12-2007

Enhancing the ability of *Panicum virgatum* to survive flooding and its effects on soil activity when used for lakeshore stabilization

Lee Edwards
Clemson University, Lee.Edwards@gvltec.edu

ENHANCING THE ABILITY OF *PANICUM VIRGATUM* TO SURVIVE FLOODING AND ITS EFFECTS ON SOIL ACTIVITY WHEN USED FOR LAKESHORE STABILIZATION

A Dissertation
Presented to
the Graduate School of
Clemson University

In Partial Fulfillment
of the Requirements for the Degree
Doctor of Philosophy
Plant and Environmental Sciences

---

by
Joan Lee Edwards
December 2007

Accepted by:
Dr. Larry A. Dyck, Committee Chair
Dr. Melissa B. Riley
Dr. Tom McInnis
Dr. Christina E. Wells

ABSTRACT

Reservoirs in upstate South Carolina are subject to erosion and vegetation has been placed to help stabilize banks, but soils are nutrient-poor and have high bulk densities. *Panicum virgatum* L. (switchgrass) has been planted in several sites, and it is hypothesized that over time, changes in microbial activity and soil quality will occur in response to the planting. As vegetated sites aged, acid phosphomonoesterase, nitrate reductase, and dehydrogenase activity increased. Low values of nitrogen fixation and substrate-induced respiration were measured at all sites with no statistical differences between sites. Fatty acids indicative of Gram negative bacteria were found in vegetated sites and the diversity of fatty acids increased in vegetated sites indicating more diverse microbial communities.

Switchgrass plants may experience periods of flooding resulting in ethylene production. Switchgrass plants were placed in a modified Hoagland's solution and treated with an ethylene producer and ethylene inhibitor to determine morphological responses. Ethylene treatment resulted in increases in the number of adventitious roots. No effects were observed on aerenchyma development, shoot elongation, root length, average root diameter, root system volume, or root surface area. Switchgrass therefore does not appear to be sensitive to ethylene which may be produced in response to flooding resulting in no effect on switchgrass growth.

Serratia ficaria, an ACC deaminase bacterium, was isolated from lakeshore soils and then added to switchgrass seeds/young plants to determine its effect as a plant growth-promoting bacterium (PGPB) under three different water regimes. Treatments

inoculated with bacteria had increased root to shoot ratios in unflooded and completely submerged water regimes, but the opposite was true for crown-flooded treatments. Shoot growth was fastest in inoculated treatments, except for completely submerged treatments, where values were not statistically different between inoculated and uninoculated groups. The bacterium was beneficial when there is no water stress, but became detrimental when water levels rose until submergence

Overall switchgrass appears to increase microbial activity and diversity over time, has limited response to ethylene due to flooding, and is able to grow in soils with minimal nutrients making it a good plant for use in lakeshore stabilization along Lake Hartwell.

DEDICATION

I absolutely could not have completed this program without the love and support of my husband, Rick Edwards. He is my anchor in the storm and the best friend I could ever wish for. I love you will all my heart and look forward to the rest of our lives together. I dedicate all the hard work it took to complete my Ph.D. to you.

ACKNOWLEDGMENTS

I want to express my sincere appreciation to my committee for guiding me through my Ph.D. program. My major advisor, Dr. Larry Dyck, is one of the best teachers I know. He has shown me not only to appreciate the details, but also to fit those details into the "big picture". I have learned so much from him, and I know I will look at the world differently from now on.

Without Dr. Lissa Riley I would never have completed the research and the writing of this dissertation. I can never thank her enough for the moral support and guidance she gave me and the countless hours she spent revising these chapters.

I want to thank Dr. Christina Wells for opening up her lab to me and allowing me to use whatever I needed. She always made me feel like part of her lab team, and I appreciate her hospitality.

Dr. Tom McInnis was always a supportive committee member and I valued his thoughts on the approach I should take with my research. I enjoyed and learned so much from the classes I took from him. Those classes gave me a very strong basis for this research.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1

LITERATURE REVIEW

Bank Stabilization of Lake Hartwell

Reservoirs in the southeastern United States are subject to high erosional forces; the erosion can be caused by waves lapping on the shores as a result of wind blowing across the surface of the water or by wakes from boat traffic in the area. Wave action erodes the shorelines which can undercut banks at the boundary of the high water mark and the tree line, resulting in the sediment between the full pond level and the high water mark being carried away. This action is much like the sand on coastal beaches between the high and low tide levels. This washing of sediment into the lakes can result in an overall decrease in the lake depth and also result in sediment build-up behind dams. Because reservoirs are artificial constructs, the erosional forces potentially cause larger problems than would occur at the boundaries of natural lakes.

Natural lakes generally have gradually sloping shorelines and are covered in vegetation with roots that help hold soils in place. The shoreline vegetation also "slows the velocity of raindrops, resulting in less impact force when they strike the ground," (www.sas.usace.army.mil/lakes/hartwell/intro.htm). When the velocity of the raindrops is decreased, there is less erosion of the finer particles, which are usually highest in nutrient content (www.sas.usace.army.mil/lakes/hartwell/intro.htm). Reservoir shorelines tend to have little, if any, vegetation to slow erosion. Additional factors increase erosion along the reservoir shoreline. Although soils of the shorelines are compacted and not very erodable on their own, they can be easily broken up by "frost-

heaving" and carried away. Also, the banks are undercut at the waterline when waves crash and the soil above slumps or caves. This action causes scarps to form. Trees at the water's edge tend to fall towards the lake either into the water or on dry shoreline following undercutting, depending on the water level when shoreline erosion occurs. This allows more sediment to be washed into the reservoir. The boundary of the reservoir may begin to extend past the present tree line destroying valuable woodland habitat.

After the Lake Hartwell reservoir was completed, the development of the Lakeshore Zoning Plan was initiated in 1972 as outlined by the United States Army Corps of Engineers (USACE) South Atlantic Division (USACE, 2007). Subsequent management plans have been outlined in order to inform landowners of guidelines issued by the USACE to "manage and protect the Hartwell Project shoreline in a manner promoting the safe and healthful use of the shoreline by the public while maintaining environmental safeguards to ensure a quality resource for future generations" (USACE, 2007).

Several methods have been used by the USACE and the general public to reinforce the Lake Hartwell shoreline and prevent creeping of the reservoir boundary. Often, large-sized quarried granite stones (rip-rap) have been placed at the lake margins to help combat the erosional forces. Rock serves as a ballast to hold down erosion control fabric or to help hold small gravel sized rocks in place. Rip-rap has been shown to be effective against the loss of shoreline due to waves, is readily available and slow to degrade, and its design can be modified for different wave or flow energies (Dorn, 2002).

However this solution has done little for the filtering of non-point source pollution or for the enrichment of habitat for fish, invertebrates, amphibians, or waterfowl that call the reservoir home.

Native vegetation planted in areas around the reservoir remedy shoreline loss and serve in a filtering capacity to trap chemicals such as pesticides and excess fertilizers. The vegetated buffer strips provide an area for decomposition of potentially harmful chemicals before they reach the water body. Introduced native species also provide habitat for animals, moderate summer temperature extremes, and improve shoreline aesthetics (USACE, 2007). The USACE has produced a list of native species approved for planting along the Lake Hartwell shoreline (within rip-rap). Some of the species include: *Chasmanthium latifolium* (River Oats), *Panicum clandestinum* (Deertongue grass), *Panicum hemitomon* (Maidencane), *Spartina patens* (Cordgrass), *Spartina pectinata* (Prairie Cordgrass), *Saccharum contortus* (Plumegrass), *Schizachyrium scoparium* (Little bluestem), and *Panicum virgatum* (switchgrass). Each has been evaluated for use on Lake Hartwell shorelines. *P. virgatum* (switchgrass) was suggested as a good candidate to survive and successfully stabilize the highly eroding shorelines, based on its tolerance for dry to very wet conditions and nutrient poor soils (Dorn, 2002). Of eleven native grasses studied, only *Chasmanthium latifolium*, *Panicum virgatum*, *Spartina patens*, *P. hemitomon*, and *S. pectinata* were suggested to have the ability to grow in clay soils with the possibility of experiencing water regimes from dry to crown inundation. Of these five native grasses, only *P. virgatum* was recommended for planting

on the plateau region as well as the slope of reconstructed banks around Lake Hartwell (Dorn, 2002).

Although vegetation buffer strips can be conducive to erosion control, the soils at these sites in the southeastern United States often tend to be nutrient poor (Brady and Weil, 2004). Historically, upstate South Carolina was used for agricultural purposes, resulting in a loss of topsoil. With the loss of topsoil, the soils of the reservoir areas are primarily B and C-horizon subsoils characteristically low in nutrient content. Because of the low nutrient content of the clay soils, planting around the shorelines and expecting plants to return season after season is a challenge. Vegetation placed in these areas must be able to withstand acidic, nutrient-poor soils with high bulk densities that are periodically inundated with water and drained at unpredictable times. As stated, switchgrass has been found to inhabit these harsh areas more successfully than other grasses, but there may be ways to aid switchgrass in its establishment and sustainability. An active and functionally diverse soil microbial community may aid the survival of vegetation in stressful habitats. Microbes have an influence over the soil environment in terms of nutrient availability (Carpenter-Boggs *et al*., 2003; Harris, 2003), pH changes (Smith and Read, 1997), and possibly pest resistance (Azcon-Aguilar and Barea, 1996).

Presently, planted switchgrass, is returning each year in the drier areas of reconstructed shoreline slopes. Swithcgrass has filled in some of the dry slopes resulting in thick stands. Switchgrass on the lower edges of the planted strips in front of the toe protecting rip-rap where the water level rises periodically have fared less well. Plants that have been flooded for several months at a time have died and the wave action has

carried soil away from the roots. If the plants had survived and reproduced vegetatively to create a thick mat, the soil may not have been easily carried away. When the soil is washed away, the grasses may be uprooted and erosion problems may begin again.

Switchgrass

Switchgrass is a native grass found over a large range from Mexico to Canada south of 55^{o}N latitude. It has been used in the United States for erosion control and to provide forage under hot and dry conditions. It is a warm season perennial herbaceous grass, employing the C-4 photosynthetic pathway, and its shoots emerge in the spring when soil temperatures rise above 10^{o}C. Switchgrass can be grown from seed and also develops from rhizomes. This grass has both shallow feeder roots and large, aerenchymous roots that can extend to 1 – 3m in depth. The erect stems can be between 0.5 – 2.7m tall and often have a reddish tint (Figure 1.1). There are two main types of switchgrass: a lowland type, found on wetter sites, and an upland type, adapted to drier habitats. The type planted on the shoreline is the lowland type, which has tall, thick, coarse stems and grows in clumps (Elberson and Christian, 2001). Flowering occurs by midsummer when up to 75% of the biomass has been formed.

Switchgrass is adapted to a wide range of soils. Unlike other grass species, switchgrass can do well under low soil fertility and acidic pH. It can tolerate pH of 4.9 to 7.6, but grows better in soils amended closer to neutrality (Elberson and Christian, 2001). This grass can tolerate low nitrogen levels and does form associations with arbuscular mycorrhizal fungi to aid in phosphorus uptake (Christian *et al*., 2001). Switchgrass is

slow to establish and may not compete well with weeds in the first year (Elberson and Christian, 2001).

Switchgrass has been proposed as a biofuel crop to help mitigate rising atmospheric carbon dioxide levels resulting from anthropogenic activities (Sanderson *et al*., 1996). Switchgrass has many attributes that make it an attractive choice for carbon sequestration, including: an extensive, deep root system (Liebig *et al*., 2005), approximately 50% greater water-use efficiency than cool season forage grasses (Ma *et al*., 2000), a relatively low nutrient requirement (Hetrick *et al*., 1988), and the potential to produce large amounts of biomass (Lee *et al*., 2007).

Soils along the Lake Hartwell shoreline are low in organic matter (approximately 2.5%). Organic matter content could increase if switchgrass established and returned over subsequent seasons. Weaver and Darland (1949) found that switchgrass roots can extend over 2.5m into the soil and Frank *et al*. (2004) reported that switchgrass roots can account for over 80% of total plant biomass when crown tissue is included with roots. Further work by Liebig *et al*. (2005) found that the organic carbon content in switchgrass soils was high not only near-surface depths, but also at depths below 30cm. Increased soil organic matter can lead to an increased microbial mineralization of plant nutrients, and soil organic carbon acts as an energy source for the microbes. The establishment of switchgrass could eventually lead to increased soil quality, but studies show it has taken up to 10 years for a soil organic matter increase of 44.8% and 28.2% at depths of 0 to 15cm and 15 to 30cm, respectively (Lee *et al*., 2007). Muir *et al*. (2001) found that

biomass production after the first year was only 40% of that obtained in the third year after establishment.

Switchgrass can grow on nutrient-poor sites partially due to its ability to effectively translocate nutrients, especially phosphorus, at the end of the growing season (Muir *et al*, 2001). Switchgrass usually grows in association with arbuscular mycorrhizae, which aids in phosphorus nutrition (Brejda *et al*., 1998). Studies have shown that switchgrass uses nitrogen from the entire profile of the root system, from 0 to 120cm (Vogel *et al*., 2002) and others have reported that switchgrass may translocate significant amounts of nitrogen to stem bases and roots at the end of the growing season (Heckathorn and DeLucia, 1995). However, switchgrass production decreases over time with no addition of nitrogen (Vogel *et al*., 2002). Therefore, to maintain mature stands of switchgrass, nitrogen fertilizer applications may be necessary. Microbes with the ability to fix nitrogen and those that mediate reactions within the nitrogen cycle could contribute to the survival of switchgrass.

Plants are known to interact with the soil microbial community in beneficial symbiotic relationships (Glick, 1995; Azcon-Aguilar and Barea, 1996). Since the interrelationship between microbes and plant roots could assist in switchgrass survival in harsh conditions, it is important to study the conditions of the soil microbial community of the sites both prior to plant establishment and in subsequent years. One way to characterize soil microbial activity is to conduct assays which measure the ability to cycle nutrients like the acid phosphomonoesterase activity, the nitrate reductase assay, and

general metabolic activity levels of the microbes present, such as the dehydrogenase assay and the substrate-induced respiration assay.

Enzymatic Activity of Lakeshore Soils

Enzyme assays can be used as a means to determine the potential of the soil microbial community to degrade or transform substrates (Dick, 1994). Soil enzyme assays indicate how well a soil can carry out important steps in nutrient cycling, nitrification, oxidation and other processes (Dick, *et al*., 1994). Enzyme assays routinely conducted on soil samples to determine their microbial activity include dehydrogenase and acid phosphomonoesterase assays, nitrate reductase assays, and substrate-induced respiration tests.

Dehydrogenase Activity

Because of the intricate relationship between dehydrogenase activity and the respiratory needs of cells, dehydrogenase measurements are used to estimate the immediate metabolic activities of soil microbes (Skujins, 1978). The dehydrogenase assay is an estimate of the potential metabolic activity of intact cells. The assay uses a tetrazolium salt, triphenyltetrazolium chloride (TTC), to act as an electron acceptor during metabolic reactions. The electron acceptor (TTC) is reduced to triphenylformazan (TPF), a colored compound whose concentration can be quantitatively measured using a spectrophotometer. The higher the conversion to TPF, the higher the dehydrogenase activity level. Values in the literature range from 9 to 1760mgTPF/grams per unit dry

weight of soil/24hr, with a mean of 337mgTPF/gdwt/24h (Camina *et al*., 1998; Casida *et al*., 1964). Because dehydrogenase activity occurs as an important part of cellular metabolism these results give an indication of the potential microbial activity, but does not necessarily agree with microbial biomass measurements (Casida, 1964; Stevenson, 1959). The results from dehydrogenase assays alone can therefore be misleading in terms of the size of the microbial community present. Dehydrogenase studies should be carried out in conjunction with other assays, like the substrate-induced respiration method to better estimate the size of the microbial community.

Substrate-Induced Respiration

The substrate-induced respiration (SIR) method can be used with the dehydrogenase assay to compare activity levels and actual biomass. Anderson and Domsch (1978) used the upper 10cm of twelve different soils, nine from agricultural plots, one from a grassland, and two from mixed oak and spruce forest from Germany. The authors used a fumigation and reinoculation method of Jenkinson and Powlson (1976) to determine the amount of carbon in the microbial biomass of the samples and also made a physiological measurement of biomass response (CO_2 evolved) with an appropriate substrate. The authors used these two methods simultaneously on the same samples to determine the correlation between respiration and microbial biomass. More recently, other researchers have further correlated carbon dioxide emission and microbial biomass (West and Sparling, 1986; Beare *et al*., 1990) that agrees with Anderson and Domsch (1978). For incubations at 22^{o}C, a substrate-induced maximal respiration rate of

1ml CO_2/hr = 40mg of microbial carbon. Literature values range from 8 – 48 mg microbial carbon/gdwt soil (Andersson *et al*., 2004). Combining the results of substrate-induced respiration and dehydrogenase assays allows one to determine whether there are a larger number of cells or whether there are fewer individual microbial cells with higher levels of metabolism by using this method.

Acid Phosphomonoesterase Activity

Phosphorus is second only to nitrogen as a limiting nutrient for plants. Limited phosphorus availability results partly from the sedimentary cycle of the nutrient. Unlike carbon and nitrogen, phosphorus never enters the atmosphere in a gaseous form. Instead of the atmosphere serving as the largest reservoir for the nutrient, the ocean sediments function as the largest reservoir. Once phosphorus reaches the sediments, it can be locked up for several thousand years (Paul and Clark, 1996). A major conduit for loss of terrestrial phosphorus to oceanic sediments is erosion; there is also significant loss of phosphorus through crop removal. Plants that take up phosphorus do not reduce it as they do nitrogen compounds, and it does not serve as a primary energy source for microbial oxidations as other elemental compounds do. Phosphorus is used as a structural component in plasma membranes and nucleic acids, and takes part in energy transformation occurring inside cells in the form of adenosine triphosphate (ATP). Without phosphorus, cells cannot divide, which can lead to stunting of plants, delayed maturity, and shriveled seed (Marschner, 1995). Plants may also be unable to convert sugars into starch which leads to the build-up of anthocyanins. The acid

phosphomonoesterase assay measures the potential for soil microbes to liberate phosphorous from organic materials present. According to the literature, the average acid phosphomonoesterase activity is 617mg p-nitrophenol/kg/h (Tabatabai, 1994).

Nitrate Reductase Activity

Nitrogen is often the most limiting nutrient in soils for plant growth. Plants use nitrogen to form proteins, nucleic acids, and secondary metabolites. Access to this extremely important nutrient is mediated by microbes in the soil whose activities transform atmospheric and organic nitrogen into plant-available forms such as nitrate and ammonium.

Microbial activity can also result in loss of soil nitrogen through denitrification in anaerobic soils. Bacteria involved in denitrification are facultative anaerobes that use nitrate as a terminal electron acceptor when oxygen is not available (Tiedje, 1994). Additionally, when soils are high in carbon and are anaerobic, an alternative end-product can be produced. Although studies have been done on the entire process of denitrification, few studies have focused on just the activity of nitrate reductase in soils (Tabatabai, 1982). Studies by Cooper and Smith (1963) showed that the rate-limiting process for denitrification in acid soils is the reduction of NO_3^-. The process of dissimilatory nitrate reduction to ammonium (DNRA) produces ammonium as an end product and yields 600 kJ or 143 kcal/mol NO_3^- (Myrold, 1999). Experiments with pure cultures and separate ecological studies have demonstrated that DNRA bacteria predominate over those denitrifiers with N_2 as an end product in carbon-rich

environments, such as sewage sludge. Respiratory dentrifiers (with N_2 as the end product) are predominant in soils (Tiedje, 1988).

Values in the literature for dissimilatory nitrate reduction ranged from 18 – 80 ug NO_2-N /gdwt/24h for soils with pH values between 5.9 to 7.8 and organic carbon levels between 0.64% and 5.59% (Abdelmagid and Tabatabai, 1987).

Nitrogen Fixation

Nitrogen fixation is a process in which highly stable nitrogen gas from the atmosphere is converted to a more accessible form for plants and soil microbes. This more accessible form is ammonia. The organisms responsible for this important transformation can be either symbiotic or can be free-living.

Free-living diazotrophs (nitrogen fixing microbes) account for a small amount of nitrogen fixed each year. For example, an estimate of nitrogen fixed by symbiotic microbes in leguminous plants is from 50 – 400 kg N/ha/yr, whereas an estimate of nitrogen fixed by free-living, heterotrophic species is 1 – 2 kg N/ha/yr (Marschner, 1995). Because the majority of free-living diazotrophs are heterotrophic, they depend on plant exudates and organic carbon residues in the soil. In soils low in organic matter, nitrogen fixation by nonsymbiotic microbial species is limited (Hubbell and Kidder, 1978). The values of nitrogen fixation found in the literature for algae and bacteria range from 6.6 to 180 nmol N_2 fixed/mgdwt soil/h (Hardy *et al*., 1972).

A widely used assay for nitrogenase activity is the acetylene reduction assay. Although other methods can measure nitrogen fixation, the acetylene reduction assay is

10^3 to 10^4 times more sensitive than ^{15}N methods. Also the product of acetylene reduction is already in the gas phase and can be immediately injected into a gas chromatograph for analysis (Hardy *et al*., 1972).

Fatty Acid Methyl Ester Analysis

Another method of studying soil microbial communities is to attempt to identify particular members present (Brock, 1975). This type of study is based on providing a single carbon source to the bacteria and/or fungi present in the soil. While this method has helped researchers understand some important cycles in nature (Foster, 1962), it is a method that works by breaking up the community instead of addressing it as a whole. Studies have shown that subculturing microbes on artificial media only leads to the growth of 0.01-10% of the total microbes present (Perfileu and Gabe, 1969; Rondon *et al*., 1999). Fatty acid methyl ester analysis (FAME) is a method that has traditionally been used on pure isolates to determine their identity. However, FAME can also be used to assess the diversity of community assemblages in samples (Kennedy and Smith, 1995).

FAME analysis works on the principle that organisms have unique fatty acids combinations present in their plasma membranes. Samples are exposed to a series of organic solvents which dissolve lipids almost instantly (White and Frerman, 1967), providing a snapshot of lipids at time of extraction. Microbial lipids extracted using the MIDI Sherlock Microbial Identification System protocol (MIDI, Inc., Newark, DE). Fatty acids were identified and quantified by comparing the sample to a library of over 300 fatty acids. Although this microbial identification system was developed for use

primarily by the medical industry for the identification of isolated bacteria (Sasser, 1990) , microbial ecologists have been able to use the technique to compare microbial communities.

Several fatty acids are associated with specific groups of microbes. Bacterial fatty acid composition is unique in that the B-hydroxy, cyclopropane, and branched-chain fatty acids are not common elsewhere in nature (Ong, 2001). Three fatty acids, 15:0 ISO and 16:0 10METHYL have been shown to be indicative of Gram positive bacteria (O'Leary and Wilkinson, 1988). The fatty acids 16:1w5c, 16:1w7c, and 18:1 are generally used as biomarkers for Gram negative bacteria (Wilkinson, 1988). Fungal fatty acid examples are 12:0, 18:2w6c, 16:00, and 18:1w9c (Kandeler, 2007).

Fatty acid analysis has been used to determine the effects of root exudates on rhizosphere microorganisms (Griffiths *et al*, 1999; Buyer *et al*., 2002), and the effects of phosphorus on arbuscular mycorrhizal fungi (Olsson *et al*, 1997). It has also been used to characterize microbial communities in agricultural soils (Zelles *et al*, 1992; Ibekwe and Kennedy, 1998). Recently, Grayston *et al*. (2001) used fatty acid analysis to study variability in soil microbial communities of temperate upland grassland ecosystems. Further studies characterizing soil microbial communities with differing management practices were performed by Grayston *et al*. (2004) on soils from around the United Kingdom. When fatty acids are extracted from soil samples they give an indication of the microorganisms currently growing in the soil because fatty acids are rapidly broken down when cells die.

Ethylene Involvement in Plant Growth

Plants typically produce ethylene in response to temperature extremes, flooding, drought, chemical exposure, and mechanical wounding, causing changes in their growth and development. Ethylene also regulates fruit ripening, senescence and abscission of plant organs, production of root hairs, seed germination, and growth and flowering of horticultural bulb crops. Ethylene has been investigated for agricultural uses since the 1920's and 1930's (Abeles *et al*., 1992). Little information is currently available on the reaction of switchgrass to ethylene exposure.

A Russian scientist, Dimitry Neljubov, working in the early 1900's, is credited with discovering that ethylene could affect pea seedlings germinated and grown in the dark. He observed that seedlings exposed to ethylene grew in a horizontal position and exhibited inhibition of elongation and swelling of the base of the stem. These characteristics are now known collectively as the "triple response" and have been used in ethylene bioassays since that time (Abeles *et al*., 1992).

Research on ethylene and its effects was difficult until the 1950's. Until that time, researchers found it complicated to determine whether ethylene was actually produced by plants or was a by-product of ripening because of technological limitations. Prior to the advent of gas chromatography, there were only crude and insensitive methods of ethylene measurement. With the introduction of gas chromatography, part-per-million levels were detectable (Srivastava, 2002). Research then showed ethylene was produced by different portions of plants and could regulate growth and development of plants, and it became known as a plant hormone in the 1950's (Srivastava, 2002).

Although research had been conducted on the effects of ethylene on plants and even the interaction of another hormone, auxin, the pathway of ethylene production was not elucidated until the 1960's and 1970's. Lieberman *et al*. (1965) identified methionine as an ethylene precursor and the next step in the pathway was found by Adams and Yang (1977). Adams and Yang (1977) showed that ethylene was synthesized from S-adenosylmethionine, which was formed from methionine. Further work determined the immediate precursor to ethylene was 1-aminocyclopropane-1-carboxylic acid (ACC) (Adams and Yang, 1979). For the past twenty years, the focus of research has been on ethylene's mode of action, its effect of different plants, and genetic control of its formation.

Ethylene's Effect on Different Plants

Hypoxia and anoxia brought on by flooding are stresses faced by switchgrass on shorelines. Some plants respond to hypoxic conditions by forming adventitious roots and the formation of aerenchyma. The literature has been contradictory on whether ethylene actually induces adventitious root formation. Wample and Reid (1979) and Yamamoto and Kozlowski (1985) reported that ethylene played only a minor role in adventitious root formation, but Drew *et al*. (1979) and Jackson (1985) found that ethylene enhanced adventitious root formation in maize. Further, Bleecker *et al*. (1987) found the same effect on deepwater rice. Studies on *Rumex* species by Visser *et al*. (1995) showed that an increase in auxin sensitivity due to high ethylene concentrations could induce

adventitious root formation. Further work on the species demonstrated adventitious root formation was a result of exposure to high ethylene concentrations (Visser *et al*. 1996).

