A MULTI-DIMENSIONAL INVESTIGATION INTO THE EFFECTS OF FLOODING ON THE PHYSICAL, CHEMICAL, AND BIOTIC PROPERTIES OF RIPARIAN SOILS

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by

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The undersigned, appointed by the Dean of the Graduate School, have examined the dissertation entitled:

A MULTI-DIMENSIONAL INVESTIGATION INTO THE EFFECTS OF FLOODING ON THE PHYSICAL, CHEMICAL AND BIOTIC PROPERTIES OF RIPARIAN SOILS

presented by Irene M. Unger

a candidate for the degree of Doctor of Philosophy,

and hereby certify that, in their opinion, it is worthy of acceptance.

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DEDICATION

This is dedicated to my father who passed away just prior to my acceptance into graduate school. Dad and mom instilled in me a love of the out-of-doors that continues to shape my life today. Thank you both for insisting that I "go play outside" and for teaching me to marvel at sunsets and star-filled skies and to appreciate nature.

I could not have completed this without support, encouragement and love from family and friends. My family have been my cheerleaders; especially Jim, who I truly could not have done this without.

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ABSTRACT

Understanding soil abiotic and biotic influences on ecosystem processes will enhance success of riparian restoration efforts. The goal of this research was to characterize changes in soil chemical and microbial community properties with periodic flooding. Simulated floods were created under greenhouse and field laboratory settings to assess these changes as well as the effect of soil chemistry changes on germination and seedling growth. In addition, riparian forests in northwest Missouri were examined to determine how flooding and microtopography affect soil chemistry and vegetation patterns across floodplains. Flood treatments did not affect soil TOC or TN; however, anoxic conditions developed and NH_4-N and total soluble polyphenolics (TSP) accumulated in soils with flooding. Germination and seedling growth were negatively correlated with soil TSP levels. Microbial community structure changed with flooding under greenhouse but not field conditions. Microbial biomass, the response of microbial groups and enzyme activity decreased under stagnant flood conditions; while stress indicators increased. In riparian forests, herbaceous and woody understory vegetation were negatively correlated and responded differently to microtopographical variables as well as TN. Site differences contribute to these results. Changes in microbial community structure and function as well as the accumulation of TSP with flooding may affect nutrient availability and thus have negative implications for plant species post-flood.

CHAPTER 1

INTRODUCTION

Periodic flooding of rivers and floodplains occurs during times of heavy rainfall, resulting in stagnant or flowing water over agricultural or riparian forest soils. Flooding may cause mechanical injury to vegetation (Streng et al., 1989; Nisson and Svedmark, 2002) or may result in sediment deposition. Thin deposits may result in reduced photosynthesis or plant growth, while thickly deposited sediment may partially or completely bury plants. Species vary in their ability to survive the stress of sedimentation; for example, species that produce adventitious roots and spread vegetatively can better survive burial (Levine and Stromberg, 2001; Nisson and Svedmark, 2002). Furthermore, seedling height and stem rigidity may play a role in seedling survival. Species with more sturdy, erect stems will be better able to remain upright with sediment deposition resulting in less physical coverage of foliage (Levine and Stromberg, 2001). The redistribution of plant material (e.g. leaves, flowers, woody debris, etc) by flood waters has similar impacts on floodplain plants; litter and debris accumulate along the high-water level and may bury plants (Nisson and Svedmark, 2002).

While sediment deposition may injure or kill existing riparian vegetation, it may also provide opportunities for the establishment of early successional species through the creation of open areas (Stromberg et al., 2007). Sediment deposition on the floodplain will vary with flood intensity creating a shifting mosaic of available patches. These patches will not only provide the bare mineral soil necessary for germination of some species, but the floods that created the patches will contribute water and nutrients that stimulate germination and subsequent growth. Riparian ruderal species have adaptations, such as short-life span,

high seed production and high allocation to reproductive output, that enhance survival in disturbed environments (Stromberg et al., 2007). Seeds of these species may disperse via wind or water following disturbance or may be present in the seed bank. However, soil seed banks are often not representative of the mature forest and thus may be important for some but not all wetland species (Hanlon et al., 1998). For example, riparian forests in the Allegheny Plateau had a strong dominance of perennials (Hanlon et al., 1998). These species may rely more heavily on vegetative means of reproduction, such as sprouting from roots or rhizomes, rather than on soil seed banks for their regeneration. For species that rely on soil seed banks or seed dispersal, factors such as floodplain hydrology (Hanlon et al., 1998), microtopography (Jones et al., 1994) and disturbance affect germination and seedling establishment. In their study of woody plant regeneration in floodplain forests, Jones et al. (1994) found that elevation was a significant predictor of seedling flux and seedling density in flooded sites with peak population densities occurring at higher elevations than peak fluxes. Jones et al. (1994) suggest that seedling populations go through two environmental filters and that species vary in their ability to pass through these filters. The first filter occurs within a few months of germination while the second filter is applied in subsequent years. It is this second filter where the ability to withstand flooding as well as other stresses, such as deep shade and intense root competition, plays a role in seedling survival (Jones et al., 1994).

Seed germination and seedling establishment may also be related to changes in soil chemistry with flooding. Flood deposited litter and other plant debris may contribute to changes in floodplain soil chemistry through the input of nutrients and/or phytotoxins following decomposition (Nisson and Svedmark, 2002). For example, phenolic compounds may accumulate in the soil during periods of inundation. Assays to determine the effects of phenolic compounds on seed germination and seedling growth have been conducted

(Reigosa et al., 1999; Muscolo et al., 2000); but these studies were limited in scope (i.e., select phenolic compounds were tested on select species) and produced mixed results. Further study of these interactions is therefore warranted. In addition, investigations into the effects of other soil chemical changes due to flooding (e.g., changes in availability of mineral ions) on seed germination or vegetative regrowth following a flood are lacking.

Research Objectives

The overall objective of this research is to characterize the changes in soil physical, chemical, and biological properties that occur with periodic flooding. Specifically, this project will:

i) characterize the effects of flooding on soil chemical properties, such as soil oxygen status and inorganic-N levels, and determine the effects of flooding and plant residue type on the accumulation of phenolic compounds within the soil,

ii) evaluate how soil chemistry changes from flooding affect germination, growth and survival of grass seedlings,

iii) characterize the effects of flooding on soil microbial community structure and function, and

iv) examine the relationships among microtopography, soil inorganic-N and polyphenolic content, and understory riparian vegetation in natural systems.

These objectives were pursued using a multi-dimensional investigation. Simulated floods were created in a greenhouse and at a field laboratory setting to examine changes in soil chemistry and microbial populations with flooding. The greenhouse experiments also addressed how flood and residue types might influence the accumulation of phenolic compounds and how, in turn, these compounds might influence germination and seedling

growth. Finally, riparian forests in northwest Missouri were examined to determine how flooding and microtopography affect soil chemistry and vegetation patterns across the floodplain.

Chapter 2 describes the effects of flooding on soil chemistry under field-laboratory conditions. This chapter focuses on an innovative field-based laboratory that allows for manipulated flooding and where various plant species are evaluated for flood tolerance. This chapter also addresses the challenges of monitoring soil systems *in situ* and contrasts automated monitoring systems with manual monitoring systems.

Chapter 3 describes the effects of flood and residue type on soil chemistry under greenhouse conditions. This chapter also addresses how phenolic compounds, along with other soil chemistry changes, affect germination and seedling growth.

Chapter 4 describes the effects of flooding on soil microbial community structure and function. Variability in the soil microbial community due to experimental conditions (i.e. greenhouse and field conditions) and sampling techniques (i.e. depth of sampling and sampling date) are explored.

Chapter 5 describes the effects of flooding on soil chemistry and vegetation in a natural system. This chapter explores the influence of microtopography on water accumulation in the landscape and how microtopography and flooding combine to affect riparian community structure.

Chapter 6 synthesizes the entire project. The findings and conclusions of previous chapters are summarized to provide a general understanding of the effects of flooding on the physical, chemical and biotic properties of riparian soils.

CHAPTER 2

MONITORING SOIL PHYSICAL AND CHEMICAL PROPERTIES IN A FIELD-BASED LABORATORY

Introduction

The increasing interest in wetland restoration and riparian forest aforestation prompted the development of the Flood Tolerance Laboratory (FTL), a unique outdoor facility designed for evaluating the flood tolerance of hardwood seedlings and ground cover plant species. At the FTL, flood water depth, duration and flow rate can be regulated to simulate experimentally the effect of flooding regimes. Flood tolerance is largely the physiological adaptation of plants to anoxic conditions, toxic substances, and other associated changes in soil properties induced by flooding. Therefore, quantifying changes in soils during flooding represents an important, but often neglected, component of flood tolerance determination.

Redox potential measurements provide one way to quantify the magnitude of reducing conditions in the soil environment and thereby assess nutrient availability in a flooded system (Patrick et al., 1996; Owens et al., 2005). Redox potential is a measure of the tendency of a biogeochemical system to receive or supply electrons (Hinchey and Schaffner, 2005). Since a number of redox couples act simultaneously within the soil system, redox potential provides a semi-qualitative assessment (Austin and Huddleston, 1999; Gao et al., 2002; van Bochove et al., 2002; Hinchey and Schaffner, 2005; Owens et al., 2005). However, this measure can allow differentiation between oxic and anoxic soil conditions and the monitoring of the progressive development of reduced conditions (Gao et.al., 2002).

