created wetlands do not become ameliorated over time (Bishel-Machung et al., 1996; Campbell et al., 2002). In traditional ecosystem studies, research shows that when plants die they are incorporated into the soil, increasing the amount of soil organic matter and decreasing bulk density (Marks and Bormann, 1972; Vepraskas, 1994; Hooper and Vitousek, 1997; Stauffer and Brooks, 1997). Root turnover also adds humus to the soil and improves general soil conditions over time, which Odum observed in successional studies (1969). However, it is not clearly understood why these processes do not occur in wetlands created for mitigation purposes (Campbell et al., 2002). More research is therefore needed to characterize the relationship between soil developmental processes and vegetation dynamics in order to determine the mechanisms behind site failure, and improve success rates in wetland construction.

Biomass Accumulation: The different parameters that comprised total tree growth (RIV_g) varied differently in both sites. Data show that in the created wetland, planted *Betula nigra* seedlings were taller, with less basal diameter, fewer roots, and less canopy cover, while in the natural wetland, seedlings were shorter, thicker at the base, and had much more well developed root structures and canopies. This variability is most likely a result of different resource limitations in the two systems, as previous studies have shown that competition for resources plays an important role in determining biomass allocation in vegetation (Wilson and Keddy, 1986; Gaudet and Keddy, 1988; Johansson and Keddy, 1991; Weiher and Keddy, 1995). In the created wetland, competition was limited, as site excavation removed all existing vegetation and seedbanks which were present prior to construction (Kusler and Kentula, 1989; Atkinson et al., 1993). It was hypothesized that this processes created an environment that offered ample rooting space, soil water, soil nutrients, and light. As such seedlings responded as R-selected individuals that grew

quickly, allocated few resources to long term survival, and concentrated primarily on producing copious amounts of seed so their progeny could take advantage of abundant resources (Pianka, 1970). In the natural wetland however, competition for rooting space, soil nutrients, and light was stringent, prompting individuals to behave more like a K-selected species, where they developed strong below-ground root structures, and a large canopy to maximize their competitive ability to capture limited resources.

With regards to total tree growth, we hypothesized that biomass would vary positively with soil organic matter; however, this was not the case. This was surprising given that others have found that higher levels of organics typically increase nutrient content and water retention, and decrease bulk density in soils, all three of which combine to create a more favorable environment for plant growth (Brouwer, 1978; Reinhartz and Warne, 1993; Stolt et al., 2000; Bruland and Richardson, 2004; Bergshneider, 2005; Daniels et al., 2005). However, our results were consistent with others who did not detect differences in total biomass along a gradient of soil organic matter in created wetlands (Cole et al., 2001; Anderson and Cowell, 2004). Our results therefore suggest that plant biomass may not be directly dependant on soil organic matter in either early successional communities like those present in the created site, or in more well-developed communities like those in the natural wetland.

We found soil C:N ratios, which were not correlated with SOM, were a better predictor of biomass, at least in the created site where growth responded negatively to elevated C:N levels. This was anticipated as past research showed macronutrient availability often represents a potential constraint on vegetative productivity (Brouwer, 1978; Lockaby and Walbridge, 1998; Green and Galatowitsch, 2001). However, this relationship was not observed in the natural site, where no correlation between soil nutrients and growth was observed. The most likely explanation for this is that in the natural site, nutrients were in excess of concentrations required by existing vegetation, and never became limiting, and that some other physical or chemical factor was acting to constrain growth such as interspecies competition.

Another factor that could potentially explain some of the observed variability in tree growth was elevation. Bailey et al. (2007) found that in created wetlands along an elevational gradient, there exists an ideal height for plant production where nutrient availability, plant available water, redox potential, etc. were optimal. Plant biomass and growth differences along elevational gradients have also been well documented in past research (Mitsch and Ewel, 1979; Brinson et al., 1981; Craft, 2001; Craft et al., 2002; Fraser and Karnezis, 2005). These studies potentially explain some of our results. Logically, plots with slightly higher elevations would be less susceptible to complete inundation during the growing season, and soils in these plots would dry more rapidly which would create optimal aerobic, yet moist, growing conditions (Brinson et al., 1981; Craft, 2001; Fraser and Karnezis, 2005). These studies, along with our research, also suggest that early seedling development does not respond to soil organic matter alone. Instead, it appears as though the combination of nutrient gradients and elevation-related hydrology may better explain differences in biomass observed in this study. However, results are inconclusive on this subject. While, the average elevation of the natural wetland was higher than the elevation of the created wetland, elevational data were only collected at the installation points of groundwater wells, and not at the plot level, rendering a more detailed analysis of these parameters impossible.

Nutrient Uptake: A significant amount of information about the content of nitrogen in plant tissues is currently available (Boyd, 1978; Hobbie, 1992; Kitterer and Andren, 1999). These studies indicate that C:N ratios in foliage are often indicative of nutrient availability in the soil matrix (Boyd, 1978). According to the Natural Resources Conservation Service Plants Database C:N ratios in *Betula nigra* are generally around 40:1 (USDA Natural Resources Conservation Service, 2008), which were consistent with values in seedlings grown in the natural wetland. However, C:N ratios were significantly higher in seedlings in the created wetland. These values indicate low nutrient availability in the local environmental, and subsequent vegetative stress; as the ratio of carbon to nitrogen rises, it shows rates of photosynthesis (carbon uptake) are remaining constant

while nitrogen uptake is decreasing (Boyd, 1978; Thormann and Bayley, 1997). Higher values therefore indicate low nutrient availability, while lower values, such as those observed in the natural wetland, potentially point to excess nutrients (Vermeer, 1986).

We hypothesized that lower levels of soil organic matter in the created wetland would limit nutrient availability and subsequent rates of nitrogen uptake. Data confirmed this hypothesis and showed that in both wetlands, soil C:N ratios and C:N ratios measured in tissues of planted seedlings were strongly correlated. This finding was supported past research which suggested that as nitrogen content in soils increase, nitrogen content in plant tissues also increases (Anderson et al., 1997). However, our results are far from conclusive as past research has also shown that the nutrient content of wetland plants is inherently variable (Boyd, 1978). This variation exists: 1) between stages of maturity, 2) between individual plants, and 3) between stands in the same environment (Boyd, 1969; Boyd, 1970; Boyd and Hess, 1970; Boyd, 1978; Gaudet, 1975).

Community Composition: Analysis indicated that community composition in both wetlands was similar to that of other created and natural wetlands in Virginia (Atkinson et al., 1993; Stolt et al., 2000; DeBerry and Perry, 2004; Atkinson et al., 2005). Prior to the start of this experiment, it was hypothesized that community composition would differ significantly between the natural and created wetlands. We expected species richness (SR), evenness (J') and the Shannon diversity index (H') to be lower in the created wetland because excavation at this site left a bare substrate to which species had to be introduced both naturally and anthropogenically (Kusler and Kentula, 1989; Atkinson et al., 1993). For the same reason it was also expected that total vegetation cover would be lower in the created wetland due to the presence of unvegetated, bare ground. Conversely, we expected measured parameters of diversity to be higher in the natural site because it remained undisturbed, and over time appeared to have developed a diverse community of bryophytes, grasses, and shrubs.

Data were inconclusive with regards to these hypotheses. While ANOVA analysis indicated significant differences existed between the two sites in terms of species richness, evenness, and the Shannon Index, the Ellenberg Community Coefficient Similarity Index indicted the communities were very similar throughout the experiment, and became ever more alike over time.

The overall similarity in vegetation between sites was likely due to the prevalent communities of *Juncus effusus* in the natural wetland, and in the areas surrounding the created site. *Juncus effusus* is an abundant, emergent macrophyte found in freshwater wetlands throughout the southeastern United States (Richards and Clapham, 1941; Mann and Wetzel, 1999; Ervin and Wetzel, 2002). It is a perennial wetland plant, adapted to full sun, which grows in clumps or tussocks and spreads by vigorous underground rhizomes or the release of seeds which can be transported by either wind or water (Richards and Clapham, 1941; Mann and Wetzel, 1999; Ervin and Wetzel, 2002). Seeds also have tiny beaks at their endpoints that also allow transport via attachment to animals as well (Richards and Clapham, 1941). *Juncus effusus* can tolerate a wide range of environmental conditions, and while it normally grows in areas that are periodically flooded, it can also withstand extended dry periods or lengthy submergence (Richards and Clapham, 1941; Mann and Wetzel, 1999; Ervin and Wetzel, 2002). This pattern was consistent with the conditions present in both the created and natural wetland during the 2009-2010 study. Both sites were inundated throughout the winter and early spring, dried out completely at the surface in early summer, and remained dry until late fall.

Past work showed that high above-ground growth rates in *Juncus effusus* allow it to out-compete other species for light by rapidly producing large quantities of photosynthetic tissues (Facelli and Pickett, 1991; Mann and Wetzel, 1999; Ervin and Wetzel, 2002). The aggregate action of thousands of arched shoots then greatly reduces the amount of available light that penetrates the *Juncus effusus* canopy, thereby significantly limiting light penetration to the surrounding sediments (Wetzel and Howe, 1999). In many studies, plant species density has been strongly correlated with light penetration to the

soil surface, rather than with plant growth rates or biomass (Facelli and Pickett, 1991; Grace, 1999; Wetzel and Howe, 1999; Ervin and Wetzel, 2002). As such we believe that light limitations caused by *Juncus effusus* strongly inhibited the establishment of other species in both wetland sites (Wetzel and Howe, 1999).

It was also hypothesized that plant communities in both wetlands were similar because of their close proximity to each other. Past research has shown that created wetland sites located adjacent to undisturbed wetlands often feature greater colonization of wetland species, and more similar communities to reference wetlands, than sites that are more geographically removed (Reinhartz and Warne, 1993; Mitsch and Wilson, 1996; Brown and Bedford, 1997; Brown, 1998; Balcombe, 2005). As the two wetlands studied in this project were directly adjacent to each, it's probable that vegetation in the natural wetland served as a potential seed source, especially given that *Juncus effusus* is so proficient at colonizing bare substrates (Thompson, 1992).

The other dominant species in the created wetland were unvegetated bare ground, followed by *Juncus acuminatus*. In the natural wetland the other dominants consisted of *Polytrichum juniperinum* and unvegetated bare ground. *Juncus acuminatus* is similar to *Juncus effusus* in that it's a perennial wetland macrophyte that prefers full sun environments (USADA Natural Resource Conservation Service, 2002). *Polytrichum juniperinum* is a bryophyte found everywhere from alpine meadows to hardwood forests to freshwater wetlands (Crum, 1972; Crum and Anderson, 1981; Schofield, 1992). This species, along with *Bryoandersonia illecebra*, another bryophyte, were among the top five dominant species in the natural wetland, though they fell much farther down the list of dominants in the created site, presumably because the removal of all *in situ* vegetation and seedbanks in the created wetland resulted in a substrate not conducive to shade intolerant mosses. Additionally, it is likely that fewer mosses were observed in the created site because near drought conditions limited their ability to survive and propagate.

The relative importance (high RIV) of bare ground decreased over time in the created site, though it still remained significantly more prevalent in this site than in the natural wetland. This result was consistent with earlier research that reported higher vegetative coverage in organically amended plots over time when compared to unamended or natural plots (Stauffer and Brooks, 1997; Bailey et al., 2007). Larger areas of bare ground, such as those observed in the created wetland, could prove to be significant in site development. Research shows bareground leads to higher, more frequent fluctuations in soil temperature due to a lack of plant shading which creates rooting stress for plants and limits productivity (Aust and Lea, 1991; Londo et al., 1999; Zak et al., 1999; Stolt et al., 2000; Reth et al., 2005). Higher soil temperatures, in conjunction with aerobic conditions, also stimulate higher microbial activity which leads to rapid organic matter decomposition (Bridgham and Richardson, 1992; Kelting et al., 1998). The combination of these events has the potential to create a negative feedback loop where created wetlands with an abundance of unvegetated bare ground become susceptible to higher soil temperatures and increasing SOM respiration rates, which in turn decrease soil organic matter and lower plant productivity, thereby leading to lower plant coverage and an increase in bare ground (Reth et al., 2005; Bailey et al., 2007).

We originally hypothesized that plant community diversity would vary with hydrology, soil organic matter, and soil nutrients, and to some extent this occurred. In the created wetland, a significant positive relationship was observed between species richness and evenness and soil moisture; however, none of the other measured parameters varied with hydrology or soil conditions. This is somewhat surprising given that in the past, research has shown strong correlations between organic matter, soil nutrients, species richness, and the Shannon Index (Tilman, 1982; Vermeer, 1986; Leps, 1999; Anderson and Cowell, 2004).

Also surprisingly, Weighted Average (WA) values in the created wetland increased over time, from a low of 1.49 (± 0.38) in the early months of the experiment, to 2.38 (± 0.35) in March, 2010 which was roughly equivalent to values measured in the natural site over

the course of the experiment. This shift most likely resulted from other species besides *Juncus effusus* (OBL), with higher indicator values (FAC, FACU, etc.), becoming established in the created wetland over the course of the experiment. It also likely resulted from the created wetland becoming increasingly dry over the course of the experiment. This result is also interesting given that WA ≤ 2.0 are used to designate wetlands on the basis of vegetation alone (Wentworth et al., 1988; Atkinson et al., 1993); values in the natural wetland exceeded this standard over the course of the trial period. and by the end of the experiment values were also higher in the created wetland.

Net Ecosystem Exchange of Carbon Dioxide: We hypothesized that net ecosystem exchange of carbon dioxide and photosynthetically active radiation (PAR) would show a positive relationship in the form of a hyperbola, with the asymptote representing P_{max}, or the highest level of photosynthesis that the plants can achieve (Streever et al., 1998; Roggero, 2003; Bailey et al., 2007). Conversely, we was expected that during periods of low PAR, measured values of net ecosystem exchange of carbon dioxide would fall below the x-axis indicating plant respiration, all of which our data confirmed. Our data also showed that in both wetlands, levels of net ecosystem exchange increased in the spring, reached a maximum during the summer, and then declined in the autumn. These fluctuations strongly corresponded to temperature and light availability, the two primary drivers of vegetative photosynthesis and respiration (Bubier et al., 1998; Clark et al., 1999; Knorr, 2000). Decreases in NEE in the late summer were also likely due to increased rates of evapotranspiration caused by high temperatures, which have negative effect on photosynthesis (Bradley and Morris, 1990).