Research supporting the importance of internal aeration for plant growth in waterlogged soils has been reported. For example, species with higher root porosity tend to form deeper roots and are more tolerant of soil waterlogging (Armstrong, 1979; Laan *et al*., 1989). Studies have been performed on maize where waterlogging has induced air-filled tissue or aerenchyma (Atwell *et al*., 1988; He *et al*., 1992). Laan *et al*. (1989) demonstrated that lateral roots of *Rumex thyrsiflorus* with a porosity of about 5% penetrated no more than 100mm when grown in waterlogged soil whereas roots of *Rumex maritimus* with a porosity of around 30% penetrated 400mm. Aerenchyma formation, which allows for gas diffusion through oxygen-limited tissues, is also attributed to ethylene exposure (Armstrong *et al*., 1994; Blom *et al*., 1990).

Work with deepwater rice demonstrated that stem elongation results from ethylene exposure. Deepwater rice can grow at rates of 20 to 25cm/day when partially submerged and can reach a length of up to 7 meters in water depths of up to 4 meters (Kende *et al*., 1998). Research has shown there is probably an interplay between gibberelic acid, abscisic acid, and ethylene that leads to internodal elongation (Azuma *et al*., 1995) in deepwater rice enabling plants to break the surface of the water and reach the atmosphere.

Ethylene Promoters and Inhibitors

Because of the physiological effects of ethylene on plants, people have manipulated ethylene exposure throughout history. Many studies have demonstrated that the presence of ripe fruits and smoke will hasten ripening (Chace and Sorber, 1936; Miller, 1947). Chemicals that generate ethylene have been used by the agricultural industry to control seed germination (Ketring, 1977), overcome dormancy in gladiolus bulbs (Halevy *et al*., 1970), and inhibit stem elongation to increase hardiness in seedlings used for transplanting (Woodrow *et al*., 1987).

Ethephon is the most common ethylene-generating compound. It can penetrate tissues and is translocated within plants. It decomposes to ethylene, and this decomposition results in ethylene, phosphate, and chloride ions in aqueous solutions above pH 4 – 5.

There are also chemicals that inhibit ethylene production. L-alpha-(aminoethoxyvinyl) glycine (AVG) is a chemical used in research studies to differentiate between conditions with and without the presence of ethylene (Penrose *et al*., 2001; Peters and Crist-Estes, 1989). Ethylene inhibition has been used to change shipping times of fruits and vegetables in order to extend their storage life (Saltveit, 2005). AVG works by inhibiting ACC synthase, an enzyme in the pathway of ethylene production (Yang and Hoffman, 1984). This compound commonly reduces ethylene production, but does not eliminate it completely.

Plant Growth-Promoting Bacteria

Plant growth promoting bacteria (PGPB) have been defined as bacteria that aggressively colonize roots and promote plant growth, regardless of the mechanism involved (Kloepper *et al*., 1980). PGPB may aid in organic material decomposition, increase P, Mn, Fe, Zn, and Cu availability, fix atmospheric nitrogen, promote plant growth through the production of hormones, and protect against root pathogens (Kennedy, 1999). The effects of PGPB have been reported in the literature, but little information has been written on the mechanism by which these bacteria aid plant growth.

A model by Glick *et al*. (1997) suggests that a bacterium selectively cultured using ACC (an ethylene precursor) as a nitrogen source will produce ACC deaminase. If this enzyme breaks down ACC in the plant, then it will not experience the effects of ethylene exposure, such as leaf senescence, epinasty, and the cessation of root elongation.

Lowering Ethylene Concentration by PGPB

The model for lowering plant ethylene levels is presented by Glick *et al*. (1997) and follows this sequence of events: a PGPB bound to either the surface of the seed or root of a developing plant secretes IAA (indoleacetic acid) in response to tryptophan and other small molecules present in seed or root exudates (Whipps, 1990). Some of the IAA is taken up by the plant, and in addition to endogenous IAA, causes stimulation of cell proliferation and/or elongation. Also, IAA can also stimulate the activity of the enzyme ACC synthase to convert S-adenosylmethionine to ACC (Kende, 1993). At this point, a significant portion of ACC may be exuded from plant roots or seeds, taken up by the

bacterium bound to plant or seed, and hydrolyzed by the enzyme ACC deaminase to ammonia and alpha-ketobutyrate. By taking up ACC released by the plant, the bacterium decreases the amount of ACC outside the plant causing the plant to exude more ACC to maintain equilibrium. The bacterium is able to exploit a nitrogen source unavailable to other bacteria. When ACC (the precursor to ethylene) is exuded outside of the plant, less ethylene is produced. The authors of this model state that longer roots and possibly longer shoots will result from reduced ethylene levels (Glick *et al*., 1997).

Evidence for the model

The authors of this model put forth several lines of evidence to support their hypothesis that bacteria containing ACC-deaminase will divert ACC to the bacterium, lowering plant ethylene levels. The lines of evidence are as follows: first, ethylene has been shown to be an inhibitor of root elongation in different systems (Abeles *et al*, 1992). This model proposes that ethylene levels are lower when bacteria with ACC deaminase are present. Hall *et al*. (1996) demonstrated that the roots of ethylene sensitive plants are longer when ACC deaminase-containing bacteria are present.

A second line of evidence comes from studies performed by Glick *et al*. (1995). Seven strains of bacteria isolated from seven different soils in two geographically disparate locations were isolated on the basis of their utilizing ACC as a nitrogen source. Each of these strains was shown to promote canola seedling root elongation. Third, three separate mutants of the well-characterized PGPB *Pseudomonas putida* GR12-2 that had no ACC deaminase activity and do not hydrolyze ACC were used to examine the role of ACC deaminase in promoting root elongation. The three mutants were unable to promote

root elongation of canola roots in growth pouches where the wild-type *P. putida* GR12-2 did promote root elongation (Lifshitz *et al*, 1987; Glick *et al*., 1994).

L-alpha-(aminoethoxyvinyl) glycine (AVG), an ethylene inhibitor, was used on young (five to seven day old) seedlings of canola, tomato, lettuce, wheat, oats, and barley. The increase in root length was similar to the response when these plants were inoculated with wild-type *P. putida* GR12-2. The plants that responded most were also found to be those that were the most sensitive to ethephon, (2-chloroethyl) phosphonic acid (Hall *et al*., 1996). Therefore, only ethylene sensitive plants respond to the presence of PGPB that contain ACC deaminase as far as root length is concerned.

Another part of the model pertains to IAA production by the bacterium in response to plant-produced tryptophan. Xie *et al*. (1996) created IAA overproducing mutants of *P. putida* GR 12-2 and found that the mutant that overproduced IAA to the greatest extent was also inhibitory to root elongation. The authors suggested that increased levels of IAA were taken up by the plant and interacted with ACC synthase, stimulating the synthesis of excess ACC which has in turn converted to ethylene (Yang and Hoffman, 1984). Devlin (1966) demonstrated the inhibitory effect of high exogenous IAA levels on root length.

Finally, a study with canola seeds and *E.coli* cells expressing a cloned *Enterobacter cloacae* ACC deaminase gene showed promotion of root elongation (Shah *et al*., 1997). This result demonstrates that, at least in this assay, the ability to promote root elongation was a direct consequence of the presence of the enzyme ACC deaminase.

Ethylene and IAA Exposure

In the Glick *et al*. (1998) model, plants are exposed to both ethylene and IAA. Even if switchgrass is insensitive to ethylene, it may still respond to IAA produced by PGPB. It has been suggested that up to 80% of bacteria isolated from the rhizosphere can produce IAA (Prikryl *et al*, 1985; Leinhos and Vacek, 1994). One consequence of IAA exposure is stem elongation. The ability for enhanced stem elongation may be necessary for survival of sudden rises in water level (Setter and Laureles, 1996). Flooding can inhibit the transport of auxin from shoots to roots because this transport requires energy. Energy may be in short supply in oxygen deprived roots, resulting in an accumulation of auxin at the base of the shoot (Wample and Reid, 1979). Studies with a known PBPG that possesses ACC deaminase showed that plants inoculated with this bacterium showed an increase in shoot length of 38% over controls (Glick *et al*., 1997). Auxin may also have an impact on adventitious root formation. Authors proposed that slowing of the polar, energy-dependent transport of auxin, followed by the accumulation of auxin at the base of the shoot caused by flooding may lead to subsequent adventitious root formation (Kramer, 1951; Visser *et al*., 1995). However, Visser *et al*. (1996) later proposed that adventitious rooting came about as a result of increased sensitivity to auxin brought on by increased levels of ethylene.

There are many stresses associated with the reservoir shoreline habitat. These stresses originate from the fact that reservoirs are human constructs and there has not been time enough to produce stable, low sloping shorelines. Because the soils are clay and made of very fine particles, erosion is a problem. A possible solution to erosion is

adding native grasses to the shoreline. However, the soils may be inhospitable for any vegetation planted resulting in a struggle to survive and spread. The study presented here involved several aspects of the sustainability of planting a native grass on eroding, nutrient-poor shorelines. The objectives of this study were to: (1) measure the levels of microbial activity and characterize the types of microbes found in Lake Hartwell shoreline sites following planting of switchgrass, (2) establish the effects of ethylene on switchgrass, and (3) determine if switchgrass can benefit from inoculation with a plant growth-promoting bacterium in control, crown flooded, and flooded conditions.

Literature Cited

Abdelmagid, H.M. and M.A. Tabatabai. 1987. Nitrate reductase activity in soils. Soil Biology and Biochemistry 19**:** 421 - 427.

Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene in Plant Biology. Academic Press, San Diego, CA.

Adams, D.O. and S.F. Yang. 1977. Methionine metabolism in apple tissue. Implication of S-adenosylmethionine as an intermediate in the conversion of methionine to ethylene. Plant Physiology 60**:** 892-896.

Adams, D.O. and S.F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of the National Academy of Science, USA 76**:** 170-174.

Anderson, J.P.E. and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10**:** 215-221.

Andersson, M., A. Kjoller, and S. Struwe. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology and Biochemistry 36**:** 1527-1537.

Armstrong, W. 1979. Aeration in higher plants. Advances in Botanical Research 7**:** 225-332.

Armstrong, W.R., Brandle, and M.B. Jackson. 1994. Mechanisms of flood tolerance in plants. Acta Botanica Neerlandica 43**:** 307-358.

Atwell, B.J., M.C. Drew, M.B. Jackson. 1988. The influence of oxygen deficiency on ethylene synthesis, 1-aminocyclopropane-1-carboxylic acid levels and aerenchyma formation in roots of *Zea mays*. Physiologia plantarum 72**:** 15-22.

Azcon-Aguilar, C. and J.M. Barea. 1996. Arbuscular mycorrhizas and biological control of soil-borne plant pathogens: an overview of the mechanisms involved. Mycorrhiza 6**:** 457 - 464.

Azuma, T., T. Hirano, Y. Deki, N. Uchida, T. Yasuda, T. Yamaguchi. 1995. Involvement of the decrease in levels of abscisic acid in the internodal elongation of submerged floating rice. Journal of Plant Physiology 146**:** 323-328.

Beare, M.H., C.L. Neely, D.C. Coleman, and W.L. Hargrove. 1990. A substrate-induced respiration (SIR) method for measurement of fungal and bacterial biomass on plant residues. Soil Biology and Biochemistry 22**:** 585-594.

Bleecker, A.B., M.A. Estelle, C. Somerville, and H. Kende. 1988. Insensitivity to ethylene conferred by a dominant mutation in *Arabidopsis thaliana*. Science 241**:** 1086-1089.

Bleecker, A.B., S. Rose-John, and H. Kende. 1987. An evaluation of 2,5-norbornadiene as a reversible inhibitor of ethylene action in deepwater rice. Plant Physiology 84**:** 395-398.

Blom, C.W.P.M., G.M. Bogeman, P. Laan, A.J.M. Van der Sman, H.M. Van de Steeg, and L.A.C.J. Voesenek. 1990. Adaptations to flooding in plants from river areas. Aquatic Botany 38**:** 29-47.

Brady, N.C. and R.R. Weil. 2004. Elements of the Nature and Properties of Soils. Second ed. Pearson Education, Inc., Upper Saddle River, New Jersey, pp 80 - 81.

Brejda, J.J., L.E. Moser, and K.P. Vogel. 1998. Evaluation of switchgrass rhizosphere microflora for enhancing yield and nutrient uptake. Agronomy Journal. 90: 753-758.

Brock, T.D. 1975. Milestones in Microbiology. American Society for Microbiology, Washington, D.C.

Buyer, J.S., D.P. Roberts, E. Russek-Cohen. 2002. Soil and plant effects of microbial community structure. Canadian Journal of Microbiology 48**:** 955-964.

Camina, R.., F. Gil-Sotres, and C. Leiros. 1998. Measurement of dehydrogenase activity in acid soils rich in organic matter. Soil Biology and Biochemistry 8**:** 1005-1011.

Carpenter-Boggs, L., P.D. Stahl, M.J. Lindstrom, and T.E. Schumacher. 2003. Soil microbial properties under permanent grass, conventional tillage, and no-till management in South Dakota. Soil and Tillage Research 71**:** 15 - 23.

Casida, L.E., Klein, D.A., and Santoro, T. 1964. Soil dehydrogenase activity. Soil Science 98**:** 371-376.

Chace, E.M. and D.G. Sorber. 1936. Treating fruits and nuts in atmospheres containing ethylene. Food Industries 8**:** 292 - 294.

Christian, D.G., H.W. Elberson, N. El Bassam, G. Sauerbeck, E. Alexopoulou, N., Sharma , P. de Visser, and D. van den Berg. 2001. Management guide for planting and production of Switchgrass as a biomass crop in Europe. *In*: Switchgrass (*Panicum virgatum*, L.) as a alternative energy crop in Europe. Initiation of a productivity network. Final Report FAIR 5-CT97-3701.

Cooper, G.S. and R.L. Smith. 1963. Sequence of products formed during denitrification in some diverse Western soils. Soil Science Society of America Proceedings 27**:** 659-662.

Devlin, R.M. 1966. Plant Physiology. Reinhold Publishing Corporation, New York.

Dick, R.P. 1994. Influence of long-term tillage and crop rotation combinations on soil enzyme activities. Soil Science Society of America Journal 48**:** 569 - 574.

Dick, R.P., D.P. Breakwell, and R.F. Turco. 1994. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. *In* Methods for Assessing Soil Quality SSSA Special Publication 49. Soil Science Society of America, Madison, WI, pp 247 - 271.

Dorn, Michael. 2002. Evaluation of eleven native grasses exposed to piedmont reservoir conditions. Masters thesis. Clemson University, Clemson, SC.

Drew, M.C., M.B. Jackson, and S. Giffard. 1979. Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. Planta 147**:** 83-88.

Elberson, H.W. and D. G. Christian. 2001. Annex: A Switchgrass bibliography. *In* Switchgrass (*Panicum virgatum*, L.) as a alternative energy crop in Europe. Initiation of a productivity network. Final Report FAIR 5-CT97-3701,

Foster, J.W. 1962. Hydrocarbons as substrates for microorganisms. Anotonie van Leeuwenhoek J. Microbiol. Serol. 28**:** 241-274.

Frank, A.B., J.D. Berdahl, J.D. Hanson, M.A. Liebig, and H.A. Johnson. 2004. Biomass and carbon partitioning in switchgrass. Crop Science. 44(4): 1391-1396.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology 41**:** 109 - 117.

Glick, B.R. and Y. Bashan. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. Biotechnological Advances 15**:** 353-378.

Glick, B.R., C.B. Jacobson, M.M. K. Schwarze, and J.J. Pasternak. 1994. 1-Aminocyclopropane -1-carboxylic deaminase mutants of the plant growth promoting rhizobacterium *Pseudomonas putida* GR12-2 do not stimulate canola root elongation. Canadian Journal of Microbiology 40**:** 911 - 915.

Glick, B.R., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth - promoting bacteria. Journal of Theoretical Biology 190**:** 63 - 68.

Grayston, S.J., C.D. Campbell, R.D. Bardgett, J.L. Mawdsley, C.D. Clegg, K. Ritz, B.S. Griffiths, J.S. Rodwell, S.J. Edwards, W.J. Davies, D.J. Eston, And P. Millard. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA, and community DNA techniques. Applied Soil Ecology 25**:** 63-84.

Grayston, S.J., G.S. Griffith, J.L. Mawdsley, C.D. Campbell, R.D. Bardgett. 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology and Biochemistry 33**:** 533-551.

Griffiths, B.S., K. Ritz, N. Ebblewhite, and G. Dobson. 1999. Soil microbial community structure: effects of substrate loading rates. Soil Biology and Biochemistry 31**:** 145-153.

Grimont, P.A.D., F. Grimont, and M. Starr. 1981. *Serratia* species isolated from plants. Current Microbiology 5**:** 317-322.

Halevy, A.H., R. Shilo, S. Simchon. 1970. Effect of 2-chloroethylphosphonic acid (Ethrel) on health, dormancy, and flower and corm yield of gladioli. J. Horticultural Sci. 45**:** 427 - 434.

Hall, J.A., D. Pierson, S. Ghosh, and B.R. Glick. 1996. Root elongation in various agronomic crops by the plant growth promoting rhizobacterium, *Pseudomonas putida* GR12-2. Isr. J. Plant Sci. 44**:** 37 - 42.

Hardy, R.W.F., R.C. Burns, and R.D. Holmes. 1972. Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry 5**:** 47 - 81.

Harris, J.A. 2003. Measurements of the soil microbial community for estimating the success of restoration. European Journal of Soil Science. 54:801 – 808.

Hartwell Dam and Lake – Introduction. U.S. Army Corp of Engineers, Savanna District. www.sas.usace.army.mil/lakes/hartwell/intro.htm.

He, C.J., P.W. Morgan, and M.C. Drew. 1992. Enhanced sensitivity to ethylene in nitrogen- or phosphate-starved roots *Zea mays* L. during aerenchyma formation. Plant Physiology 98**:** 137-142.

Heckathorn, S.A. and E.H. DeLucia. 1995. Ammonia volatilization during drought in perennial C4 grasses of tallgrass prairie. Oecologia 101: 361-365.

Hetrick, B.A.D., D.G. Kitt, and G.W.T. Wilson. 1988. Mycorrhizal dependency and growth habit of warm-season and cool-season tallgrass prairie plants. Canadian Journal of Botany. 86: 1376-1380.

Hubbel, D.H., and G. Kidder. 1978. Biological Nitrogen Fixation. *In* University of Florida Extension, ed. University of Florida

Ibekwe, A.M. and A.C. Kennedy. 1998. Phospholipid fatty acid profiles and carbon utilization patterns for analysis of microbial community structure under field and greenhouse conditions. FEMS Microbiology and Ecology 26**:** 151-163.

Jackson, M.B. 1985. Ethylene and responses of plants to soil waterlogging and submergence. Annual Review of Plant Physiology 36**:** 145-174.

Jenkinson, D.S. and D.S. Powlson. 1976. The effects of biocidal treatments on metabolism in soil. V. A method for measuring soil biomass. Soil Biol. Biochem. 8: 209-213.

Kandeler, E. 2007. Physiological and Biochemical Methods for Studying Soil Biota and Their Function. *In* EA Paul, ed, Soil Microbiology, Ecology, and Biochemistry, Ed 3rd. Academic Press, Oxford, UK, pp 53-83.

Kende, H. 1993. Ethylene biosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 44**:** 283-307.

Kende, H., E. van der Knaap, and H.T. Cho. 1998. Deepwater rice: a model plant to study stem elongation. Plant Physiology 118**:** 1105-1110.

Kennedy, A.C. 1999. The rhizosphere and spermosphere. *In* DM Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Inc., Upper Saddle River, NJ, pp 389-407.

Kennedy, A.C. and K.L. Smith. 1995. Soil microbial diversity and the sustainability of agricultural soils. Plant and Soil 170:75-86.

Ketring, D.L. 1977. Physiology of oil seeds. VI. A means to break dormancy of peanut (*Arachis hypogaea* L.) seeds in the field. Peanut Sci. 4**:** 42 - 45.

Kloepper, J.W., M.N. Schroth, and T.D. Miller. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70**:** 1078-1082.

Kramer, P.J. 1951. Causes of injury to plants resulting from flooding the soil. Plant Physiology 26**:** 722 - 733.

Laan, P., M.J. Berrevoets, S. Lythe, W. Armstrong, and C.W.P.M. Blom. 1989. Root morphology and aerenchyma formation as indicators of flood tolerance of *Rumex* species. Journal of Ecology 77**:** 693-703.

Lee, D.K., V.N. Owens, and J.J. Doolittle. 2007. Switchgrass and soil carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. Agronomy Journal. 99: 462-468.

Leinhos, V. and O.Vacek. 1994. Biosynthesis of auxins by phosphate-solubilizing rhizobacteria from wheat and rye. Microbial Research 149**:** 31-35.

Lieberman, M., A.T. Kunishi, L.W. Mapson, and D.A. Wardale. 1965. Ethylene production from methionine. Journal of Biochemistry 97**:** 449 - 459.

Liebig, M.A., H.A. Johnson, J.D. Hanson, and A.B. Frank. 2005. Soil carbon under switchgrass stands and cultivated cropland. Biomass and Bioenergy. 28: 347-354.

Lifshitz, R., J.W. Kloepper, M. Kozlowski, C. Simonson, J. Carlson, E.M. Tipping, and I. Zaleska. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Canadian Journal of Microbiology 33**:** 390-395.

Ma, A., C.W. Wood, and D.J. Bransby. 2000. Soil management impacts on soil carbon sequestration by switchgrass. Biomass Bioenergy. 18: 469-477.

Marschner, H. 1995. Mineral Nutrition of Higher Plants, Second edition. Academic Press Inc., San Diego, CA.

Muir, J.P., M.A. Sanderson, W.R. Ocumpaugh, R.M. Jones, and R.L. Reed. 2001. Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. Agronomy Journal. 93: 896-901.

Myrold, D.D. 1999.Transformations of Nitrogen. *In* DM Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ

Okamoto, H., M. Sato, Y. Miyata, M. Yoshikawa, and M. Isaka. 2000. Biocontrol of *Phytophthora* root rot of Angelica trees by *Enterobacter cloacae* and *Serratia ficaria* strains. Journal of General Plant Pathology 66**:** 86-94.

O'Leary, W.M. and S.G. Wilkinson. 1988. Gram-positive bacteria. *In* CaSGW Ratledge, ed, Microbial Lipids, Vol 1. Academic Press, London, pp 117 - 202.

Olsson, P.A., E. Baath, and I. Jakobsen. 1997. Phosphorus effects of the mycelium and storage structures of an arbuscular mycroohizal fungus as studied in the soil and roots by analysis of fatty acid signatures. Applied and Environmental Microbiology 63**:** 3531-3538.

Ong, Kevin L. 2001. Bacterial wilt in South Carolina tobacco production system: winter cover-summer crop rotation, microbial communities, and the diversity of the causal agent, *Ralstonia solanacearum*, in the southeastern United States. Ph.D. dissertation. Clemson University.

Paul, E.A. and F.E. Clark. 1996. Soil Microbiology and Biochemistry, Ed 2nd. Academic Press, San Diego.

Penrose, D.M., B.A. Moffatt, and B.R. Glick. 2001. Determination of 1-aminocyclopropane-1-carboxylic acid (ACC) to assess the effects of ACC deaminase-containing bacteria on roots of canola seedlings. Canadian Journal of Microbiology 47**:** 77 - 80.

Perfilev, B.V. and D.R. Gabe. 1969. Capillary Methods of Investigating Microorganisms. Oliver and Boyd, Edinburgh, UK.

Peters, N.K. and K. Crist-Estes. 1989. Nodule formation is stimulated by the ethylene inhibitor aminoethoxyvinylglycine. Plant Physiology 91**:** 690-693.

Prikryl, Z., V. Vancura, and M. Wurst. 1985. Auxin formation by rhizosphere bacteria as a factor of root growth. Biol. Plant. 27**:** 159-163.

Rondon, M.R., Goodman, R.M., and Handelsman, J. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. Trends Biotechnol. 17**:** 403-409.

Saltveit, M.E. 2005. Aminoethoxyvinylglycine (AVG) reduces ethylene and protein biosynthesis in excised discs of mature-green tomato pericarp tissue. Postharvest Biology and Technology 35**:** 183 - 190.

Sanderson, M.A., R.L. Reed, S.B. McLaughlin, S.D. Wullschleger, B.V. Conger, D.J. Parrish, D.D. Wolf, C. Taliaferro, A.A. Hopkins, W.R. Ocumpaugh, M.A. Hussey, J.C. Read, and C.R. Tischler. 1996. Switchgrass as a sustainable bioenergy crop. Bioresource Technology 56: 83-93.

Setter, T.L. and E.V. Laureles. 1996. The beneficial effect of reduced elongation growth on submergence tolerance of rice. Journal of Experimental Botany 47**:** 1551 - 1559.

Shah, S., J. Li, B.A. Moffatt, and B.R. Glick. 1997. ACC deaminase genes from plant growth promoting rhizobacteria. *In* : A. Ogoshi, K. Kobayashi, Y. Homma, F. Kodama, N. Kondo, and S. Akino, ed, Plant Growth-Promoting Rhizobacteria. Present Status and Future Prospects. OECD, Paris, pp 320 - 324.

Sharma, I. Piscioneri, P. de Visser, and D. van den Berg. 2001. Management guide for planting and production of Switchgrass as a biomass crop in Europe. In: Switchgrass (*Panicum virgatum*, L.) as a alternative energy crop in Europe. Initiation of a productivity network. Final Report FAIR 5-CT97-3701 "Switchgrass". www.switchgrass.nl.

Skujins, J. 1978. History of Abiontic Soil Enzyme Research. *In* RG Burns, ed, Soil Enzymes. Academic Press, London, pp 1 - 33.

Smith, S.E. and D.J. Read. 1997. Mycorrhizal Symbiosis. 2nd Edition. San Diego. Academic Press.

Srivastava, L.M. 2002. Plant Growth and Development Hormones and Environment. Academic Press, San Diego, CA.

Stevenson, I.L. 1959. Dehydrogenase activity in soils. Canadian Journal of Microbiology 5**:** 229-235.

Tabatabai, M.A. 1982. Soil Enzymes. *In* AL Page, R.H. Miller, and D.R. Keeney, ed, Methods of Soil Analysis, Vol Part 2.

Tabatabai, M.A. 1994. Soil Enzymes. *In* RW Weaver, ed, Methods of soil analysis, Vol 2. SSSA and ASA, Madison, WI, pp Pages 775 - 833.

Tiedje, J.M. 1994. Denitrifiers. *In* R.W. Weaver *et al*. Editorial committee, ed, Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, pp 245-267.

United States Army Corps of Engineers. 2007. Project Operation Shoreline Management Plan for Hartwell Lake Project Georgia and South Carolina. District Pamphlet No. 1130-2-18.