A number of studies (Austin and Huddleston, 1999; van Bochove et al., 2002; Mansfeldt, 2003; Wafer et al., 2004) have shown limited success in long-term monitoring of soil redox conditions with platinum electrodes. Wafer et al. (2004) demonstrated that after 19 months in the field, 98% of their electrodes were functioning satisfactorily and Austin and Huddleston (1999) had a similar rate of success 3 and 5 years after installation of electrodes. However, these studies and others caution that permanently installed electrodes may become contaminated over time leading to erroneous readings (Austin and Huddleston, 1999; Mansfeldt, 2003; Hinchey and Schaffner, 2005). Electrode rupture or leakage also results in unreliable readings (Mansfeldt, 2003). Other potential problems that may arise during long-term monitoring include: calibration drift, signal disruption and electronic failure from water leakage into the system, lack of circulation around an electrode, and maintenance and monitoring costs.

Despite some obvious pitfalls, permanent installation and automated monitoring of electrodes may have advantages over manual measurements. Manual measurements may be impractical due to location and accessibility of field sites during periods of inundation; fluctuations that occur with weather and flooding may therefore be overlooked (Vorenhout et.al., 2004). In addition, manual measurements may be affected by operator presence which may change soil pressure, thereby affecting redox readings (Vorenhout et al., 2004). Permanent installation of electrodes permits frequent measurements over short intervals, allowing for identification of diurnal fluctuations. In addition, the system can be monitored from a distance, creating fewer disturbances. Other advantages of automated systems include the ability to store data, adjust the time of sampling, control sensor operation, and monitor operation of each sensor.

Objectives and Expectations:

The objectives of this study were: to characterize the soils of the FTL, to compare the effects of flood duration and flow rate on soil physical and chemical properties in the FTL, and to examine the performance of an automated system for *in situ* monitoring of the changes in redox potential and pH as well as other soil properties affected by flooding including soil water content, temperature and dissolved oxygen content. Since the FTL is a large (total area: 72 m x 180 m), field-based facility, variation in soil properties, such as color, texture, pH, and CEC, across the lab were expected. Redox potential was expected to decrease with flooding and long duration floods (i.e., 5 week floods) were expected to have lower average redox potentials than other treatments. Flowing flood waters should have higher dissolved oxygen than stagnant flood waters and consequently soil dissolved oxygen should be higher under flowing flood conditions than under stagnant flood conditions. Total N was expected to decrease; however, ammonium levels were expected to increase relative to nitrate levels. Accumulation of polyphenolic compounds was also expected, especially under floods of longer duration.

Methods

The Study Site

This study was conducted at the Flood Tolerance Laboratory (FTL) at the Horticulture and Agroforestry Research Center (HARC) in New Franklin, Missouri (39° 0' 0" N, 92° 46' 0" W) as a part of research examining flood tolerance of hardwood seedlings and ground cover plant species. The FTL is an outdoor research facility constructed in 1999 on a wide terrace floodplain adjacent to Sulphur Creek. A limestone-covered county road runs on the north side of the research facility, allowing access. The FTL consists of twelve 6

m x 180 m parallel channels; each channel can be manipulated independently of adjacent channels to allow for various flood treatments (i.e. changing depth, flow rate and duration of flooding). Water is pumped underground from the retention pond to the inlet end of the channels, and in flowing-flood treatments, water circulates between the pond and the channels, flowing from the inlet to the outlet (pond) end. Channels are numbered sequentially (1-12) from north to south. Soil removed in the creation of a retention pond for the facility was used to create berms (2 m high and 6 m wide) between the channels; this allowed for minimal disturbance to the soils within the channels. Channels have been disked or rototilled prior to plantings. Generally tillage is shallow (i.e., to a depth of 4") and is concentrated in the portions of the channels that will be planted that season. No tillage has occurred in the area surrounding the automated sensors since their installation in 2005.

Soil Characterization

In September, 2005 soil samples were collected from the FTL for characterization. Each channel was separated into three sampling zones (one-third of the area closest to the inlet, the middle one-third, and the one-third closest to the outlet of the channel); within each of these sampling zones, soil core samples to a depth of 120 cm were taken using a Giddings probe. Soil morphology (i.e. soil texture, structure, color, redoximorphic features, etc.) of these core samples was determined in the field; soil samples from each horizon designation were sent to the Soil Characterization Lab at the University of Missouri. Characterization included analysis for soil texture, soil pH, total organic carbon (TOC), exchangeable bases (Ca^{+2}, Mg^{+2}, Na^{+} and K^{+}), extractable Al^{+3} and Al^{+3} saturation, CEC and base saturation.

Pre- and post-flood soil samples were also collected from the sampling zones for analysis of total organic carbon (TOC), total N (TN), C:N ratio, inorganic N content, and total soluble polyphenolic (TSP) content. Pre-flood samples were collected in May and post-flood samples were collected in July. In each of the three sampling zones, composite soil samples were created by collecting 10 random soil samples with a push probe. These samples were divided into two depths (0-10 cm and 10-20 cm) and cores from the same depth within the sampling zone were combined to create a single soil sample. Samples were placed in coolers while in the field and frozen upon return to campus. Soils were later freeze-dried and ground to pass through a 2 mm sieve.

Soil samples from the sensor /outlet-end of the channel were of primary interest. These samples were taken from an area that was not planted with experimental species; however, a number of "weedy species" became established during the growing season. These species were generally wetland adapted species (i.e., able to tolerate frequent flooding and/or soils with poor drainage) that were common to the Sulphur Creek floodplain. Identified species included Pennsylvania smartweed (*Polygonum* sp.), yellow nutsedge (*Cyperus esculentus* L.), curly dock (*Rumex* sp.) and cinquefoil (*Potentilla* sp.).

Soil samples from the sensor/ outlet-end of the channel were analyzed by dry combustion for TOC and TN using a TruSpec CN analyzer (LECO, St. Joseph, MI); C:N ratios were calculated from these measures. Inorganic N was determined using the Lachet QuickChem Method 12-107-04-1-B for nitrate (NO_3-N) determination (Appendix A) and the Lachet QuickChem Method 12-107-06-2-A for ammonium (NH_4-N) determination (Appendix B). Total soluble polyphenolics were determined using the Folin-Ciocalteau technique generally following Suominen et al. (2003). Soil extracts for this test were obtained by mixing 15 g soil with 15 ml of distilled water. This mixture was shaken for 8 hours

followed by 30-min centrifugation and the supernatants were filtered with Whatman #1 filter paper. The Folin-Ciocalteau test was conducted by mixing 2 ml distilled water, 2 ml soil extract, 5 ml Na_2CO_3 and 1 ml Folin-Ciocalteau reagent (Sigma-Aldrich). This mixture was incubated for 30 minutes at room temperature to allow for color development and absorbance was measured at 735 nm using a spectrophotometer. Tannic acid (reagent grade) was used as the standard.

Flood Treatments

Four flood treatments were evaluated: i) no flood (control), ii) three weeks of flowing water maintained at 15 cm depth (3WF), iii) five weeks of flowing water maintained at 15 cm depth (5WF), and iv) five weeks of stagnant water maintained at 15 cm depth (5WS). Flooding of all experimental channels was initiated on May 23, 2005; 3-week-flowing channels were drained on June 13th, and 5-week-flowing and 5-week-stagnant channels were drained on June 27th. Automated monitoring began with the flooding of the channels and continued until July 4th allowing for an assessment of the changes that occurred not only with the flooding but also with dry-down of the channels. The experimental design was a randomized complete block with three blocks arranged in a north-south direction; each block contained each of the four treatments.

Automated Monitoring

In order to automate the monitoring of changes in soil volumetric water content, temperature, redox potential, dissolved oxygen content, and pH in response to flood treatments, electronic sensors connected to dataloggers were installed in each channel in May, 2005. We installed three CR23X micrologger (Campbell Scientific, Logan, UT) stations

in waterproof enclosures, one between channels 2 and 3, one between channels 6 and 7 and one between channels 10 and 11. Each micrologger received signals from 4 channels and was connected to an AM16/32A 16 channel multiplexer. The multiplexer increased the capacity of the datalogger, allowing for connection of more sensors for possible expansion of the system. The microloggers were programmed such that measurements were taken every 30 seconds; readings were then averaged for the hour and recorded in the datalogger memory. The micrologger installed between channels 10 and 11 was also equipped to measure ambient air temperature and rainfall. A 107-L temperature probe, housed within a 6-plate radiation shield, was used to measure ambient temperature (Campbell Scientific, Logan, UT). The radiation shield reflects solar radiation, keeping the probe at or near ambient temperature. Precipitation was collected using a Texas Electronics 15.24 cm tipping bucket rain gage (0.0254 cm tip) (Campbell Scientific, Logan, UT). Sensors for the monitoring of soil volumetric water content, temperature, redox potential, dissolved oxygen content, and pH were installed near the outlet end of the channels. To protect the cables from rodents, cables were threaded through PVC pipe that was buried in berms separating the channels.