While monthly patterns of NEE, and GPP and R, were very similar in the both wetlands, significant differences existed in the magnitude of carbon exchange. Rates of gross primary production were much higher in the created wetland during the spring and summer. However, as the growing season began to wane, productivity in both sites equilibrated and became approximately equal. The large difference between GPP at the two sites during the growing season was surprising. DeBerry and Perry (2004) compared

standing crop biomass, another measure of annual plant productivity, between natural and created wetlands and found no significant differences among them. Additionally, research using gas exchange field measurements similar to those used in this study, found no significant differences between created and natural wetlands in terms of GPP (Roggero, 2003; Cornell et al., 2006). In our experiment over the same time period (spring and summer), total respiration in the natural wetland greatly exceeded respiration in the created site. The balance of these two parameters resulted in much higher NEE values in the created wetland, where higher rates of productivity outweighed relatively low levels of respiration. Conversely, in the natural site, high rates of respiration negated lower levels of primary productivity. Additionally, from May to September in the created site, net ecosystem exchange was positive, indicating that the site served as a net carbon sink. The natural wetland behaved similarly, except NEE was negligible in August, and negative in September.

Negative NEE values at both sites likely resulted from spiking rates of respiration coupled with a simultaneous decrease in GPP during the fall. These results are similar to those reported in past studies which reported R exceeding GPP in the late summer, and throughout the fall, in tidal freshwater wetlands in Virginia (Neubauer et al. 2000). Differences in the timing and magnitude of these shifts were likely due to higher levels of soil organic matter in the natural wetland which fueled higher rates of soil respiration (Bridgham and Richardson, 1992; Bowden et al., 1993; Kelting et al., 1998; Craft, 2001). In addition to providing an ample fuel source for heterotrophic microbes, increased soil organic matter in the natural site also likely increased respiration by lowering bulk density (Kelley et al., 1995). Lower levels of bulk density improve conditions conducive to high levels of respiration by increasing the depth of aeration in the soil profile, and exposing more of the available organic matter to oxidation (Bridgham and Richardson, 1992; Bowden et al., 1993; Kelting et al., 1998). Finally, differences in site elevations likely also drove differing rates of respiration. On average the natural site was higher than the created site, resulting in a decrease in the frequency and duration of inundation. This is significant because past studies have shown flooding strongly inhibits

heterotrophic activity in wetland ecosystems (Bubier et al., 1998; Wickland et al., 2001). Other studies support this finding and show that even slight changes in surface elevation have the potential to change a wetland from a net carbon sink to a net source (Shurpali et al., 1995; Lafleur et al., 1997; Suyker et al., 1997). However, ecosystem respiration, as measured in this experiment, is also a product of the metabolic respiration of plants, and not solely a result of soil respiration (Trumbore, 2000). Plant respiration is generally thought to be approximately equal to 50% of carbon uptake (Ryan, 1991). As such, plant respiration is a far less significant factor in explaining differences in total respiration observed between the two wetlands. Competition for resources between herbaceous vegetation and the established, mature over-story also likely limited primary productivity in this wetland; while limited competition for available resources allowed high levels of GPP in emergent vegetation in the created wetland (Grime, 1973; Connor and Simberloff, 1979; Hooper, 1998).

The range of annual GPP and R rates measured in the created and natural wetlands highlighted a significant difference between the two sites in terms of their ability to act as carbon sinks. When all plots were summed over the course of the year, GPP and R data showed the created wetland was net autrophic, sequestering 1375.75 (± 155.49) g C in the 36 selected plots, while the natural wetland was net heterotrophic releasing 366.38 (± 126.62) g C from the 36 study plots. These differences are likely due to the same factors that drove monthly variation between the two sites, i.e. increased levels of microbial respiration due to soil organic matter and limited levels of productivity due to competition in the natural site.

Differences in NEE may also have resulted from differences in the age of the two wetlands used in this study. Past research examining gas exchange in wetlands found that negative, or near zero, NEEs are often characteristic of mature systems while early successional systems often feature positive NEE fluxes (Hirota et al., 2006; Clark et al., 1999; Neubauer et al., 2000). These differences are also reflected in the ratio of GPP to R, which in mature wetlands is approximately one (Odum, 1969). Conversely, in

younger systems the GPP to R ratio is significantly greater than and decreases over time (Odum, 1969). This relationship results from higher levels of primary production in young, developing ecosystems, and lower levels of ecosystem respiration; in mature sites however, lower levels of photosynthesis are balanced by higher rates of respiration.

Our results showed GPP:R equaled 1.71 (± 0.32) in the created wetland, while in the natural site GPP:R equaled 0.88 ((± 0.22). Based on past studies indicating both low organic content in created wetland soils, and quickly accumulating plant biomass, the ratio measured in the created site is likely representative of many newly created palustrine wetlands in southeastern Virginia which act as primary successional systems and rapidly accumulate carbon (Whittecar and Daniels, 1999; Stolt et al., 2000; DeBerry and Perry, 2004; Bailey et al., 2007). GPP:R ratios below one are typically only produced in cases of organic pollution (Odum, 1969); naturally functioning ecosystems cannot maintain such a ratio, as the respiration is proportional to the amount of organic matter produced in the system. Without an external input of organic material, on an annual basis, respiration cannot exceed primary production (Smith, 1996). Several anthropogenic circumstances, such as forest clearing, can contribute to unusually low GPP:R ratios (Smith, 1996). In this experiment, higher levels of respiration likely resulted from dumping of debris cleared from the adjacent created site during construction. These inputs likely caused higher rates of organic matter decomposition.

Summary and Recommendations

Our results showed generally higher levels of ecosystem function in the natural site. Planted vegetation featured higher growth rates, seedlings sequestered more nutrients from the soil, and plots were more diverse in terms of richness, evenness, and the Shannon index. However, being an early successional system, the created wetland sequestered more carbon during the growing season, and over the course of the year long sampling period.

A combination of hydrological conditions, soil organic matter, and available nitrogen in the soil matrix exerted some control on growth and community composition at both sites. Our analysis indicated that the most important of these factors was hydrology, which is not surprising given that most documented failures in wetland construction are related to hydrologic deficiencies (Mitsch and Wilson, 1996). Despite the strong connection between hydrology and vegetative dynamics, it appears that other species which had been naturally introduced to the created site, such as *Juncus effusus*, play just as important of a role in determining ecosystem function. This highlights the importance of active site management following construction, not just in maintaining planted stock, but also in limiting the introduction of potentially invasive native and non-native species. We therefore recommend that hydrological conditions in created wetlands more closely match conditions in natural reference wetlands and that project managers devote more resources to active site management following construction.

Results of this study indicated that the created wetland sequestered more carbon dioxide than the natural wetland. However, this result was expected since the created wetland was an early successional system. More research is therefore needed to investigate if over time, production and respiration will become balanced such that the created wetland will neither add nor remove carbon from atmospheric pools as successional theory predicts.

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Figure Captions

Figure 3.1: Diagram of the net ecosystem exchange sampling equipment. Air is pulled from the chamber into the IRGA, which measures carbon dioxide (CO_2) content. The air is then returned to the chamber. A PAR meter, air temperature probe, and soil temperature probe also gather information.

Figure 3.2: Graphical representation of the tree height in *Betula nigra* seedlings planted in the created and natural wetlands observed in this study. Tree height increased in both treatment groups; however seedlings in the created wetland achieved a greater increase in height than those planted in the natural site.

Figure 3.3: Stem diameter of seedlings differed significantly between both sites over the course of the experiment. Significant differences were also observed in both sites over time.

Figure 3.4: Canopy cover of seedlings differed significantly by both site and month.

Figure 3.5: Below-ground root structures in seedlings differed significantly between both sites over the course of the experiment, with an increase in the dried weight of these structures increasing over time.

Figure 3.6: Mean monthly percent change in growth parameters.

Figure 3.7: Graphical representation of tree growth (RIV_g) which was calculated as a weighted average of all parameters of growth measured in this experiment. Data indicate significant differences in size between seedlings planted in the created and natural wetland with greater size being observed in the natural site.

Figure 3.8: Rates of growth in both wetlands increased dramatically following transplanting, and then tapered off as the growing season progressed.

Figure 3.9: Regression analysis indicated a significant positive correlation between soil moisture and growth. This suggests that rather than an over abundance of water, it was a lack of water which strongly limited tree sizes.

Figure 3.10: Regression analysis indicated a weak, yet significant correlation between nutrient availability, measured as soil C:N ratios, and tree size in the created wetland. This relationship was not observed in the natural wetland, most likely because C:N ratios remained low, indicating no nitrogen limitations.

Figure 3.11: C:N ratios in *Betula nigra* seedlings planted in the two treatment groups were approximately equal at the beginning of the experiment. Over the course of the year long trial period however, these ratios increased in the created site while they remained constant in the natural wetland.

Figure 3.12: Regression analysis indicated that the increase in C:N ratios measured in *Betula nigra* seedlings sampled from the created wetland was significant over time. Increasing C:N ratios in plant tissues highlight a nitrogen deficiency in the soil matrix.

Figure 3.13: Raw C:N ratios were converted to percent change relative to initial baseline C:N values for seedlings planted in both treatment groups. Relative increases in C:N were then used to calculate to monthly rates of change. Analysis of this data indicated that in the created wetland, C:N ratios increased at an increasing rate over the course of the growing season. This showed that by the end of the growing season, plants had utilized all available *in situ* nitrogen stores in the created site. The rate of increase then began to slow down during the fall and winter months as growth declined and decomposition of the fall litter layer added labile nitrogen to the soil matrix.

Figure 3.14: Regression analysis indicated a significant, positive correlation between soil C:N and C:N ratios measured in plant tissues at both wetland sites.

Figure 3.15: Linear regression indicated a significant increase in species richness in the created and natural wetland sites over time.

Figure 3.16: Linear regression indicated a significant increase in species evenness in the created and natural wetland sites over time.

Figure 3.17: Linear regression indicated a significant increase in calculated values of the Shannon Index in the created and natural wetland sites over time.

Figure 3.18: Species richness was the only calculated diversity index which varied with an environmental parameter. Regression analysis indicated a positive correlation between it and soil moisture. It's likely that other measures of diversity fluctuated based on complex interactions between species, nutrients, and hydrology which all acted together to influence community composition.

Figure 3.19: Surprisingly, calculated weighted averages increased significantly in the created wetland over time, while values slightly decreased in the natural site. This dynamic is most likely a result of upland species moving in to the created site over time, as it become drier over the course of the trial period.

Figure 3.20: The Ellenberg Community Similarity Index was high between the two sites throughout the experiment. Linear regression also showed that this value increased over time, indicating that over the course of the year, the two sites became more similar.

Figure 3.21: Gross primary productivity was significantly different in the created and natural wetland over the course of the year; however GPP showed similar seasonality in both sites.

Figure 3.22: Total ecosystem respiration also differed significantly over the course of the year while showing similar seasonality driven by both organic matter inputs and temperature.

Figure 3.23: Net ecosystem exchange of carbon dioxide, was significantly different in the created and natural wetland over the course of the year despite showing similar seasonal fluctuations in response to temperature and light.

Figure 3.24: Calculated GPP:R ratios indicate whether communities are net autrophic or net heterotrophic. In the case of our experiment, data indicate that the created site remained autrophic two months longer than the natural site, where respiration began to exceed photosynthesis in August. This shift in productivity did not occur in the created wetland until October.

Table Captions

Table 3.1: Annual importance values (RIV_d) of all species found in the created and natural wetland over the course of the year long trial period.

Table 3.2: Annual dominant species, ranked based on relative importance values (RIV_d).

Table 3.3: Monthly diversity indices in both wetland sites.

Table 3.4: Annual diversity indices.

Table 3.5: Monthly and annual Ellenberg Community Similarity Indices.

Table 3.6: Results of ANOVA.