Visser, E.J.W., C.J. Heijink, K.J.G.M. Van Hout, L.A.C.J. Voesenek, G.W.M. Barendse, and C.W.P.M. Blom. 1995. Regulatory role of auxin in adventitious root formation in two species of *Rumex* differing in their sensitivity to waterlogging. Physiologia Plantarum 93 (1) : 116-122.

Visser, E.J.W., G.M. Bogemann, C.W.P.M. Blom, and A.C.J. Voesnek. 1996. Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots. Journal of Experimental Botany 47**:** 403-410.

Vogel, K.P., J.J. Brejda, D.T. Walters, and D.R. Buxton. 2002. Switchgrass biomass production in the Midwest USA: harvest and nitrogen management. Agronomy Journal. 94: 413-420.

Wample, R.L. and D.M. Reid. 1979. The role of endogenous auxins and ethylene in the formation of adventitious roots and hypocotyl hypertrophy in flooded sunflower plants (*Helianthus annus*). Physiologia Plantarum 45**:** 219-260.

Weaver, J.E. and R.W. Darland. 1949. Soil-root relationships of certain native grasses in various soil types. Ecological Monographs. 19: 303-338.

West, A.W. and G.P. Sparling. 1986. Correlation between four methods to estimate total microbial biomass in stored, air-dried and glucose amended soils. Soil Biology and Biochemistry 18**:** 569-576.

Whipps, J.M. 1990. Carbon Utilization. *In* JM Lynch, ed, The Rhizosphere. Wiley Interscience, Chichester, pp 59 - 97.

White, D.C. and F.E. Frerman. 1967. Extraction, characterization, and cellular localization of the lipids of *Staphyloccous aureus*. Journal of Bacteriology 94**:** 1854-1867.

Wilkinson, S.G. 1988. Gram-negative bacteria. *In* C Ratledge, and S.G. Wilkinson, ed, Microbial Lipids, Vol 1. Academic Press, London, pp 299 - 408.

Woodrow, L., A. Liptay, and B. Grodzinski. 1987. The effects of CO_2 enrichment and ethephon application on the production of tomato transplants. Acta Hortic. 201**:** 133 - 140.

Xie, H., J.J. Pasternak, and B.R. Glick. 1996. Isolation and characterization of mutants of the plant growth-promoting rhizobacterium Pseudomonas putida GR12-2 that overproduce indoleacetic acid. Current Microbiology 32**:** 67-71.

Yamamoto, F. and T.T. Kozlowski. 1987. Regulation by auxin and ethylene of responses of *Acer negundo* seedlings to flooding of the soil. Environmental and Experimental Botany 27**:** 329-340.

Yang, S.F. and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35**:** 155-189.

Zelles, L., Q.Y. Bai, T. Beck, and F. Beese. 1992. Signature fatty acids in phospholipids and lipopolysacharides as indicators of microbial biomass and community structure in agricultural soils. Soil Biology and Biochemistry 24**:** 317-323.

Figure 1.1. *Panicum virgatum* L. ‘Alamo’ (switchgrass)

CHAPTER 2

SITE MODIFICATION AND ITS EFFECTS ON ENZYMATIC ACTIVITY OF SHORELINE SOILS ON LAKE HARTWELL

Abstract

Plants on the shoreline of upstate South Carolina reservoirs grow in nutrient-poor soils whose microbial activity has not been characterized. It was hypothesized that as plants used for lakeshore stabilization age, microbial activity associated with soils will increase and microbial community will change. Soil samples were taken from *Panicum virgatum* (switchgrass) stands of different ages (1, 4, and 5 years old) on upstate SC reservoirs shorelines. Acid phosphomonoesterase, nitrate reductase, nitrogen fixation by acetylene reduction, dehydrogenase, substrate-induced respiration, and fatty acid methyl ester analyses were performed. As planted sites matured, acid phosphomonoesterase, nitrate reductase, and dehydrogenase activity increased. Acid phosphomonoesterase average activity ranged from 99.31 (1 year old site) to 231.92 (5 year old site) mg p-nitrophenol produced/kg/h in planted sites and was 80.77 and 108.7 mg p-nitrophenol produced/kg/h in non-vegetated sites. Nitrate reductase average activity went from 0.0065 to 0.0198 μg NO_2-N/gdwt/24h in vegetated sites of 1 year and 4 years old, respectively, and was not detected in most samples from non-vegetated sites. In vegetated sites, dehydrogenase values ranged from 592.45 (1 year old site) to 1564.15 mg TPF/ gdwt/24h (4 year old site), but were only 131.69 and 180.16 mgTPF/gdwt/24h in non-vegetated sites. Very low values of nitrogen fixation (<1.0 nmol/N_2-fixed/gdwt/h) and substrate-induced respiration (0.23 to 0.63 mg microbial C/g dwt soil) were measured

at all sites with no statistical differences among sites. Fatty acids indicative of Gram negative bacteria were found at higher concentrations in planted sites and fatty acid concentrations differed between sites. There was an increase in the diversity of fatty acids associated with planted sites indicating a more diverse microbial community when compared to non-vegetated sites. Overall the results indicated that switchgrass altered aoil activities and the microbial community. It is not know if the soil activity and microbial community would continue to change past the four years associated with this study.

Introduction

Lake Hartwell is a reservoir operated by the United States Army Corps of Engineers located in Pickens County, South Carolina. The clay soils of this area have high bulk densities, and are low in nutrients, especially nitrogen and phosphorus. Shorelines of the reservoir are steep and high intensity winds cause waves to "eat away" at the base of the slope. Site conditions have been improved by rebuilding the slope, amending soils with larger particle size soils to alter bulk density, and applying fertilizer to increase nutrient levels. However, fertilizer application adjacent to a body of water poses two problems: increased amounts of phosphorus cause eutrophication which leads to hypoxia and anoxia in the water column, and shorelines of the reservoirs in South Carolina are extensive. Applying and reapplying nutrients is costly and time consuming.

A hardy, native grass, *Panicum virgatum* (switchgrass), has been planted in areas of the shoreline. Switchgrass is naturally found in regions with hardpan clay soils with high bulk densities (Schwendiman and Hawk, 1973). This grass has an extensive root system which can aid in bank stabilization (Liebig, 2005). Adding switchgrass to these areas could increase the soil microbial activity which in turn could have a positive effect on the permanent establishment of vegetation through improvement of soil quality.

Microbial activity level is presumed to be low due to the lack of an energy source, which could be provided by plant root exudates and root decomposition. Although some microbial activity is detrimental to plants, most is beneficial. Soil bacteria mediate nutrient cycles, making nutrients available for plant uptake. Soil enzyme assays provide information on the level of nutrient cycling occurring, as well as nutrient availability.

The data from soil enzyme studies can also demonstrate the microbial activity changes as sites mature.

The effects of the soil microbial community have been investigated for years. Researchers have tried to make a connection between a soil fertility index and the level of various soil enzyme activities. By the 1970's, this idea was abandoned due to enzyme-substrate specificity and the unique properties of each type of soil (Skujins, 1978). Soil enzyme assays, however, are still used to help understand some processes occurring in the soil, like decomposition and the state of nutrient cycling (Skujins, 1978).

Members of the soil microbial community can extract phosphorus from the soil at concentrations lower than those available to plant roots. A healthy soil microbial community can access concentrations of phosphorus compounds unavailable to plants by enzymatic activity. Because the soils in upstate South Carolina are highly weathered, acidic clays high in aluminum and iron (which characteristically adsorb phosphorus compounds), multiple applications of fertilizers may be required (Brady and Weil, 2004). Eventually the adsorption sites are filled and the phosphorus is available for plant use. Much of the fertilizer not bound to the clay charges is washed into the nearby streams and lakes. This problem can theoretically by solved by enlisting the help of soil microbes.

Microbes are responsible for some immobilization of phosphorus when the element is taken up and converted to an organic form. Enzymes are important in the mineralization of phosphate, especially the phosphomonoesterases, which was the enzyme investigated here. Phosphomonoesterases hydrolyze the phosphate from monoester forms of phosphorus, like those in nucleotides or phospholipids, to release

HPO_4^{-2}. Inadequate supplies of phosphorus stimulate the production of phosphatase by plants, bacteria, or both, which in turn, increases the mineralization of organic phosphorus. However, when soluble phosphorus concentrations rise, phosphatases become inhibited, actually by orthophosphate which is a competitive inhibitor of acid phosphatases (Juma and Tabatabai, 1978). For example, when soluble phosphorus levels rise above P>0.55mM or 87mg/kg, phosphatase activity is decreased. Increased phosphatase activity has been shown to be related to organic carbon content of the soil (Stevenson, 1986). It is hypothesized that as a stand of switchgrass matures, phosphomonoesterase activity increases, either from an increase in the demand from plants which take phosphorus from the soil solution or by an increase in the individuals in the heterotrophic microbial community able to produce these enzymes, which live on the carbon exuded by the plants in the stand.

Nitrogen is one of the essential elements but is often the most limiting nutrient in soils. Plants use nitrogen in the formation of proteins, nucleic acids, and secondary metabolites. Access to this extremely important nutrient is mediated by soil microbes that convert nitrogen compounds from one form to another. Some microbes are responsible for fixing atmospheric nitrogen into organic forms, while others are responsible for the release of gaseous nitrogen under anaerobic conditions.

To investigate the status of nitrogen cycling in soils of the reservoir shorelines, the activity levels of both the nitrogenase enzyme complex involved in nitrogen fixation and the first enzyme involved in denitrification, nitrate reductase, were examined. Sampling areas were planted with native species and plants were supplied with starter

fertilizer to give the plants a solid introduction to the harsh environment, but no additional fertilizer was applied after establishment. Knowing the soils are low in nitrogen, it was hypothesized that the plants would depend on the microbial community to acquire necessary nitrogen. Therefore, there would be some nitrogen fixation occurring in the soil by nonsymbiotic diazotrophs and rates of nitrogen fixation would be higher in older stands. Nitrogen is in short supply in these soils, minimal levels of nitrate reduction were expected. Any nitrate reduction occurring would mean there was excess nitrate in the soil not taken up by vegetation or microbes. Older sites with more vegetation were expected to have higher activity values because there would be more time for denitrifiers to move into the sampling area and there would be more organic residue breaking down from previous seasons of plant material at sites. Also, sections of sites that were either inundated at sampling time or were closest to the water line would have higher nitrate reductase values because the reaction occurs under anaerobic conditions.

Measurement of dehydrogenase activity is used to reflect the total oxidative activities of the soil microflora. These numbers are used in conjunction with biomass calculations to estimate not just the total quantity of soil microbes that may be present, active or inactive, but more importantly the amount of active microbial cells since active dehydrogenases are considered to be an integral part of intact cells (Dick *et al*., 1996).

The method for determination of dehydrogenase activity was the TTC (triphenyltetrazolium chloride) method (Tabatabai, 1994) . Triphenyltetrazolium chloride (TTC) is used as an artificial electron acceptor because it can serve as an electron

acceptor for several dehydrogenases at a redox potential of - 0.08V. When TTC accepts electrons through dehydrogenase activity, it forms triphenyl formazan (TPF) which is measured spectrophotometrically. Triphenyltetrazolium chloride is a good choice as an electron acceptor because nearly all microbes can reduce TTC (Mattson *et al*., 1947). The dehydrogenase activity of plants and animals can also be evaluated using this method. It is important to remember that dehydrogenase activity of plants and animals can also be evaluated using this method

Because the dehydrogenase method is used to measure the oxidative activity occurring in a sample, it was hypothesized that older stands of switchgrass, DNR I and DNR II, would have a higher activity than the site established more recently, Camp Hope. Older stands have more organic material from successive years of plant material dying back and becoming incorporated into the soil and carbon exudates from root systems. The literature on dehydrogenase activity supports the idea that higher organic material in the soil results in higher oxidative activities (Bandick, 1999; Dick, 1996; Yang, 2003). Increased organic material can support higher oxidative activity, but may not indicate increased numbers of microbial cells. Dehydrogenase activity must be compared to substrate-induced respiration assays to determine if there is a correlation between increased dehydrogenase activity and number of microbial cells present using glucose as a substrate.

Bacteria present in soil often live off carbon exudates from plant roots and decaying organic material aiding growth of plant in the area (Kennedy, 1999; Mantelin and Touraine, 2004). Knowing how much microbial biomass is in soil can help

understand more about the soil community in addition to determine bacterial species present. Substrate-induced respiration (SIR) can be used as a measure of microbial biomass, as higher respiration indicates higher microbial biomass.

The method used here combines a previous approach (Jenkinson, 1966) and work by Anderson and Domsch (1975) on microbial weight to estimate microbial biomass by carbon dioxide emission. Anderson and Domsch (1975) used the upper 10cm of twelve different soils, nine from agricultural plots, one from a grassland, and two from mixed oak and spruce forest in Germany to study the correlation between respiration and microbial biomass. More recently, other researchers have further correlated carbon dioxide emission and microbial biomass (West and Sparling, 1986; Beare *et al*., 1990). For incubations at 22^{o}C, a substrate-induced maximal respiration rate of 1ml CO_2/h was found to equal 40mg of microbial carbon biomass (Anderson and Domsch, 1978). As with the previous assays, it is hypothesized that with increasing age of vegetated sites there would be an increase in microbial carbon.

The study of soil communities can be challenging due to the sheer number and diversity of microbes present. It is also limited by the fact that only 0.01 to 10% of the total microbes present in soil can be cultured on artificial media (Perfileu and Gabe, 1969; Rondon *et al*., 1999). Due to the limitations of classical approaches of culturing organisms, other methods have been developed to attempt to describe microbial communities, including nucleic acid analysis and fatty acid analysis. Fatty acid methyl ester (FAME) analyses of soils differentiate microbial communities based on differences in fatty acid profiles (Sasser, 1990). This assay can help researchers understand more

about microbial community assemblages without subculturing, and more members of the community can potentially be identified.

The length, level of saturation, and branching patterns of fatty acids can be characteristic of specific genera (Miller, 1982; Deboer and Sasser, 1986). Odd-numbered, branched chain fatty acids have been found to be produced by Gram positive bacteria. The even-numbered, straight chain and cyclopropyl fatty acids tend to be associated with Gram negative bacteria. The level of saturation can aid in identification of the organism, and unsaturated fatty acids are usually associated with anaerobic environments (Kandeler, 2007). A few fatty acids have a close association with specific organisms, such as the fatty acid 18:2ω6, which accounts for 43% of the total fatty acids of 47 species of soil fungi (Kandeler, 2007). The literature suggests that environments with low nutrient status are dominated by fungi and that the community shifts towards bacteria as the nutrient levels improve (Grayston *et al*., 2004; Bardgett *et al*., 1996). The planted sites in this study are hypothesized to have more diverse fatty acids associated with bacterial communities, perhaps due to improvement of nutrient status through the establishment of vegetation. Control soils from barren shoreline and shoreline fill soil are hypothesized to contain more fatty acids associated with fungi.

The objectives of this study were to (1) measure acid phosphomonoesterase, dehydrogenase, nitrate reductase, nitrogen fixation, and substrate-induced respiration values for 1, 4, and 5 year old switchgrass stands, and (2) to conduct fatty acid methyl ester analyses on the same soil samples to determine if microbial activity and microbial communities change in soils with switchgrass over time.

Materials and Methods

Sites

Three sites were chosen along the shoreline of Lake Hartwell in Pickens County in the northwest portion of South Carolina (Fig. 2.1). The original soils of this area are Ultisols or alumino-silicate clays resulting from the weathering of granite bedrock. These soils are nutrient-poor and acidic (Brady and Weil, 2004). The sites were chosen based on previous shoreline treatment and establishment of switchgrass. All planted sites began as highly eroded scarps that were reconstructed and protected with rock at the toe. Most of the plants were placed in the rock and above to aid in stabilization and naturalization of the reconstructed bank (Fig. 2.2).

The first site (DNR I) was established in 2000 and is located on Lake Hartwell behind the South Carolina Department of Natural Resources, Pickens County Office (Fig. 2.3). At the bottom of the site (nearest the water line), plugs of switchgrass were planted in holes that contained slow release fertilizer and lime. Moving upward along the bank, rip-rap was used to protect the toe of the bank from high energy erosional forces. Soil taken from the area where rip-rap was placed was moved in front of the granite rocks. Some incidental switchgrass seed which had washed down the bank or spilled over germinated in this soil. Under the granite rocks a filter fabric-backed turf reinforcement mat (TRM) was placed, which was seeded only. The mat was backfilled with soil from an old flood plain of the Seneca River that formerly traveled through a portion of Clemson University. Fill dirt of the same type as the original alumino-silicate clay was brought in from a nearby source to lessen the slope of the eroded bank. This soil is

referred to as “Smith” soil in this study and is a B-horizon red clay. The reconstructed slope received plugs and seed, and was covered with a synthetic mat, TRM-50, with no backing. Plugs were cut to the ground before the mat was applied and plug holes received slow release fertilizer and lime. The rest of the reconstructed slope received starter fertilizer and lime. At the top of the plateau, the area was leveled, starter fertilizer was added, the soil was limed, and finally seeded. A coir erosion control fabric covered the plateau. This site was approximately 5 years old at time of sampling.

The following year (2001), another site was added to the DNR Pickens County Office area, which is referred to as DNR II in this research (Fig. 2.4). This site had much less area in front of the toe, so there were no plantings out away from the rip-rap as had been done at the earlier DNR I site. The excavation spoils from the rip-rap toe footing, located in front of the rip-rap, were plugged with slow release fertilizer and lime. However, some incidental seed had probably washed in from the seeded area above. Next, rip-rap was placed on top of a backed TRM similar to the mat at the other DNR site and the TRM extended to cover the lower portion of the reconstructed bank that was formed from fill dirt obtained from the same source as in the first DNR site. This reconstructed bank was seeded with switchgrass and starter fertilizer and lime were added. The area was also plugged with the holes receiving slow release fertilizer and lime. There were no plantings on top of the plateau. The DNR II site was approximately 4 years old at time of sampling.

The final planted site is located at Camp Hope on Lake Hartwell in Pickens County, SC and is referred to as Camp Hope (Fig. 2.5). This area was modified in 2004

and is a smaller stand than the DNR sites. This site was approximately 1 year old at time of sampling. Soil was brought in from the control site, using "Smith" soil to lessen the slope of the original bank. Once the slope was modified, a TRM was placed on the soil with rip-rap covering the entire mat. This slope was plugged with switchgrass using well-established plants in 6 inch pots, and the holes received slow release fertilizer and lime.

Control samples were taken from an area of shoreline soil approximately 200 feet from the DNR I site which was completely devoid of vegetation and had not been modified. This sample is referred to as "Shoreline" soil and is considered to be a control sample. The area represented the soil present in the planted sites (DNR I, DNR II, and Camp Hope) before any plantings or amendments of any kind occurred. Also, as referred to above, samples were taken from a nearby soil source used to reconstruct DNR I, DNR II, and Camp Hope slopes and are referred to as "Smith" soil, a B-horizon red clay. This soil came from a site with no vegetation and was also considered a control sample.

Sampling

All soil samples for enzyme assays, substrate-induced respiration analysis, and fatty acid methyl ester analysis were dug with a shovel from the top 10cm of each sampling area, removing the top 1cm of soil to avoid cyanobacterial contamination. Samples were taken at least 10cm from switchgrass plants to avoid the nursery mix and amendments that surrounded the original plug. DNR I and DNR II were sampled in three columns across and three rows from the top of the stand to the bottom, evenly spaced, for

a total of nine samples each (Fig. 2.6). The water level of the lake at the times of sampling was approximately 3 inches below the bottom row of samples taken. There was approximately 80 feet between rows. Camp Hope was similarly sampled, but with only two rows vertically due to the smaller dimensions of the stand (Fig. 2.7). The water level of the lake was approximately 3 inches below the bottom row of samples taken and there were approximately 50 feet between rows.

One sample was taken during each sampling event and designated "Shoreline" from an area adjacent to the DNR stands, with no vegetation present. Another sample was taken during each sampling event from material which was used to build the slopes at DNR I, DNR II, and Camp Hope. This soil was designated "Smith".

Soil samples were sieved through 3mm mesh to remove roots and litter before being placed in plastic, resealable bags and labeled with site, column, and row information. Samples were kept separate and were not pooled for analysis. The bags were transported in coolers back to the lab where they were stored at $4^{o}C$ until analyzed. Samples were taken on June 4, August 1, and October 8, 2005.

Weather Data

Weather data was taken from a weather station at Clemson University, Clemson, SC, just off of Cherry Road maintained by Dr. Dale Linvill. Temperature highs and lows for each day were recorded and precipitation levels were monitored daily.

Soil Analysis

Composite samples from each site were taken on June 4, 2005 and submitted to the Clemson University Agricultural Service Laboratory (Clemson, SC). Soil analyses included determination of pH, phosphorus, organic matter, and nitrate nitrogen levels.

Statistical Analysis

All enzyme assay values, substrate-induced respiration values, and fatty acid methyl ester analysis values for the three experimental sites, DNR I, DNR II, and Camp Hope, were analyzed using an analysis of variance (ANOVA), run on SAS statistical software (SAS Institute Inc., 2002). The values for the Shoreline soil and Smith soils were included in the yearly ANOVA analysis but not included in the monthly ANOVA analysis due to small sample sizes. Those control values can serve only as values with which to compare yearly trends, but not to determine statistical differences from month to month. Statistical differences between site values were determined using Fisher's least significant differences ($p<0.05$). Values from each site were checked for normality and homogeneity of variance using t-tests. If values from within sites were not statistically different, averages of values from within site were used.

Enzyme Assays

All chemicals used in enzyme assays were obtained from Fisher Scientific (Fair Lawn, NJ) or Sigma-Aldrich, Co. (St. Louis, MO) except 1,3,5 triphenyl formazan, which was obtained from TCI America (Portland, OR).

Phosphomonoesterase Assay

The phosphomonoesterase assay is based on a colorimetric estimation of the p-nitrophenol released by phosphatase activity when soil is incubated with buffered (pH 6.5) sodium nitrophenyl phosphate solution and toluene (Tabatabai, 1994). A modified universal buffer (MUB) stock solution was prepared by dissolving 12.1g of tris (hydroxymethyl) aminomethane (THAM), 11.6g maleic acid, 14.0g citric acid, and 6.3g of boric acid (H_3BO_3) in 488ml of 1N NaOH bringing solution up to 1L with water. To prepare the pH 6.5 MUB, 200ml of MUB stock solution was adjusted to pH 6.5 using 0.1 N HCl and the volume was adjusted to 1L with water.

One gram of soil was put in a 50mL Erlenmeyer flask to which 0.2mL of toluene, 4mL of modified universal buffer (pH 6.5), and 1mL of p-nitrophenyl phosphate solution in MUB, (pH 6.5) were added. The flask was swirled to mix the contents, was stoppered and placed in an incubator at 37^{o}C. The sample was incubated for one hour. After incubation, 1mL of 0.5M $CaCl_2$ and 4mL of 0.5M NaOH were added. The soil suspension was filtered through a Whatman no.2 folded filter paper. Absorbance was measured at 410nm using a Jasco V550 spectrophotometer (Jasco, Inc., Easton, MD). A blank was needed for each sample and was prepared as above except 1mL of p-nitrophenyl phosphate was added after incubation to stop the reaction.

The p-nitrophenol content of the filtrate was determined by comparing the absorbance values to a calibration graph prepared using 0, 10, 20, 30, 40 and 50ug of p-nitrophenol which were added in a total volume of 5mL water. If the absorbance exceeded that of 50ug p-nitrophenol standard, the soil filtrate was diluted with water.

Nitrate Reduction Assay

This assay investigates dissimilatory nitrate reduction due to anaerobic conditions. The procedure followed is from Abdelmagid and Tabatabai (1987). The assay measures only the activity of nitrate reductase because the energy required for the following steps in denitrification are inhibited by 2,4-dinitrophenol (DNP). DNP is an uncoupler of oxidative phosphorylation that interferes with mitochondrial electron transport in eukaryotic organisms and plays a similar part in this assay (Abdelmagid and Tabatabai, 1987).

Soil samples (5g) were placed in screw cap test tubes with 4ml 0.9mM DNP solution, 1mL of nitrate solution (25mM), and 5mL distilled water. The contents were mixed well, stoppered, and incubated for 24 hours at 25^{o}C. After incubation, 10mL of 2M KCl solution was added. The test tube contents was mixed thoroughly and filtered through Whatman no. 1 filter paper. Nitrite concentration was measured using the modified Griess-Ilsovay method (Bremner, 1965). Every soil sample required a corresponding blank which consisted of 5g soil, 6mL water, and 4mL 0.9mM DNP. Blanks were treated as samples above.

Modified Griess-Ilsovay Method (Bremner, 1965)

An aliquot (2mL) of the filtrate was transferred to a 50mL volumetric flask, and water was added to total volume of approximately 45mL. Diazotizing reagent (1mL) was added and contents of flask was swirled to mix. (Diazotizing reagent was prepared by dissolving 0.5g of sulfanilamide in 100mL of 2.4N HCl). After 5 minutes, 1mL of

coupling reagent was added. (Coupling reagent was prepared by dissolving 0.3g of N-(1-naphthyl)-ethylenediamine hydrochloride in 100mL of 0.12N HCl and stored in an amber bottle at 4^{o}C). The solution was mixed and allowed to stand at room temperature for 20 minutes. The solution was brought up to 50mL with distilled water. The absorbance was measured at 535nm with a Jasco V550 spectrophotometer (Jasco Inc., Easton, MD). A blank was prepared following the above steps except no soil was included.

The standard curve was prepared according to recommendations by Bremner (1965). A stock nitrite solution (1) was made by dissolving 4.9257g $NaNO_2$ in 1000mL of distilled water. A second stock nitrite solution (2) consisted of stock nitrite solution 1 (20mL) diluted to 1L. Stock solution 2 was used to make a working stock solution (1mL diluted to 100mL). For the standard curve, 0, 250, 500, 750, and 1000µL volumes of working stock were transferred to 50ml volumetric flasks and brought to volume with distilled water. These volumes corresponded to concentrations of 0, 0.25, 0.50, 0.75, and 1.0µg of nitrite-N. The amount of nitrite-N per gram/24hours was determined using the following formula:

$$NO_2\text{-N } \mu g/g \text{ dwt}/24h = \frac{NO_2\text{-N } (\mu g/ml)\text{filtrate}*2}{5*\text{dwt}}$$

where,

dwt = dry weight of 1g moist soil,

2 = volume of filtrate (ml), and

5 = weight of moist soil (g).

Nitrogen Fixation Assay By Acetylene Reduction Method

The capacity for nitrogen fixation by soil microbes was measured using a widely used acetylene reduction assay (Hardy *et al*., 1973). Nitrogen fixation samples were prepared by placing 1g of soil into a 20mL screw-top vial with a Teflon septum. Acetylene, supplied by National Welders Supply Co. (Charlotte, NC), (200µL) was injected into the screw-top vial. Samples were incubated at 25^{o}C for 48 hours. A 1mL sample removed from the vial and was injected into a gas chromatograph (Shimadzu GC-9A). A blank for each sample was prepared and incubated as above omitting addition of 200µL of acetylene.