Soil volumetric water content was monitored at two depths (10 and 20 cm) using a water content reflectometer (Campbell Scientific, Logan, UT). This sensor which consisted of two 30 cm stainless steel rods connected to measurement electronics was buried parallel to the surface. The measuring range of volumetric water content was from 0% to saturation. Soil temperature was also monitored at two depths (10 and 20 cm). The probe selected for this parameter can be used to measure the temperature of a variety of media, including soil. It consists of a thermistor encapsulated in a cylindrical housing and is capable of measuring temperatures from -35 to +50°C.

Dissolved oxygen was measured at the soil/water interface using submersible galvanic dissolved oxygen sensors (Sensorex, Garden Grove, CA). This sensor was equipped with a zinc anode and a silver cathode (both internal) covered by a teflon membrane. The range of the sensor was 0-20 mg L^{-1} or 0 to 200% saturation. Sensor output was 1.65 mV per mg L^{-1} (+/- 0.45 mV) and this factor was used to convert mV to mg L^{-1} for reporting. Since this sensor was equipped with an in-line thermistor, temperature related conversions were unnecessary.

Soil redox potential and soil pH were measured at a depth of 5 cm using flat-surface, self-cleaning electrodes (models: S650CD-ORP and S650CD respectively; Sensorex, Garden Grove, CA). These sensors are designed for measurements in systems with high concentrations of suspended solids, making them also suitable for soil systems. Both electrodes are combination electrodes (ORP/reference or pH/reference) with double reference junctions; the ORP electrode has a platinum standard while the pH electrode is equipped with a pH-responsive flat glass surface. The ORP electrode has a range of +/- 2000 mV, and the pH electrode has a range of 0-14 pH. Due to the travel distance of the signal (approximately 90 m), both ORP and pH electrodes were attached to PHAMP-1 Battery-Powered pH/ORP pre-amplifiers (Sensorex, Garden Grove, CA) in order to amplify the signals. Replacement of the electrodes is facilitated by plug-in cable connections.

Prior to installation, both ORP and pH electrodes were tested to establish if the system was functioning properly. Tests were conducted to determine if the signal was reaching the datalogger and whether the electrode was providing accurate readings. An ORP standard solution and tap water were used to test the ORP electrodes. Electrodes with an Ag/AgCl reference should produce a reading of approximately 215 mV for this solution; tap water was tested to determine if the electrodes responded to changes in the system. The

ORP standard solution was also used to calibrate redox potentials to the standard hydrogen electrode. The pH electrodes were calibrated using pH 4 and pH 7 buffers.

Manual Monitoring

In addition to the automated monitoring system, manual measurements of soil redox, soil pH, and dissolved oxygen at the soil/water interface were taken twice weekly (Monday and Friday mornings) during the flood treatments and the post-flood recovery period. For the redox and pH monitoring, portable, waterproof Oakton 300 series meters (Cole-Parmer, Vernon Hills, IL) were used. These meters have a pH range of -2 to 16 pH with a resolution of 0.1/0.01 pH and an ORP millivolt range of +/- 2000 mV with a resolution of 0.1 to +/- 399.9 mV and a resolution of 1 mV when outside this range. Submersible, double junction, 90 cm pH and ORP electrodes were used to measure pH and ORP (models: 27001-80 and 27001-69 respectively; Cole-Parmer, Vernon Hills, IL). Manual monitoring of dissolved oxygen levels at the soil/water interface was conducted using the Oakton DO 300 (Cole-Parmer, Vernon Hills, IL). This is a waterproof, hand-held dissolved oxygen/temperature meter with a range of 0-19.99 mg L^{-1} and a resolution of 0.01 mg L^{-1}.

Data Analysis

The effect of flood treatment on soil chemical characteristics (TOC, TN, C:N ratio, NO_3-N, NH_4-N, and TSP) was evaluated using ANOVA (Proc GLM). One-way ANOVA was used for each sample date (pre- and post-flood) and depth (upper (0-10 cm) and lower (10-20 cm)) combination. T-tests were used to compare the pre- and post-flood samples for changes in these soil chemical characteristics.

Changes in soil parameters (volumetric water content, temperature, redox potential, dissolved oxygen content, and pH) over time due to flood treatments as monitored by the automated system and the manual approach were analyzed using ANOVA (Proc MIXED) with a repeated statement. This procedure represents a simplification of the generalized linear model (Proc GLM) but with a wider class of covariance structures and improved ability to handle missing values. The compound symmetry covariance structure was used for this analysis. Data were reported as least square means; comparisons of least square means were made using PDIFF ($\alpha = 0.05$).

The exact time of the manual readings was not recorded (i.e. all readings were taken before noon but the precise hour was not documented); therefore, direct comparisons between the manual and automated readings were not possible. Automated readings recorded between 7 am and noon on the days of the manual readings were averaged and 95% confidence intervals were calculated. Manual readings were examined to see if values were within these confidence intervals. Correlation and regression analysis (Proc CORR and Proc REG) were used to determine the association between average automated and manual sensor readings. All analyses were conducted using SAS 9.1 Statistical Software (SAS Institute Inc., 2002-2003).

Results

Soil Characterization

Thirty-six soil cores (each 120 cm long) were collected from the FTL channels for analysis. Of these, the ones that corresponded most closely with the electronic sensors are of most interest; therefore, only the characteristics of the upper 50-60 cm of soil for each of the 12 cores that were collected from the one-third of the channel closest to the outlet are

reported (Appendix C). Most soils were silt loam, although a few horizons were either silty clay loam or silty clay. The silt fraction generally increased with proximity to the creek while clay fraction generally decreased.

The upper 50-60 cm of each core had 3 delineated horizons of varying depths. All cores revealed evidence of plowing (i.e. all had an Ap horizon) and 8 of the 12 cores had Bg layers (gleyed condition) while 3 of the 12 channels had Bt layers (clay accumulations); channels with Bg layers were near the creek. Soil color varied little; most commonly the soils were dark and dull, identified as 10 YR 4/3 (brown/dark brown), 10 YR 4/2 (dark grayish brown) or 10 YR 4/1 (dark gray). Masses of oxidized iron (Fe^{3+}) accumulation were the most common redoximorphic feature; these masses could be found in all layers of all cores analyzed. Iron depletions, iron-manganese concretions and masses of manganese accumulations were also present.

Soil pH, CEC and base saturation varied across the channels. Each of these was slightly higher in the channels adjacent to the road, possibly because it was limestone-covered, and decreased with increasing proximity to the stream. Total organic carbon (TOC) was consistently low across the channels.

Soil Nutrient Analysis

Type of flood treatment (i.e. duration and/or condition (stagnant vs flowing)) had no effect on the TOC, TN or C:N ratio of the soils in the FTL at either sampling depth (Tables 2.1-2.3). However, pre- vs post-flood comparisons using T-tests revealed changes in some of these characteristics over time (Table 2.4). When upper and lower samples were pooled for each flood treatment, TOC decreased in the control treatment over time. Pooling of all lower samples revealed a decrease in TOC; this result was also observed when all samples

were pooled (Table 2.4). The C:N ratio decreased in the upper (0-10 cm) sample for the 3WF treatment and in the lower (10-20 cm) sample for the 5WF treatment. Pooling of upper and lower samples revealed a significant decrease in C:N ratio the 3WF treatment. Pooling of all lower samples revealed a decrease in C:N ratio; this result was also observed when all samples were pooled (Table 2.4). Pre- vs post-flood comparisons however revealed no differences for TN over time (Table 2.4).

Table 2.1. Means and standard deviations for TOC by sample date and depth for each treatment for soil samples from the FTL. Overall averages summarize all sample dates and depths for a particular flood treatment. ANOVA results were not significant. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant.

	Pre-Flood 0-10 cm		**Pre-Flood 10-20 cm**		**Post-Flood 0-10 cm**		**Post Flood 10-20 cm**		**Overall Average**	
	Mean %	**SD**	**Mean %**	**SD**	**Mean %**	**SD**	**Mean %**	**SD**	**Mean %**	**SD**
3WF	1.21	0.10	0.98	0.13	1.15	0.05	0.99	0.15	1.08	0.14
5WF	1.25	0.11	1.16	0.05	1.27	0.09	1.06	0.11	1.19	0.17
5WS	1.23	0.24	1.10	0.21	1.20	0.23	1.03	0.18	1.14	0.21
Control	1.26	0.16	1.11	0.09	1.16	0.21	1.06	0.10	1.14	0.15

Table 2.2. Means and standard deviations for TN by sample date and depth for each treatment for soil samples from the FTL. Overall averages summarize all sample dates and depths for a particular flood treatment. ANOVA results were not significant. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant.