Figures

Figure 3.1

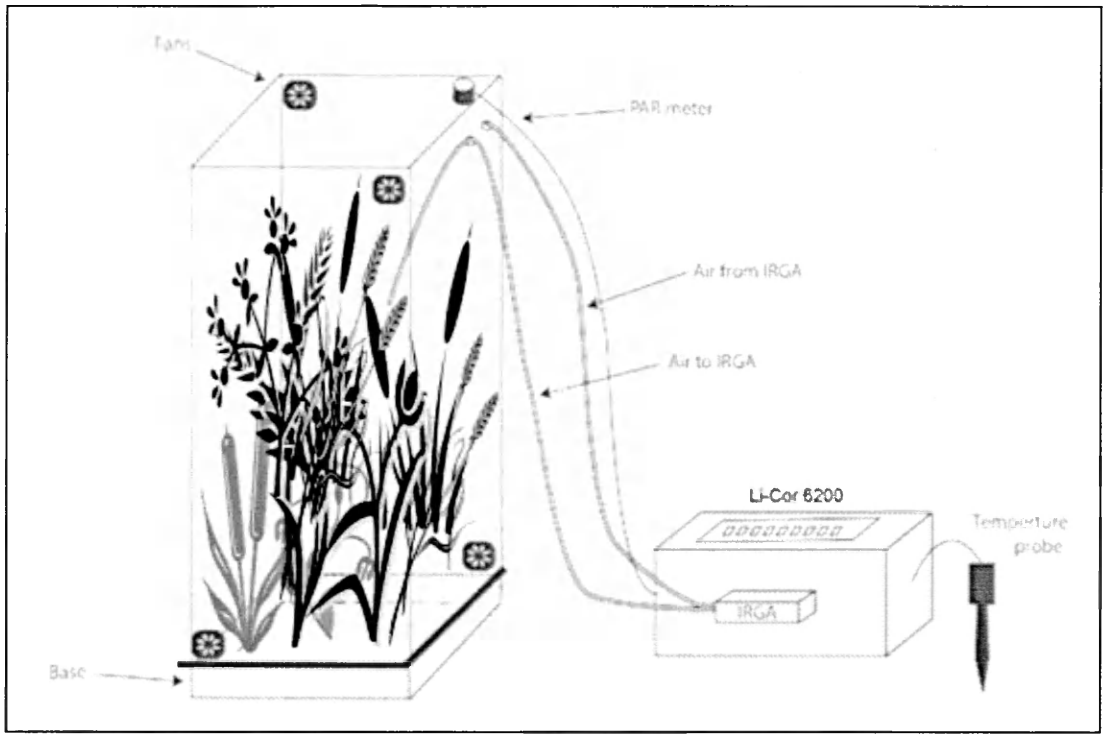


Figure 3.2

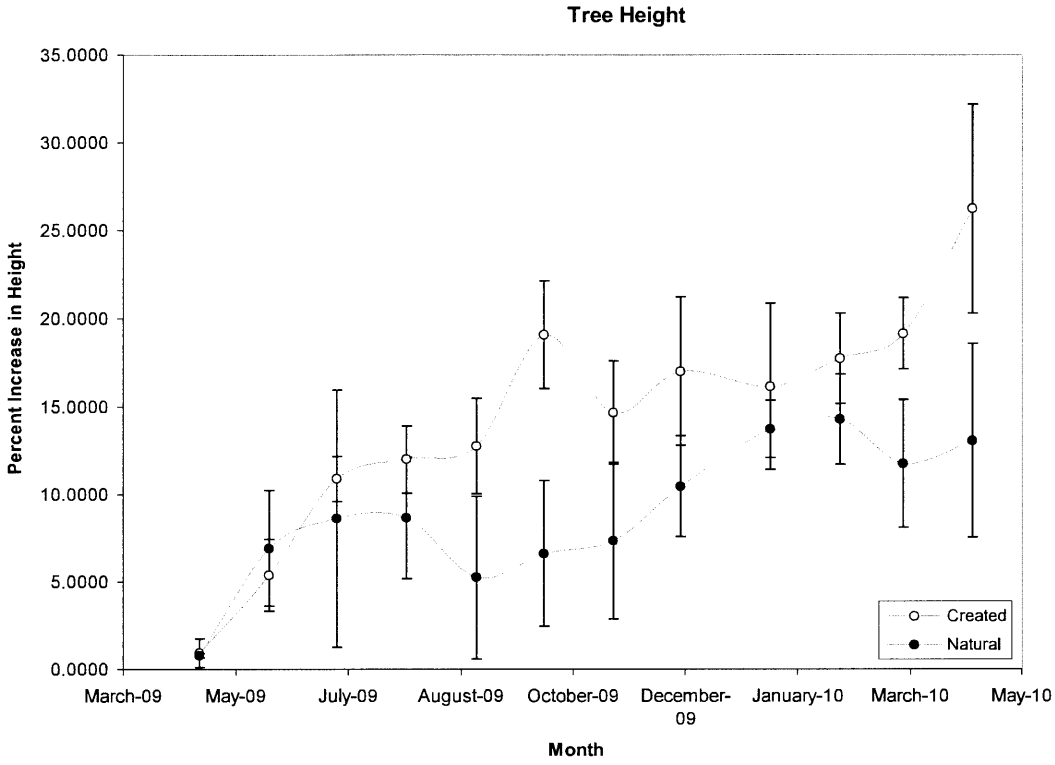

Tree Height
35.0000
30.0000
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
Percent Increase in Height
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 3.3

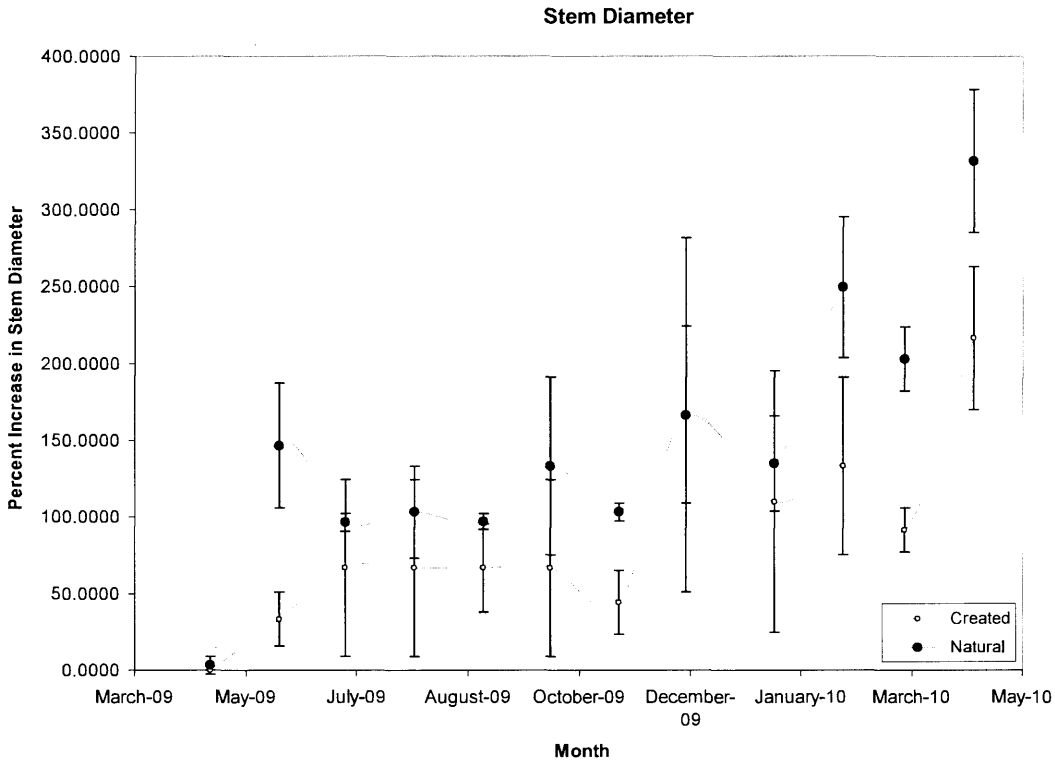

Stem Diameter
400.0000
350.0000
300.0000
250.0000
200.0000
150.0000
100.0000
50.0000
0.0000
Percent Increase in Stem Diameter
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 3.4

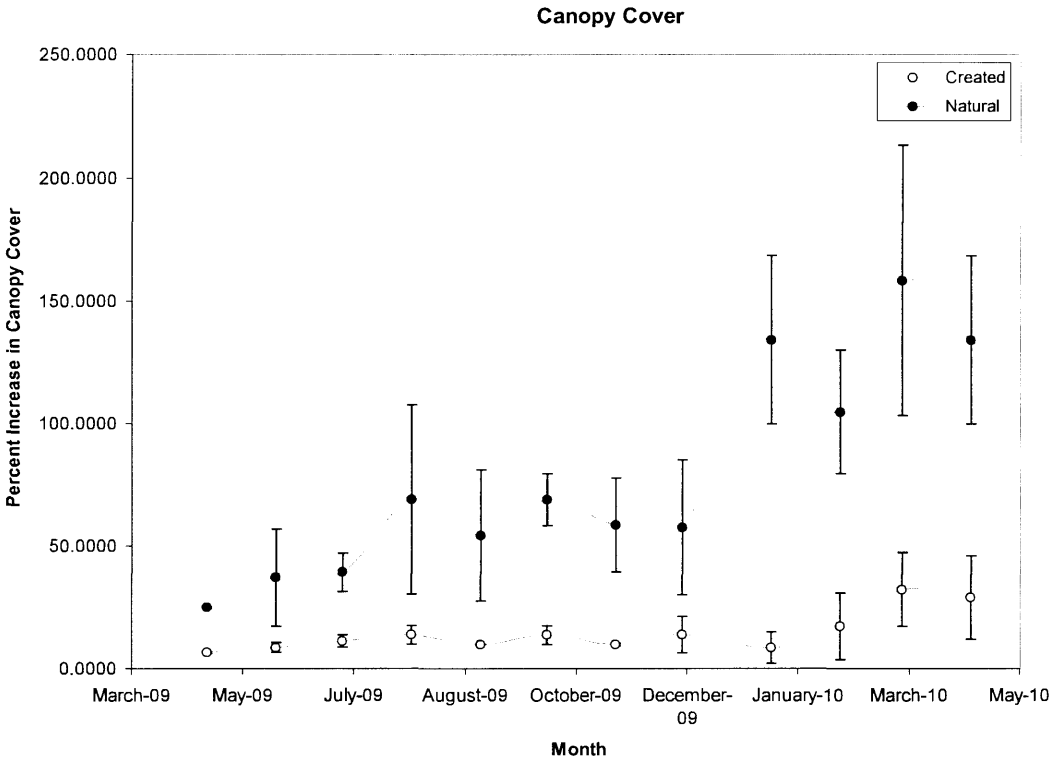

Canopy Cover
250.0000
200.0000
150.0000
100.0000
50.0000
0.0000
Percent Increase in Canopy Cover
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.5

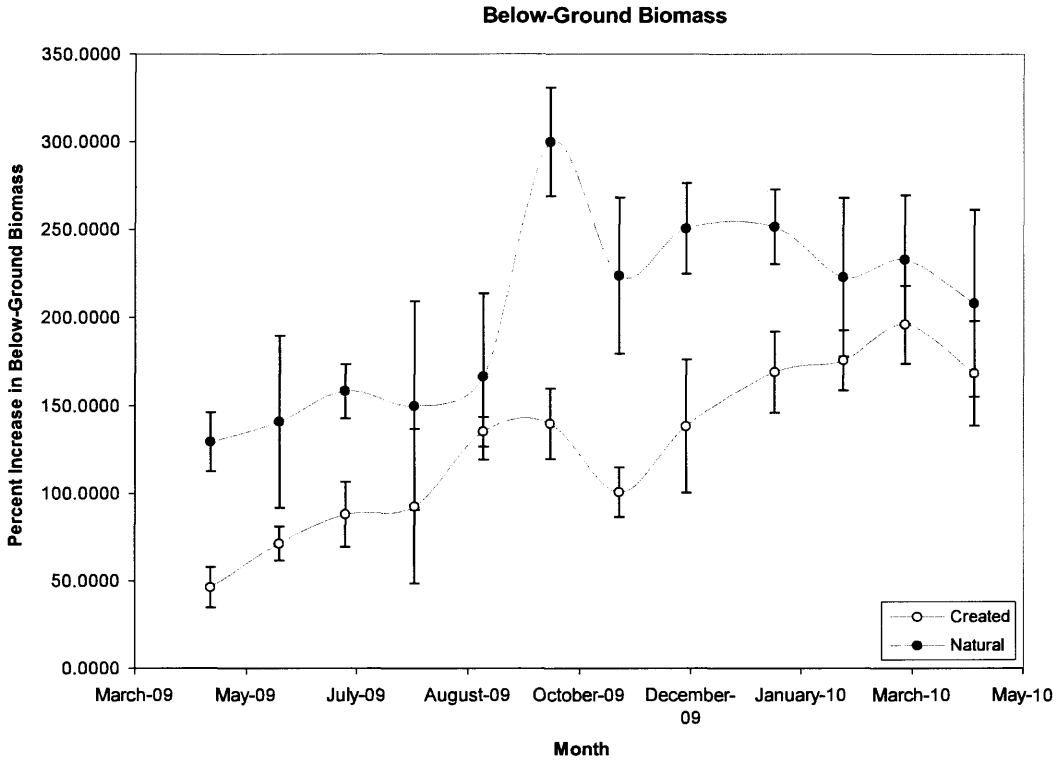

Below-Ground Biomass
Percent Increase in Below-Ground Biomass
350.0000
300.0000
250.0000
200.0000
150.0000
100.0000
50.0000
0.0000
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 3.6

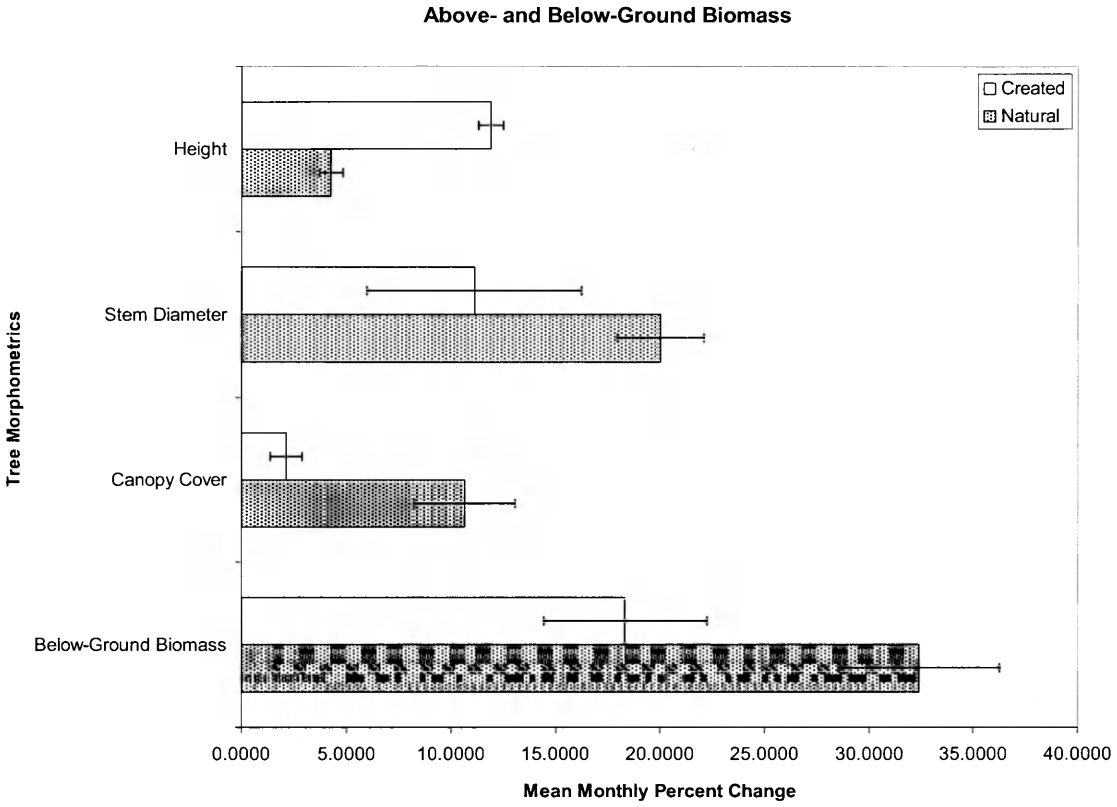

Above- and Below-Ground Biomass
Created
Natural
Height
Stem Diameter
Canopy Cover
Below-Ground Biomass
Tree Morphometrics
0.0000
5.0000
10.0000
15.0000
20.0000
25.0000
30.0000
35.0000
40.0000
Mean Monthly Percent Change