Calibration Curve

A calibration curve was constructed by injecting 2, 4, 6, 8, and 10ug of a 10ppm ethylene standard (Scott Specialty Gases, Plumsteadville, PA) into the gas chromatograph. The gas chromatograph was a Shimadzu GC-9A (Shimadzu Scientific Instruments, Inc., Columbia, MD) with a flame ionization detector, using nitrogen as a carrier gas at 50ml/minutes and a phenylisocyanate Porasil C column (2M x 1/8") with 80/100 mesh). The injector temperature was 140^{o}C and the column temperature was 30^{o}C. Since acetylene to ethylene conversion by nitrogenase is a 1:1 measure, the amount of ethylene is equivalent to the amount of acetylene fixed.

Dehydrogenase Assay

The dehydrogenase assay involved using tetrazolium salts as indicators of dehydrogenase activity (Tabatabai, 1994). The tetrazolium salt, TTC, is colorless or pale-colored and is easily transformed into intensely colored, water insoluble, methanol soluble TPF by reduction (Tabatabai, 1994). The absorbance of TPF was measured at 485nm in a Jasco V550 spectrophotometer (Jasco Inc., Easton, MD).

For each sample, 7.5g of soil was weighed and combined with 0.075g $CaCO_3$, which was mixed thoroughly. A 1.5g aliquot of this mixture was placed in each of 4 test tubes (16 x 150mm). To 3 test tubes, 250µL of 3% TTC (in water) and 625µL of distilled water were added. The contents of the tubes were mixed with a glass rod, stoppered, and incubated at 37^{o}C for 24 hours. The fourth tube was the blank and had 875µL of distilled water added. After 24 hours of incubation, 3mL methanol were added to all tubes. Tubes were stoppered and shaken for 1 minute. The soil suspensions were filtered through glass funnels plugged with absorbent cotton into 25mL volumetric flasks. The tubes were rinsed with methanol. The soil was washed until the reddish color had disappeared from the cotton plug. The filtrate was brought to 25mL with methanol. With clay samples, centrifugation for 10 minutes at 10,000 x g of the filtrate was necessary. Centrifugation was performed after filtration. Concentrations of 1.25, 2.50, 3.75, and 5.0ug of TPF/25mL methanol were used for a standard curve.

Substrate-induced Respiration Assay

The method of Anderson and Domsch (1975) was followed. Prior to analyzing the respiratory rate of the soil samples, the glucose amendment level had to be determined for the type of soil being examined. The amendment level was ascertained by adding varying concentrations of glucose (5, 10, 25, 50, 100, and 200µMol) to samples in 100µL aliquots. The soil was placed in a gastight container for CO_2 headspace analysis and incubated at 22^{o}C for 1 hour. The amount of CO_2 evolved was analyzed using gas chromatography (Shimadzu GC-8A). The minimum amount of glucose needed to invoke the maximum respiratory response, expressed as mL CO_2/h/g of dry weight of soil, was used in all sample analyses.

The glucose concentration (10µMol) was added to 10g soil samples (in gas tight containers) in 100uL aliquots. One replicate per sample was prepared. For control samples, 100uL of water without glucose was added. Samples were incubated at 22^{o}C in the dark to avoid interference from cyanobacteria. With a gas tight syringe, 0.5mL were withdrawn every hour for each replicate and injected into a gas chromatograph (Shimadzu GC-8A equipped with a thermal conductivity detector, Porapak R column (6' x 1/8"), carrier gas helium with a flow rate of 50ml/min, column temperature 40^{o}C, detector temperature 100^{o}C, Shimazdu Scientific Instruments, Inc., Columbia, MD). Samples were taken every hour for 3 hours until a linear increase in CO_2 evolution was determined. Hourly measurements were taken to accommodate increases, decreases, or lags in CO_2 efflux. The minimum hourly rate of linear CO_2 production was used to determine biomass. A positive tendency or increase in CO_2 production over time is

interpreted as new biomass synthesis indicated that measurements must precede this event (Anderson and Domsch, 1975).

To calculate the microbial carbon present in samples, the following equation was used (Anderson and Domsch, 1978):

$$x = 40.04y + 0.37$$

x = total microbial biomass carbon

y = maximum initial rate of CO_2 respired (mL CO_2/g dwt)

Fatty Acid Methyl Ester Analysis

One gram of soil was placed in a screw cap tube (13mm x 100mm) and fatty acids were extracted as described for bacterial isolates (Sasser, 1990). One screw cap tube had no soil and was used as a solvent blank. The organic layer (200µL) was concentrated 4-fold (50µL) by blowing dry nitrogen over samples in autosampler vials prior to analysis on the gas chromatograph. Fatty acid samples were analyzed by gas chromatography on a Hewlett-Packard 5890 gas chromatograph (GC) (Agilent Technologies, Wilmington, DE) equipped with Sherlock Microbial Identification System (MIDI Inc., Newark, DE). The system was equipped with an Agilent Ultra 2 column (crosslinked 5% phenyl methyl silicone, 25m x 0.25mm, 0.33µm film) and a flame ionization detector at 300^{o}C. Samples were run using an amended AEROBE method which had an additional 5 minutes at the end of the temperature program to make sure all components within sample had eluted off the column. The amended temperature program started at 170^{o}C and was increased to 260^{o}C, at a rate of 5^{o}C/minute, then increased to 310^{o}C at a rate of

40°C/minute. The final temperature was held for 5 minutes (total run time, 25 minutes). The carrier gas was hydrogen, the injected volume was 2μL, and the injector temperature was 250°C. HP3365 Chemstation software and computer were utilized for GC control. Fatty acids were identified by Microbial Identification software (MIDI, Inc. Newark, DE) based on a comparison with a peak table of fatty acids and a calibration mixture.

Results

Soil Analysis

Soil analyses (Table 2.1) were obtained from the Agricultural Service Lab, Clemson, SC for the composite samples from each site. The vegetated sites had higher phosphorus and calcium levels. Potassium levels were higher in the unvegetated soils and the 1 year old vegetated site. DNR I and DNR II sites had the highest magnesium levels. Nitrate nitrogen levels were low for vegetated and unvegetated sites with Camp Hope values being the highest. All soils had slightly acidic soil pH.

Weather Data

Weather data (Table 2.2) were obtained from Dr. Dale Linvill, Clemson University. Just prior to the August sampling (July 30, 2005), 2.89 inches of precipitation were recorded. Approximately 0.5 inches of rain was recorded for the entire month of September. Immediately before the October sampling (October 6 – October 8, 2005), 2.82 inches of precipitation were recorded.

Enzyme Assays

Phosphomonesterase Assay

Average values across all sites and sampling dates ranged from a low of 67.15mg p-nitrophenol produced/kg/h in October at Camp Hope to a high of 315.84mg p-nitrophenol produced/kg/h in October at DNR I. Values for different sampling dates

within each site were normally distributed as determined by t-tests, so an average for all sampling periods for the sites were determined (Table 2.3).

Across all sampling dates, DNR I had higher rates of acid phosphatase activity when compared to other sites and control soil ($p<0.05$) (Table 2.3). Because of the sampling scheme used, there was only one sample for the control soil and in the planted sites there were 6 – 9 separate samples per month. Differences over time could be evaluated for the planted sites only. The highest acid phosphatase activity occurred in October at DNR I. This value was significantly higher than the June and August values at the DNR I site. The other sites showed no statistical differences in acid phosphatase values over time. In June, DNR I and DNR II acid phosphatase activity was higher than that of Camp Hope values. In October, DNR I was had higher activity than Camp Hope, but DNR II was not different from either DNR I or Camp Hope.

Nitrate Reductase Assay

Values calculated for the disimilatory nitrate reductase assay were much lower than has been previously reported in the literature (Abdelmagid and Tabatabai, 1987). The values ranged from "none detected" to 0.04ug NO_2-N/g dwt/24h (Table 2.4). The highest value was recorded from DNR II in October which was 0.0419ug NO_2-N/g dwt/24hr. The lowest values were recorded from Smith soil, where no nitrate reductase activity was detected on any date. No ethylene was detected in any of the samples (Table 2.4).

Averaged across all sampling dates (Table 2.4), had greater nitrate reductase activity than Shoreline and Smith soil. However, values from DNR I and Camp Hope did not differ significantly from that of DNR I. The highest value recorded at DNR II was 0.041 NO_2-N/gdwt/day in October, and this value was significantly greater than those of DNR I and Camp Hope on this date.

Nitrogen Fixation Assay by Acetylene Reduction Method

There were no differences in average yearly nitrogen fixation rates among sites (Table 2.5), nor were there differences on individual sampling dates. The highest yearly average was measured in the Smith soil, which is the fill soil used to construct the slopes at DNR I, DNR II, and Camp Hope. There was one value that skewed the results in favor of Smith soil. Omitting that high value, DNR I has the highest yearly average, but not statistically different from DNR II, which was second highest or from Camp Hope, which was third highest in nitrogen fixation.

Calculations of nitrogen fixation rates over the three planted sites showed no statistical differences between the vegetated sites(Table 2.5). When looking at each site over the growing season, there were no differences between June and October for DNR I, DNR II, or Camp Hope. Also, there were no significant differences in June, August, or October over all planted sites.

Dehydrogenase Assay

Dehydrogenase activity was in October for four out of five soils evaluated. This trend was more pronounced DNR I and DNR II (Table 2.6). The difference in dehydrogenase activity could be attributed to precipitation and temperature values prior to sampling in October 2005 because the sites were undisturbed. Both the monthly precipitation and average high and low temperatures for the growing season between May and October are given in Table 2.1.

There are significant differences among the yearly averages, with DNR II having the highest average of 1564.15 TPF/g dwt/24h and the Shoreline sample having the lowest average of 131.69 TPF. The Smith soil was also very low at 180.16 TPF/g dwt/24h. Among the three experimental sites, there were few statistical differences by month or by sampling site. In DNR I and DNR II, the October samples had significantly higher activity than the June and August samples shown in Table 2.7. Camp Hope showed similar activity across all sampling dates. DNR I and DNR II had significantly higher dehydrogenase rates than Camp Hope in October.

Substrate-Induced Respiration Assay

Camp Hope soils had the highest yearly average substrate-induced respiration value but this value was only significantly different from that of the Smith site (Table 2.7). DNR I, DNR II, Camp Hope, and Shoreline soils were not statistically different from each other.

There was a trend with August values having the lowest average and October values being in the middle. Both Camp Hope and DNR I showed the same pattern, with June having higher, statistically significant averages, values dropping August, and values increasing in October, although not to the height of the June values. However, DNR II had its lowest values in June and highest values in October.

Fatty Acid Methyl Ester Analysis

The results of the FAME analysis are presented in two parts. Table 2.8 shows those fatty acids that were present in concentrations of >5% of the corrected area of the peak (CA) divided by the total corrected area (TCA) (Table 2.8). Table 2.9 shows fatty acids that were present at lower concentrations, between 1.0 and 4.99% CA/TCA. The two fatty acids indicative of bacteria, 16:1ω5c and 19:1ω6c/19:0cyc ω9, were not present in Shoreline or Smith soils. Fatty acids indicative of fungi, 16:0 and 18:1ω9c, were not present in the Smith soil.

The fatty acids listed in Table 2.9 are present in smaller amounts than the fatty acids listed in Table 2.8. None of the fatty acids listed in Table 2.9 are found in the Shoreline soil, and many are not found in the Smith soils. Fatty acids 12:0 and 18:2ω6c, associated with fungi are found in the DNR I, DNR II, and Camp Hope soils. Fatty acids 16:1ω7c and i17:1ω10c, associated with bacteria, were present in DNR I, DNR II, and Camp Hope soils. There were no significant differences between planted sites in any of these fatty acids.

Discussion

Shoreline soils of reservoirs in upstate South Carolina are primarily clays with low nutrient content. Soil microbial activity may help shoreline plants to obtain nutrients and survive in the harsh conditions. Enzyme assays, nitrogen fixation measurements, CO_2 evolution measurements, and fatty acid methyl ester analyses were conducted to better understand the interactions between soil microbes and plants at these sites.

Since phosphorus is an essential nutrient, but also a limited nutrient in many soils, an assay to determine the potential activity of phosphomonoesterase was employed. The assay measured the potential of soil microbes to hydrolyze phosphate from monoester forms, like those in nucleotides and phospholipids.

The results of the phosphomonoesterase assay demonstrate that the older, 5 year old site, DNRI, had the highest acid phosphomonoesterase potential, followed by DNR II (4 year old site), and Camp Hope, the 1 year old site. However, every individual value obtained in this study was below the average of 617mg p-nitrophenol/kg/h found in the literature and was in the low range of reported values of 23 to 2100mg p-nitrophenol/kg/h (Tabatabai, 1994). The hypothesis that older sites would have higher activity was supported by the data. Only the DNR I site showed a change in acid phosphatase activity during the growing season: activity was higher in October than in June or August, perhaps due to changes in weather or seasonal phenology.

To investigate why the DNR I October value was higher , the precipitation and temperature data for this time period was evaluated. September temperature highs were very similar to August and the lows remained above 63^{o}F. Also, 2.82 inches of rain were

recorded between October 6 and October 8, 2005, which immediately preceded sampling (Linvill, 2005). The warmer temperatures would have favored the C-4 *Panicum virgatum* which would have continued its growth and need for phosphorus. The plants themselves could have produced phosphatases to gain phosphorus to support flowering. With the continued growth of the plant stand, the microbial community would benefit as well. The plant roots would have continued to exude materials from the root system ramping up metabolism of soil microbes. Increased activity of the microbial community in turn increases the need for phosphorus and phosphomonoesterase activity. When precipitation data were evaluated, it was noted that there was a significant rainfall event which occurred prior to the October sampling period. A total of 2.82 inches of rain was recorded immediately before sampling October 2005. The high temperatures, flowering activity, and the rainfall event could have caused not only the vegetation, but also the microbial community to increase its activity, which would create a need for phosphorus.

From these data, we can conclude that as sites age, acid phosphomonoesterase activity increases. Compared to the Shoreline and Smith fill control soils, soils with plants showed higher values. A conclusion drawn from these data is that if plants can survive for five years, the interaction between the plants and the soil microbes results in higher acid phosphomonoesterase activity which is beneficial for the plant in phosphorus limited soils. The literature shows the presence of plants has effects on soil phosphatase activity (Neal, 1973, Kiss *et al*., 1974). The effect may be indirect: increased soil organic matter may bring about higher activity due to the availability of an energy source. This situation could help explain why DNR I had high values, as DNR I had the highest

organic matter percentage (Table 2.1). But Shoreline soil also had a high phosphatase average over the sampling period. This high value may be attributed to the presence of fungi which could have produced large amounts of phosphatase.

Nitrogen plays a significant role in the nutritional demands of plants, but can be the most limiting nutrient in soils. Microbial activity converts nitrogen from a stable gas to a plant-available form in the soil. Both denitrification, which puts nitrogen back into the atmosphere, and nitrogen fixation, which brings nitrogen from the atmosphere to the soil, were evaluated in this study. Both processes proceed in anaerobic environments and are increased in soils with higher organic material. Therefore, it was hypothesized that in both cases, the older planted areas would have higher measured values and that samples from inundated soils would have higher values.

The values found in this study demonstrated minimal denitrification occurring in any site evaluated. Values reported in the literature are from 18 – 80 μg NO_2-N /g dwt/24h (Abdelgid and Tabatabai, 1987). Sites in this study showed values less than 1.0 μg NO_2-N /g dwt/24h over the entire growing season. If no denitrification is measured, presumably no nitrogen is being liberated from the soil to the atmosphere. Possible limitations to denitrification could be a lack of the substrate, nitrate. DNR I and DNR II had no detectable levels of NO_3-N. Smith and Shoreline soils only had 1 ppm. Camp Hope had 5 ppm of NO_3-N (Table 2). The higher value of NO_3-N in Camp Hope soil may be an artifact from the fertilizer applied to the site when it was modified one year before. According to Myrold (1999), in many wildland soils, such as grasslands, net nitrogen mineralization and net production of nitrate are small. The plants in older sites

(DNR I and DNR II) may have bound up all the available nitrogen leading to undetectable nitrate nitrogen levels.

The highest value seen in this study was from an October sample at DNR II. The October samples were taken after a rain event that deposited 2.82 inches of precipitation. The literature states that rainwater can saturate the soil, setting up an anaerobic environment in soil spaces (Brady and Weil, 2004). Since nitrate reductase functions in an anaerobic environment, the filling of pore spaces between soil particles may have stimulated production of the enzyme. Oxygen not only inhibits the synthesis of nitrate reductase, but also inhibits enzyme activity (Myrold, 1999).

It is clear that there is very little denitrification occurring on any of the sampled sites. The hypothesis that the older sites would have a higher level of denitrification has not been supported, and there is very little difference in the sites over the entire sampling period. There was only one sample that had a significantly higher value and that value was at the DNR II site, which is one of the older sites. When the individual sites were evaluated, there were no differences within the sites in terms of distance from the water and whether soils were routinely flooded or not. It was hypothesized that the soil samples taken closer to the water line or those that were routinely flooded would have higher values of denitrification because the soils would have been anaerobic more than other soil samples. The data did not support this hypothesis.

Little nitrogen fixation was occurring in the switchgrass stands. Hardy *et al*., (1973) stated that for algae and bacteria, the range of values of nitrogen fixation was between 6.6 to 180 nmol/N_2 fixed/g dwt/h. The values from the sites of this study were

well below the lowest values of this reported range. All values from all sites were less than 1.0 nmol/N_2 fixed/g dwt/hr. When looking at the yearly averages, no site was statistically different from any other site. The one value that stands out from the others is the value obtained from the October sample of Smith soil. That value was the highest of all the values for the study period of all sites. This site was sampled because it is the source of the fill dirt to make the slopes of the planted sites, DNR I, DNR II, and Camp Hope. However, the sample taken from the same source in August had no detected nitrogen fixation. The reason for the increased nitrogen fixation could be a result of a rainfall event immediately before the sample was taken.

The lowest values were recorded from the Shoreline sample. Both June and October samples demonstrated no nitrogen fixation detected. The Shoreline sample is representative of what values would have been measured if no plants or no new soil had been brought in. It is postulated that the Smith soil added to the amount of nitrogen fixation occurring on the planted sites, along with the grasses planted in these areas which could help support free-living heterotrophic nitrogen fixers.

The hypothesis that the older sites would have higher nitrogen fixation values was not supported because the values for the planted sites were not statistically different from one another. Samples taken from the areas closer to the water line were no different than samples taken further away from the water line. Also, samples that were taken from inundated soils were not higher than other samples.

The soils evaluated in this study are Ultisols, which are characteristically low in organic carbon. The sites in this study had organic carbon values between 2.5%

(Shoreline) and 3.6% (DNR I), as shown in Table 2.1. Free-living diazotrophs are heterotrophic and depend on outside carbon sources for their metabolic needs. These soils may be unable to support large populations of heterotrophic bacteria. According to Hubbell and Kidder (1978), the soils must be enriched in carbon before a large heterotrophic bacterial population can be sustained. Over time, the soils in the sites studied may become colonized by free-living diazotrophs. The bacteria will benefit from increased levels of organic material, which will in turn, support larger populations of diazotrophs, leading to increased nitrogen fixation.

Because of the intricate relationship of dehydrogenases and the respiratory needs of cells, dehydrogenase activity measurements are used to measure the immediate metabolic activities of soil microbes at the time of sample (Skujins, 1978). The dehydrogenase assay is a measure of the potential metabolic activity of intact living cells. Values in the literature range from 9 to 1760mgTPF/g dwt/24h, with a mean of 337mgTPF/g dwt/24h (Camina *et al*., 1998; Casida *et al*., 1964). The planted sites' values were well above reported averages, but the Shoreline and Smith values were lower. Table 2.6 shows that the older sites, DNR I and DNR II, have higher dehydrogenase values than the younger site Camp Hope site, and unplanted control sites for the growing season of 2005.

Because dehydrogenase activity occurs as an important part of cellular metabolism these results give an indication of the potential microbial activity, but do not necessarily agree with biomass measurements (Casida, 1964; Stevenson, 1959). This demonstrates that older sites, DNR I and DNR II, have higher potential metabolic

activity. Increased organic matter may contribute to higher dehydrogenase values, but is not the whole story. Table 2.1 shows that DNR I and Camp Hope have higher organic matter percentages, but DNR II and Smith have identical organic matter percentages. The presence of actively growing vegetation seemed to have an effect, also. This conclusion is evidenced by the comparison of planted versus unplanted sites. The unplanted sites have yearly average values of 131.69 and 180.16 mgTPF/g dwt/24h. These values are an order of magnitude lower than DNR I and DNR II, which have values of 1283.26 and 1564.15 mgTPF/g dwt/24h over the growing season, respectively. DNR I has had 5 years of growth and DNR II has had 4 years of growth in order to accumulate organic material, and the plants of these sites have had these extra years to elongate roots which in turn not only increases organic materials when roots die, but also these roots can secrete organic materials which microbes can use. Camp Hope results also support the above conclusion. The Camp Hope site had one year to establish itself at the time of sampling and had a yearly average value of 592.15 mgTPF/gdwt/24hrs, which is almost half-way between the nonplanted and planted sites, indicating vegetation does have an effect on dehydrogenase activity. The literature has shown increased organic material and vegetation present increases dehydrogenase activity values (Camina R., 1998; Wlodarczyk, 2002).

When evaluating the planted sites, it is evident that both DNR I and DNR II October values were significantly higher than the June and August samples. Camp Hope October values were higher than both the June and August samples of the same site, but were not significantly different. Because the sites were not manipulated in any way

during the growing season (tilling, mowing, etc.), the difference is most likely due to environmental conditions. According to the precipitation data (Table 2.2), immediately previous to the October sampling, there were 2.82 inches of rain ending a month long period with very little rain. This pulse of water may have had indirect effects on the microbial population due to increased metabolic activity of switchgrass. Switchgrass was able to increase photosynthesis and cellular respiration which may lead to organic materials being exuded from the root system (Taiz and Zeiger, 1999). Just as the increased precipitation led to increased acid phosphatase activity, dehydrogenase activity increased as well. Temperatures remained high through September which could have benefited the microbial community. However, the reason for the October values for all sites except Smith soil being above previous values is unknown.

Substrate induced respiration values were evaluated along with dehydrogenase values to further investigate the numbers of active and inactive microbial organisms present. The hypothesis was that the highest substrate induced respiration values would be from the oldest planted sites, DNR I and DNR II because of the amount of time these sites had to accumulate organic material in the soil. However, this hypothesis was not supported. Actually, Camp Hope had the highest average microbial carbon levels over the length of the study. The Camp Hope numbers were only statistically higher than Smith soils. The lowest values came from the Smith soil, while the hypothesis had stated that the Shoreline soil would actually have the lowest values due to the lack of vegetation at that sampling site. The highest values for all sites sampled were measured in June. The values fell in August, and rebounded in October although to lower values than were

measured in June. Because each site exhibited the same pattern it is reasoned the pattern is a result of temperature and precipitation over the sampling period. Higher values were recorded in June due to temperatures increasing, moving into the summer months. Although May was a relatively dry month, there was a rain event just prior to the June sampling that could have caused the microbial population to increase and therefore cause microbial carbon values to increase. High mid-summer temperatures could have suppressed microbial growth, whereas when temperatures decreased as the seasons moved toward fall and microbial carbon numbers increased slightly (Wagner and Wolfe, 1995). However, all values reported in this study are much lower than a majority of those reported in the literature. Andersson *et al*. (2004) reported values are from 8 – 48 mg microbial carbon/g dwt soil; however, Lohmus reported values of 0.24 – 2.89 mg microbial carbon/g dwt soil which are more closely aligned to the values in this research, <1 mg microbial carbon/g dwt soil. Since these soils are relatively low in nutrients, it is not surprising that the values obtained (0.23 to 0.63mg microbial carbon/g dwt soil) are on the low side of previously reported values.

Analysis of the soil microbial community is difficult using traditional culture techniques because only an estimated 10% of the soil microorganisms are culturable (Kennedy, 1999). By extracting key molecules from membranes of soil microorganisms, researchers can determine a higher percentage of members of the community. Fatty acids were identified using a gas chromatograph system equipped with a library of known fatty acid signatures. By determining fatty acids present and comparing the fatty acid

combinations to known fatty acid signatures, the composition of the community can be assessed.

The sites studied in this research are hypothesized to have differing nutrient levels. Three of the sites, DNR I, DNR II, and Camp Hope sites have been planted with switchgrass for 5, 4, and 1 years, respectively, at the time of sampling. Because of the growing seasons and decay of organic material occurring in the soils, these planted sites were thought to have higher nutrient levels than the other two sites included in this study, Shoreline and Smith soils. Information from Table 2.1 shows this to possibly be true for phosphorus and calcium and organic matter.

Soils low in nutrients tend to support fungi, whereas soils with higher nutrient levels tend to support bacteria (Grayston *et al*., 2004; Bardgett *et al*., 1996). With this information, a hypothesis was formed stating that the Shoreline soil should exhibit more fatty acids representative of fungi and the planted sites should show more fatty acids representative of bacteria. Also, the planted sites should be statistically different in their assemblage of fatty acids, again because of the difference in nutrient status of the soils. Results of this study show that there may was a statistically higher percentage of fatty acids 16:0 and 18:1w9c which are indicative of fungi in the shoreline soil. These fatty acids are indicative of fungi. Other studies have demonstrated a definite successional progression of fungi to bacteria in microbial communities. Cavigelli *et al*. (1995) stated that polyunsaturated molecules typically belong to eukaryotic species.

Unfortunately, many of the fatty acids found in this study are not unique to certain microbes. Therefore, it is difficult to determine if there are more bacteria in the planted

soils versus fungi in the Shoreline and Smith soils. However, it is interesting to note that the fatty acids associated with Gram negative bacteria are found only in the planted sites. The fungal associated fatty acids were found in a majority of planted sites, but not in the Smith soil. Fungal associated fatty acids were found in statistically higher amounts in the Shoreline soil than in the other soils, as well. The hypothesis that the Shoreline soil will contain higher amounts of fungal associated fatty acids and the planted sites will have more bacterial associated fatty acids is supported, but more research is needed to further support this hypothesis.

Table 2.9 shows fatty acids found in lower concentrations. Most of the fatty acids from this table were not found in either the Shoreline soil or the Smith soil and showed no statistical differences between any site in terms of prevalence. These data demonstrate the lack of diversity of fatty acids present in the Shoreline and Smith soils as well as lower microbial biomass since many of these fatty acids were not detected in the nonvegetated sites. Table 2.8 shows there were statistical differences between sites, but not clear cut differences. The differences were not clear cut because to see distinct patterns in the assemblages, the fatty acids need to be associated with either fungi or bacteria. It would be beneficial to look at the microbial communities of the planted sites several years later to determine if differences between the vegetated and nonvegetated sites increased.