	Pre-Flood 0-10 cm		**Pre-Flood 10-20 cm**		**Post-Flood 0-10 cm**		**Post Flood 10-20 cm**		**Overall Average**	
	Mean %	**SD**	**Mean %**	**SD**	**Mean %**	**SD**	**Mean %**	**SD**	**Mean %**	**SD**
3WF	0.13	0.01	0.11	0.02	0.13	0.002	0.11	0.02	0.12	0.01
5WF	0.13	0.01	0.12	0.003	0.14	0.01	0.12	0.01	0.13	0.01
5WS	0.13	0.02	0.12	0.03	0.13	0.03	0.11	0.01	0.12	0.02
Control	0.13	0.01	0.12	0.01	0.12	0.03	0.12	0.01	0.12	0.02

Table 2.3. Means and standard deviations for C:N ratio by sample date and depth for each treatment for soil samples from the FTL. Overall averages summarize all sample dates and depths for a particular flood treatment. ANOVA results were not significant. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant.

	Pre-Flood 0-10 cm		Pre-Flood 10-20 cm		Post-Flood 0-10 cm		Post Flood 10-20 cm		Overall Average	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
3WF	9.48	0.41	9.07	0.28	8.89	0.31	8.72	0.27	9.04	0.40
5WF	9.36	0.84	9.38	0.12	9.33	0.15	8.75	0.16	9.21	0.46
5WS	9.63	0.54	9.38	0.28	9.11	0.34	9.18	0.57	9.33	0.44
Control	9.58	0.49	9.11	0.34	9.53	0.65	8.63	0.15	9.21	0.55

Table 2.4. T-test results for TOC, TN and C:N ratio for soil samples from the FTL. Positive t-values indicate an increase in the variable over time, while negative t-values indicate a decrease in the variable overtime. T-values significant at $\alpha < 0.05$ level are highlighted in bold. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant, "upper" = samples from 0-10 cm depth, and "lower" = samples from 10-20 cm depth.

		TOC		TN		C:N ratio	
		t value	**p**	**t value**	**p**	**t value**	**p**
Control	**Upper**	-2.44	0.13	-0.78	0.52	-0.08	0.94
	Lower	-1.11	0.38	0.10	0.93	-2.77	0.11
	Both	**-2.54**	**0.05**	-0.63	0.56	-0.89	0.42
3WF	**Upper**	-1.58	0.25	0.70	0.55	**-5.97**	**0.03**
	Lower	0.32	0.78	0.93	0.45	-1.18	0.36
	Both	-0.99	0.37	1.24	0.27	**-3.14**	**0.03**
5WF	**Upper**	0.20	0.86	0.25	0.82	-0.06	0.95
	Lower	-2.43	0.14	-0.56	0.63	**-11.92**	**0.007**
	Both	-0.75	0.48	-0.11	0.92	-1.15	0.30
5WS	**Upper**	-0.76	0.53	0.68	0.57	-1.28	0.33
	Lower	-3.81	0.06	-0.82	0.50	-0.41	0.72
	Both	-2.28	0.07	-0.17	0.87	-1.24	0.27
All	**Upper**	-1.51	0.16	-0.04	0.97	-1.41	0.19
All	**Lower**	**-2.73**	**0.02**	-0.31	0.76	**-3.07**	**0.01**
All	**Both**	**-2.82**	**0.01**	-0.23	0.82	**-2.88**	**0.01**

There were differences in NO_3-N in the post-flood samples at the lower sampling depth (10-20 cm) (Table 2.5). The control treatment had significantly higher levels of NO_3-N than the flood treatments post-flood, but the flood treatments were not significantly different. Pre- vs post-flood comparisons using t-tests revealed a significant increase in NO_3-N in the control channels when upper and lower samples were combined (Table 2.6).

Table 2.5. Means and standard deviations for NO_3-N by sample date and depth for each treatment for soil samples from the FTL. Overall averages summarize all sample dates and depths for a particular flood treatment. Means with the same letter are not significantly different at $\alpha = 0.05$. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant.

	Pre-Flood 0-10 cm		**Pre-Flood 10-20 cm**		**Post-Flood 0-10 cm**		**Post Flood 10-20 cm**		**Overall Average**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
3WF	1.56	0.38	2.53	0.84	8.02	9.56	2.50 **b**	0.72	3.65	4.90
5WF	3.69	4.07	3.98	1.58	1.71	0.64	2.30 **b**	0.60	2.93	2.14
5WS	11.55	9.03	5.71	3.01	1.64	0.56	1.93 **b**	1.04	5.21	5.84
Control	1.11	0.16	2.27	0.73	6.94	4.27	4.57 **a**	0.99	3.72	3.01

Table 2.6. T-test results for NO_3-N, NH_4-N and TSP for soil samples from the FTL. Positive t-values indicate an increase in the variable over time, while negative t-values indicate a decrease in the variable overtime. T-values significant at $\alpha < 0.05$ level are highlighted in bold. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant, "upper" = samples from 0-10 cm depth, and "lower" = samples from 10-20 cm depth.

		NO_3-N		NH_4-N		TSP	
		t value	p	t value	p	t value	p
Control	**Upper**	2.44	0.13	2.14	0.17	-1.68	0.23
	Lower	2.98	0.10	2.54	0.13	-1.88	0.20
	Both	**2.96**	**0.03**	**2.93**	**0.03**	**-2.79**	**0.04**
3WF	**Upper**	1.20	0.35	1.50	0.27	-3.89	0.06
	Lower	-0.06	0.96	0.11	0.92	-0.90	0.46
	Both	1.14	0.31	0.82	0.45	**-2.66**	**0.05**
5WF	**Upper**	-1.00	0.42	3.18	0.09	0.14	0.90
	Lower	-2.31	0.15	**19.97**	**0.003**	0.44	0.70
	Both	-1.93	0.11	**5.68**	**0.002**	0.35	0.74
5WS	**Upper**	-1.97	0.19	3.35	0.08	0.37	0.74
	Lower	-1.61	0.25	1.63	0.25	**-4.63**	**0.04**
	Both	-2.42	0.06	**3.24**	**0.02**	-0.24	0.82
All	**Upper**	0.04	0.97	**5.07**	**0.0004**	-1.11	0.29
All	**Lower**	-0.91	0.38	**2.87**	**0.02**	**-2.21**	**0.05**
All	**Both**	-0.25	0.80	**5.41**	**<0.0001**	**-2.10**	**0.05**

Flood treatment type did not affect NH_4-N levels (Table 2.7); but pre- vs. post-flood analysis revealed changes in NH_4-N over time (Table 2.6). Ammonium increased in lower sampling depth (10-20 cm) for the 5WF flood treatment. Pooling of upper and lower samples revealed an increase in NH_4-N for the control and both of the 5-week flood treatments. No significant changes were observed in the 3WF treatment. There was a general trend of NH_4-N increasing in both soil depths with flooding.

Table 2.7. Means and standard deviations for NH_4-N by sample date and depth for each treatment for soil samples from the FTL. Overall averages summarize all sample dates and depths for a particular flood treatment. ANOVA results were not significant. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant.

	Pre-Flood 0-10 cm		**Pre-Flood 10-20 cm**		**Post-Flood 0-10 cm**		**Post Flood 10-20 cm**		**Overall Average**	
	Mean	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**	**Mean**	**SD**
	mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}		mg kg^{-1}	
3WF	10.55	2.08	11.35	6.35	13.68	4.44	11.83	1.92	11.85	3.71
5WF	9.61	3.70	7.54	0.90	18.29	2.83	13.94	1.42	12.34	4.81
5WS	7.59	1.32	10.43	3.99	17.30	3.98	14.52	0.50	12.46	4.62
Control	8.81	0.95	8.33	0.91	16.17	5.11	11.90	3.34	11.30	4.21

ANOVA revealed flood treatment type differences for TSP in the post-flood samples at the upper sampling depth (0-10 cm) (Table 2.8). The control and the 3WF treatments were not different from each other; likewise the 5-week flood treatments were not different from each other. The 5-week flood treatments had higher levels of TSP than the control and the 3WF treatments. Pre- and post-flood analysis also revealed differences (Table 2.6). Decreases in TSP were observed for the 5WS treatment at the lower sampling depth (10-20 cm). Pooling of upper and lower samples revealed a decrease in TSP for the control and the 3WF treatments. Pooling of all lower (10-20 cm) samples revealed a decrease in TSP. There was a general trend of TSP decreasing over time.

Table 2.8. Means and standard deviation for TSP (μg TA g^{-1} soil) by sample date and depth for each treatment for soil samples from the FTL. Overall averages summarize all sample dates and depths for a particular flood treatment. Means with the same letter are not significantly different at $\alpha = 0.05$. Note: 3WF = 3-week flowing, 5WF = 5-week flowing, 5WS = 5-week stagnant

	Pre-Flood 0-4 Depth		**Pre-Flood 4-8 Depth**		**Post-Flood 0-4 Depth**		**Post Flood 4-8 Depth**		**Overall Average**	
	Mean μg TA g^{-1} soil	**SD**	**Mean** μg TA g^{-1} soil	**SD**	**Mean** μg TA g^{-1} soil	**SD**	**Mean** μg TA g^{-1} soil	**SD**	**Mean** μg TA g^{-1} soil	**SD**
3WF	3.54	0.91	1.97	1.95	1.87 **a**	0.29	1.46	1.61	2.21	1.42
5WF	4.79	1.05	1.81	0.47	4.96 **b**	1.12	2.06	0.82	3.41	1.72
5WS	4.28	1.32	2.06	0.60	4.92 **b**	1.74	1.01	0.28	3.07	1.93
Control	4.63	2.19	2.51	1.30	2.74 **a**	0.48	0.74	1.13	2.65	1.88

Volumetric Water Content

Soils within the flooded channels became saturated soon after experimental treatments began (Figure 2.1). Once these soils were saturated, water content varied little for each treatment over time, decreasing only slightly with removal of flood treatments. Differences in soil water content were observed between the two depths; these differences were likely caused by variation in bulk density due to changes in pore volume or soil texture with depth. Soil texture, in particular smectitic clay content, has been shown to affect the accuracy of water content reflectometers (Udawatta, et al. 2005). Calibration curves have been developed to correct for smectitic clay content (Udawatta, et al. 2005); however, these curves were not applied to the results reported here.