Figure 3.7

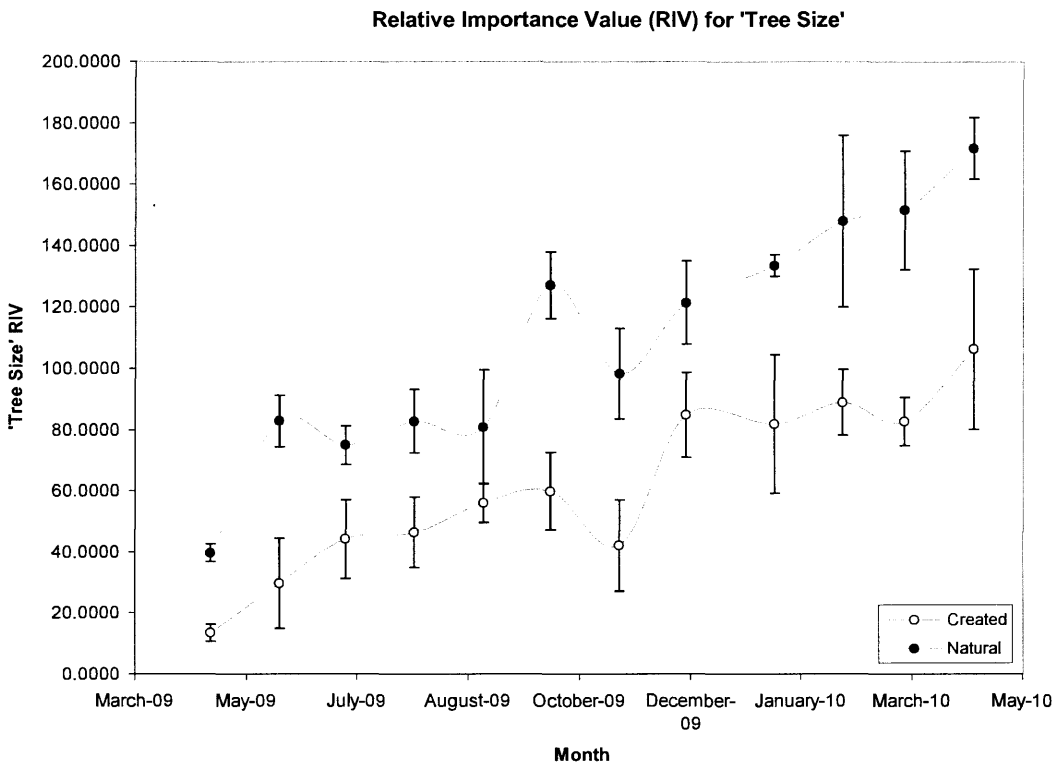

Relative Importance Value (RIV) for 'Tree Size'
200.0000
180.0000
160.0000
140.0000
120.0000
100.0000
80.0000
60.0000
40.0000
20.0000
0.0000
'Tree Size' RIV
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 3.8

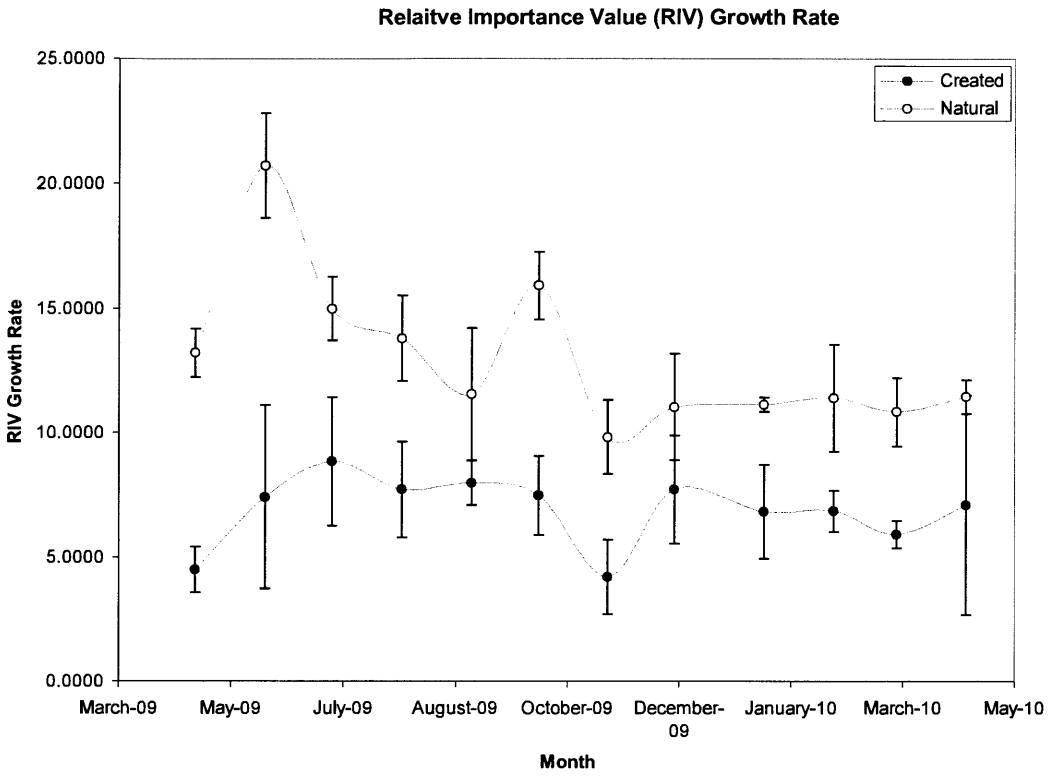

Relaitve Importance Value (RIV) Growth Rate
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
RIV Growth Rate
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.9

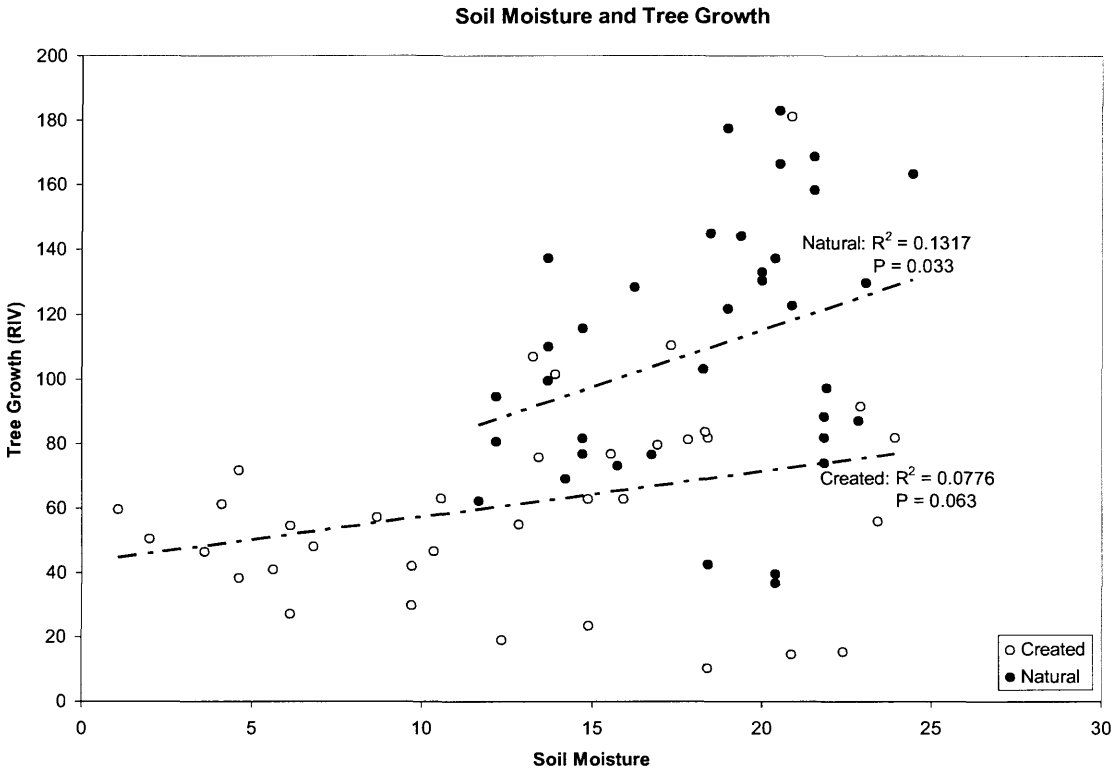

Soil Moisture and Tree Growth
Tree Growth (RIV)
Soil Moisture
Natural: R2 = 0.1317
P = 0.033
Created: R2 = 0.0776
P = 0.063
Created
Natural
0
20
40
60
80
100
120
140
160
180
200
0
5
10
15
20
25
30

Figure 3.10

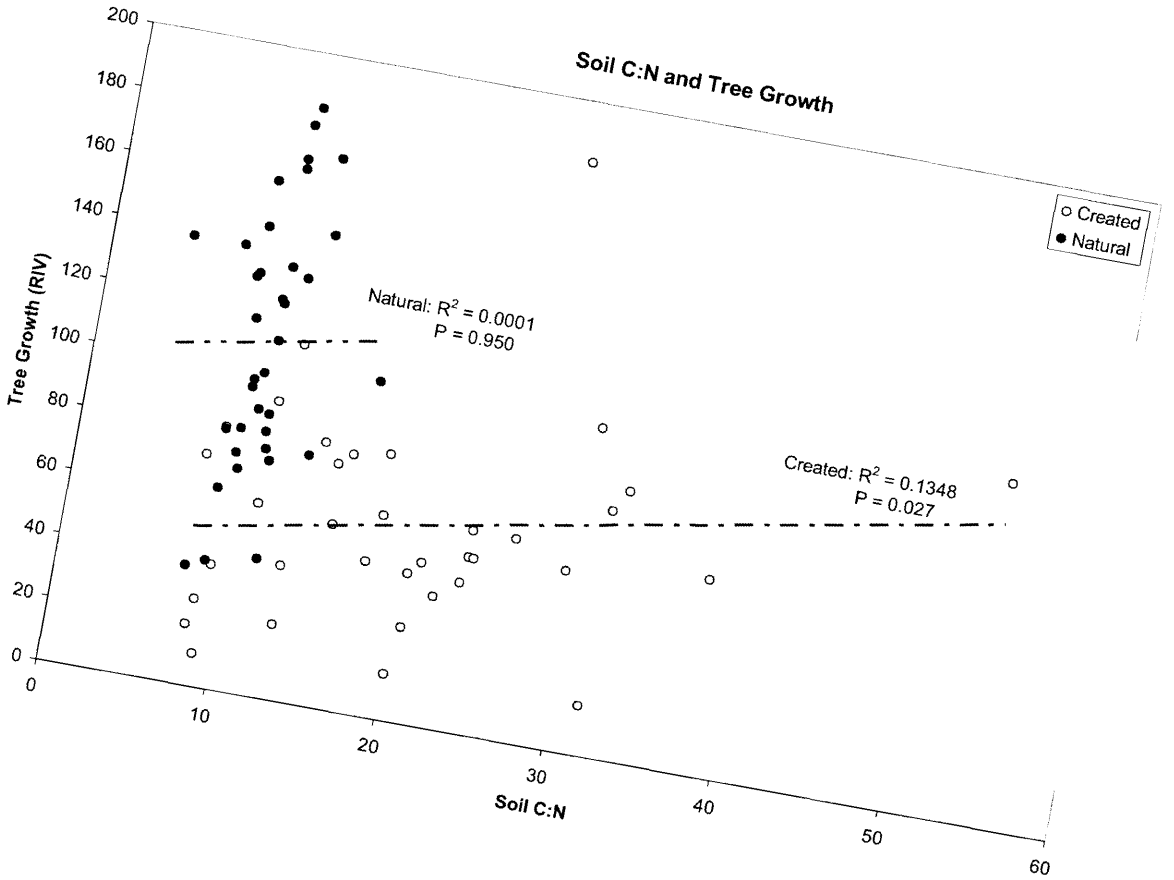

Soil C:N and Tree Growth
Created
Natural
Natural: R^2 = 0.0001
P = 0.950
Created: R^2 = 0.1348
P = 0.027
Tree Growth (RIV)
Soil C:N
0
20
40
60
80
100
120
140
160
180
200
0
10
20
30
40
50
60