After investigating soils with several enzyme assays, a respiratory analysis, and fatty acid methyl ester analysis, the results demonstrated that for every test, except acid phosphatase and dehydrogenase assays, values were much lower than those reported in

the literature. Acid phosphomonoesterase, nitrate reduction, and nitrogen fixation assays are indicative of the activity of nutrient cycling occurring in the soil. The low results further indicate the poor nutrient status of these soils.

The literature states that soil microbial biomass increases as soil fertility decreases in grasslands (Grayston *et al*., 2004; Grayston *et al*., 2001). Also, the literature states that fungi are found in soils with lower nutrient status (Bardgett *et al*., 1996). These findings directly relate to the interpretation of this research. Literature states that bacteria have a much lower biomass but higher respiratory rate than fungi (Anderson and Domsch, 1975). This could account for the higher substrate induced respiration values in the control samples, especially Shoreline soil and the lower dehydrogenase values of Shoreline and Smith soils. These conclusions are supported by the FAME analysis which found fungal fatty acids in sites with lower nutrient potential and higher microbial biomass. Bacterial markers were more evident in soils with vegetation. Increased diversity of fatty acids was also associated with the planted sites indicated a more diverse microbial community as well as higher levels of microorganisms.

Shoreline soils were evaluated in this study to determine nutrient levels and microbial activity. It was hypothesized that soils with vegetation would have higher nutrient levels and higher microbial activity due to the accumulation of plant residue and these values would increase over time. Increased acid phosphomonoesterase, nitrate reduction, and nitrogen fixation values could result in an advantage for plants growing in the soil due to nutrient availability. Surprisingly, organic matter percentages of soil samples taken in June were very similar with vegetation nor with increasing age. But

phosphorus, calcium, and magnesium values did increase in vegetated stands compared to barren soils in these same soil samples. Nitrate nitrogen values were at zero for the oldest stands, DNR I and DNR II, (5 and 4 years old at time of sampling, respectively), highest for Camp Hope (1 year old at time of sampling), and at 1 ppm for barren soils. These nitrogen values seem contradictory, but the literature supports these data. Switchgrass has been reported as extremely efficient at nutrient remobilization, especially nitrogen. Anex *et al*. (2007) reported that switchgrass had a 78% recovery potential when nitrogen fertilizer was applied. Part of this high recovery was due to low nitrogen loss as NH_3 volatilization during senescence. The nitrogen was translocated to belowground rhizomes and roots during shoot senescence (Heckathorn and DeLucia, 1995).

Other research on switchgrass reiterates that the plant efficiently translocates nutrients at the end of the growing season to stem bases and roots (Vogel *et al*., 2002). Therefore, switchgrass may be able to sustain itself for successive growing seasons with very little available nitrogen. However, Lee *et al*. (2007) and Muir *et al*. (2002) both concluded that switchgrass biomass deteriorated over time without the addition of nitrogen. The authors stated "sustainable production of switchgrass biomass is not feasible without N fertilization" (Muir *et al*., 2002). However, in the same study, Muir *et al*. (2002) concluded that even in soils characteristically low in nutrients, switchgrass was able to extract soil-bound phosphorus in quantities sufficient for sustainability. Therefore, it appears that as time progresses, there will be a need to supplement switchgrass stands on Lake Hartwell with nitrogen for optimal growth. It will not be

necessary to add additional phosphorus which possibly would have been detrimental to the lake adjacent to switchgrass stands.

Data from this supports supports the hypothesis that the presence of vegetation increases microbial activity in soils over time and this microbial activity can benefit the nutrient status of the soil resulting in increased plant growth. However, for sustainability of switchgrass stands for years to come, some intervention will be required. Surface fertilizers can be added to supplement nutrients in the soil, especially nitrogen.

Literature Cited

Abdelmagid, H. M., and M.A. Tabatabai. 1987. Nitrate reductase activity in soils. Soil Biology and Biochemistry 19(4): 421 - 427.

Alef, K. 1995. Dehydrogenase Activity. In: Methods in Applied Soil Microbiology and Biochemistry. K. Alef, P. Nannipieri. London, Academic Press**:** 228 - 229.

Anderson, J. P. E., and K.H. Domsch. 1975. Measurement of bacterial and fungal contributions to soil respiration of selected agricultural and forest soils. Canadian Journal of Microbiology 21: 314 - 322.

Anderson, J. P. E., and Domsch, K.H. 1978. A physiological method for the quantitative measurement of microbial biomass in soils. Soil Biology and Biochemistry 10: 215-221.

Andersson, M., A. Kjoller, and S. Struwe. 2004. Microbial enzyme activities in leaf litter, humus and mineral soil layers of European forests. Soil Biology and Biochemistry 36: 1527-1537.

Anex, R.P., L.R. Lynd, M.S. Laser, A.H. Heggenstaller, and M. Liebman. 2007. Potential for enhanced nutrient cycling through coupling of agricultural and bioenergy systems. Crop Science 47**:** 1327-1335.

Bandick, A. K., R.P. Dick. 1999. Field management effects on soil enzyme activities. Soil Biology and Biochemistry 31: 1471-1479.

Bardgett, R. D., P.J. Hobbs, and A. Frostegard. 1996. Changes in soil fungal:bacterial biomass ratios following reductions in the intensity of management of an upland grassland. Biology and Fertility of Soils 22: 261-264.

Brady, N.C., and R.R. Weil. 2004. Elements of the Nature and Properties of Soils. Second Ed.. Pearson Education, Inc., Upper Saddle River, New Jersey, pp 80 - 81.

Bremner, J.M., ed. 1965. "Inorganic forms of nitrogen" In: Methods of Soil Anaylsis, Vol 2

Calderon, F.J., L.E. Jackson, K.M. Scow, and D.E. Rolston. 2001. Short-term dynamics of nitrogen, microbial activity, and phospholipids fatty acids after tillage. Soil Scientists Society of America Journal 65**:** 118 - 126.

Camina, R., F. Gil-Sotres, and C. Leiros. 1998. Measurement of dehydrogenase activity in acid soils rich in organic matter. Soil Biology and Biochemistry 8**:** 1005-1011.

Casida, L.E., Klein, D.A., and Santoro, T. 1964. Soil dehydrogenase activity. Soil Science 98**:** 371-376.

Cavigelli, M.A., Robertson, G.P., and M.J. Klug. 1995. Fatty acid methyl ester (FAME) profiles as measures of soil microbial community structure. Plant Soil 170**:** 99-113.

DeBoer, S.H., and M. Sasser. 1986. Differentiation of *Erwinia carotovora* spp. carotovora and *E. carotovora* spp. atrosptica on the basis of fatty acid composition. Canadian Journal of Microbiology 32**:** 796-800.

Dick, R.P., D.P. Breakwell, and R.F. Turco. 1996. Soil enzyme activities and biodiversity measurements as integrative microbiological indicators. *In* Methods for Assessing Soil Quality SSSA Special Publication 49. Soil Science Society of America, Madison, WI, pp 247 - 271.

Grayston, S.J., G.S. Griffith, J.L. Mawdsley, C.D. Campbell, R.D. Bardgett. 2001. Accounting for variability in soil microbial communities of temperate upland grassland ecosystems. Soil Biology and Biochemistry 33**:** 533-551.

Grayston, S.J., C.D. Campbell, R.D. Bardgett, J.L. Mawdsley, C.D. Clegg, K. Ritz, B.S. Griffiths, J.S. Rodwell, S.J. Edwards, W.J. Davies, D.J. Eston, And P. Millard. 2004. Assessing shifts in microbial community structure across a range of grasslands of differing management intensity using CLPP, PLFA, and community DNA techniques. Applied Soil Ecology 25**:** 63-84.

Haack, S.K., H. Garchow, D.L. Odelson, L.J. Forney, and M.Klug. 1994. Accuracy, reproducibility and interpretation of fatty acid methyl ester profiles of model bacterial communities. Applied and Environmental Microbiology 60**:** 2483-2493.

Hardy RWF, R.C. Burns, and R.D. Holmes (1973) Applications of the acetylene-ethylene assay for measurement of nitrogen fixation. Soil Biology and Biochemistry 5**:** 47 - 81.

Heckathorn, S.A., and E.H. DeLucia. 1995. Ammonia volatilization during drought in perennial C4 grasses of tallgrass prairie. Oecologia 101**:** 361-365.

Horwath, W.R., and E.A. Paul. 1994. “Microbial Biomass" in: *Methods of Soil Analysis, Part 2*. *In* RW Weaver, J.S. Angle, and P.S. Bottomley, ed, Microbiological and Biochemical Properties., pp Pages 753 - 773.

Hubbell, D.H., and G. Kidder. 1978. Biological Nitrogen Fixation. *In* University of Florida Extension, ed. University of Florida.

Jenkinson, D.S. 1966. Studies on the decomposition of plant material in soil. Partial sterilization of soil and the soil biomass. Journal of Soil Science 17**:** 280-302.

Juma, N.G., and M.A. Tabatabai. 1978. Distribution of phosphomonoesterases in soils. Soil Science 126**:** 101-108.

Kandeler, E. 2007. Physiological and Biochemical Methods for Studying Soil Biota and Their Function. *In* EA Paul, ed, Soil Microbiology, Ecology, and Biochemistry, Ed 3rd. Academic Press, Oxford, UK, pp 53-83.

Kennedy, A.C. 1994. Carbon Utilization and Fatty Acid Profiles for Characterization of Bacteria. *In* RW Weaver, ed, Methods of Soil Analysis, Part 2 - Microbiological and Biochemical Properties, Vol 5. Soil Science Society of America, Madison, WI

Kennedy, A.C. 1999. The rhizosphere and spermosphere. *In* D.M. Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Inc., Upper Saddle River, NJ, pp 389-407.

Kraigher, B., B. Stres, J. Hacin, L. Ausec, I. Mahne, J. van Elsas, I. Mandic-Mulec. 2006. Microbial activity and community structure in two drained fen soils in the Ljubljana Marsh. Soil Biology and Biochemistry 38**:** 2762-2771.

Lee, D.K., V.N. Owens, and J.J. Doolittle. 2007. Switchgrass and carbon sequestration response to ammonium nitrate, manure, and harvest frequency on conservation reserve program land. Agronomy Journal 99**:** 462-468.

Liebig, M.A., H.A. Johnson, J.D. Hanson, and A.B. Frank. 2005. Soil carbon under switchgrass stands and cultivated cropland. Biomass and Bioenergy 28**:** 347-354.

Lohmus, K., M.Truu, J. Truu, I. Ostonen, E. Kaar, A. Vares, V. Uri, S. Alama, and A. Kanal. 2006. Functional diversity of culturable bacterial communities in the rhizosphere in relation to fine-root and soil parameters in alder stands on forest, abandoned agricultural, and oil-shale mining areas. Plant and Soil 283**:** 1-10.

Mantelin, S., and B. Touraine. 2004. Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. Journal of Experimental Botany 55**:** 27-34.

Mattson, A.M., C.O. Jensen, A.R. Dutcher. 1947. Triphenyltetrazolium chloride as a dye for vital tissue. Science 106**:** 294 - 295.

Miller, L.T. 1982. A single derivitization method for bacterial fatty acid methyl esters including hydroxy acids. J. Clin. Microbiol. 16**:** 584-586.

Muir, J.P., M.A. Sanderson, W.R. Ocumpaugh, R.M. Jones, and R.L. Reed. 2001. Biomass production of 'Alamo' switchgrass in response to nitrogen, phosphorus, and row spacing. Agronomy Journal 93**:** 896-901.

Mullen, M.D. 1999. Transformations of Other Elements. *In* DM Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Inc., Upper Saddle River, NJ, pp 369-386.

Myrold, D.D. 1999. Transformations of Nitrogen. *In* DM Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Upper Saddle River, NJ

Nannipieri, K., L. Muccini, and C. Ciardi. 1983. Microbial biomass and enzyme activities: production and persistence. Soil Biology and Biochemistry 15**:** 679 - 685.

Perfileu, B.V., and D.R. Gabe. 1969. Capillary Methods of Investigating Microorganisms. Oliver and Boyd, Edinburgh, UK.

Rondon, M.R., Goodman, R.M., and Handelsman, J. 1999. The Earth's bounty: assessing and accessing soil microbial diversity. Trends Biotechnol. 17**:** 403-409.

Sasser, M. 1990. Bacterial Identification by Gas Chromatographic Analysis of Fatty Acids Methyl Esters (GC-FAME). Technical Note #101. *In*. MIDI, Inc.403-409.

Schwendiman, J.L., and V.B. Hawk. 1973. Chapter 22: Other grasses for the north and west. *In* ME Heath, D.S. Metcalfe, and R.F. Barnes, eds., Forages, The Science of Grassland Agriculture, 3rd Ed. The Iowa State University Press, Ames, IA, p 755.

Skujins, J. 1978. History of Abiontic Soil Enzyme Research. *In* RG Burns, ed, Soil Enzymes. Academic Press, London, pp 1 - 33.

Sparling, G.P. 1992. Ratio of microbial biomass carbon to soil organic carbon as a sensitive indicator of changes in soil organic matter. Australian Journal of Soil Research 30**:** 195-207.

Stevenson, F.J. 1986. Cycles of Soil: Carbon, Nitrogen, Phosphorus, Sulfur, and Micronutrients. Wiley, New York.

Stevenson, I.L. 1959. Dehydrogenase activity in soils. Canadian Journal of Microbiology 5**:** 229-235.

Tabatabai, M.A.. 1994. Soil Enzymes. *In* RW Weaver, ed, Methods of soil analysis, Vol 2. SSSA and ASA, Madison, WI, pp Pages 775 - 833.

Taiz L, and E. Zeiger. 1998. Plant Physiology, Ed 2nd. Sinauer Associates, Inc., Sunderland, MA.

Tiedje, J.M. 1988. Ecology of denitrification and dissimilatory nitrate reduction to ammonium. *In* AJB Zehnder, ed, Biology of anaerobic microorganisms. John Wiley and Sons, New York, pp 179-244.

Tiedje, J.M. 1994. Denitrifiers. *In* R.W. Weaver *et al*. Editorial committee, ed, Methods of Soil Analysis. Part 2, Microbiological and Biochemical Properties. Soil Science Society of America, Madison, pp 245-267.

Vogel, K.P., J.J. Brejda, D.T. Walters, and D.R. Buxton. 2002. Switchgrass biomass in the midwest USA: harvest and nitrogen management. Agronomy Journal 94**:** 413-420.

Wagner, G.H., and D.C. Wolf. 1999. Carbon transformations and soil organic matter formation. *In* DM Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Inc., Upper Saddle River, NJ.

West, A.W., and G.P. Sparling. 1986. Correlation between four methods to estimate total microbial biomass in stored, air-dried and glucose amended soils. Soil Biology and Biochemistry 18**:** 569-576.

Wlodarczyk, T., W. Stepniewski, and M. Brzezinska. 2002. Dehydrogenase activity, redox potential, and emissions of carbon dioxide and nitrous oxide from Cambisols under flooding conditions. Biology and Fertility of Soils 36**:** 200-206.

Figure 2.1. Map of South Carolina showing location of experimental sites along Lake Hartwell as indicated by the star.

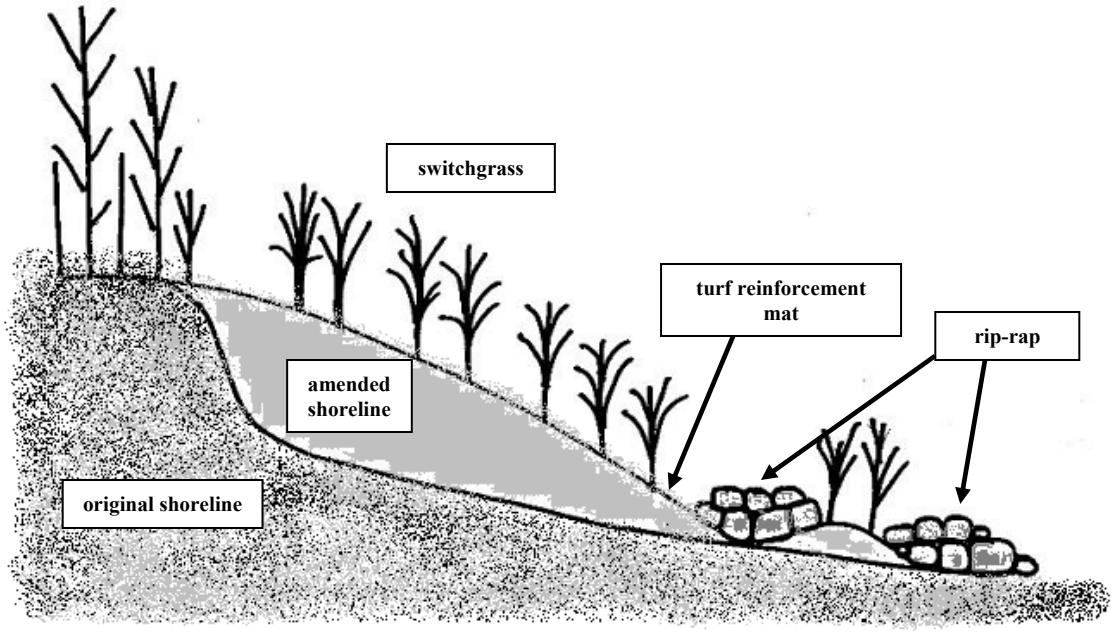

Fig. 2.2. Cross-section of shoreline showing reconstructed slope and amendments.

Figure 2.3. DNR I site with vegetation, established in 2000 (5 years old).

Figure 2.4. DNR II site with vegetation, established in 2001 (4 years old).

Figure 2.5. Camp Hope site with vegetation, established in 2004 (1 year old).

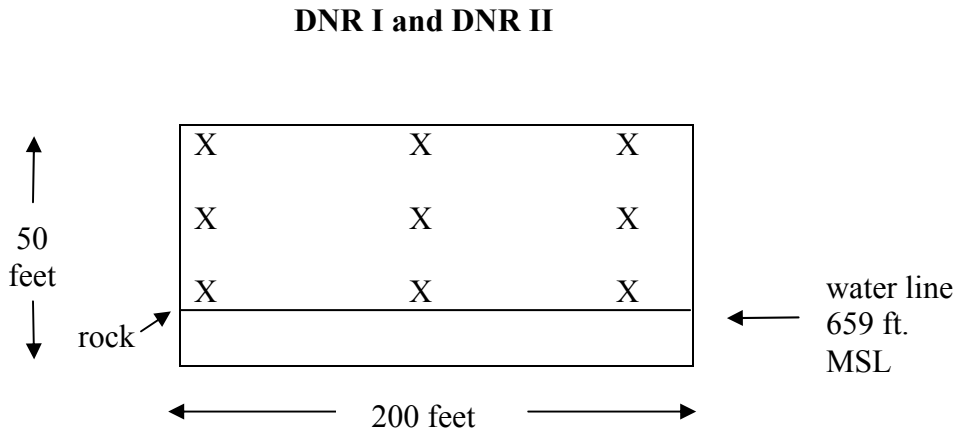

Figure 2.6. Sampling scheme used in DNR I and DNR II stands of *Panicum virgatum*.

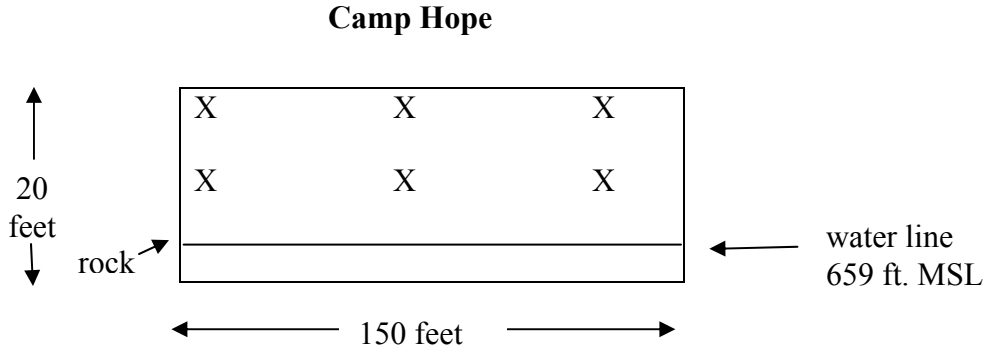

Figure 2.7. Sampling scheme used in Camp Hope stand of *Panicum virgatum*.

Table 2.1. Soil analyses for composite samples all sites.

	Soil pH	Phosphorus (lbs/Acre)	Potassium (lbs/Acre)	Calcium (lbs/Acre)	Magnesium (lbs/Acre)	NO_3-N (ppm)	Organic Matter %
DNR I (5 years)	6.6	11	54	1019	438	0	3.6
DNR II (4 years)	6.5	4	43	724	304	0	3
Camp Hope (1 year)	6.0	10	94	1020	287	5	3.5
Smith (control)	5.5	2	80	250	288	1	3
Shoreline (control)	5.3	1	112	393	161	1	2.5

Table 2.2. Temperature averages and total precipitation for growing season 2005.

	May	June	July	August	September	October
High ^{o}F	79.29	83.07	88.16	88.58	87.70	75.16
Low ^{o}F	54.13	65.00	69.87	69.55	63.37	52.71
Precipitation (inches)	3.88	9.68	9.07	3.71	0.66	2.82

Table 2.3. Acid phosphatase values for all sites over sampling period (2005).

Site	June	August	October	Average for sampling period
DNR I (5 years)	203.95^{b,x} (s.e. = 21.32)	175.98^{b,x} (s.e. = 22.93)	315.84^{a,y} (s.e. = 22.93)	**231.92^{A} (s.e.=28.66) N=27**
DNR II (4 years)	174.05^{a,x} (s.e. = 21.32)	166.27^{a,x} (s.e. = 21.32)	147.02$^{a,x/y}$ (s.e. = 21.32)	**162.44$^{A/B}$ (s.e.=28.66) N=27**
Camp Hope (1 year)	111.28^{a,y} (s.e. = 26.11)	119.5^{a,x} (s.e. = 26.11)	67.15^{a,x} (s.e. = 26.11)	**99.31^{B} (s.e.=28.66) N=18**
Shoreline (control)	93.38	161.09	71.62	**108.7^{B} (s.e.=28.66) N=3**
Smith (control)	54.11	132.71	55.5	**80.77^{B} (s.e.=28.66) N=3**

All values reported as mg p-nitrophenol produced/kg/hr. Upper case letters denote differences between overall averages. First lower-case letter denotes differences between months (rows), second lower-case letter denotes differences between sites for that month (columns). Differences are at an alpha = 0.05 significance.

Table 2.4. Nitrate reductase values for all sites over sampling period (2005).

Site	June	August	October	Average for sampling period
DNR I (5 years)	0.001850^{a,x} (s.e.=0.008072)	0.000200^{a,x} (s.e.=0.008680)	0.023090^{a,y} (s.e.=0.008680)	**0.008380$^{A/B}$ (s.e.=0.005177) N=27**
DNR II (4 years)	0.000520^{b,x} (s.e.=0.008072)	0.016900^{b,x} (s.e.=0.008072)	0.041880^{a,x} (s.e.=0.008072)	**0.019766^{A} (s.e.=0.005177) N=27**
Camp Hope (1 year)	0.000180^{a,x} (s.e.=0.009886)	n.d.	0.019200^{a,y} (s.e.=0.009886)	**0.006460$^{A/B}$ (s.e.=0.005177) N=18**
Shoreline (control)	n.d.	n.d.	0.001898	**0.000632^{B} (s.e.= 0.005177) N=3**
Smith (control)	n.d.	n.d.	n.d.	**0.000000^{B} (s.e.= 0.005177) N=3**

All values reported as µg NO_2-N /gdwt/24hrs. Upper-case letters denote differences between overall averages. First lower-case denotes differences between months (rows), second lower-case letter denotes differences between sites for that month (columns). Differences are at an alpha = 0.05 significance.

Table 2.5. Nitrogen fixation values for all sites over sampling period (2005).

Site	June	August	October	Average for sampling period
DNR I (5 years)	0.09080[a,x] (s.e.=0.06749)	0.12330[a,x] (s.e.=0.07257)	0.24190[a,x] (s.e.=0.07257)	**0.15200[A] (s.e.=0.07820) N=27**
DNR II (4 years)	0.12470[a,x] (s.e.=0.06749)	0.06640[a,x] (s.e.=0.06749)	0.14600[a,x] (s.e.=0.06749)	**0.11237[A] (s.e.= 0.07820) N=27**
Camp Hope (1 year)	0.24300[a,x] (s.e.=0.24300)	0.02590[a,x] (s.e.=0.08265)	0.16330[a,x] (s.e.=0.08265)	**0.14407[A] (s.e.= 0.07820) N=18**
Shoreline (control)	n.d.	0.01632	n.d.	**0.00544[A] (s.e.= 0.07820) N=3**
Smith (control)	0.11622	n.d.	0.58093	**0.23238[A] (s.e.= 0.07820) N=3**

All values reported a nmol N_2fixed/mg dwt/h. Upper-case letters denote differences between overall averages. First lower-case letter denotes differences between months (rows), second lower-case denotes differences between sites for that month (columns). Differences are at an alpha = 0.05 significance.

Table 2.6. Dehydrogenase values for all sites over sampling period (2005).

Site	June	August	October	Average for sampling period
DNR I (5 years)	$635.91^{b,x}$ (s.e.=339.42)	$1149.16^{a/b,x}$ (s.e.=364.98)	$2081.78^{a,y}$ (s.e.=364.98)	**$1283.26^{A/B}$ (s.e.=250.25) N=27**
DNR II (4 years)	$1089.93^{b,x}$ (s.e.=339.42)	$1003.75^{b,x}$ (s.e.=339.42)	$2598.77^{a,y}$ (s.e.=339.42)	**1564.15^{A} (s.e.=250.25) N=27**
Camp Hope (1 year)	$632.78^{a,x}$ (s.e.=415.71)	$298.79^{a,x}$ (s.e.=415.71)	$845.77^{a,x}$ (s.e.=415.71)	**$592.45^{B/C}$ (s.e.=250.25) N=18**
Shoreline (control)	47.36	20.86	326.84	**131.69^{C} (s.e.=250.25) N=3**
Smith (control)	231.11	168.00	141.38	**180.16^{C} (s.e.=250.25) N=3**

All values reported as mgTPF/gdwt/24hr. Upper case letters denote differences between overall averages. First lower-case letter denotes differences between months (rows), second lower-case letter denotes differences between sites for that month (columns). Differences are at an alpha = 0.05 significance.

Table 2.7. Substrate induced respiration values for all sites over sampling period (2005).