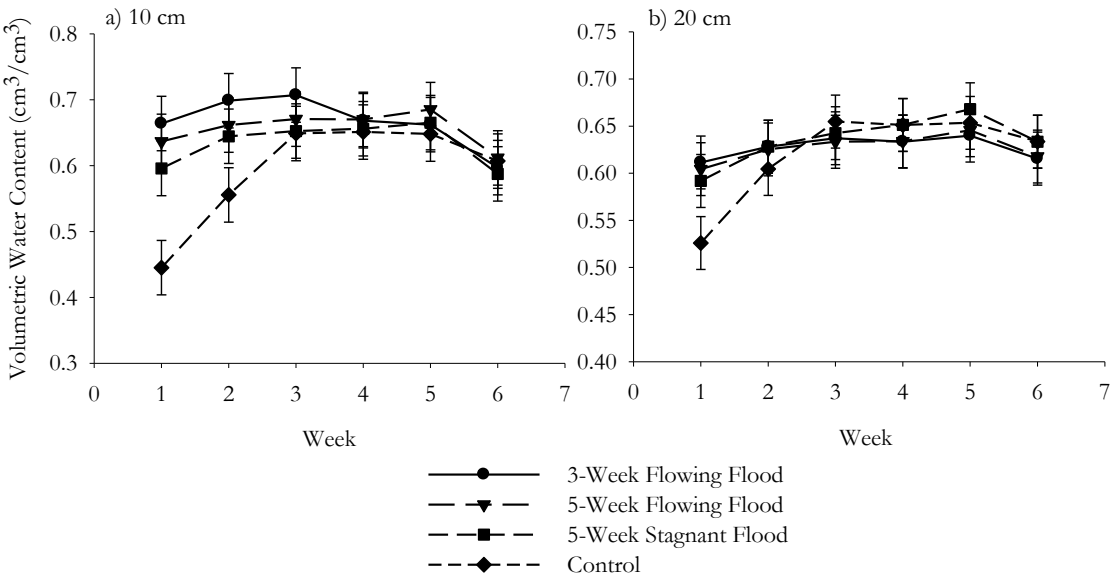

Figure 2.1. Soil volumetric water content at 10 cm (a) and 20 cm (b) by treatment measured by automated sensors for a 6 week period (May 23th – July 4th 2005). Weekly averages for each treatment were calculated by combining hourly data from replicate channels; error bars represent ± SE.

Soils from the flooded channels had significantly higher volumetric water content than the control channels during the first week of the experiment. During this period, the water content of the flooded channels was 0.14-0.21 cm^3 cm^{-3} higher than the control channels at 10 cm and 0.07-0.09 cm^3 cm^{-3} higher at 20 cm. Differences were less pronounced during the second week, with the water content of the flooded channels 0.09-0.14 cm^3 cm^{-3} higher than the control channels at 10 cm and 0.02 cm^3 cm^{-3} higher at 20 cm. By week 3 of flooding, differences between the control and flooded channels were absent regardless of depth (Figure 2.1). The increase in soil water content of the control channels at week 3 may be attributed to a period of abundant rainfall (June 3-13th) (Figure 2.2a) or to seepage from adjacent flooded channels. Since increases in soil water content in the control channels were observed between week 1 and 2 when rainfall was slight, seepage from

adjacent channels cannot be ruled out. Control channels were notably moist by the termination of the experiment; for example, indentations in the soil created from walking in the channels would slowly fill with water. There were significant main effects (flood treatment and time) but no significant interaction for soil water content at 10 cm (flood treatment: $F= 3.75$, $P < 0.02$; time: $F= 3.02$, $P < 0.02$). Soil water content varied significantly over time at 20 cm ($F=3.06$, $P <0.02$), but there was no significant flood treatment effect or flood treatment × time interaction.

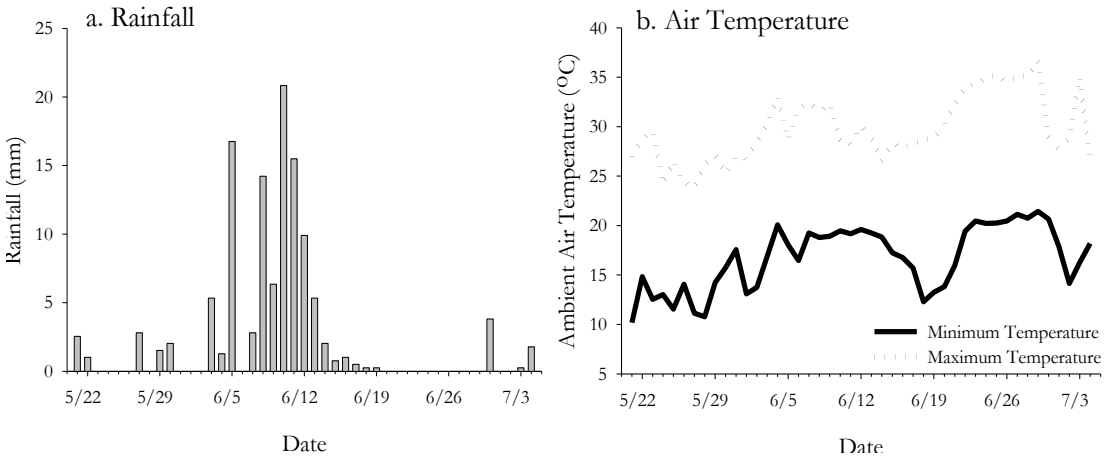

Figure 2.2. Rainfall (a) and ambient air temperature (b) for 6 week period (May 22nd – July 4th 2005). Automated sensors recorded data every 30 seconds and averaged hourly.

Soil and Air Temperature

Ambient air temperatures gradually increased over the monitoring period (Figure 2.2b). Likewise, soil temperatures at both depths (10 cm and 20 cm) gradually increased over the course of the experiment (Figure 2.3). Since the experiment spanned 6 weeks beginning in late May and ending in early July, this increasing trend was not surprising.

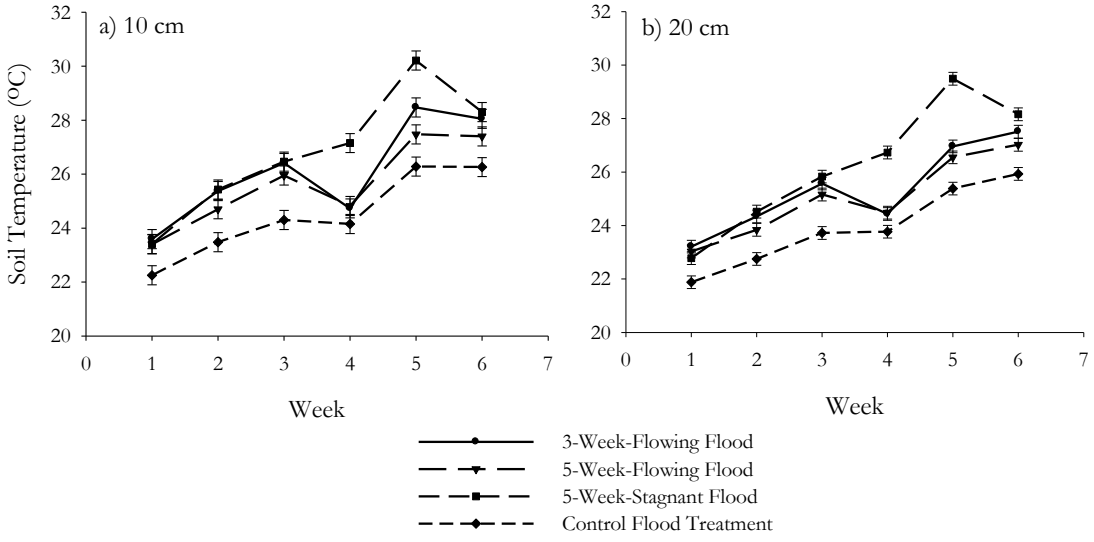

Figure 2.3. Soil temperature at 10 cm (a) and 20 cm (b) by treatment measured by automated sensors for a 6 week period (May 23th – July 4th 2005). Weekly averages for each treatment were calculated by combining hourly data from replicate channels; error bars represent ± SE.

Regardless of depth, soils in the control channels were slightly cooler than soils in the flooded channels (Figure 2.3). A combination of factors contributed to higher soil temperatures in the flooded channels. The flooded channels had darker surfaces because they supported less vegetation than the controls, presumably causing the flooded channels to absorb more solar radiation and to become warmer. Also, the higher specific heat of the flooded channels caused them to retain more heat during the night.