Figure 3.11

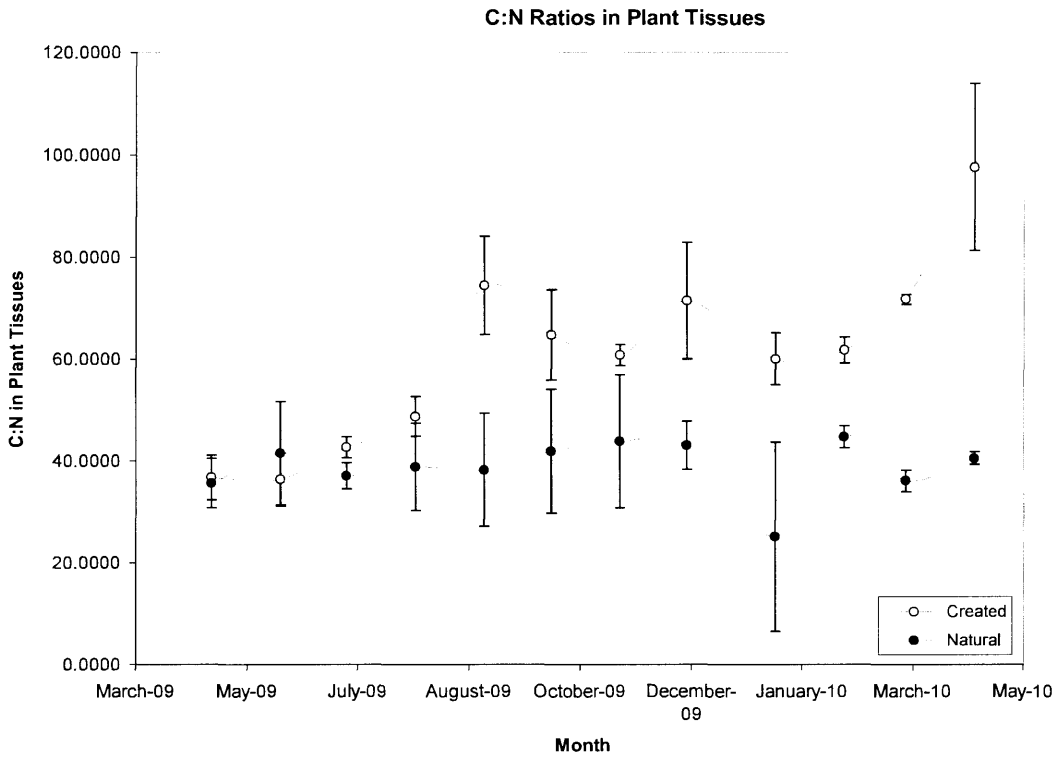

C:N Ratios in Plant Tissues
120.0000
100.0000
80.0000
60.0000
40.0000
20.0000
0.0000
C:N in Plant Tissues
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month
Created
Natural

Figure 3.12

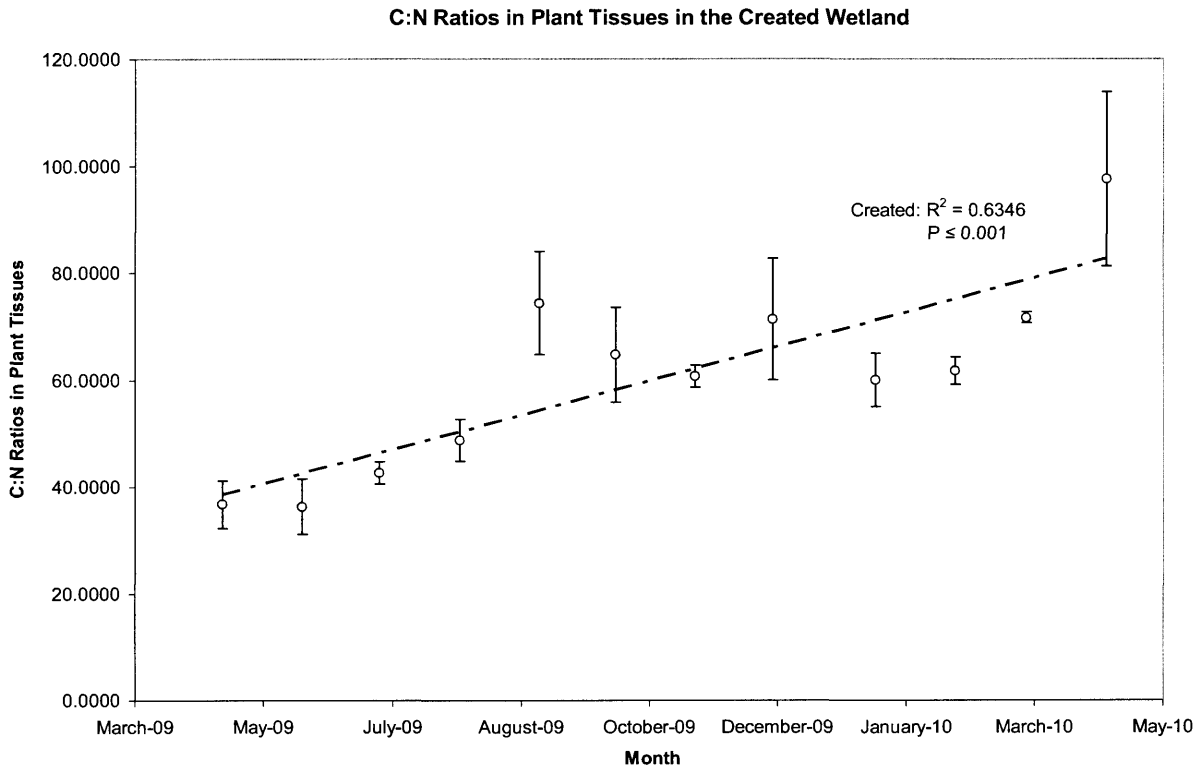

C:N Ratios in Plant Tissues in the Created Wetland
120.0000
100.0000
80.0000
60.0000
40.0000
20.0000
0.0000
C:N Ratios in Plant Tissues
Created: R2 = 0.6346
P ≤ 0.001
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.13

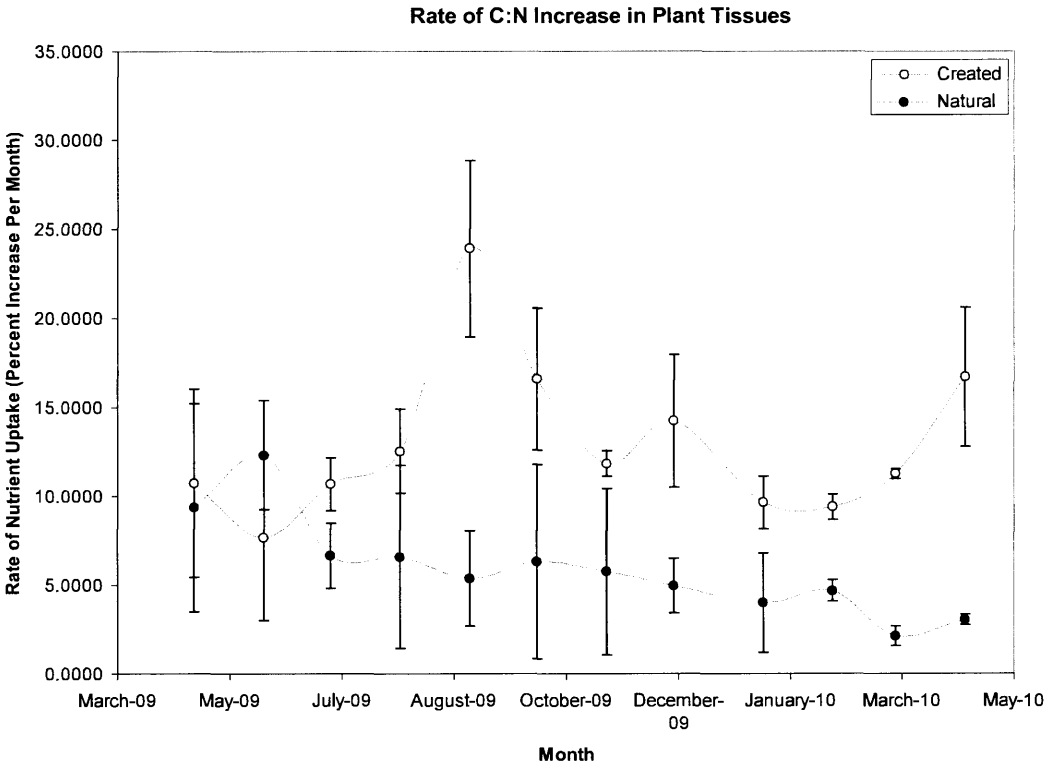

Rate of C:N Increase in Plant Tissues
Rate of Nutrient Uptake (Percent Increase Per Month)
35.0000
30.0000
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.14

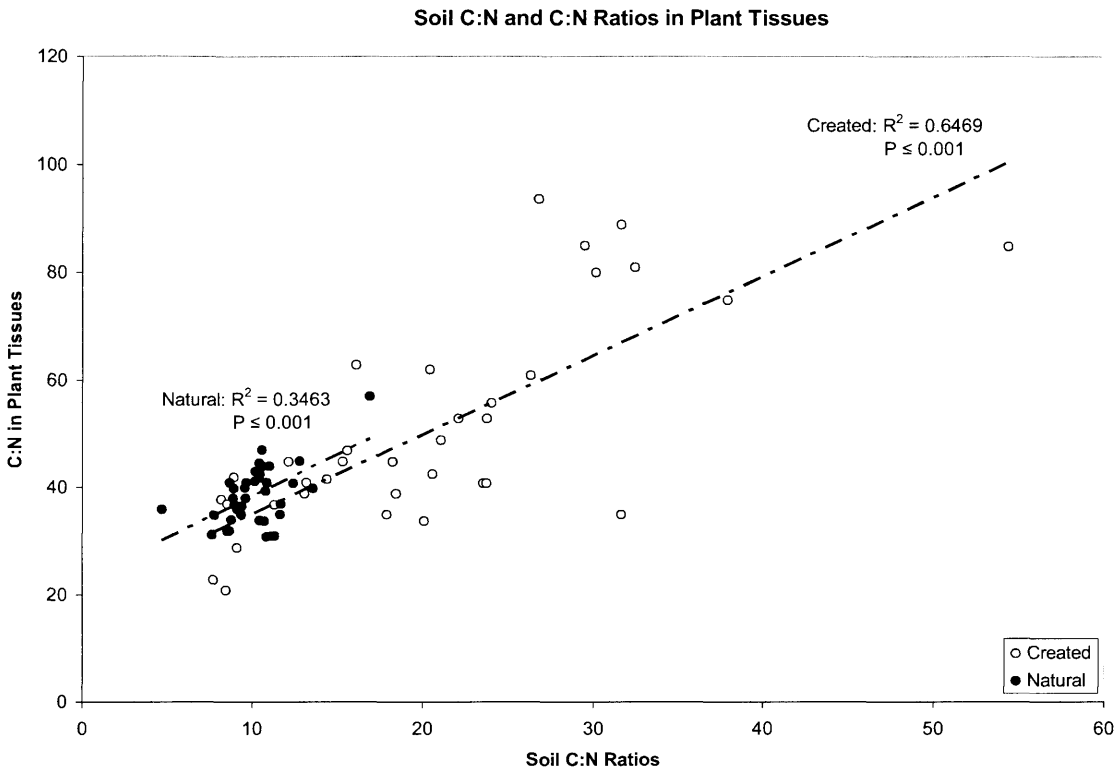

Soil C:N and C:N Ratios in Plant Tissues
C:N in Plant Tissues
Soil C:N Ratios
Created: R2 = 0.6469
P ≤ 0.001
Natural: R2 = 0.3463
P ≤ 0.001
Created
Natural
0
20
40
60
80
100
120
0
10
20
30
40
50
60

Figure 3.15

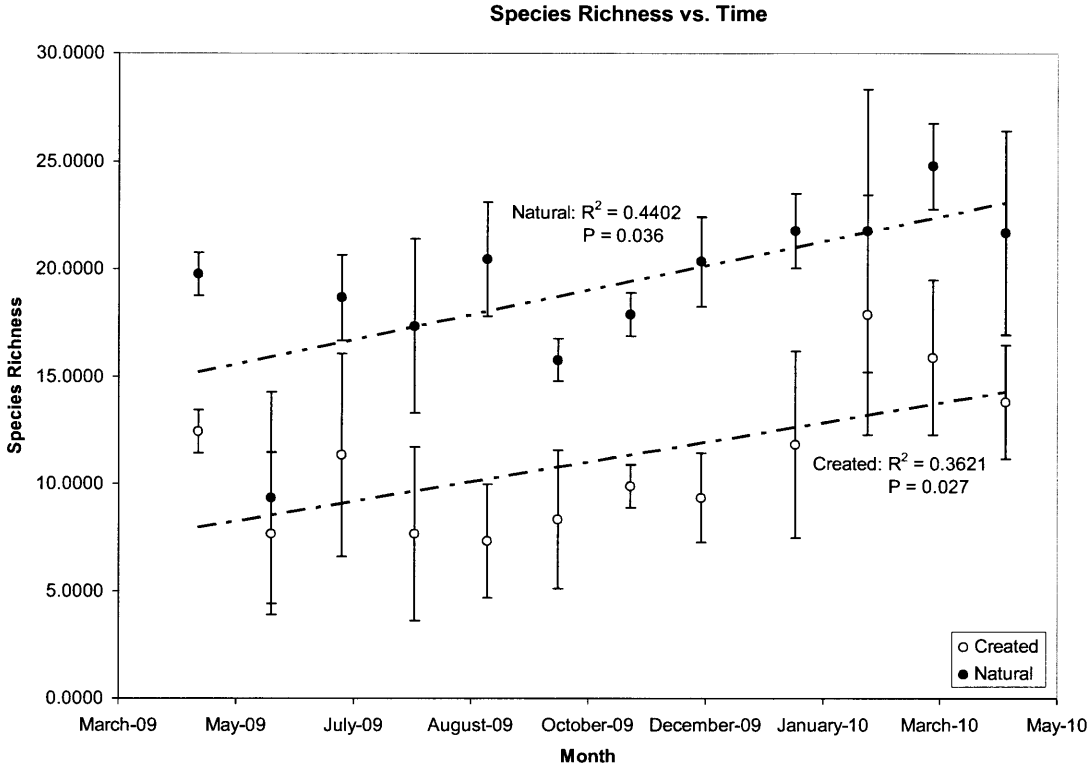

Species Richness vs. Time
30.0000
25.0000
20.0000
15.0000
10.0000
5.0000
0.0000
Species Richness
Natural: R2 = 0.4402
P = 0.036
Created: R2 = 0.3621
P = 0.027
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.16