Site	June	August	October	Average for sampling period
DNR I (5 years)	$0.63003^{a,x}$ (s.e.=0.05342)	$0.38450^{b,x}$ (s.e.=0.05744)	$0.41526^{b,x}$ (s.e.=0.05744)	**$0.48557^{A/B}$ (s.e.=0.05864) N=27**
DNR II (4 years)	$0.36061^{a,y}$ (s.e.=0.05342)	$0.34896^{a,x}$ (s.e.=0.05342)	$0.47732^{a,x}$ (0.05342)	**$0.39563^{A/B}$ (s.e.=0.05864) N=27**
Camp Hope (1 year)	$0.79664^{a,x}$ (s.e.=0.06543)	$0.37829^{b,x}$ (s.e.=0.06543)	$0.41634^{b,x}$ (s.e.=0.06543)	**0.53040^{A} (s.e.=0.05864) N=18**
Shoreline (control)	0.49463	0.31666	0.43955	**$0.41694^{A/B}$ (s.e.=0.05864) N=3**
Smith (control)	0.37825	0.25967	0.37206	**0.33485^{B} (s.e.=0.05864) N=3**

All values reported as mg microbial C/g dry wt. soil. Upper-case letters denote differences between overall averages. First lower-case letter denotes differences between months (rows), second lower-case letter denotes differences between sites for that month (columns). Differences are at an alpha = 0.05 significance.

Table 2.8. Fatty acids in sites sampled in June 2005.

Site	Fatty Acid	i11:0	C16 N alcohol	16:1w5c	16:00	i/a17:1	18:1w9c	cis 9,10 epoxy 18:0	19:1w6c or 19:0cycw9	i21:0	21:2w6c
	Indicator of:	**-**	**-**	**Gram -**	**Fungi**	**-**	**Fungi**		**Anaerobic Gram -**		
DNR I (5 years)		0.1737^{C}	0.4704^{B}	$3.118^{A/B}$	3.348^{B}	2.557^{A}	2.438^{B}	0.665^{B}	14.052^{A}	6.558^{A}	2.487^{B}
DNR II (4 years)		0.2138^{C}	0.2572^{B}	6.003^{A}	3.881^{B}	3.683^{A}	1.429^{B}	$1.282^{A/B}$	10.963^{A}	9.344^{A}	3.003^{B}
Camp Hope (1 year)		0.4481^{C}	0.3925^{B}	$3.808^{A/B}$	2.125^{B}	7.522^{A}	0.428^{B}	$1.966^{A/B}$	9.967^{A}	3.648^{A}	$4.075^{A/B}$
Smith (control)		1.1035^{B}	n.d.	n.d.	1.539^{B}	9.441^{A}	n.d.	3.216^{A}	n.d.	n.d.	8.417^{A}
Shoreline (control)		5.2868^{A}	8.4569^{A}	n.d.	8.842^{A}	10.418^{A}	10.161^{A}	n.d.	n.d.	n.d.	n.d.

Fatty acids listed above are >5.0% Corrected area/Total Corrected Area. Upper case letters denote differences between sites for fatty acids. n.d. = none detected.

Table 2.9. Fatty acids in sites sampled in June 2005.

Site	Fatty Acid	12:0	C9 dicarboxylic acid	13:1ω7c	14:0	15:1ω8c	16:2ω6c or a16:0	16:1ω7c	i17:1ω10c	17:1ω7c	18:2ω6c	18:1ω3c	ECL 18.46[1]	19:2ω6c
	Indicator of:	Fungi	-	-	-	-	-	Aerobe Gram -	Eubacteria	-	Fungi	-	-	-
DNR I (5 years)		1.190^{A}	2.0785^{A}	1.3576^{A}	0.937^{A}	1.343^{A}	0.5673^{B}	3.290^{A}	0.2272^{A}	1.1970^{A}	1.822^{A}	0.2535^{A}	0.942$^{A/B}$	0.5067^{B}
DNR II (4 years)		1.942^{A}	1.0696$^{A/B}$	0.1339^{A}	1.301^{A}	0.753^{A}	0.6795^{B}	2.062^{A}	0.2962^{A}	0.7434^{A}	0.962^{A}	0.3918^{A}	1.039$^{A/B}$	0.6730$^{A/B}$
Camp Hope (1 year)		1.099^{A}	1.2910$^{A/B}$	n.d.	0.765^{A}	2.101^{A}	1.7541$^{A/B}$	0.593^{A}	1.1873^{A}	1.8884^{A}	0.515^{A}	0.7232^{A}	2.342^{A}	1.5358$^{A/B}$
Smith (control)		n.d.	n.d.	n.d.	n.d.	n.d.	2.4866^{A}	n.d.	n.d.	n.d.	n.d.	1.3529^{A}	2.942^{A}	2.5968^{A}
Shoreline (control)		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.

Fatty acids listed above are between 1.0 and 4.99% Corrected Area/Total Corrected Area. Upper case letters denote differences between sites for fatty acids. n.d. = none detected.

[1]ECL = equivalent chain length of unidentified fatty acid.

CHAPTER 3

EFFECTS OF ETHYLENE ON THE MORPHOLOGICAL CHARACTERISTICS OF *PANICUM VIRGATUM* L.

Abstract

Panicum virgatum (switchgrass) plants used along upstate South Carolina reservoir shorelines for stabilization may experience periods of inundation which can result in plant stress. Under stress, plants typically produce ethylene, a hormone which may alter morphological responses. Switchgrass plants (34 days old) were placed in a modified Hoagland's solution for 84h, and exposed to either 1mM ethephon solution (ethylene inducer), or 10uM aminoethoxyvinylglycine (AVG, ethylene inhibitor) and were compared to or modified Hoagland's solution as a control. Plants were enclosed in resealable bags and ethylene determinations were made every 12h. At the end of 84h, plants were harvested and total root length, root average diameter, root volume, and root surface area measurements were determined using WinRhizo Professional 2002a root scanning softward. Aerenchyma presence and shoot length were also determined. Ethephon treatments resulted in an increase in the number of adventitious roots, but there was no effect on aerenchyma development, shoot elongation, root length, average root diameter, root system volume, or root surface area when compared to either the AVG or control treatment. These results suggest that switchgrass is not as sensitive to ethylene exposure as other plants. The insensitivity to ethylene indicates that morphological changes of switchgrass experiencing inundation are possibly under more genetic control than hormonal control.

Introduction

Lake Hartwell is a reservoir operated by the United States Army Corps of Engineers and is used for power generation, flood control, and public recreation. It is subject to fluctuations in water levels that can affect plants along the water edge. From mid-October through mid-December, water is drawn down to 656 ft MSL, exposing more of the banks whereas for the rest of the year, water levels are generally higher (660 ft MSL) and may actually inundate plants along banks. These areas can be flooded for extended periods of time. Some switchgrass plants experience several weeks of flooded conditions. The banks of Lake Hartwell are also subject to erosive forces and the soils are often nutrient poor. Plants have been utilized to aid in bank stabilization and for soil improvement. Plants used for these purposes have to be able to survive flooding and drying events, as well as low nutrient and clay soils with high bulk density. *Panicum virgatum* L. (switchgrass) is on of the plants that have been planted in stands along the shoreline of Lake Hartwell in Pickens County, SC to aid in bank stabilization and is on the list of plants approved by the Army Corps of Engineers.

The Switchgrass cultivar 'Alamo', was chosen for these stands. It is a lowland variety of grass normally found in wet and dry areas. The lowland type has tall, thick, coarse stems and grows in bunches instead of a turf-like growth habit (Elberson and Christian, 2001). This variety should be able to withstand flooding conditions better than others. This grass is also known for its production of large root systems, both in volume and length, with aerenchyma present. In many plants, the formation of aerenchyma has been attributed to exposure to the plant hormone ethylene. It is possible that Switchgrass

may produce aerenchyma as a result of exposure to ethylene when plants experience stressful conditions, like inundation or reduced oxygen tension in deep soils. No literature was found pertaining to the responses of switchgrass to ethylene; therefore, this study was conducted to investigate morphological responses of this plant to ethylene.

Ethylene has been of interest as a plant growth regulator ever since its discovery in the nineteenth century, when it was noticed that illuminating gas from gaslights damaged adjacent plants (Abeles *et al*., 1992). Ethylene is considered to be a stress hormone, produced when plants experience extremes in temperature, drought, flooding, or mechanical wounding. This gaseous compound reported to be produced by all parts of the plant (Visser and Pierik, 2007) and is commonly reported to be involved in plant morphological responses, such as aerenchyma development, changes in root elongation, shoot length suppression, and epinasty (Jackson, 1985).

The connection between ethylene production and morphological responses seen in plants has been investigated. An important area of ethylene research integral to this study is the impact of inundation on vegetation. Flooding of vegetation ultimately leads to a decrease in gas diffusion into and out of plants. The diffusion of oxygen is 10,000 times slower in water than air (Dat *et al*., 2004; Colmer, 2003). Slower diffusion of gases soon leads to hypoxic environments around plant roots in sediments, slowing respiratory metabolism. If flooding continues, hypoxia leads to anoxia, and plants run a greater risk of death. Complete inundation not only restricts the root system, but can also starve the shoot tissues of gas exchange, hampering photosynthesis. In an attempt to alleviate these issues, plants produce hormones like ethylene which redirects the growth of stems and

roots in order to maximize the probability of survival. The biosynthesis of ethylene increases rapidly (within 4 hours) during hypoxia in several species (Drew *et al*., 1979; Lorbiecke and Sauter, 1999). Ethylene is also known to accumulate in root tissues of flooded plants because of the slow diffusion of gases into water. Visser *et al*. (1996) measured $2cm^3/m^3$ ethylene in *Rumex palustris*, a flood-tolerant species, and $9cm^3/m^3$ in *Rumex thyrsiflorus*, a flood-intolerant species when submerged.

Plants exposed to ethylene typically show inhibited shoot growth. The triple response, first described in 1901, is still used as a reliable bioassay for ethylene exposure. When plants are exposed to various concentrations of ethylene, inhibition of stem and root elongation, increased lateral growth, and horizontal growth are observed (Taiz and Zeiger, 1998). An opposite response has been found in some aquatic and semiaquatic plants which produce ethylene in amounts that accelerate shoot elongation under water (Jackson, 1985; Kende, 1998). Stem elongation for these plants has been suggested as a mechanism for shoots to regain contact with aerated conditions and compete for light (Summers *et al*., 1996).

Part of the classic triple response is the inhibition of root growth as well as shoot growth. However, ethylene effects on root growth have been contradictory and it has been difficult to find a correlation between root extension and ethylene exposure. Research on crop plants, such as barley (Hall *et al*., 1977) and maize (Abeles *et al*., 1992), demonstrated ethylene inhibits root elongation. However, other studies have shown that ethylene does not inhibit root elongation in species such as *Carex* or *Thalictrum* (Visser and Pierek, 2007).

Ethylene effects on the formation of adventitious roots has also been unclear (Jackson, 1985). Visser *et al*. (1996) stated that research on ethylene's impact on the formation of adventitious roots was contradictory. Some studies attribute only a minor ethylene effect on adventitious root formation (Wample and Reid, 1979; Yamamoto and Kozlowski, 1987) while in maize and deep-water rice, ethylene had a positive effect on adventitious root formation (Drew *et al*., 1979; Bleeker *et al*., 1987). In species like *Rumex*, the formation of adventitious roots enables the survival of waterlogged plants, but the extent of adventitious root formation varies greatly between species (Laan *et al*., 1989). Visser *et al*. (1996) found that the formation of adventitious roots in *Rumex* species was strongly enhanced by both flooding and ethylene treatments. *Rumex* plants were found to produce the maximum number of adventitious roots in response to 1 – 3 μL/L ethylene (Visser *et al*., 1996). There is also evidence that ethylene treatment of 5μL/L promotes adventitious roots in maize (Jackson *et al*., 1981).

Probably one of the most important responses to ethylene in aquatic and semiaquatic plants is aerenchyma development. Aerenchyma is the internal, longitudinally-connected, gas-filled, intercellular spaces found in the cortex of roots and shoots that is caused by cell separation (schizogenous) or by breakdown of cells (lysigenous) (Jackson and Armstrong, 1999). Aerenchyma provides a low resistance pathway for the diffusion of gases through the plant. Aerenchyma formation is crucial to a plant's survival when inundated because of the slow diffusion of gases in water. Research supporting the importance of internal aeration for plant growth in waterlogged soils has been reported. For example, species with higher root porosity tend to form

deeper roots and are more tolerant of soil waterlogging (Armstrong, 1979; Laan *et al*., 1989). Laan *et al*. (1989) demonstrated that lateral roots of *Rumex thyrsiflorus* with a porosity of about 5% penetrated no more than 100mm when grown in waterlogged soil whereas roots of *Rumex maritimus* with a porosity of around 30% penetrated 400mm.

Several chemicals are available to study the effects of waterlogging and ethylene. Ethephon (2-chloroethylphosphonic acid) is an ethylene producing chemical. It decomposes to ethylene, phosphate, and a chloride ion in aqueous solutions above pH 4 – 5. Ethephon can be added to a nutrient solution and plants enclosed to allow for ethylene accumulation. An ethylene inhibitor, aminoethoxyvinylglycine (AVG) ([S]-trans-2-amino-4-(2-aminoethoxy)-3-butenoic acid hydrochloride) can be added to a nutrient solution to inhibit ethylene production by plants. This inhibitor acts by inhibiting the enzyme 1-aminocyclopropane-1-carboxylic acid synthase (ACC synthase) which converts S-adenosyl methionine to 1-aminocyclopropane-1carboxylic acid – the immediate precursor of ethylene (Adams and Yang, 1979).

The objectives of this study were to determine the morphological effects (shoot length, root length, root volume, average root diameter, root surface area, production of adventitious roots, and aerenchyma formation) of ethylene exposure on switchgrass. Based on the literature, it was hypothesized that the variety of switchgrass (Alamo) planted on the shorelines would react like other flood-tolerant species when exposed to ethylene. It was hypothesized that the shoot would elongate, more adventitious roots would develop, and an increased amount of aerenchyma would be present in plants exposed to ethylene as compared to controls and those exposed to AVG, an ethylene

inhibitor. Although there was some conflict in the literature (Hall *et al*., 1977; Abeles *et al*., 1992; Visser and Peirek, 2007) pertaining to root elongation when exposed to ethylene, it was hypothesized that ethylene exposure would result in no root length suppression.

Materials and Methods

Panicum virgatum ('Alamo) seeds, obtained from (Sharp Brothers Seed, Clinton, MO), were started in Fafard Super-fine Germinating Mix (Fafard, Inc., Anderson, SC) on a misting table in the Clemson University Biosystems Research Complex greenhouse propagation room. Once the seedlings had sprouted and reached the 3-leaf stage (after 26 days), with an average of 8mm in height, the seedlings were transferred to a modified Hoagland's solution (Epstein, 1972), shown in Table 3.1. All chemicals used were obtained from Fisher Scientific (Raleigh, NC) or Sigma-Aldrich (St. Louis, MO).

Autoclaved canning jars (500mL) were filled with modified Hoagland's solution. Jars were covered with aluminum foil to prevent growth of cyanobacteria or algae. Five plants were inserted through black plastic placed over the mouth of the jar and secured with a metal ring screwed onto the jar. Jars were placed in a greenhouse with deionized water added as needed through a small hole in the black plastic. Plants were left in the Jordan Hall greenhouse, Clemson University, for 8 days to become acclimated to the hydroponic solution.

Once plants had become acclimated to the hydroponic solution, 10µM aminoethoxyvinyl glycine (AVG) (Sigma-Aldrich, St. Louis, MO) was added to the solution of two of the jars (5 plants each), 1mM solution of ethephon (Sigma-Aldrich, St. Louis, MO) was added to the solution of two jars (5 plants each). Peters and Crist-Estes (1989) was successful at inhibiting ethylene production up to 5 days after addition of 10µM AVG to a liquid medium, and a 1mM ethephon solution was used by Goudey *et al*., (1987) to produce ethylene. Two jars with 5 plants each were treated as controls with

only nutrient solution. To contain the ethylene that would be formed, jars were placed in 9.45L resealable bags (Figure 3.1). There were two jars per bag. The bags with jars were sealed and placed in the Jordan Hall greenhouse. Two initial gas samples were taken from each bag by opening each bag as little as possible and inserting the syringe to the height of the shoots and withdrawing 1mL. Initial heights of each plant were measured.

Ethylene samples were taken every 12h for a total of 84h. Ethylene samples were analyzed with a Shimadzu GC-9A (Shimadzu Scientific Instruments, Inc., Columbia, MD) with a flame ionization detector, using nitrogen as the carrier gas at 50mL/minute and a phenylisocyanate Porasil C column (2m x 1/8") with 80/100 mesh (Alltech Associates, Inc., Deerfield, IL). The injector temperature was 140^{o}C and the column temperature was 30^{o}C.

At the end of 84h, plant shoot heights were measured. The number of adventitious roots were measured by using a dissecting microscope (Wild Heerbrugg, Switzerland) at 90X. Roots were placed in 50% ethanol until further analyzed. Roots were further examined using WinRhizo Professional 2002a root scanning program (Regent Instruments, Inc.,) for total root length, total root system average diameter, total root volume, and total root surface area. For aerenchyma development, roots were sectioned by hand using a single edge razor blade 20mm from root tips on seminal root and two adventitious roots. Root sections were observed under a dissecting microscope (Wild Heerbrugg, Switzerland) at 180X. Presence (1) or absence (0) of aerenchyma was recorded for each root sectioned and values were averaged for each plant. There were 5 plants in each of two bottles per treatment and the experiment was conducted twice.

Statistical Analysis

Significant differences between treatments were tested with one-way ANOVA and Fisher's least significant differences were examined using SAS software version 9.0 (SAS Institute Inc., Cary, NC, 2002). Probability of $p<0.05$ was used to indicate significant difference.

Results

Ethylene levels

Figure 3.2 shows the ethylene levels for the treatments (control, ethephon, and AVG) for each set of plants for the duration of the experiment. The AVG-treated plants were exposed to no more than 0.12ppm of ethylene and the control plants maximum ethylene exposure was 0.09ppm. Plants exposed to the ethylene producer, ethephon, had ethylene levels of 1.13ppm to 2.7ppm from 24 hours into the study to the end of the study at 84 hours.

Morphological responses

Seven different growth parameters were measured in this study. They were root length, root average diameter, root volume, shoot growth, number of adventitious roots, and presence of aerenchyma. No experimental differences were noted between trials of four of the growth parameters measured so the data were merged (Table 3.2). Root surface area resulted in differences between AVG and ethephon treatments, but neither were different from the control. Shoot growth showed no statistical differences between any treatments. The ethephon treatment had statistically higher numbers of adventitious roots than both the control and AVG treatments. The control had statistically lower amounts of aerenchyma tissue than both the AVG and ethephon treatments (Table 3.2).

The remaining three parameters, root length, root average diameter, and root volume, showed experimental effects. In trial 1, total root length was the longest in the control treatment and statistically different from the AVG and ethephon treatments. However, in trial 2, the control treatment had the shortest root length, but was not

statistically different than either the AVG or ethephon treatments (Table 3.3). Total root average diameter in trial 1 was the largest in the AVG treatment and smallest in the control treatment. The largest total root average diameters in trial 2 were found in both the control and the ethephon treatment, where the AVG treatment had the smallest diameter and was statistically different from the other two treatments (Table 3.3). Total root volume in trial 1 showed that the AVG treatment was statistically higher than the control and the ethephon treatments, but in trial 2 there were no statistical differences between any of the treatments Table 3.3).

The remaining three parameters, root length, root average diameter, and root volume, showed experimental effects. In trial 1, total root length was the longest in the control treatment and statistically different from the AVG and ethephon treatments. However, in trial 2, the control treatment had the shortest root length, but was not statistically different than either the AVG or ethephon treatments (Table 3.3). Total root average diameter in trial 1 was the largest in the AVG treatment and smallest in the control treatment. The largest total root average diameters in trial 2 were found in both the control and the ethephon treatment, where the AVG treatment had the smallest diameter and was statistically different from the other two treatments (Table 3.3). Total root volume in trial 1 showed that the AVG treatment was statistically higher than the control and the ethephon treatments, but in trial 2 there were no statistical differences between any of the treatments Table 3.3).

Discussion

Plants exposed to ethylene typically exhibit similar symptoms. These symptoms include root and shoot growth inhibition (Visser *et al*., 1997; Jackson, 1985), epinasty (Taiz and Zeiger, 1999), aerenchyma formation (Armstrong, 1979), and the formation of adventitious roots (Laan *et al*., 1989). Recent research has shown that plants can respond in different ways, sometimes depending on their environment. Flood tolerant plants, like deepwater rice, can actually elongate their shoots when submerged (Kende *et al*., 1998). Other plants, both wetland and typically dry land species, show no root growth inhibition when exposed to ethylene (Visser and Pieruk, 2007). There is even a plant, *Potamogeton pectinatus*, that has been shown unable to constitutively synthesize ethylene due to the lack of the enzyme, 1-aminocyclopropane-1-carboxylic acid oxidase, which is responsible for converting 1-aminocyclopropane-1-carboxylic acid (ACC) to ethylene (Summers *et al*., 1996). It is impossible to definitively say that ethylene is responsible for particular morphological response in all plants.

Switchgrass (*Panicum virgatum* L. 'Alamo') has been used as a shoreline soil stabilizer. It has an extensive fibrous root system with the ability to slow erosional forces. Stands of switchgrass along reservoir shorelines can be periodically inundated with water. Inundation may increase ethylene production (Taiz and Zeiger, 1998), so this study was conducted to determine the response of switchgrass to ethylene.

Ethephon treatments resulted in increased numbers of adventitious roots. There were significantly more adventitious roots in the ethephon treatment than either the control or AVG treatments. Adventitious roots are typical of wetland plants and often

contain more aerenchyma than the primary lateral roots (Visser *et al*., 1997). It was hypothesized that this switchgrass variety would develop more adventitious roots in response to ethylene exposure, as had been found by others investigating wetland plants (Visser *et al*., 1997; Visser *et al*., 1996). The plants exposed to ethylene showed significantly more adventitious roots than the control and AVG plants. This indicates that ethylene has an impact on adventitious root formation in switchgrass.

Values for aerenchyma presence showed no statistical differences between the ethephon and AVG treatments, suggesting that ethylene may not be the only factor which results in increased aerenchyma tissue in switchgrass. Aerenchyma was evaluated in this experiment solely on its presence or its absence. From each plant, the seminal root was assessed along with two adventitious roots. The average of the values from roots of each treatment were compared. Aerenchyma development has been reported to result from ethylene exposure (Gunawardena *et al*., 2001; Colmer *et al*., 2003) Roots need ready access to oxygen and also need to be able to get rid of carbon dioxide and other gases that can accumulate. When roots are waterlogged, the capacity for diffusion decreases markedly. Aerenchyma can serve as a way to ventilate the plants in waterlogged soils (Visser and Pieruk, 2007). The hypothesis that switchgrass would develop aerenchyma in response to ethylene, especially since the variety chosen is typically found in lowland areas prone to waterlogged soils, is not supported. The ethephon-treated plants formed significantly more aerenchyma than the control treatment, but the AVG-treated plants resulted in values statistically similar to ethephon treatments (Table 3.2).

Shoot elongation has been inhibited by levels of ethylene in some plants and has been thought of as a classical ethylene response. However, work with deepwater rice and other wetland species have shown that in some plants, inundation leads to ethylene entrapment in the surrounding waters (Kende *et al*., 1998; Jackson 1985) can cause shoot elongation (Kende *et al*., 1998). It was hypothesized that switchgrass of this variety ('Alamo'), would respond as other flood-tolerant species and elongate its shoots. However, switchgrass showed no significant differences between treatments related to shoot growth (Table 3.3).

Scanning WinRhizo 2002a software (Regent Instruments, Inc.) was used for the evaluation of root length, root system average diameter, root system volume, and root surface area. Typically, previous research has only reported root length, but since switchgrass has such an extensive root system, there was a question as to whether ethylene had an effect on the architecture of the root system as well. Root length, which has been reported in the literature as being inhibited by the presence of ethylene (Abeles *et al*., 1992) was not affected by ethylene in switchgrass. In trial 1, the control treatment root length was much longer than the other treatments, but after review of the raw data, this discrepancy may have been a result of some plants' roots in the control treatment may have been larger than the other plant roots at the beginning of the experiment, which potentially skewed the final results (Table 3.3). The results of trial 2 confirm that no conclusions can be drawn from this study pertaining to root length and ethylene exposure. In trial 2, the control treatment had the shortest root system. The total root system

average diameter had inconsistent values from trial 1 and trial 2, as did total root volume (Table 3.3).

From the results of this study, it is possible that *P. virgatum* 'Alamo', may be less sensitive to ethylene than other plants. It showed no recognizable patterns in morphological responses to ethylene except presence of aerenchyma and adventitious root formation. Recent literature has reported two plants, *Juncus effuses* and *Potamogeton pectinatus* have the ability to develop structures without the presence of ethylene. These studies have shown that aerenchyma can be formed independently of ethylene exposure in *Juncus effuses* (Visser and Bogeman, 2006) and the entire developmental process can proceed without ethylene because the plant is incapable of producing it (Summers *et al*., 1996). Visser and Bogeman (2006) found that as *Juncus effuses*, grown hydroponically, had root elongation rates of 0.55 – 0.65 mm/h and ethylene levels of 0.1 – 50 μL/L. The root elongation rates were not statistically different from those plants exposed to air. Also, when *Juncus effuses* was exposed to relatively high concentrations of ethylene (50 μL/L), there was no increase in root porosity (Visser and Bogeman, 2006). It is not inconceivable that switchgrass of this variety may only be slightly affected by ethylene exposure. This low level of sensitivity may be protective in nature for wetland plants. This plant produces more adventitious roots and more aerenchyma when exposed to ethylene which may increase its survival during waterlogged soil conditions.

Literature Cited

Abeles, F.B., P.W. Morgan, and M.E. Saltveit, Jr. 1992. Ethylene in Plant Biology. Academic Press, San Diego, CA.

Adams, D.O., and S.F. Yang. 1979. Ethylene biosynthesis: identification of 1-aminocyclopropane-1-carboxylic acid as an intermediate in the conversion of methionine to ethylene. Proceedings of the National Academy of Science, USA 76**:** 170-174.

Armstrong, W. 1979. Aeration in higher plants. Advances in Botanical Research 7**:** 225-332.

Bleeker, A.B., S. Rose-John, and H. Kende. 1987. An evaluation of 2,5-norbornadiene as a reversible inhibitor of ethylene action in deepwater rice. Plant Physiology 84**:** 395-398.

Colmer, T.D. 2003. Long-distance transport of gases in plants: a perspective on internal aeration and radial oxygen loss from roots. Plant, Cell and Environment 26**:** 17-36.

Dat, J.F., N. Capelli, H. Folzer, P.Bourgeade, and P.M. Badot. 2004. Sensing and signaling during plant flooding. Plant Physiology and Biochemistry 42**:** 273-282.