Soil temperature data for the 3WF and the 5WS treatments were similar at both depths early in the recording period, while the 5WF treatment exhibited slightly lower temperatures. At week 4, temperatures in the flowing treatments decreased while the 5WS temperatures remained consistent with the general trend of increasing soil temperature readings. The observed drop in temperature for the flowing channels may be related to the

heavy rains recorded during the previous 10 days (Figure 2.2a). Soils in channels under the flowing flood treatments would be expected to be cooler than those under stagnant flood treatments because of dissipation of energy due to water movement. ANOVA results revealed a significant flood treatment × time interaction for soil temperature at both depths (10 cm: $F=2.03$, $P < 0.04$; 20 cm: $F=4.91$, $P <0.0001$); both main effects were also significant at both depths.

Soil Redox Potential

In general, redox levels decreased after initiation of flooding and increased following dry-down of the channels (Figure 2.4). In the control channels, the average redox levels remained relatively constant over time (Figure 2.4a). Average redox levels recorded with the automated sensors ranged from 217-287 mV over the course of 6 weeks, while those recorded with handheld meters were considerably higher and ranged from 405-530 mV (except approximately 3 weeks into the experiment). The differences between the automated and manual readings were notable because the automated sensor readings for the control channels were within the "suboxic" range, indicating that the soils of these channels were neither anaerobic nor well-oxygenated. However, manual readings for these channels generally fell within the oxic range, indicating well-oxygenated soils.

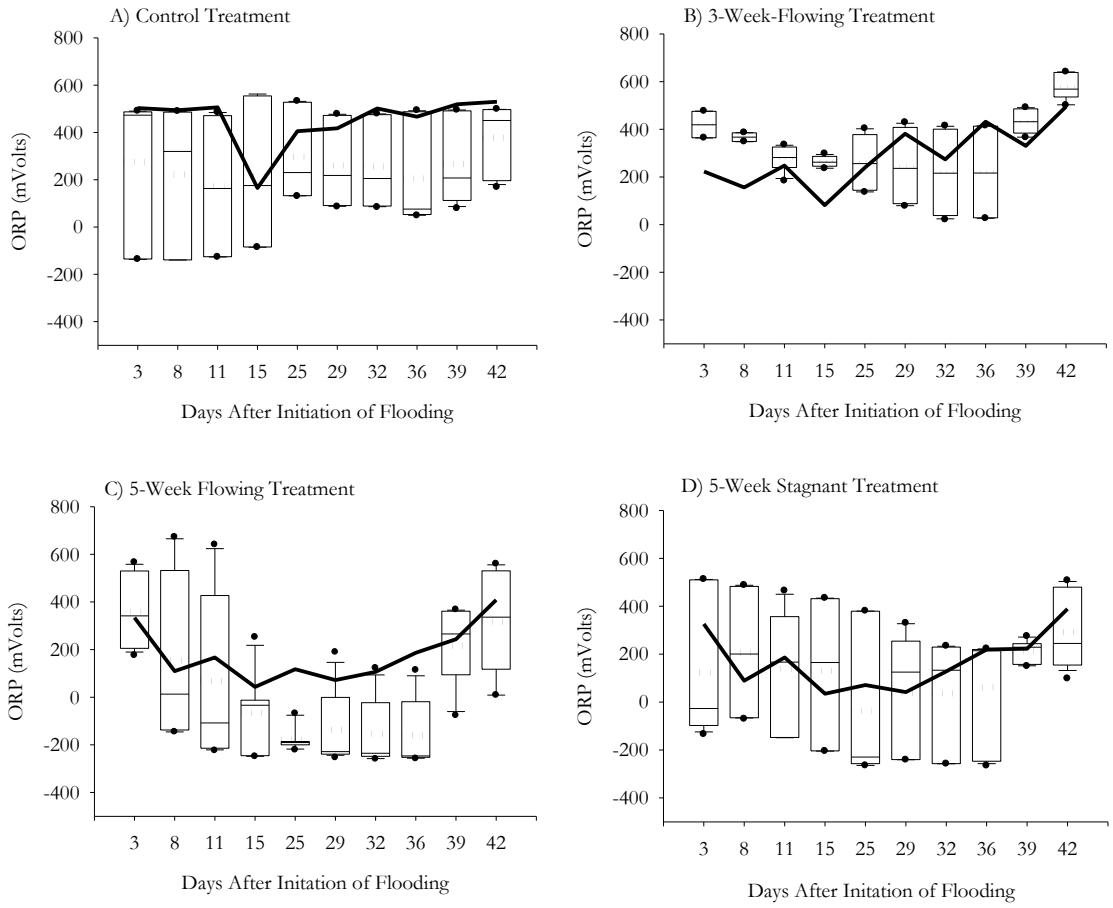

Figure 2.4. Soil redox potential measured by the automated sensors (box plots) and manual sensors (line) for a 6 week period (May 26th – July 4th 2005). For automated sensors, weekly averages were calculated from hourly values between 7 am and 12:00 pm from replicate channels. For replicate channels, the boxes depict the 25th and 75th percentile, the whiskers indicate 10th and 90th percentiles, the solid line represents the median, and the dotted line represents the mean. Manual readings were measured once during the period sampled by the automated sensors; data points represent the average reading for replicated channels under the designated treatments.

Average redox potentials for 3WF treatment were lower than the control channels (Figure 2.4b). Initial average redox potentials revealed suboxic conditions in soils under this treatment regards of monitoring method (automated average = 167 mV; manual average = 223 mV). Redox potentials decreased to their lowest points during the second and third week of the flood (lowest automated average = 58 mV; lowest manual average = 81.5 mV); and anoxic conditions occurred during this time period. With the removal of the flood treatment at the end of week 3/ beginning of week 4, the redox levels increased; however, this increase was not consistent and some fluctuation occurred. At termination of the experiment, automated readings indicated suboxic soils (average reading = 155 mV), while manual readings indicated oxic soils (average reading = 518 mV). As was the case with the control channels, the manual readings were higher than those recorded by the automated sensors.

As expected, the lowest redox potentials were recorded and anoxic conditions were observed in the 5WS and 5WF channels (Figure 2.4c & 2.4d). The manual readings for these treatments were similar and considerably higher than the automated readings (Figure 2.4c and 2.4d). The average manual readings ranged from 326-334 mV and decreased to 34-43 mV during the first two weeks of flooding; at termination manual readings ranged from 506-539 mV. Average automated readings for the 5WS treatment decreased from 301 mV to 24 mV by the fourth week and increased to 156 mV at the termination of monitoring (Figure 2.4c). The automated readings in the 5WF treatment were lower than in the 5WS treatment. Redox readings for the 5WF treatment decreased from 326 mV to -173.68 by the fourth week and subsequently increased to 120 mV at the termination of monitoring (Figure 2.4d).

Redox levels for the automated sensors did not differ due to flood treatment, time or flood treatment × time interaction. The manual sensors, however, showed a significant

flood treatment × time interaction ($F = 1.7$; $P < 0.03$) as well as significant main effects (flood treatment: $F=38.08$, $P<0.0001$, time: $F=16.98$, $P <0.0001$). Nearly every manual reading for redox potential fell outside the calculated confidence intervals, indicating a significant difference in the two methods of data collection. Pearson correlation coefficients were generally low with the exception of the 5WS comparison (Table 2.9). Automated and manual readings for other flood treatments were not correlated.

Table 2.9. Pearson correlations between ORP measurements using automated and manual ORP systems with different flood treatments.

Flood Treatment	N	r^2	P value
3-Week-Flowing	10	0.20	0.20
5-Week-Flowing	10	0.33	0.08
5-Week-Stagnant	10	0.63	**0.01**
Control	10	0.07	0.47

Dissolved Oxygen

Dissolved oxygen readings differed between the control soils and the flooded soils. Dissolved oxygen levels in control channels averaged 12-14 mg L^{-1}, with the exception of weeks 2 and 3 where dissolved oxygen fell to 7.21 mg L^{-1} and 9.81 mg L^{-1} respectively (Figure 2.5). Dissolved oxygen readings for the flooded channels averaged 1.77-5.90 mg L^{-1}. Similar to the control channels, the lowest dissolved oxygen readings for the flooded channels occurring during weeks 2 and 3. ANOVA results indicated a significant treatment effect ($F = 12.28$, $P <0.0001$), however time and the flood treatment × time interaction were not significant. Although dissolved oxygen levels in the flooded channels did not vary

notably by treatment, the 5WF treatment had consistently lower dissolved oxygen readings than the other flood treatments (Figure 2.5).