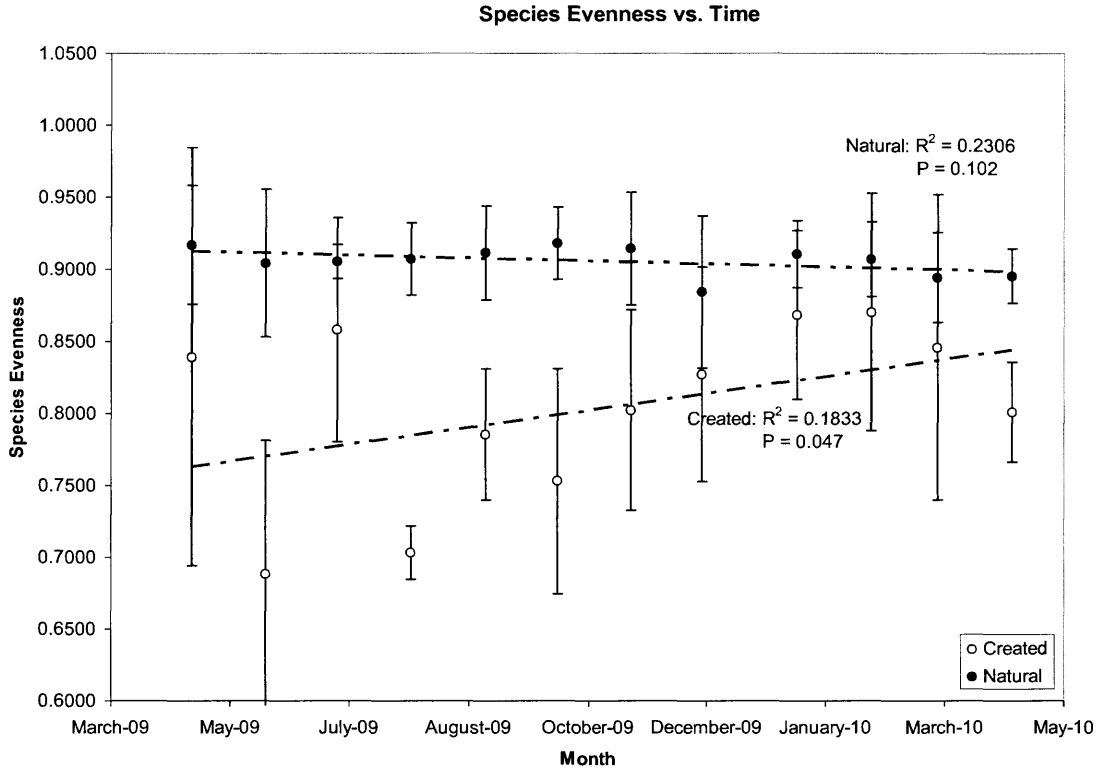

Species Evenness vs. Time
1.0500
1.0000
0.9500
0.9000
0.8500
0.8000
0.7500
0.7000
0.6500
0.6000
Species Evenness
Natural: R2 = 0.2306
P = 0.102
Created: R2 = 0.1833
P = 0.047
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.17

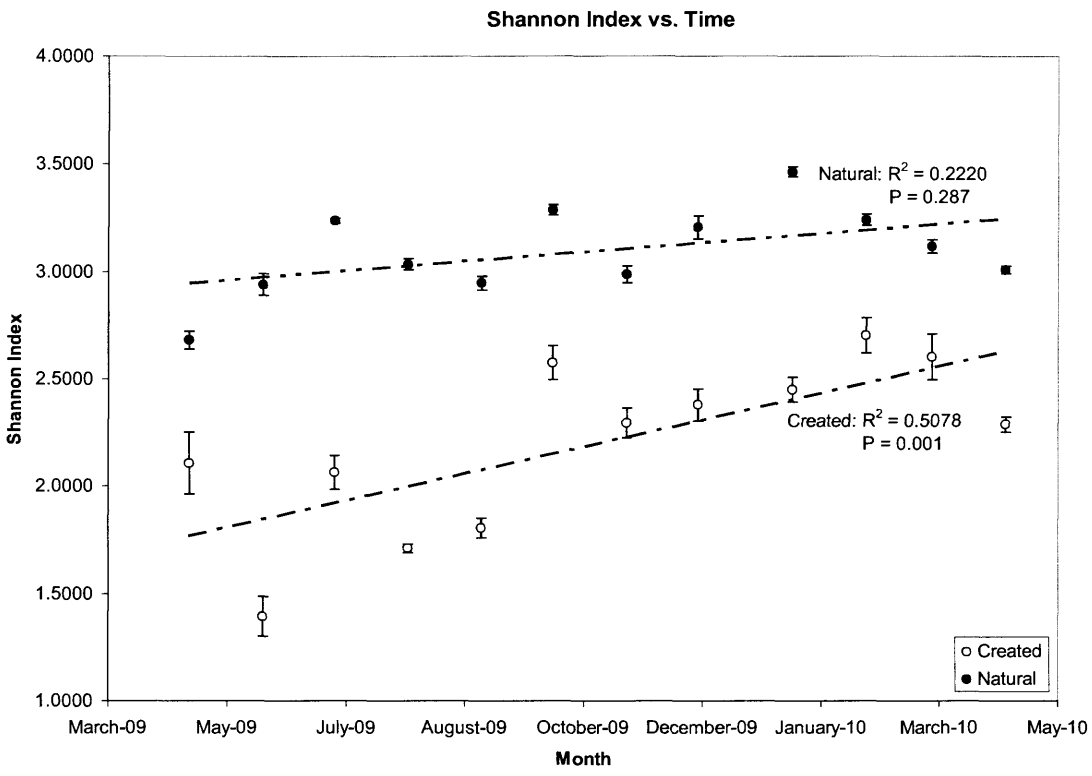

Shannon Index vs. Time
4.0000
3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
Shannon Index
Natural: R2 = 0.2220
P = 0.287
Created: R2 = 0.5078
P = 0.001
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.18

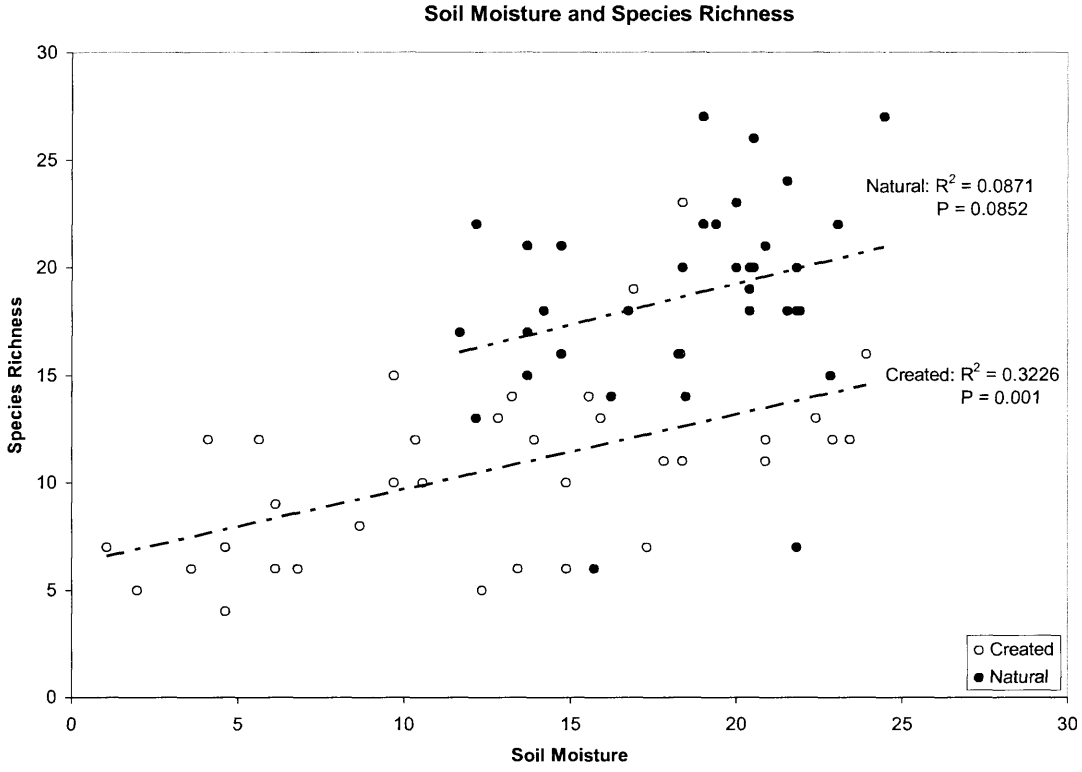

Soil Moisture and Species Richness
Species Richness
Soil Moisture
Natural: R^2 = 0.0871
P = 0.0852
Created: R^2 = 0.3226
P = 0.001
Created
Natural
0
5
10
15
20
25
30

Figure 3.19

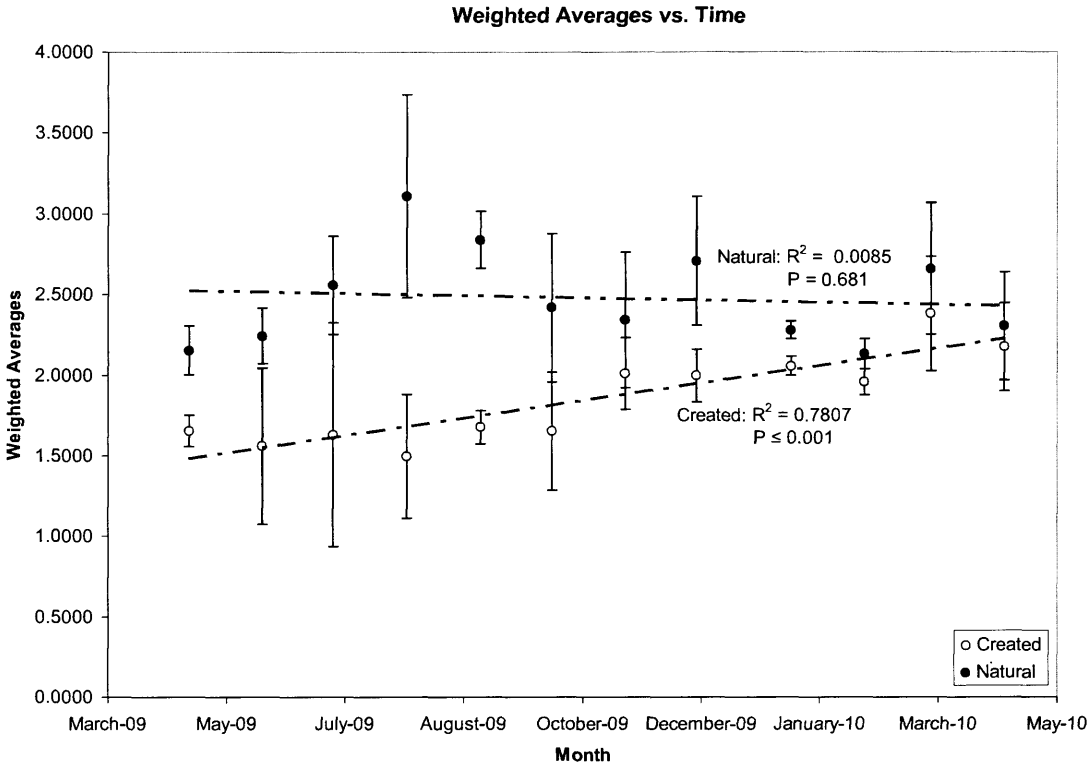

Weighted Averages vs. Time
4.0000
3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
Weighted Averages
Natural: R2 = 0.0085
P = 0.681
Created: R2 = 0.7807
P ≤ 0.001
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.20

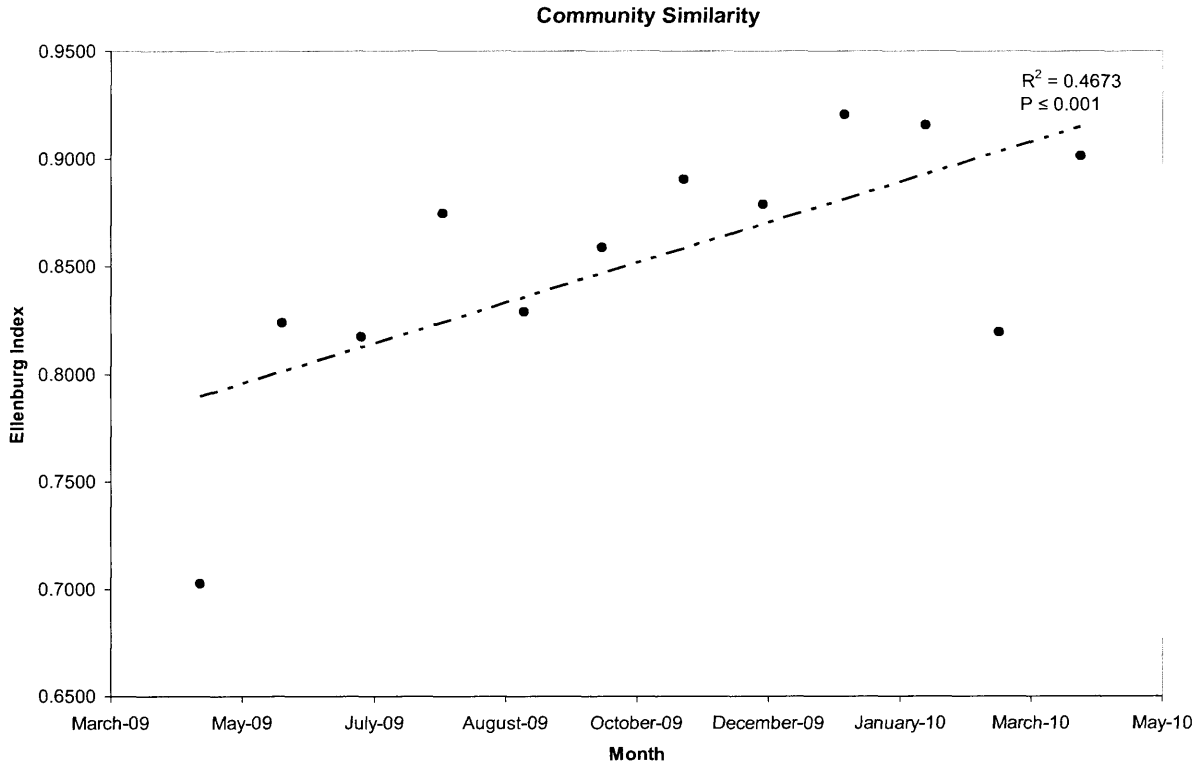

Community Similarity
0.9500
0.9000
0.8500
0.8000
0.7500
0.7000
0.6500
Ellenburg Index
R^2 = 0.4673
P ≤ 0.001
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.21