Drew, M.C., M.B. Jackson, and S. Giffard. 1979. Ethylene-promoted adventitious rooting and development of cortical air spaces (aerenchyma) in roots may be adaptive responses to flooding in *Zea mays* L. Planta 147**:** 83-88.

Elberson, H.W., and D. G. Christian. 2001. Annex: A Switchgrass bibliography. *In* Switchgrass (*Panicum virgatum*, L.) as a alternative energy crop in Europe. Initiation of a productivity network. Final Report FAIR 5-CT97-3701,

Epstein, E. 1972. Mineral Nutrition of Plants: Principles and Perspectives. John Wiley, New York.

Goudey, J.S., H.S. Saini, and M.S. Spencer. 1987. Uptake and fate of ethephon (2-chloroethylphosphonic acid) in dormant weed seeds. Plant Physiology 85**:** 155-157.

Gunawardena, A.H.L.A.N., D.M.E. Pearce, M.B. Jackson, C.R. Hawes, and D.E. Evans. 2001. Rapid changes in cell wall pectic polysaccharides are closely associated with early stages of aerenchyma formation, a spatially localized form of programmed cell death in roots of maize (*Zea mays* L.) promoted by ethylene. Plant, Cell and Environment 24**:** 1369-1375.

Hall, M.A., J.A. Kapuyz, S. Sivakumaran, and J. Sivakumaran. 1977. The role of ethylene in the response of plants to stress. Pesticide Science 8**:** 217-223.

Jackson, M.B. 1985. Ethylene and responses of plants to soil waterlogging and submergence. Annual Review of Plant Physiology 36**:** 145-174.

Jackson, M.B., and W. Armstrong. 1999. Formation of aerenchyma and the processes of plant ventilation in relation to soil flooding an submergence. Plant Biology 1**:** 274-287.

Jackson, M.B., M.C. Drew, and S.C. Gifford. 1981. Effects of applying ethylene to the root system of *Zea mays* on growth and nutrient concentration in relation to flooding. Physiologia Plantarum 52**:** 23-28.

Kende, H., E. van der Knaap, and H.T. Cho. 1998. Deepwater rice: a model plant to study stem elongation. Plant Physiology 118**:** 1105-1110.

Laan, P., M.J. Berrevoets, S. Lythe, W. Armstrong, and C.W.P.M. Blom. 1989. Root morphology and aerenchyma formation as indicators of flood tolerance of *Rumex* species. Journal of Ecology 77**:** 693-703.

Lorbiecke, R., and M. Sauter. 1999. Adventitious root grow and cell-cycle induction in deep-water rice. Plant Physiology 119**:** 21-29.

Peters, N.K., and K. Crist-Estes. 1989. Nodule formation is stimulated by the ethylene inhibitor aminoethoxyvinylglycine. Plant Physiology 91**:** 690-693.

Summers, J.E., L.A.C.J. Voesenek, C.W.P.M. Blom, M.J. Lewis, and M.B. Jackson. 1996. *Potamogeton pectinatus* is constitutively incapable of synthesizing ethylene and lacks 1-aminocyclopropane-1-carboxylic acid oxidase. Plant Physiology 111**:** 901-908.

Taiz, L., and E. Zeiger. 1998. Plant Physiology, Ed 2nd. Sinauer Associates, Inc., Sunderland, MA.

Visser, E.J.W. and G.M. Bogemann. 2006. Aerenchyma formation in the wetland plant *Juncus effusus* is independent of ethylene. New Phytologist 171**:** 305-314.

Visser, E.J.W., and R. Pierik. 2007. Inhibition of root elongation by ethylene in wetland and nonwetland plant species and the impact of longitudinal ventilation. Plant, Cell and Environment 30**:** 31-38.

Visser, E.J.W., G.M. Bogemann, C.W.P.M. Blom, and A.C.J. Voesnek. 1996. Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventitious roots. Journal of Experimental Botany 47**:** 403-410.

Visser, E.J.W., R.H.M. Nabben, C.W.P.M. Blom, and L.A.C.J. Voesenek. 1997. Elongation of primary lateral roots and adventitious roots during conditions of hypoxia and high ethylene concentrations. Plant, Cell and Environment 20**:** 647-653.

Wample, R.L., and D.M. Reid. 1979. The role of endogenous auxins and ethylene in the formation of adventitious roots and hypocotyl hypertrophy in flooded sunflower plants (*Helianthus annus*). Physiologia Plantarum 45**:** 219-260.

Yamamoto, F., and T.T. Kozlowski. 1987. Regulation by auxin and ethylene of responses of *Acer negundo* seedlings to flooding of the soil. Environmental and Experimental Botany 27**:** 329-340.

Table 3.1. Modified Hoagland's solution.

	Concentration of Stock Solution	**Volume of stock solution per liter of final solution**
Macronutrients		
KNO_3	101.10g/L	6.0mL
$Ca(NO_3)_2 \cdot 4H_2O$	236.16g/L	4.0mL
$NH_4H_2PO_4$	115.08g/L	2.0mL
$MgSO_4 \cdot 7H_2O$	246.49g/L	1.0mL
Micronutrients		
KCl	1.864g/L	2.0mL
H_3BO_3	0.773g/L	
$MnSO_4 \cdot H_2O$	0.169g/L	
$ZnSO_4 \cdot 7H_2O$	0.288g/L	
$CuSO_4 \cdot 5H_2O$	0.062g/L	
H_2MoO_4	0.040g/L	
NaFeDTPA	30.0g/L	0.5mL

Figure 3.1. Experimental set-up showing plastic enclosures.

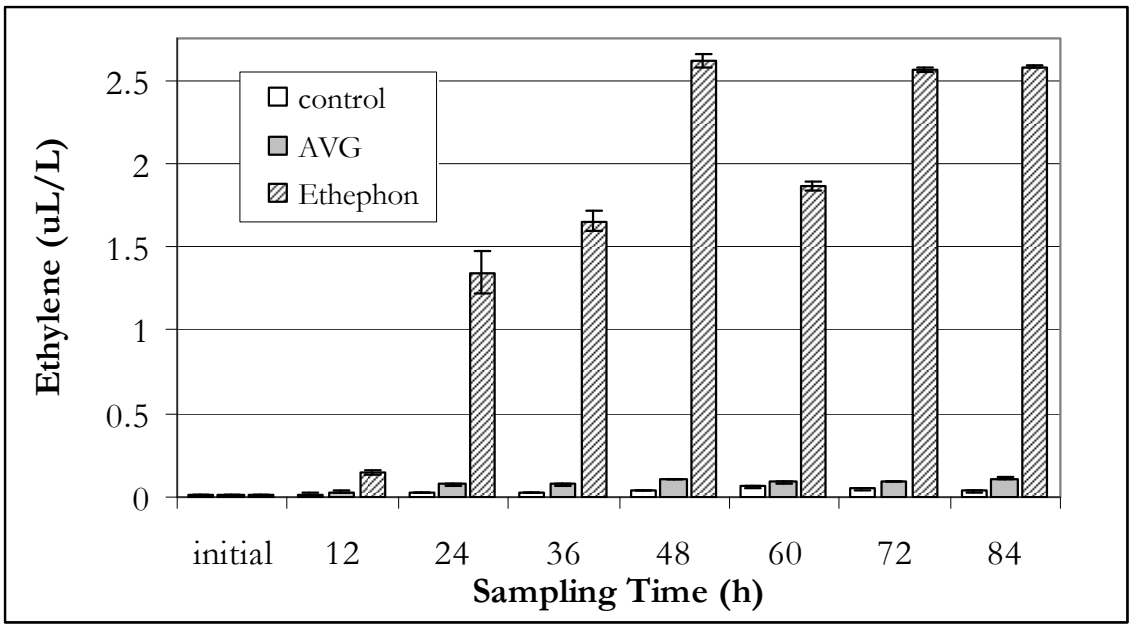

Figure 3.2. Ethylene levels associated with control, ethephon, and AVG treatments for all treatments. Drop in ethylene values at 60h for ethephon-added treatment are a result of opening bags to add water to nutrient solution. Ethylene levels experienced by the plants are an average of two experiments with a total of four replicates per experiment. Bars indicate standard error.

Table 3.2. Morphological values of switchgrass in response to AVG and ethephon treatment. Compilation of trials 1 and 2.

Group	Total Root Surface Area (cm^2)	Total Shoot Growth (mm)	Number of Adventitious Roots	Presence of Aerenchyma (% of root system)
Control (n=20)	$11.58^{A/B}$ (s.e.=0.79)	1.35^{A} (s.e.=0.189)	1.9^{B} (s.e.=0.13)	41.7^{B} (s.e.=0.065)
AVG added (n=20)	13.01^{A} (s.e.=0.79)	1.13^{A} (s.e.=0.189)	2.1^{B} (s.e.=0.13)	65.0^{A} (s.e.=0.065)
Ethephon added (n=20)	10.47^{B} (s.e.=0.79)	0.885^{A} (s.e.=0.189)	2.5^{A} (s.e.=0.13)	70.8^{A} (s.e.=0.065)

Letters following values indicate significant differences. s.e. = standard error.

Table 3.3. Morphological values of switchgrass in response to AVG and ethephon treatment.

Group	Total Root Length (mm)		Total Root Average Diameter (cm)		Total Root Volume (cm^3)	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
Control (n=10)	75.01^{A} (s.e.=6.47)	41.8^{B} (s.e.=5.47)	0.54^{C} (s.e.=0.02)	0.77^{A} (s.e.=0.01)	0.18^{B} (s.e.=0.02)	0.19^{A} (s.e.=0.02)
AVG added (n=10)	53.11^{B} (s.e.=6.47)	58.5^{A} (s.e.=5.47)	0.84^{A} (s.e.=0.02)	0.69^{B} (s.e.=0.01)	0.28^{A} (s.e.=0.02)	0.21^{A} (s.e.=0.02)
Ethephon added (n=10)	47.96^{B} (s.e.=6.47)	$43.1^{A/B}$ (s.e.=5.47)	0.76^{B} (s.e.=0.02)	0.73^{A} (s.e.=0.01)	0.21^{B} (s.e.=0.02)	0.17^{A} (s.e.=0.02)

Letters following values indicate significant differences. s.e. = standard error.

CHAPTER 4

THE IMPACT OF *SERRATIA FICARIA* ON THE GROWTH OF *PANICUM VIRGATUM* UNDER THREE DIFFERENT WATER REGIMES

Abstract

The effect of a plant growth-promoting bacterium (PGPB) on *Panicum virgatum* (switchgrass) in three different water regimes, unflooded, crown-flooded, and completely submerged was determined. A Gram negative, facultative, rod-shaped, native bacterium was isolated using selective media with 1-aminocyclopropane-1-carboxylic acid (ACC) for bacteria containing ACC deaminase from switchgrass stands. The bacterium was identified as *Serratia ficaria*. Two hormones are believed to be involved in communication between PGPB and inoculated plants, indoleacetic acid (IAA) and ethylene. Both were hypothesized to have an effect on morphological characteristics of switchgrass. Seeds of switchgrass were inoculated with *Serratia ficaria* prior to planting and reinoculated seven weeks later before transfer to larger pots. Plants were allowed to grow for an additional two weeks before being placed in their respective water treatments. All plants were kept in their respective water treatments for three weeks and were then evaluated. *Serratia ficaria* positively impacted root system measurements in terms of total root length, root surface area, root volume, and number of adventitious roots of unflooded plants. Treatments with bacteria had increased root to shoot ratios in unflooded and completely submerged water regimes, but the opposite was true for crown-flooded treatments. Shoot growth was fastest in inoculated treatments, except for completely submerged treatments, where values were not statistically significant between

inoculated and uninoculated groups. Overall treatments with *Serratia ficaria* were beneficial to unflodded plants suggesting that the bacterium is beneficial when there is no water stress. It does however become detrimental to the plant when water levels rise until completely submerged. Completely submerged, the bacteria did not have any effect on plant growth. Other PGPB may be isolated from flooded switchgrass which may be more beneficial to the plants under these flooded conditions. The PGPB isolations for this study were made from sites that were not under flooded conditions and as a result may have resulted in the isolation of bacteria that were more beneficial under nonflooded conditions.

Introduction

The reservoirs in the upstate of South Carolina have severely eroding shorelines. To remedy this problem, *Panicum virgatum* 'Alamo' (switchgrass) was planted on artificially constructed slopes to revegetate depauperate soils to lessen the erosive power of waves which carry away fine clay particles that make up the soils of this area. This grass has an extensive root system both in root number and root length and is a successful bank stabilizer. In addition to its considerable root system, this grass is extremely hardy and has the ability to withstand extreme conditions such as low nutrient content in soils and fluctuations in water levels from flooded to drought conditions.

Due to the challenging nature of the soils in the Lake Hartwell area, which are low in nitrogen and phosphorus, and water fluctuations endured by the plants on these shorelines, this research was conducted to investigate how plant growth promoting bacteria (PGPB) isolated from Lake Hartwell sites could affect the morphological responses of switchgrass PGPB have been identified as important factors in the survivability of some plants (Grichko and Glick, 2001; Mantelin and Touraine, 2004). These bacteria are defined as those that aggressively colonize roots and cause plant growth promotion, regardless of the mechanisms involved (Kloepper *et al*., 1980). Typically PGPB are classified into two groups, either indirectly or directly involved with plant growth, according to how they encourage the growth and development of the plant. Indirectly, these bacteria decrease or prevent some of the deleterious effects of phytopathogenic organisms by one or more different mechanisms (Glick and Bashan, 1997). Directly, PGPB synthesize compounds, such as the plant growth hormones

indoleacetic acid and gibberellins. The bacteria can also increase nutrient availability by solubilizing phosphorus, producing chelating agents to sequester iron, or through nitrogen fixation (Kennedy, 1999). Of particular importance to flooded plants is the enzymatic lowering of plant ethylene concentrations (Glick, 1995).

The exposure of plants to ethylene can be reduced when a bacterium (PGPB) containing an 1-aminocyclopropane-1-carboxylic acid (ACC) deaminase enzyme is added to the rhizosphere (Grichko and Glick, 2001). ACC deaminase is responsible for breaking down ACC into ammonia and alpha-ketobutyrate inside of the bacterium (Glick *et al*., 1998). This decreases the ACC available for conversion to ethylene inside the plant. The model proposed by Glick *et al*. (1998) states that the bacterium is bound to the surface of the plant seed or root. The bacterium secretes indoleacetic acid (IAA) in response to tryptophan and other small molecules present in the seed or root exudates. IAA is taken up and can direct cell elongation and/or proliferation. Some IAA can be used to stimulate the production of ACC synthase which is responsible for converting S-adenosylmethionine to ACC (Kende, 1993). Much of the ACC is exuded by the plant and taken up by the bacterium, which acts as a sink for ACC. Inside the bacterium, ACC is hydrolyzed by ACC deaminase. Because the bacterium has taken up ACC, there is little outside the plant causing the plant to exude more ACC to maintain equilibrium. The overall result is that less ethylene is formed by the plant.

In addition to decreasing the ethylene produced by the plant, the model suggests another valuable point: PGPB may produce IAA in response to molecules produced by the seed or root. *P. virgatum* has been suggested to be relatively insensitive to high

concentrations of ethylene (Chapter 3, this study), but it may be sensitive to IAA which would result in shoot elongation and production of adventitious roots (Taiz and Zeiger, 1998). IAA is known to cause cell division and elongation, produce lateral roots at cut ends of stems, and stimulate ethylene production(Cleland, 1995). In plants, IAA is produced in shoot apical meristems, young leaves, and developing fruits and is transported basipetally, toward roots, in a process that requires metabolic energy. IAA in the root is transported mainly from the base of the root towards the root tip, again in a process requiring energy. Shoot growth is promoted by concentrations of IAA between 10^{-6}M to 10^{-5}M but root elongation is promoted at much lower levels, like 10^{-10}M to 10^{-9}M. Concentrations of IAA that promote shoot growth are actually inhibitory to root growth (Taiz and Zeiger, 1998). However, high levels of IAA have been shown to cause initiation of lateral roots and adventitious roots. Visser *et al*. (1995) proposed that flooded plants in hypoxic conditions were unable to actively transport IAA because of the lowered ATP levels in roots, and IAA concentrations rose at the base of the shoot causing adventitious roots to develop. Calenza *et al*. (1995) developed a model on the impacts of IAA on root growth working with *Arabidopsis* mutants which proposed that IAA is required for at least two steps in the formation of lateral roots. First, IAA transported acropetally (from the base to the tip) in the stele is required to initiate cell division in the pericycle. Second, IAA is required to promote cell division and maintain cell viability in the developing lateral root.

Two of the hormones involved in plant growth and development in the research presented here are ethylene and IAA, investigated because of the proposed idea of Glick

et al. (1998) that PGPB adhering to the root system secrete IAA, causing a series of events that results in lowered ethylene levels. Because of the lack of information on switchgrass in the literature, this study was undertaken to attempt to elucidate the effects a bacterium, cultured from a stand of switchgrass with ACC deaminase activity, would have on the grass during three different water regimes. Previous studies (Chapter 3) showed the only correlation between ethylene exposure and young switchgrass plants was an increase in adventitious roots and an increase in aerenchyma present in the root system. Other growth parameters studied including root elongation, root volume, and shoot growth, were not significantly affected by ethephon treatments. Furthermore, the inhibitor AVG could not inhibit these effects over background influences. It was hypothesized that plants inoculated with an ACC deaminase bacterium would lower ethylene levels and as a result fewer adventitious roots and a lower percentage of aerenchyma would be found in the root system.

Water regimes may also play a part in changing morphological responses of plants inoculated with PBPR. It is hypothesized that all plants, whether inoculated or not, will show signs of hypoxic stress when either crown-flooded or completely submerged. Plants inoculated with PGPB may have decreased ethylene-related stress symptoms. All plants would be expected to have less biomass, both above and below the soil level, but inoculated plants should have more adventitious roots, longer root systems, and higher surface area because of the increase in lateral roots in response to IAA. Inoculated plants in both crown-flooded and completely submerged water treatments will exhibit less

aerenchyma, which is most likely detrimental to those plants at the bottom of the stands of the lakeshore that periodically experience flooded conditions.

The objectives of this study were to: (1.) isolate and identify possible PGPB from switchgrass stands, (2.) investigate the effects of inoculation of switchgrass with PGPB on root length, root surface area, root volume, number of adventitious roots, shoot growth, and root:shoot ratio, and (3.) determine the response of PGPB inoculated and non-inoculated switchgrass to semi-flooded and flooded conditions.

Materials and Methods

Soil samples for isolation of PGPB were taken from a switchgrass stand (site DNR II, chapter 3) behind the Department of Natural Resources Office for Pickens County, SC, located on Lake Hartwell. Samples were stored at 4^{o}C until used in isolation studies. All chemicals used are from Sigma-Aldrich Co. (St. Louis, MO) or Fisher Scientific (Fair Lawn, NJ).

PGPB Isolation

One gram of soil was added to 50mL of sterile medium containing 10g proteose peptone, 10g casein hydrolysate, 1.5g $MgSO_4$ heptahydrate, 1.5g K_2HPO_4, and 10mL glycerol in a 300mL flask. Flasks were incubated at 30^{o}C for 24 hours with constant shaking. One mL of cultures was transferred to 50mL of the same medium as above in a 300mL flask and incubated 24hours at 30^{o}C. An aliquot (1 mL) was removed from the second growing culture and was transferred to 50mL of sterile DF salts minimal medium (Dworkin and Foster, 1958) with 2g/L $(NH_4)_2SO_4$ as a nitrogen source, contained in a 300mL flask. The flasks were incubated at 30^{o}C with constant shaking for 24 hours, then a 1mL aliquot was removed from the third growing culture and transferred to 50mL of sterile DF salts minimal medium with 0.3033g/L ACC as a nitrogen source contained in a 300mL flask. ACC was filter-sterilized (0.2µm membrane, Fisher Scientific, Fair Lawn, NJ) into the medium after autoclaving. The flasks were incubated at 30^{o}C for 24 hours with constant shaking. Aliquots (100uL) were plated onto solid DF salts minimal

medium containing 3mM ACC and 1.8% Bacto-Agar (Difco Laboratories, Detriot, MI) were incubated at 28^{o}C for 48 – 72 hours.

Isolates were first identified using fatty acid methyl ester analysis (FAME) (MIDI Inc., Newark, DE). Isolate identification was verified using the Biolog Identification System (Biolog, Inc., Hayward, CA) at the Plant Problem Clinic at Clemson University.

Inoculation of seeds and planting

Switchgrass seeds were obtained from Sharp Brothers Seed (Clinton, MO). Half of the switchgrass seeds were inoculated using the method of Grichko and Glick (2001). Bacterial suspensions, inoculated from a single colony from the above culturing instructions, were grown overnight in 300mL tryptic soy broth medium at 30^{o}C on a shaker table. Bacterial cells were separated from the culture medium by centrifugation at 12,000 x g for 10 minutes at room temperature and then washed twice, suspended in 5mL sterile distilled water, followed by centrifugation for 10 minutes at 12,000 x g. Resuspended cells were adjusted to an absorbance of 0.5 OD at 600nm. Switchgrass seeds were surface sterilized in 70% ethanol for 1 minute and then 1% sodium hypochlorite for 10 minutes. Seeds were then washed five times with sterile distilled water to remove residual bleach. Seeds were transferred to sterile polystyrene Petri dishes and incubated with 5mL suspended bacteria or water (uninoculated controls) for 1 hour at room temperature to allow bacteria to bind to the seed coat.

All seeds were planted in Fafard, Inc. (Anderson, SC) Superfine Germinating Mix and started in the propagation room in the Biosystems Research Complex greenhouse at

Clemson University. All germinating mix was autoclaved for 20 minutes at 121^{o}C to reduce bacteria and trays were rinsed in 10% bleach solution. Inoculated seeds were planted in a separate tray from noninoculated seeds. Seeds were kept in the propagation room for 7 weeks. At the end of 7 weeks, seedlings were approximately 5cm and were transferred to 6 inch pots.

Seedlings were transferred to 6 inch pots that had been rinsed in 10% bleach solution and filled with Fafard 3B (Fafard, Inc., Anderson, SC). The soil-less mixture had been autoclaved at 121^{o}C for 20 minutes to reduce bacteria present in the mixture. Inoculated seedlings were re-inoculated with 60ml bacterial suspension, prepared as described previously. All plants were transferred to the Jordan Hall greenhouse (Clemson University) in trays that had been rinsed in 10% bleach solution. This study was completed between June 16 through July 6, 2007 and the temperature in the greenhouse varied between 20^{o}C and 45^{o}C. Trials were conducted simultaneously to take advantage of peak growing season. Plants were exposed to natural light only.

After 2 weeks, inoculated and non-inoculated plants were placed in their respective water treatment (Figure 4.1). Nonflooded plants were kept with 3cm of water in the tray, crown-flooded plants were kept with 7 cm of water in the tray, and the flooded plants were in 27cm of water with 20cm of water above the soil surface. Shoot height was measured every 3 to 4 days for 3 weeks.

Plants were harvested at the end of 3 weeks. Shoots were placed in brown paper bags in a drying oven at 90^{o}C for 24h to determine shoot dry weight. Soil mix was

washed off roots and the number of adventitious roots were counted. Roots were then placed in plastic bags which were placed in cold storage (4^{o}C) until further analysis.

Roots from cold storage were further examined using WinRhizo Professional 2002a root scanning program (Regent Instruments, Inc.,) for total root length, total root system average diameter, total root volume, and total root surface area. For aerenchyma development, roots were sectioned by hand using a single edge razor blade 40mm from root tips on five roots. Root sections were observed under a Wild Heerbrugg (Switzerland) dissecting microscope at 90X. Presence (1) or absence (0) of aerenchyma was recorded for each root sectioned and values were averaged for each plant. After all measurements were obtained, roots were dried in a 90^{o}C oven for 24 hours and root dry weights were determined.

Statistical Analysis

Significant differences between treatments were tested with one-way ANOVA and Fisher's least significant differences were examined using SAS software version 9.0 (SAS Institute Inc., Cary, NC, 2002). Probability of $p<0.05$ was used to indicate significant difference. Each treatment used 10 plants and the experiment was conducted twice. If no experimental difference occurred between experiments, the data was combined and re-evaluated with 20 plants.

Results

A soil sample was taken from an established switchgrass stand and cultured specifically to isolate bacteria that contained ACC deaminase. The bacterium cultured from this sample was *Serratia ficaria*, a bacterium of the family Enterobacteriaceae. Minimal information is published concerning this bacterium, but according to Grimont *et al*. (1981), this species was found in figs, mushrooms, trees and shrubs, small plants, and grasses. Okamoto *et al*. (2000) showed this same bacterium could aid in the control of *Phytophthora* root rot in Angelica trees. Switchgrass was inoculated with *S. ficaria* and several morphological parameters were evaluated over a three week period with plants in semi-flooded and flooded conditions and compared with control.

All root growth parameters were measured using WinRhizo root scanning software (Regent Instruments, Inc.). Total root length describes the combined length of all roots of the entire root system of each plant. Inoculated plants in the nonflooded conditions had higher values than nonflooded, uninoculated plants, but the values were only significantly different in trial 1 (Table 4.1). Uninoculated plants had higher total root length values in the crown-flooded conditions, but neither trial 1 not trial 2 values were statistically different between inoculated and uninoculated plants. Root lengths of the completely submerged plants were much lower than in the other two water treatments. There were no differences observed between inoculated and uninoculated plants between trial 1 or trial 2 (Table 4.1) in completely submerged plants.

Total root surface area measurements and total root volume measurements followed the same pattern as total root length measurements in terms of statistical

significance in both trials 1 and 2. In both total root surface area and total root volume in nonflooded treatments, the inoculated plants had higher values, but only statistically significant in trial 1. The crown-flooded plants exhibited no discernable patterns. In trial 1 inoculated plants had smaller surface areas and smaller root volumes, but the reverse was true for trial 2. Completely submerged plants showed the same pattern between trial 1 and trial 2 with inoculated plants having higher root volume values than the uninoculated plants. No statistical differences were observed between plants with bacteria and plants without bacteria (Table 4.1).

The number of adventitious roots produced were counted for each plant. Inoculated nonflooded plants had higher numbers of adventitious roots than uninoculated plants in both trial 1 and trial 2. There was a statistical difference in nonflooded, uninoculated and nonflooded inoculated treatments for trial 1 but not in trial 2. Crown-flooded, inoculated plants had more adventitious roots than noninoculated plants in the same water treatment of both trials, but there was no statistical difference. There was no difference in number of adventitious roots of the completely submerged plants. Trial 1 had noninoculated plants with more adventitious roots where trial 2 showed that the inoculated plants had more adventitious roots (Table 4.1).