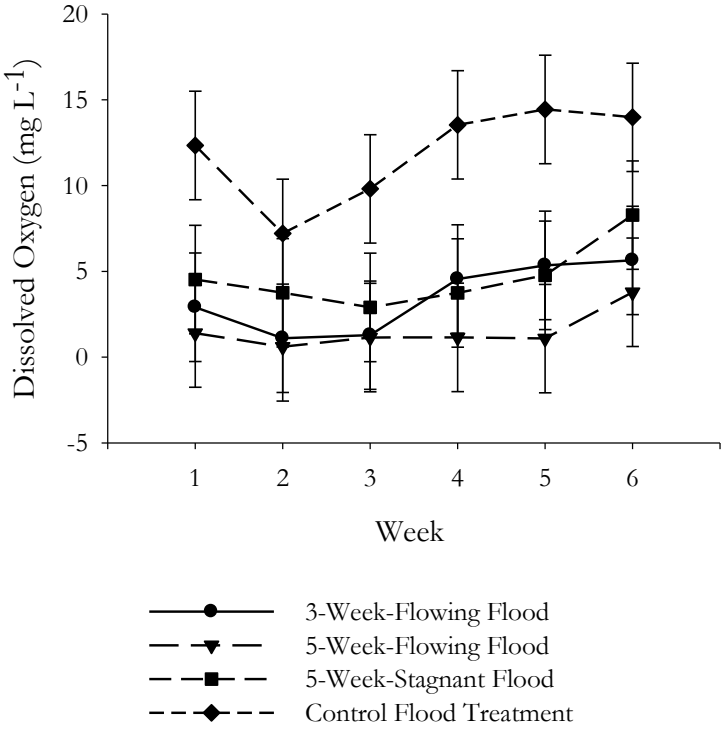

Figure 2.5. Dissolved oxygen content by treatments measured by automated sensors for a 6 week period (May 23th – July 4th 2005). Weekly averages for each treatment were calculated by combining hourly data from replicate channels; error bars represent ± SE.

Manual monitoring of dissolved oxygen occurred at the soil/water interface, providing a measure of flood water (as opposed to soil) dissolved oxygen content. Since this reading could be obtained only in flooded channels, comparisons are limited to the 5-week flood treatments. Although the dissolved oxygen content of the 5WF flood water is slightly higher than that of the 5WS flood water (Figure 2.6), this difference is not significant (F=

3.87, P = 0.07). Flood water dissolved oxygen content for both the 5WF and 5WS treatments decreased over the course of the monitoring period. Initial DO readings were 7.62 mg L^{-1} and 6.37 mg L^{-1} respectively, while final readings were 3.10 mg L^{-1} and 4.25 mg L^{-1} respectively. These data constitute a significant flood treatment x time interaction (F = 2.89, P = 0.04) and a significant time effect (F = 8.15, P < 0.001).

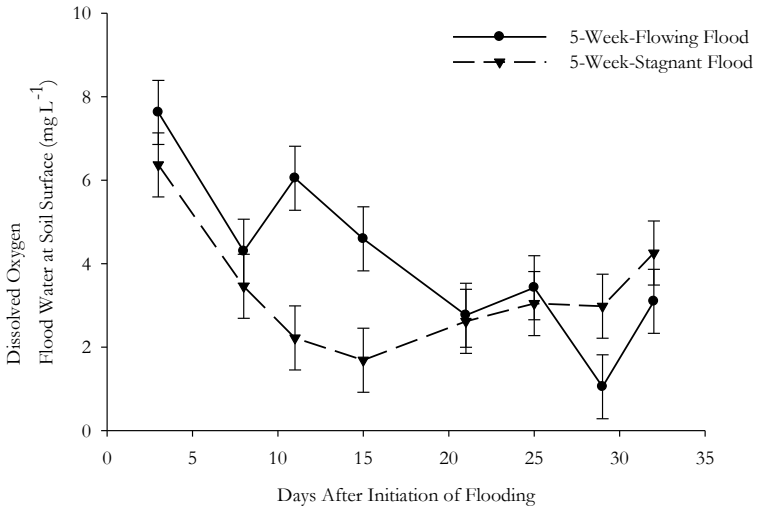

Figure 2.6. Dissolved oxygen content of the flood water at the soil/water interface for the 5-week flood treatments as measured by manual sensors. Sampling began on May 26th, 3 days after the initiation of flooding and continued until June 24th, 3 days prior to the removal of the flood water. Data points represent average readings for replicate channels for each treatment; error bars represent ± 1 standard deviation.

Soil pH

Preliminary examination of the automated pH data revealed unexpected treatment effects for several channels. Flooding should result in the convergence of pH values to neutrality (i.e. pH between 6.7 and 7.2) (Mitsch and Gosselink, 2000); however, in some cases, increases in pH to values ≥8 were observed. In addition, anomalous values were detected in several of the channels; these anomalies included negative pH values as well as pH values > 14. The anomalous data, i.e. all pH readings < 4 or > 8, were removed from the data set prior to analysis.

No general convergence toward neutrality was observed for this system regardless of sensor type (Figure 2.7). In fact, no trends in pH with flood treatment or over time were obvious. Both manual and automated readings of pH showed high variability; of note is the wide range of values recorded by the automated sensors (Figure 2.7). Mean pH varied little from treatment to treatment or from week to week.

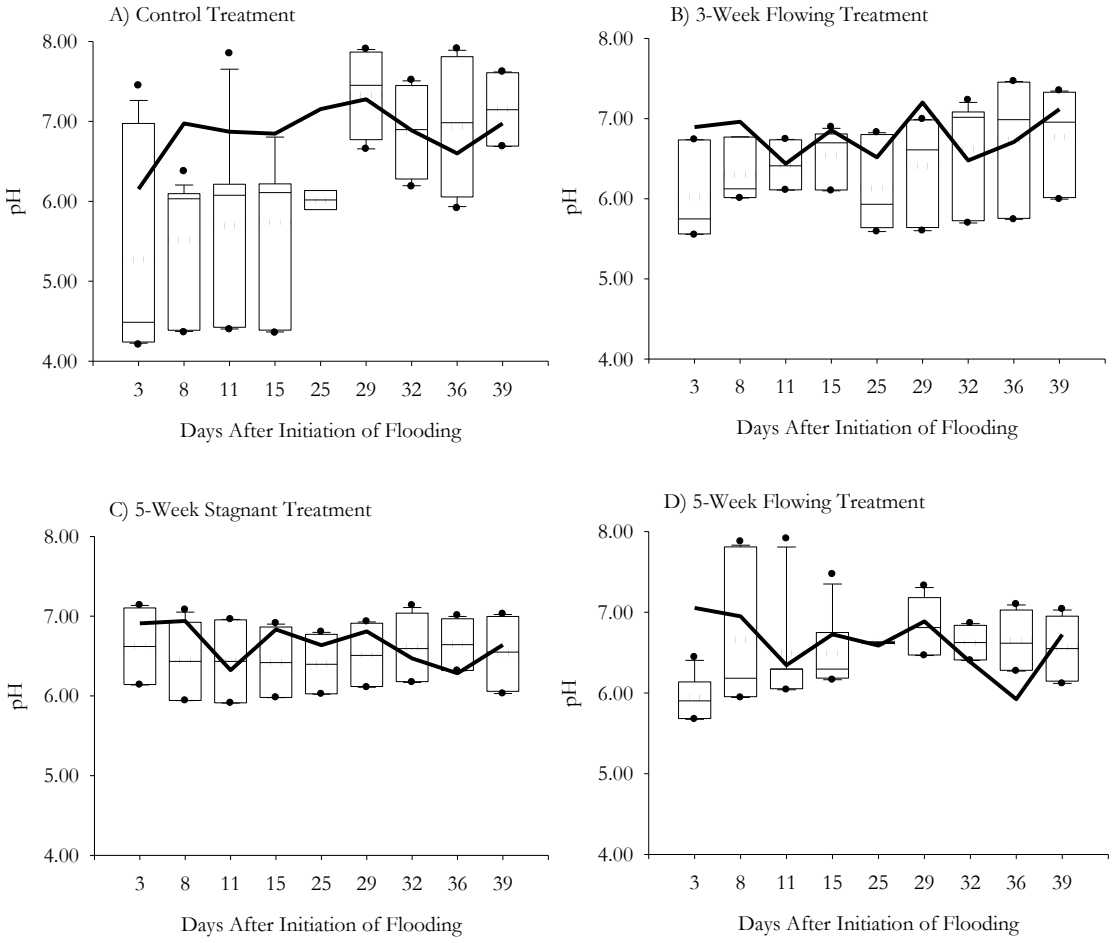

Figure 2.7. Soil pH measured by the automated sensors (box plots) and manual sensors (line) for a 6 week period (May 26th – July 4th 2005). For automated sensors, anomalous data points were removed (i.e. pH readings < 4 or > 8) then weekly averages were calculated from hourly values between 7 am and 12:00 pm from replicate channels. For replicate channels, the boxes depict the 25th and 75th percentile, the whiskers indicate 10th and 90th percentiles, the solid line represents the median, and the dotted line represents the mean. Manual readings were measured once during the period sampled by the automated sensors; data points represent the average reading for replicated channels under the designated treatments.

ANOVA results for the automated sensor data revealed no significant differences in pH due to flood treatment, time or flood treatment × time interaction. However, ANOVA results for the manual sensor data revealed a significant effect of time ($F = 2.99$; $P < 0.01$), but there was no significant differences among flood treatments and no interaction effect. Nearly every manual pH reading fell outside the calculated confidence intervals, indicating a significant difference in the two methods of data collection. Pearson correlation coefficients were generally low (Table 2.10), indicating that these measures were not correlated with each other.