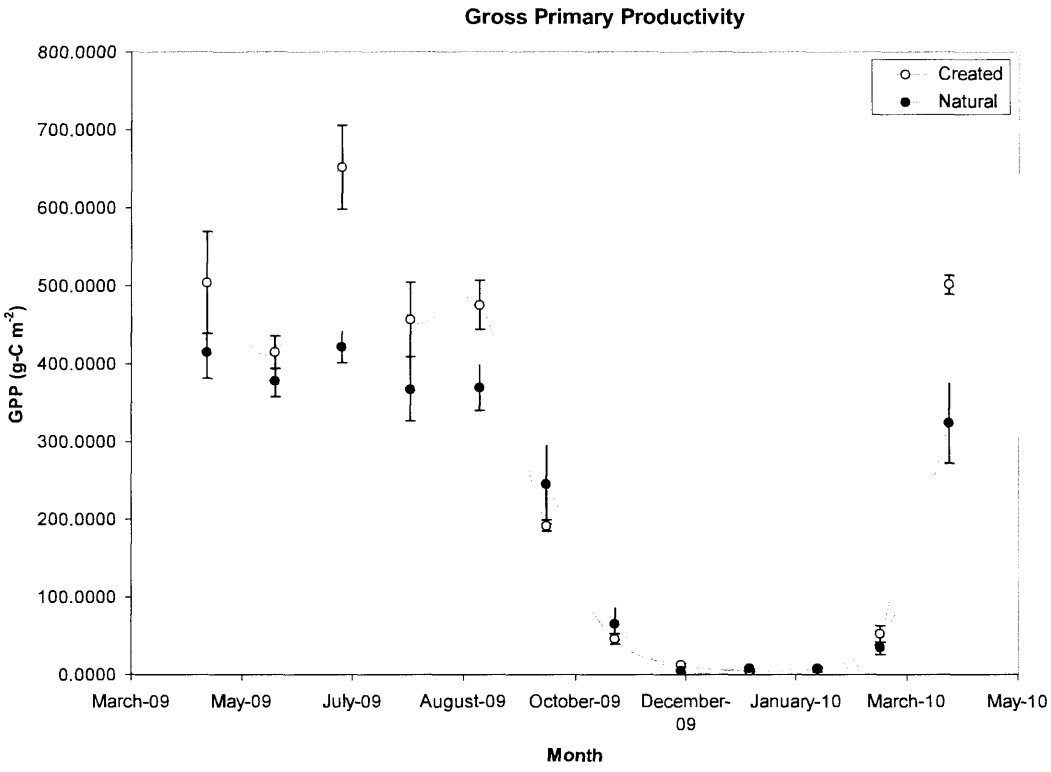

Gross Primary Productivity
800.0000
700.0000
600.0000
500.0000
400.0000
300.0000
200.0000
100.0000
0.0000
GPP (g-C m-2)
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.22

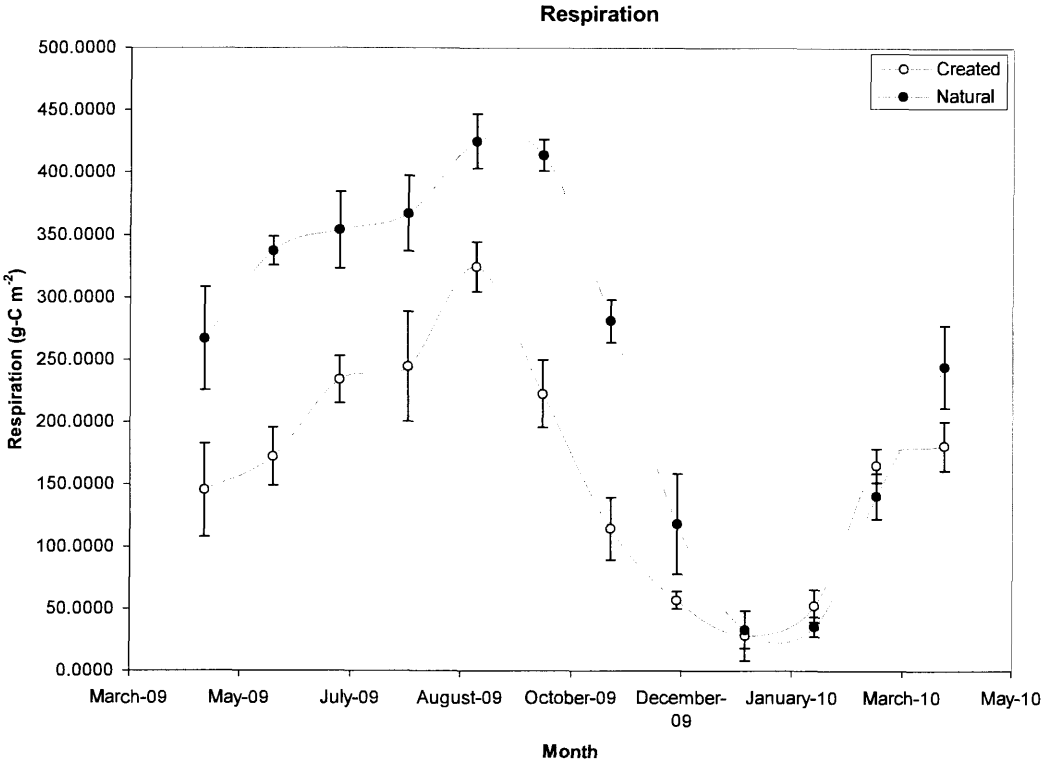

Respiration
Created
Natural
500.0000
450.0000
400.0000
350.0000
300.0000
250.0000
200.0000
150.0000
100.0000
50.0000
0.0000
Respiration (g-C m-2)
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.23

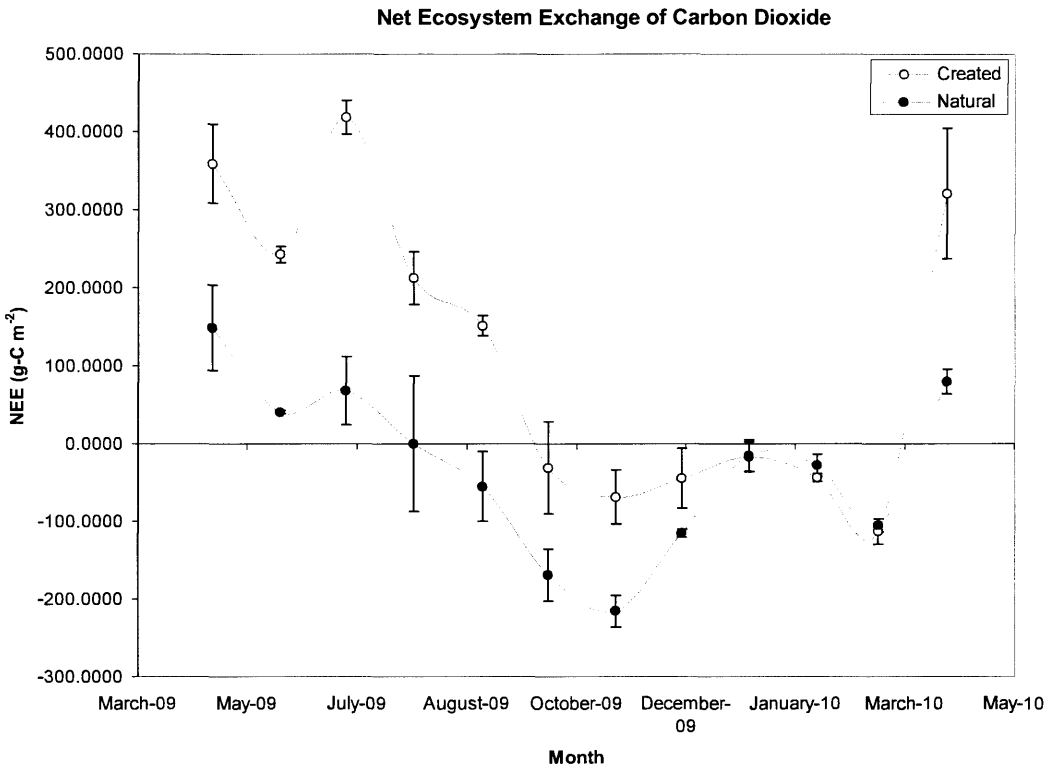
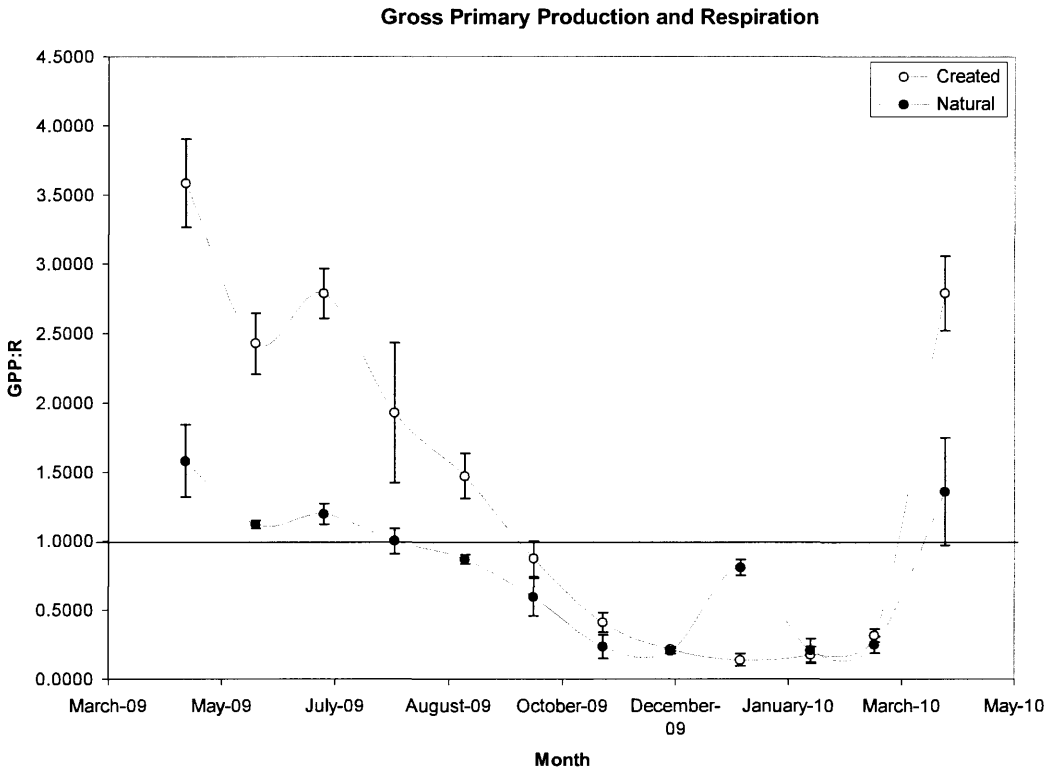

Net Ecosystem Exchange of Carbon Dioxide
500.0000
400.0000
300.0000
200.0000
100.0000
0.0000
-100.0000
-200.0000
-300.0000
NEE (g-C m-2)
Created
Natural
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Figure 3.24


Gross Primary Production and Respiration
Created
Natural
GPP:R
4.5000
4.0000
3.5000
3.0000
2.5000
2.0000
1.5000
1.0000
0.5000
0.0000
March-09
May-09
July-09
August-09
October-09
December-09
January-10
March-10
May-10
Month

Tables

Table 3.1

Family and Species	Indicator	Duration	Created IV	Natural IV
Apocynaceae				
Apocynum cannabinum L.	FACU	Perennial	0.90	1.81
Asteraceae				
Ambrosia artemisiifolia L.	FACU	Perennial	1.32	1.65
Baccharis halimifolia L.	FACW	Perennial	0.98	0.95
Bidens frondosa L.	FACW	Annual		1.84
Eclipta prostrata (L.) L.	FAC	Annual/Perennial	1.45	1.44
Eupatorium perfoliatum L.	FACW+	Perennial	1.39	3.03
Euthamia graminifolia (L.) Nutt.	FAC	Perennial	1.04	1.91
Solidago canadensis L.	FACU	Perennial	1.76	1.13
Brachytheciaceae				
Bryoandersonia illecebra		Perennial	2.35	6.32
Betulaceae				
Betula nigra L.		Perennial	0.96	0.90
Clusiaceae				
Hypericum hypericoides (L.) Crantz	FACU	Perennial		1.79
Cyperaceae				
Carex lurida Wahlenb.	OBL	Perennial	1.92	1.95
Carex vulpinoidea Michx.	OBL	Perennial	1.20	2.53
Cyperus pseudovegetus Steud.	FACW	Perennial	1.48	1.72
Cyperus strigosus L.	FACW	Perennial	1.60	2.37
Eleocharis obtusa (Willd.) J.A. Schultes	OBL	Annual	1.83	1.36
Rhynchospora capitellata (Michx.) Vahl	OBL	Perennial	1.37	1.28
Rhynchospora corniculata (Lam.)	OBL	Perennial		1.38