Root to shoot ratios were calculated for each plant in each water treatment. In both trials, the uninoculated, crown-flooded plants had the highest values, corresponding to the highest root mass value compared to shoot mass (Table 4.2). In trial 1, the root to shoot ratio of the uninoculated, crown-flooded plants was statistically different from all other values, but this was not true in trial 2. In trial 1, the inoculated, nonflooded plants

and the inoculated, crown-flooded plants had the second highest values and were statistically similar to each other. In trial 2, nonflooded, inoculated and crown-flooded inoculated root to shoot ratios were statistically similar to both the nonflooded, uninoculated and crown-flooded, uninoculated plants. Within each trial, the completely submerged plants had the lowest values. In trial 1, uninoculated completely submerged had the lowest values, with completely submerged inoculated plants being slightly higher. In trial 2, completely submerged plants whether inoculated or not, were statistically similar to each other in root to shoot ratios with inoculated plants having higher values (Table 4.2).

Each plant's shoot growth was measured throughout the study and plotted. The slope of each graph was calculated and the average of all slopes was reported in Table 4.2. The slopes for inoculated control plants were higher than the noninoculated plants for nonflooded and crown-flooded plants. Shoot growth was the only parameter where there was no experimental difference, and the data was combined and analyzed. The nonflooded, inoculated treatment had the greatest slope and the value was statistically higher than the uninoculated, nonflooded treatment. Inoculated, crown-flooded plants had the third highest slope, but the value was not statistically different from uninoculated, crown-flooded plants. Flooded plants (uninoculated and inoculated) were statistically lower than all other treatments, but not statistically different from each other (Table 4.3).

When sectioned, all roots were positive for aerenchymatous tissue. No differences were observed between any of the treatments inoculated or uninoculated with the nonflooded, crown-flooded, or flooded plants, therefore no data is reported.

Discussion

Although Panicum virgatum 'Alamo' (switchgrass) is well-suited to difficult landscapes, such as the Lake Hartwell shoreline, it was hypothesized that a symbiont might aid the plant's survival. Plant growth promoting bacteria have been shown to promote growth of plants due to their association (Grichko and Glick, 2001; Mantelin and Touraine, 2004). A bacterium was isolated from native soils planted with switchgrass in order to not introduce unforeseen problems. A Gram-negative, facultative, rod-shaped bacterium, *Serratia ficaria*, was isolated from the study site soil using a selective nitrogen source, 1-aminocyclopropane-1-carboxylic acid (ACC). Only bacteria with an ACC deaminase enzyme would be able to use this compound as a nitrogen source. Previous researchers have found bacteria with ACC deaminase activity are able to benefit plants either indirectly by decreasing or preventing some of the deleterious effects including pathogenic interactions (Glick and Bashan, 1997) or directly by synthesizing hormones, solubilizing phosphorus, fixing nitrogen (Kennedy, 1999), or by lowering ethylene levels (Glick, 1995).

This study was conducted to determine if a native bacterium with ACC deaminase activity could increase the probability that switchgrass would survive periodic inundation and even increase certain growth parameters. When root systems were scanned to measure total root system length, volume, and surface area, some interesting points were observed. First, it was obvious that just water treatments had a big impact on the overall growth of the plants. It was not surprising when the completely submerged plants had statistically lower values in terms of root measurements than the other water treatments.

However, the unflooded water treatments showed that inoculated plants had higher root length, root volume, and root surface area. Some values were not statistically different, but the inoculation seemed to benefit the growth of the root system. Inoculation of plant root systems may have resulted in a lowering of ethylene levels which would allow root systems to continue growth (Glick *et al*., 1998). Unflooded water levels did not induce stress and previous work (chapter 3, this study) suggested that switchgrass may be insensitive to ethylene exposure. The increase in root length, root surface area, and root volume could have resulted from IAA produced by *Serratia ficaria* used for inoculation which has been suggested to be produced in many previously reported PGPB's (Patten and Glick, 1995). It has been suggested that up to 80% of bacteria isolated from the rhizosphere can produce IAA (Prikryl *et al*, 1985; Leinhos and Vacek, 1994) and microbial-produced IAA is identical to IAA produced by plants (Patten and Glick, 1995). One bacterium isolated from the rhizosphere of cucumbers, *Enterobacter cloacae*, accelerates the growth of various agriculturally useful plants by producing IAA (Koga *et al*., 1991). Also, an increase in the initiation rate of lateral roots resulting in a more branched root system architecture when inoculated by a plant growth promoting bacterium has been proposed (Kapulnik *et al*.,1985; Lifshitz *et al*., 1987). When crowns were flooded, root measurements decreased (except when comparing to uninoculated nonflooded values of trial 1). Having water completely saturating the soil slowed root growth, and there was no consistency between trial 1 and trial 2 values. Therefore, no conclusions could be drawn about interactions between saturated soil, plant root growth, and inoculation with *Serratia ficaria*. Root length of completely submerged plants was

low compared to other water treatments, and no pattern was seen between trial 1 and trial 2. Inoculated completely submerged plants had higher surface area and higher root volume than the uninoculated plants. The bacteria may have been blocking production of ethylene while still providing IAA, but at low levels. These bacteria may actually benefit root growth when plants are completely submerged. Plants inoculated with bacteria having ACC deaminase activity can partially prevent ethylene-mediated decreases in plant root and shoot length, and biomass, especially under stressful conditions (Mayak *et al*., 2004). Increased surface area is important in the root system in order to transport substances from soil to root and vice versa. Increased surface area and root volume may have arisen from increased branching of the root system which would be advantageous in contacting as much soil as possible. Crown-flooded plants were similar (but not always statistically similar) to nonflooded plants in length, volume, and surface area probably because the variety of switchgrass chosen is naturally found in lowland, wet areas. Although having their crowns flooded may not be ideal for extended periods of time, this experiment may not have lasted long enough to impact crown-flooded plants.

Plants found in wet areas tend to have increased abilities to form adventitious roots (Laan *et al*., 1989; Visser *et al*., 1996). Switchgrass is known to produce large quantities of adventitious roots. It was hypothesized that this plant would produce more adventitious roots when inoculated because of the IAA to which the plant would possibly be exposed. This plant produced the highest number of adventitious roots in the nonflooded water treatment with plants which had been inoculated. Only plants in trial 1 were statistically higher in number of adventitious roots produced, but inoculated plants

(nonflooded water treatment) were consistently higher. *Serratia ficaria* had an impact on adventitious rooting due to the possible production of IAA by the bacterium. However, uninoculated crown-flooded plants had greater number of adventitious roots than the inoculated plants of the same water treatment. It appears as though soil saturation had a negative impact on adventitious root formation and bacterial inoculation did not overcome this negative impact. Visser *et al*. (1996) showed that *Rumex palustris*, a wetland species, formed adventitious roots in response to flooding. Adventitious rooting occurred only after increased endogenous ethylene concentration in the root systems which caused an increase in the sensitivity of root-forming tissues to endogenous indoleacetic acid, initiating the formation of adventitious roots. Possibly, the bacteria decreased ethylene in the rhizosphere and therefore no increase in sensitivity to IAA occurred. There was no reproducible pattern between inoculated and noninoculated, completely submerged plants. All completely submerged plants produced fewer adventitious roots than all other treatments. Complete inundation appears to be very stressful for *P. virgatum* L. and the bacteria had no effect.

Due to the possible exposure to IAA in the inoculated plants, it was hypothesized that they would have faster shoot growth and this was the case in most instances. Shaharoona *et al*. (2006) found that when *Zea mays* was inoculated with bacterial strains possessing ACC deaminase activity, shoot length of seedlings significantly increased up to 1.9-fold over uninoculated controls. Canola plants inoculated with *Pseudomonas putida* GR12-2, which possesses ACC deaminase and is known to produce IAA, shoot elongation increased 38% over uninoculated controls (Glick *et al*., 1997). In the

nonflooded water treatment, inoculated plants exhibited the highest rate of shoot growth. The difference in the inoculated versus uninoculated plants in the nonflooded water treatment was significant. The same pattern was seen in crown-flooded plants, but not at a statistical significance. The movement of endogenous IAA through the stem, supplemented by IAA supplied by the bacterium, may have caused the increase in shoot growth of inoculated plants. Completely submerged plants showed no statistical differences between inoculated and noninoculated plants.

Root to shoot ratios were calculated based on biomass to understand how plants were allocating their resources. A higher number meant that plants were putting more energy into building a root system and a smaller number meant less energy was distributed to the root system. The treatment that had more energy used to build a root system were crown-flooded, uninoculated plants. In trial 1, these plants were statistically higher than all other plants. Crown-flooded, uninoculated plants in trial 2 had the highest value, but were not statistically different from inoculated, nonflooded plants and inoculated crown-flooded plants. Plants that had slightly lower values were the inoculated nonflooded plants and inoculated crown-flooded plants in both trials. Completely submerged plants that had been inoculated had higher values than those without bacteria, but not statistically higher. Only in the crown-flooded plants did the uninoculated plants put more resources into the root system. This fact most likely comes from crown-flooded, uninoculated plants formed high numbers of adventitious roots coupled with slower rates of shoot growth. As mentioned above, endogenous IAA may

have been supplemented by bacterial IAA in inoculated plants but uninoculated plants would not have been supplemented.

All plants of every water treatment, whether inoculated or not, produced aerenchyma in all roots sampled. The literature states that plants exposed to ethylene produce aerenchyma (Drew, He, and Morgan, 2000). However aerenchyma is found in many wetland species and its presence may not depend on ethylene exposure (Visser and Bogeman, 2006). In the case of switchgrass, it appears that formation of aerenchyma is not influenced by the presence of ethylene since it was found in all plants of the study under all conditions. Perhaps this plant, native to lowland areas, developed constitutive aerenchyma formation since it would have been advantageous for its survival. It may be part of the developmental process and is independent of environmental conditions as was found in *Juncus effusus* (Visser and Bogeman, 2006).

This study shows that switchgrass survives well in water levels up to soil saturation, but complete submergence affects its growth and survival severely. Complete submergence for plants of this age slows growth of both shoots and roots and leaves the plant barely surviving. Bacterial inoculation of these plants did not aid much in the survival of these plants when crown-flooded or submerged. However, when flooding was not a factor, bacterial inoculation increased both root and shoot growth. In nonstressful conditions, *S. ficaria* enhanced the growth of this plant. When stress is added in the form of flooding, the bacteria may actually begin to additionally stress the plant. The only parameter where inoculated, crown-flooded plants excelled was shoot growth.

Several future studies still need to be conducted. An area that needs investigation is whether *Serratia ficaria* produces IAA. According to the model of Glick *et al*., (1998), bacteria with ACC deaminase activity produce IAA, but this property was not specifically tested for *Serratia ficaria*. The levels of IAA produced by switchgrass need to be investigated. It may be that additional IAA provided by microbes proves inhibitory to plant growth. Also, *Serratia ficaria* was the bacterium isolated from the switchgrass stand in this study, but there may be other species that possess ACC deaminase activity in these sites. Those bacteria should be investigated with switchgrass to determine if they have effects, either positive or negative, on survivorship of the plants. A sequence of fluctuating water levels are important to further investigate because plants on the shoreline experience drained, semi-flooded, and flooded conditions over the course of a year. Finally, it should be determined whether switchgrass produces aerenchyma constitutively. Research presented here supports the hypothesis that this plant produces aerenchyma without cues from its environment, but more studies should be performed to further support this idea.

Literature Cited

Celenza, J.L., P.L. Grisafi, and G.R. Fink. 1995. A pathway for lateral root formation in *Arabidopsis thaliana*. Genes and Development 9**:** 2131-2142.

Cleland, R.E. 1995. Auxin and Cell Elongation. *In* PJ Davies, ed, Plant Hormones and Their Role in Plant Growth Development, Ed 2nd. Kluwer, Dordrecht, Netherlands, pp 214-227.

Drew, M.C., C.J. He, and P.W. Morgan. 2000. Programmed cell death and aerenchyma formation in roots. Trends in Plant Science 5**:** 123-127.

Dworkin, M. and J. Foster. 1958. Experiments with some microorganisms which utilize ethane and hydrogen. Journal of Bacteriology 75**:** 592-601.

Glick, B.R. 1995. The enhancement of plant growth by free-living bacteria. Canadian Journal of Microbiology 41**:** 109 - 117.

Glick, BR, C. Liu, S. Ghosh, and E.B. Dumbroff. 1997. Early development of canola seedlings in the presence of the plant-growth promoting rhizobacterium *Pseudomonas putida* GR12-2. Soil Biology and Biochemistry 29**:** 1233-1239.

Glick, B.R., and Y. Bashan. 1997. Genetic manipulation of plant growth-promoting bacteria to enhance biocontrol of fungal phytopathogens. Biotechnological Advances 15**:** 353-378.

Glick, B.R., D.M. Penrose, and J. Li. 1998. A model for the lowering of plant ethylene concentrations by plant growth - promoting bacteria. Journal of Theoretical Biology 190**:** 63 - 68.

Grichko, V.P., and B.R. Glick. 2001. Amelioration of flooding stress by ACC deaminase-containing plant growth-promoting bacteria. Plant Physiology and Biochemistry 39**:** 11 - 17.

Grimont, P.A.D., F. Grimont, and M. Starr. 1981. *Serratia* species isolated from plants. Current Microbiology 5**:** 317-322.

Kapulnik, Y., Y. Okon, and Y. Henis. 1985. Changes in root morphology of wheat caused by *Azospirillum* inoculation. Canadian Journal of Microbiology 31**:** 881-887.

Kende, H. 1993. Ethylene biosynthesis. Annual Review of Plant Physiology and Plant Molecular Biology 44**:** 283-307.

Kende, H., E. van der Knaap, and H.T. Cho. 1998. Deepwater rice: a model plant to study stem elongation. Plant Physiology 118**:** 1105-1110.

Kennedy, A.C. 1999. The rhizosphere and spermosphere. *In* DM Sylvia, J.J. Fuhrmann, P.G. Jartel, and D.A. Zuberer, ed, Principles and Applications of Soil Microbiology. Prentice Hall, Inc., Upper Saddle River, NJ, pp 389-407.

Kloepper, J.W., M.N. Schroth, and T.D. Miller. 1980. Effects of rhizosphere colonization by plant growth-promoting rhizobacteria on potato plant development and yield. Phytopathology 70**:** 1078-1082.

Koga, J, T. Adachi, and H. Hidaka. 1991. IAA biosynthesis pathway from tryptophan via indole-3-pyruvic acid in *Enterobacter cloacae*. Agric. Biol. Chem. 55**:** 701-706.

Laan, P., M.J. Berrevoets, S. Lythe, W. Armstrong, and C.W.P.M. Blom. 1989. Root morphology and aerenchyma formation as indicators of flood tolerance of *Rumex* species. Journal of Ecology 77**:** 693-703.

Leinhos, V, and O.Vacek. 1994. Biosynthesis of auxins by phosphate-solubilizing rhizobacteria from wheat and rye. Microbial Research 149**:** 31-35.

Lifshitz, R., J.W. Kloepper, M. Kozlowski, C. Simonson, J. Carlson, E.M. Tipping, and I. Zaleska. 1987. Growth promotion of canola (rapeseed) seedlings by a strain of *Pseudomonas putida* under gnotobiotic conditions. Canadian Journal of Microbiology 33**:** 390-395.

Mantelin, S., and B. Touraine. 2004. Plant growth-promoting bacteria and nitrate availability: impacts on root development and nitrate uptake. Journal of Experimental Botany 55**:** 27-34.

Mayak, S., T. Tirosh, and B.R. Glick. 2004. Plant growth promoting bacteria that confer resistance to water stress in tomato and pepper. Plant Science 166**:** 525-530.

Miller, E.V. 1947. The story of ethylene. Scientific Monthly 65**:** 335 - 342.

Okamoto, H., M. Sato, Y. Miyata, M. Yoshikawa, and M. Isaka. 2000. Biocontrol of *Phytophthora* root rot of Angelica trees by *Enterobacter cloacae* and *Serratia ficaria* strains. Journal of General Plant Pathology 66**:** 86-94.

Patten, CL, and B.R. Glick. 1995. Bacterial biosynthesis of indole-3-acetic acid. Canadian Journal of Microbiology 42**:** 207-220.

Prikryl, Z, V. Vancura, and M. Wurst. 1985. Auxin formation by rhizosphere bacteria as a factor of root growth. Biol. Plant. 27**:** 159-163.

Shaharoona, B., M. Arshad, and Z.A. Zahir. 2006. Effect of plant growth promoting rhizobacteria containing ACC-deaminase on maize (*Zea mays* L.) growth under axenic conditions and on nodulation in mung bean (*Vigna radiata* L.). Letters in Applied Microbiology 42**:** 155-159.

Taiz, L., and E. Zeiger. 1998. Plant Physiology, Ed 2nd. Sinauer Associates, Inc., Sunderland, MA.

Visser, E.J.W., and G.M. Bogemann. 2006. Aerenchyma formation in the wetland plant *Juncus effusus* is independent of ethylene. New Phytologist 171**:** 305-314.

Visser, E.J.W., C.J. Heijink, K.J.G.M. Van Hout, L.A.C.J. Voesenek, G.W.M. Barendse, and C.W.P.M. Blom. 1995. Regulatory role of auxin in adventitious root formation in two species of *Rumex* differing in their sensitivity to waterlogging. Journal of Experimental Botany 47**:** 403-410.

Visser, E.J.W., G.M. Bogemann, C.W.P.M. Blom, and A.C.J. Voesnek. 1996. Ethylene accumulation in waterlogged *Rumex* plants promotes formation of adventious roots. Journal of Experimental Botany 47**:** 403-410.

Yang, S.F., and N.E. Hoffman. 1984. Ethylene biosynthesis and its regulation in higher plants. Annual Review of Plant Physiology 35**:** 155-189.

Figure 4.1. Experimental set-up showing *Panicum virgatum* in three different water treatments.

Table 4.1. Morphological parameters for inoculated and uninoculated *Panicum virgatum* L. plants in three different water treatments.

	Total root length (mm)		**Total root surface area (cm^2)**		**Total root volume (cm^3)**		**# adventitious roots**	
	Trial 1	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**	**Trial 1**	**Trial 2**
Nonflooded/- (n=10)	$429.43^{B/C}$ (s.e.=127.52)	1215.84^{A} (s.e.=199.31)	$157.41^{B/C}$ (s.e.=39.32)	393.33^{A} (s.e.=63.62)	$4.60^{B/C}$ (s.e.=1.03)	10.29^{A} (s.e.=1.71)	$9.4^{B/C}$ (s.e.=1.9)	14.6^{A} (s.e.=1.9)
Nonflooded/+ (n=10)	1130.08^{A} (s.e.=127.52)	1366.56^{A} (s.e.=199.31)	377.77^{A} (s.e.=39.32)	424.37^{A} (s.e.=63.62)	10.08^{A} (s.e.=1.03)	10.51^{A} (s.e.=1.71)	20.0^{A} (s.e.=1.9)	16.0^{A} (s.e.=1.9)
Crown flooded/- (n=10)	676.16^{B} (s.e.=127.52)	1222.09^{A} (s.e.=199.31)	210.43^{B} (s.e.=39.32)	383.25^{A} (s.e.=63.62)	5.35^{B} (s.e.=1.03)	9.61^{A} (s.e.=1.71)	14.0^{B} (s.e.=1.9)	16.8^{A} (s.e.=1.9)
Crown flooded/+ (n=10)	663.12^{B} (s.e.=127.52)	972.23^{A} (s.e.=199.31)	220.86^{B} (s.e.=39.32)	315.66^{A} (s.e.=63.62)	5.86^{B} (s.e.=1.03)	8.23^{A} (s.e.=1.71)	$10.4^{B/C}$ (s.e.=1.9)	14.2^{A} (s.e.=1.9)
Completely submerged/- (n=10)	164.68^{C} (s.e.=127.52)	145.50^{B} (s.e.=199.31)	58.67^{C} (s.e.=39.32)	55.71^{B} (s.e.=63.62)	1.68^{C} (s.e.=1.03)	1.71^{B} (s.e.=1.71)	7.0^{C} (s.e.=1.9)	4.6^{B} (s.e.=1.9)
Completely submerged/+ (n=10)	146.10^{C} (s.e.=127.52)	192.16^{B} (s.e.=199.31)	62.82^{C} (s.e.=39.32)	67.06^{B} (s.e.=63.62)	2.15^{C} (s.e.=1.03)	1.89^{B} (s.e.=1.71)	6.4^{C} (s.e.=1.9)	7.4^{B} (s.e.=1.9)

Letters indicate significant differences at $p<0.05$. s.e. = standard error between values of each column. Plus (+) indicates inoculated with *Serratia ficaria*, minus (-) indicates no inoculation

Table 4.2. Root:shoot ratios for *Panicum virgatum* when exposed to 3 different water regimes.

	Root:Shoot ratio	
	Trial 1	**Trial 2**
Nonflooded/- (n=10)	0.1182^{C} (s.e.=0.0126)	0.1687^{B} (s.e.=0.0127)
Nonflooded/+ (n=10)	0.1655^{B} (s.e.=0.0126)	$0.1806^{A/B}$ (s.e.=0.0127)
Crown flooded/- (n=10)	0.2026^{A} (s.e.=0.0126)	0.2105^{A} (s.e.=0.0127)
Crown flooded/+ (n=10)	0.1643^{B} (s.e.=0.0126)	$0.1802^{A/B}$ (s.e.=0.0127)
Completely submerged/- (n=10)	0.0728^{D} (s.e.=0.0126)	0.0998^{C} (s.e.=0.0127)
Completely submerged/+ (n=10)	$0.0990^{C/D}$ (s.e.=0.0126)	0.1236^{C} (s.e.=0.0127)

Letters indicate significant differences at $p<0.05$. s.e. = standard error between values for each column. Plus (+) indicates inoculated with *Serratia ficaria*, minus (-) indicates no inoculation.

Table 4.3. Shoot growth slopes for *Panicum virgatum* when exposed to 3 different water regimes.

	Shoot growth slope
Nonflooded/- (n=20)	9.13^{B} (s.e.=0.51)
Nonflooded/+ (n=20)	11.22^{A} (s.e.=0.51)
Crown flooded/- (n=20)	7.24^{C} (s.e.=0.51)
Crown flooded/+ (n=20)	$8.25^{B/C}$ (s.e.=0.51)
Completely submerged/- (n=20)	4.11^{D} (s.e.=0.51)
Completely submerged/+ (n=20)	4.07^{D} (s.e.=0.51)

Data from trial 1 and trial 2 was merged. Letters indicate significant differences at $p<0.05$. s.e. = standard error between values for each column. Plus (+) indicates inoculated with *Serratia ficaria*, minus (-) indicates no inoculation.

CONCLUSION

The previous chapters outlined studies performed on different aspects of *Panicum virgatum* (switchgrass) and its survivability on the shorelines of Lake Hartwell in Pickens County, South Carolina. Little research has been performed on soils planted with switchgrass over periods of years and also, little information exists on how switchgrass reacts to low nutrient soils and periodic inundation. These chapters serve as the beginning of research needed in these areas.

The soils in which the plants are placed are nutrient-poor and were hypothesized to have low microbial activity levels. Several microbial activity assays were performed on soils samples from three different planted sites, ranging from 1 to 5 years old. These analyses included: acid phosphomonoesterase, denitrification (nitrate reductase), nitrogen fixation by acetylene reduction, dehydrogenase, and substrate-induced respiration (SIR). To compare microbial community assemblages, fatty acid methyl ester (FAME) analysis was also performed. Low microbial activity was found in dentrification, nitrogen fixation, and substrate-induced respiration assays and only dentrification values were impacted by the presence of plants over time. Acid phosphomonoesterase and dehydrogenase values were high, compared to the literature, and values increased as stands aged. Fatty acid analysis indicated the presence of Gram negative bacteria in planted sites while the fatty acids associated with Gram negative bacteria were not detected in non-vegetated sites. Diversity appeared to increase as stands aged as indicated by an increase in the number of different fatty acids detected.

From these results, it can be inferred that once switchgrass plants have established themselves, microbial activity will increase and will possibly aid in the acquisition of nutrients from the soil. Microbes may also aid in nutrient recycling after shoot and root growth from previous seasons die.

Because switchgrass is periodically inundated, it was hypothesized that the plants would produce, and be exposed to, ethylene during flooding. Little information was found in the literature pertaining specifically to the effects of ethylene on switchgrass. In this study, young plants were exposed hydroponically to ethylene and ethylene inhibitors in closed containers. These plants showed very little consistent response of root growth parameters when exposed to either ethylene or ethylene inhibitors. An increase in adventitious root formation was the only parameter that significantly increase in response to ethylene.

Without further research, there can be no definitive statement made as to the sensitivity of switchgrass to ethylene. However, these results seem to indicate that switchgrass is relatively insensitive to ethylene. Further, it appears that aerenchyma development in this species is not, or at least not entirely, under the control of ethylene as it has been reported in many other plants. Aerenchyma was produced regardless of environmental conditions or treatment.

Soil bacteria are under investigation to determine which species aid plant growth and could possibly be added to the rhizosphere for this purpose. *Serratia ficaria* was selectively isolated from switchgrass stands based on the presence of ACC deaminase, which has been shown to be present in plant growth-promoting bacteria. Plants were

either inoculated or not inoculated with *S. ficaria* and were then subjected to no flooding, crown-flooding or submergence. It appeared that *S. ficaria* aided shoot growth, total root length, root surface area, root volume, and number of adventitious roots of unflooded plants. Submerged, inoculated plants had increased root to shoot ratios, but the opposite was true in crown flooded treatments.

It is possible that *S. ficaria* can help switchgrass plants become established faster if the plants are not subjected to flooding. Because the bacterium was isolated from established switchgrass stands, there would be no danger of adding this bacterium to the environment. It is possible that because the isolation was made from non-flooded sites the bacteria that was isolated was better for unflooded conditions. An isolate from flooded sites could result in the isolation and detection of a PGPB that would be more beneficial under flooded conditions.

To conclude, this research provides some basic information on using switchgrass for stabilization of shorelines and one option that can be used to help ensure establishment of these plants in these area. Switchgrass may be relatively insensitive to ethylene exposure and may not respond as acutely to plant growth-promoting bacteria as other plants, but the addition of *S. ficaria* may aid in giving switchgrass an advantage in nutrient poor soils during initial establishment.

Further research is needed in all areas of the study presented. Five years may be too short of a time period to determine if the presence of switchgrass in shoreline soils results in a difference in microbial activity. In an additional five years, nutrient level measurements and enzyme assays should be conducted again and compared to the results

presented in chapter 2 of this research. Another area that needs more investigation is the response of switchgrass to ethylene. Is the sensitivity of switchgrass to ethylene different from the ethylene sensitivity of other plants? Is the ethylene receptor different in switchgrass compared to ethylene receptors of other plants? Are the morphological differences seen in switchgrass, typically attributed to ethylene exposure, completely under genetic control? Finally, are there other plant growth promoting bacteria in Lake Hartwell shoreline soils that could positively impact switchgrass growth and sustainability especially under flooded conditions? Answering these questions will give us a better understanding of switchgrass and its relationship to the microbial community and its environment.