Gray				
Scirpus cyperinus (L.) Kunth	FACW+	Perennial	1.40	1.47
Euphorbiaceae				
Acalypha rhomboidea Raf.	FACU-	Annual	1.94	1.62
Fabaceae				
Lespedeza cuneata (Dum.-Cours.) G. Don	NI	Perennial	1.28	1.58
Juncaceae				
Juncus acuminatus Michx.	OBL	Perennial	2.53	2.06
Juncus effusus L.	OBL	Perennial	22.40	18.80
Juncus tenuus Willd.	FAC-	Perennial	1.75	2.96
Lythraceae				
Rotala ramosior (L.) Koehne	OBL	Annual	0.75	1.06
Oxalidaceae				
Oxalis stricta L.	UPL	Perennial	1.74	1.39
Poaceae				
Andropogon virginicus L.	FACU	Perennial	1.53	2.03
Arthraxon hispidus (Thunb.) Makino	None	Annual	0.75	1.47
Dichanthelium scoparium (Lam.) Gould	FACW	Perennial	2.67	1.18
Digitaria ischaemum (Schreb.) Schreb Muhl.	UPL	Annual	1.46	0.96
Echinochloa muricata (Beauv.) Fern.	FACW+	Annual		1.95
Panicum dichotomiflorum Michx.	FACW-	Annual	1.28	1.59
Saccharum giganteum (Walt.) Pers.	FACW+	Perennial	1.69	1.71
Setaria parviflora (Poir.) Kerguelen	FAC	Perennial	1.39	1.74
Polygonaceae				
Polygonum Hydropiperoides Michx.	OBL	Perennial	1.98	1.50

Polygonum lapathifolium L.	FACU+	Annual	1.56	1.05
Polygonum pensylvanicum L.	FACW	Annual	1.39	1.38
Polygonum persicaria L.	FACW	Annual/Perennial	1.18	1.59
Polygonum punctatum Ell.	OBL	Annual/Perennial	1.95	1.02
Rumex crispus L.	FACU	Perennial	1.66	2.12
Polytrichaceae				
Polytrichum juniperinum Hedw.		Perennial	2.51	12.22
Non-Categorical				
Bareground			22.56	7.12

Table 3.2

Species	Created IV	Natural IV
Juncus effusus L.	22.40	18.80
Bareground	22.56	7.12
Juncus acuminatus Michx.	2.53	2.06
Polytrichum juniperinum Hedw.	2.51	12.22
Bryoandersonia illecebra	2.35	6.32

Table 3.3

Species Richness (SR)		
Month	**Created**	**Natural**
May 2009	12.43 ± 1.00	19.75 ± 1.00
June 2009	7.66 ± 3.78	9.33 ± 4.93
July 2009	11.33 ± 4.72	18.65 ± 2.00
August 2009	7.66 ± 4.04	17.33 ± 4.04
September 2009	7.31 ± 2.64	20.43 ± 2.64
October 2009	8.33 ± 3.21	15.75 ± 1.00
November 2009	9.87 ± 1.00	17.87 ± 1.00
December 2009	9.33 ± 2.08	20.33 ± 2.08
January 2010	11.82 ± 4.35	21.75 ± 1.73
February 2010	17.85 ± 5.56	21.75 ± 6.55
March 2010	15.87 ± 3.60	24.75 ± 2.00
April 2010	13.80 ± 2.64	21.66 ± 4.72
Evenness (J')		
Month	**Created**	**Natural**
May 2009	0.83 ± 0.14	0.91 ± 0.04
June 2009	0.68 ± 0.09	0.90 ± 0.05
July 2009	0.85 ± 0.07	0.90 ± 0.01
August 2009	0.70 ± 0.01	0.90 ± 0.02
September 2009	0.78 ± 0.04	0.91 ± 0.03
October 2009	0.75 ± 0.07	0.91 ± 0.02
November 2009	0.80 ± 0.06	0.91 ± 0.03
December 2009	0.82 ± 0.07	0.88 ± 0.05
January 2010	0.86 ± 0.05	0.91 ± 0.02
February 2010	0.87 ± 0.08	0.90 ± 0.02
March 2010	0.84 ± 0.10	0.89 ± 0.03
April 2010	0.80 ± 0.03	0.89 ± 0.01
Shannon Index (H')		
Month	**Created**	**Natural**
May 2009	2.10 ± 0.37	2.68 ± 0.30
June 2009	1.39 ± 0.37	2.93 ± 0.32
July 2009	2.06 ± 0.28	3.23 ± 0.27
August 2009	1.71 ± 0.26	3.03 ± 0.17
September 2009	1.80 ± 0.11	2.94 ± 0.14
October 2009	2.57 ± 0.56	3.28 ± 0.17
November 2009	2.29 ± 0.53	2.98 ± 0.10
December 2009	2.38 ± 0.46	3.20 ± 1.12
January 2010	2.45 ± 0.45	3.46 ± 0.58
February 2010	2.70 ± 0.64	3.23 ± 0.35
March 2010	2.60 ± 0.55	3.11 ± 0.58
April 2010	2.28 ± 0.54	3.00 ± 1.03

Weighted Averages (WA)		
Month	**Created**	**Natural**
May 2009	1.65 ± 0.09	2.15 ± 0.15
June 2009	1.55 ± 0.48	2.24 ± 0.17
July 2009	1.63 ± 0.69	2.55 ± 0.30
August 2009	1.49 ± 0.38	3.10 ± 0.62
September 2009	1.67 ± 0.10	2.83 ± 0.17
October 2009	1.65 ± 0.36	2.41 ± 0.45
November 2009	2.01 ± 0.22	2.34 ± 0.42
December 2009	1.99 ± 0.16	2.70 ± 0.39
January 2010	2.05 ± 0.16	2.27 ± 0.05
February 2010	1.95 ± 0.08	2.13 ± 0.09
March 2010	2.38 ± 0.35	2.65 ± 0.40
April 2010	2.17 ± 0.27	2.30 ± 0.33

Table 3.4

Parameter	Created	Natural	P-Value
Species Richness (SR)	35.00 ± 1.10	39.66 ± 0.57	0.002
Evenness (J')	2.55 ± 0.44	3.14 ± 0.28	0.124
Shannon Index (H')	0.75 ± 0.03	0.91 ± 0.04	0.003
Weighted Averages (WA)	1.51 ± 0.14	2.21 ± 0.11	0.002

Table 3.5

Month	Ellenburg Index
May 2009	0.7025
June 2009	0.8238
July 2009	0.8175
August 2009	0.8745
September 2009	0.8289
October 2009	0.8589
November 2009	0.8905
December 2009	0.8789
January 2010	0.9204
February 2010	0.9156
March 2010	0.8196
April 2010	0.9014
Year	0.9182

Table 3.6

Parameter	*Factor*	*Df*	*F*	*P*
Height	Site	1	13.309	< 0.001
	Month	11	49.834	< 0.001
	Site × month	11	2.5466	0.012
Stem Diameter	Site	1	5.982	< 0.001
	Month	11	12.859	< 0.001
	Site × month	11	0.620	0.802
Canopy	Site	1	8.385	< 0.001
	Month	11	156.749	< 0.001
	Site × month	11	4.690	< 0.001
Roots	Site	1	11.918	< 0.001
	Month	11	97.315	< 0.001
	Site × month	11	2.134	0.035
Total Biomass	Site	1	16.565	< 0.001
	Month	11	107.006	< 0.001
	Site × month	11	1.025	0.439
Growth Rate	Site	1	5.580	< 0.001
	Month	11	175.306	< 0.001
	Site × month	11	3.150	0.003
Nutrient Uptake	Site	1	1.942	0.056
	Month	11	15.427	< 0.001
	Site × month	11	1.976	0.052
Rate of Nutrient Uptake	Site	1	1.868	0.068
	Month	11	57.030	< 0.001
	Site × month	11	3.368	0.001
Richness	Site	1	5.067	< 0.001
	Month	11	98.076	< 0.001
	Site × month	11	1.210	0.305
Evenness	Site	1	5.067	< 0.001
	Month	11	98.076	< 0.001
	Site × month	11	1.210	0.305
Shannon Index	Site	1	5.067	< 0.001
	Month	11	98.076	< 0.001
	Site × month	11	1.210	0.305
Weighted Averages	Site	1	5.067	< 0.001
	Month	11	98.076	< 0.001
	Site × month	11	1.210	0.305
Annual Richness	Site	1	49.000	0.002
Annual Evenness	Site	1	3.762	0.124
Annual Shannon Index	Site	1	39.005	0.003
Annual Weighted	Site	1	44.918	0.003

Averages				
GPP	Site	1	296.848	< 0.001
	Month	11	64.289	< 0.001
	Site × month	11	11.948	< 0.001
R	Site	1	119.530	< 0.001
	Month	11	227.931	< 0.001
	Site × month	11	12.538	< 0.001
NEE	Site	1	83.671	< 0.001
	Month	11	261.641	< 0.001
	Site × month	11	13.324	< 0.001
GPP:R	Site	1	44.487	< 0.001
	Month	11	77.977	< 0.001
	Site × month	11	9.540	< 0.001
Annual GPP	Site	1	1.306	0.256
Annual R	Site	1	10.632	0.002
Annual NEE	Site	1	16.427	< 0.001
Annual GPP:R	Site	1	8.498	0.004

CONCLUSION:

The Need for Further Research in Palustrine, Forested Wetlands Created for Mitigation Purposes

Introduction

Chapter one provided a detailed review of current construction practices for mitigation wetlands, wetland biogeochemistry, and vegetation dynamics in wetland systems. Chapters two and three covered a suite of parameters that constitute a wetland ecosystem. Between the analysis of hydrology, soils, biogeochemistry, and vegetation, data present a clear picture of the differences that exist between a created and natural palustrine wetland in southeastern Virginia. Data also show how various environmental parameters impact ecosystem function in these two wetland types.

Our data showed that soil moisture, soil organic matter, total carbon, and total nitrogen were all lower in the created wetland than in the natural wetland, while bulk density was higher in the created wetland. Moreover, microbial mediated biogeochemical processes varied significantly between the two wetlands. Soil respiration, nitrogen mineralization, and denitrification were all lower in the created wetland. Conversely, nitrogen fixation was greater in the created wetland while methane emissions were negligible from both sites. These analyses indicate that, even when constructed based on current recommendations, environmental conditions in created wetlands produce a challenging environment for the establishment of vegetation.

Analysis of vegetational communities supported this finding. Above- and below-ground biomass production and nutrient uptake were significantly lower in the created wetland, likely as a result of environmental conditions. Additionally, our results showed the created wetland was less diverse in terms of richness, evenness, and the Shannon index. However, being an early successional system, the created wetland featured higher levels of primary production and sequestered more carbon both during the growing season and over the course of the year long sampling period.

In order to minimize these differences we therefore recommend that hydrological conditions in created wetlands more closely match conditions in natural reference wetlands. We also recommend that more care be taken to ensure that nutrient conditions in created wetlands meet vegetational demands for growth.

Suggestions for Further Research

One of the main differences observed in this study was that the created wetland had significantly more bareground than the natural wetland. Past studies have shown bareground has the potential to create a negative feedback loop where wetlands with an abundance of unvegetated ground become susceptible to higher soil temperatures and increasing organic matter respiration rates, which in turn decrease SOM and lower plant productivity, thereby leading to lower plant coverage and an increase in bare ground (Aust and Lea, 1991; Londo et al., 1999; Zak et al., 1999; Stolt et al., 2000; Reth et al., 2005).

More research is needed to investigate the potential use of top-application of stockpiled topsoil to circumvent this series of events. Most current recommendations suggest fully incorporating topsoil directly into the soil profile; however, when organic matter is incorporated into the soil all at once, there is a rapid, short release of a large quantity of nutrients that cannot be captured by vegetation (DeGregorio and Ashley, 1986; Hartwig, 1987; Hofsretter, 1988). As such, many of the nutrients are lost from the system, and the benefits of adding organics are minimized. Conversely, the natural incorporation of surface topsoil residues from the soil surface into deeper layers is a slow process, primarily regulated by the activity of the macrofauna, which results in the long, slow release of nutrients (Langdale and Leonard, 1983; Elliot et al., 1987; Hofsretter, 1988). Therefore perhaps top application of organic material should be considered.

Research already done on this topic suggests surface mulch should consist of material characterized by both low and high C:N ratios (Anderson and Cowell, 2004). Anderson and Cowell (2004) showed this combination increased labile nitrogen, nutrient

immobilization, organic matter accumulation, and humus formation during the growing season, which improved soil structure in both the short and long term. An additional benefit of using surface mulches is that they cover the soil surface and protect against the impact of rain, and desiccation from the sun (Langdale and Leonard, 1983; Hartwig, 1987; Corak et al., 1991). Surface residues have also been shown control invasive weed species, and enhance soil aggregation and intact large pores, improve water infiltration, and reduce runoff and erosion (Pimentel et al., 1995).

Finally, our study highlights the need for further research into the performance of mitigation wetlands following construction. To our knowledge, few studies have addressed the connections between microbial communities and vegetation, particularly in created wetlands. Our research provided just a cursory overview of this topic, and tended to treat both factors independently. However, past research has shown that by-products from roots and plant residues feed soil microorganisms; in turn, soil organisms support plant health as they decompose organic matter, recycle nutrients, and enhance soil structure (Drew and Lynch, 1980; Neue, 1985; Garbeva et al., 2004). More research is needed to address these connections, and investigate how to improve linkages between soil microbial communities and plants to improve created wetland success.

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VITA

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