Table 2.10. Pearson correlations between pH measurements using automated and manual pH systems with different flood treatments.

Flood Treatment	N	r^2	P value
3-Week-Flowing	9	0.01	0.83
5-Week-Flowing	9	0.10	0.40
5-Week-Stagnant	9	0.25	0.17
Control	9	0.21	0.22

Discussion

Soil Characterization

Examination of the soil profile may provide clues as to the reduced conditions of the soil. The soil solution of reduced soils will have reduced forms of O, N, Mn, Fe or S, which will be manifested in morphological features, such as soil color patterns, soil color changes, and odors (Vepraskas, 2001). For example, when Fe is reduced it becomes colorless, soluble and mobile within the soil. As the Fe moves off the ped surfaces, the color of the sand and clay is revealed and the soil appears grey (value >4, chroma <2) (Vepraskas, 2001).

Overwhelmingly, soil in the FTL channels exhibited gleyed colors. The soils were dark and dull, identified as 10 YR 4/3 (brown/dark brown), 10 YR 4/2 (dark grayish brown) or 10 YR 4/1 (dark gray). Another common feature, found in nearly every horizon of every soil core was masses of oxidized iron (Fe^{3+}) accumulation. Accumulation of Fe^{3+} occurs when Fe moves into an area, is oxidized and then precipitates; these accumulations created a mottle appearance in the soil, with the mottle colors varying (any shade of red, orange, yellow or brown is possible) with type of Fe present (Vepraskas, 2001). Presence of these colors and features indicated that the soil has been reduced. Since the time required to form such features is variable and depends on factors such as soil temperature and % organic matter (Vepraskas, 2001), it is unknown whether the current flood treatments have resulted in the patterns observed or if these patterns are relict features from flooding that occurred prior to the construction of the facility.

Soil Nutrients

In general, soil NO_3-N concentrations decreased and NH_4-N concentrations increased with flooding. These trends were expected based on the assumption that flooding results in anaerobic soil conditions. Under anaerobic conditions, three main N transformations occur: i) ammonification (the conversion of organic N into NH_4-N), ii) nitrate reduction (conversion of NO_3-N into NH_4-N), or iii) denitrification (conversion of NO_3-N into N_2 or other N gases). Under flooded conditions, NH_4-N is therefore produced (ammonification and nitrate reduction) while NO_3-N is lost (nitrate reduction and denitrification). Nitrate, with its negative charge is a more mobile form of N and thus it can also be leached from the system under flooded conditions. These trends (a decrease in NO_3-N and an increase in NH_4-N) were strongest in the 5WF and 5WS treatments; however, no

differences were observed between the flowing and stagnant treatments indicating that flowing conditions did not effectively change the anaerobic status of these soils.

The control flood treatment showed an increase in both NO_3-N and NH_4-N. The observed increase in NH_4-N is contrary to what is expected based on the observed redox potentials of these soils (soils remained in the suboxic to oxic range). Ammonification will occur under aerobic conditions, but nitrification (the conversion of NH_4-N into NO_3-N) is the more dominant process in well-aerated soils. However, soil volumetric water content in the control channels increased over the course of the study period, and the observed increases in NH_4-N may be related to these increases in soil water content and subsequent decreases in soil oxygen content.

Loss of N from the soil or a shift in the type of inorganic-N found in the soil with inundation has been reported in other studies. For example, Lockaby et al. (1996) reported a general loss of N under flooding treatments; the greatest loss of N occurred under continuous flooding for three months, however, all flood treatments resulted in a loss of N relative to the control. Hefting et al. (2004) demonstrated that the height of the water table can affect the outcome of N mineralization. In their study, when the water table was within 10 cm of the soil surface, ammonification was the primary N mineralization reaction taking place while net nitrification was insignificant. Water table fluctuations due to cycles of inundation and drying allow aerobic and anaerobic N conversions to occur in close proximity to one another; this may in turn result in the large removal of N from riparian soils due to denitrification processes. For example, Reddy and Patrick (1975) observed a greater loss of N under treatments that alternated between aerobic and anaerobic conditions every two days as opposed to treatments with longer cycles or non-fluctuating conditions (i.e. complete aerobic or completely anaerobic). Completely aerobic conditions resulted in

the accumulation of NO_3-N, while NH_4-N accumulated under complete anaerobic conditions. In all of the alternating aerobic-anaerobic treatments, except the 2 day cycle, all inorganic-N was present as NH_4-N (Reddy and Patrick 1975). While changes in TN were not observed in the current study, changes in the relative amounts of NO_3-N and NH_4-N were observed. These changes in inorganic-N are similar to observations reported by others (Reddy and Patrick, 1975; Hefting et al., 2004).

Investigations in rice systems have shown an increase in phenolic compounds with intensified cropping (Olk et al., 1996; Olofsdotter et al., 2002) or with incorporation of plant residue materials (Tsutsuki and Ponnamperuma, 1987). In the current study, TSP content was affected by flood treatment with higher levels observed in the 5WF and 5WS floods than in the control and the 3WF flood. Pre- vs post-flood comparisons revealed decreases in TSP levels in the control and 3WF treatment, while TSP levels in the 5WF and 5WS floods remained relatively unchanged. Similar results were discussed by Olofsdotter et al. (2002). In their study, the phenolic acid content of irrigated soils under rice was considerably higher than that of upland soils. Olofsdotter el al. (2002) reasoned that this difference was due to reduced degradation of phenolic acids under anaerobic conditions. In the case of the soils of the FTL, there is not an accumulation of phenolic acids as much as an apparent decrease in phenolic acid decomposition.

Accumulation of phenolic acids in the soil may have an impact on plant available N and subsequently on net primary productivity. Polyphenolic compounds are known to bind to N, rendering it unavailable for microbial conversion or plant uptake (Gaunt et al., 1995; Inderjit and Mallik, 1997; Kruse et al., 2004; Schmidt-Rohr et al., 2004). For example, Inderjit and Mallik (1997) observed lower available N in soils amended with different phenolic compounds compared to an unamended control soil. Likewise, Schmidt-Rohr et al.

(2004), in their study of triple-cropped rice soil, found a significant fraction of N bonded to aromatic carbons. However, while Olk et al. (1996) did observe yield declines in rice systems that were associated with decreased effective soil N, they were unable to link this decreased N availability to the accumulation of phenolic compounds in the soil under field conditions. Phenolic acids may either degrade quickly under field conditions or may be leached from the system (Olofsdotter et al. 2002). Therefore, the effects of increased phenolic compounds on nutrient availability under flooded conditions may be dependent on the residence time of the phenolic compounds in the soil (Inderjit and Mallik 1997); thus these effects may be transient.

Soil Redox Potential

The overall patterns of observed redox potential response to different flood treatments were similar to those described in other systems. For example, Austin and Huddleston (1999) observed that in non-saturated soils, the redox potential fluctuated in response to rainfall; likewise, Schuur and Matson (2001) observed a dramatic decrease in redox potential with increased precipitation. While redox potentials in the control channels were relatively constant over the course of the experimental period, with manual monitoring we detected a decline in redox potential during a time of heavy rainfall. Austin and Huddleston (1999) and D'Amore et al. (2004) both described a rapid decline in redox potentials with saturation and a subsequent rise in redox potentials with desaturation and reaeration of the soil. In addition, D'Amore et al. (2004) and Mansfeldt (2003) indicated that lower redox potentials were associated with longer periods of saturation. Similarly, a decline in redox potential with inundation and a rise in redox potentials with the dry-down of the

channels were observed in the current study. In addition, redox potentials were lower in the 5-week-floods than the 3-week-flood.

Differences in redox potential between the 5WF and 5WS treatments were expected but not observed. Lower redox levels were anticipated for the 5WS treatment than for the 5WF treatment because the movement of water through the flowing channels should maintain higher oxygen levels in the flood water, thereby increasing the diffusion of dissolved oxygen into the soil. Dissolved oxygen readings may help explain the redox potential results. Lower soil dissolved oxygen levels were expected and observed under flood treatments. As water-filled pore space increases, a corresponding decrease in air-filled pore space would be expected. Although soil dissolved oxygen levels in the flooded channels did not vary notably by treatment, the 5WF treatment had consistently lower soil dissolved oxygen readings than the other flood treatments. These lower soil dissolved oxygen readings could help explain the lower redox potential readings of the 5WF treatment. Dissolved oxygen content of the flood water did not differ between the 5WF and 5WS treatments. Lower than expected DO readings for the 5WF treatment may be due the placement of the sensors at the outlet end of the channel and thus due to the long distance that the flood water traveled before reaching the sensor.

The average automated readings were usually lower and much more variable than the average manual readings. This variation may be due to sensor irregularities. For example, channel 12, a channel under the 5WS treatment, had consistently high redox potentials and failed to show the decrease in redox potential expected with inundation. On the other hand, all three channels under the 5WF treatment showed the expected downward trend in redox potential with flooding. Sensor irregularities may also explain why the automated readings for the control and 3WF treatments were lower than the manual readings. Channel 7 (a

control channel) and channel 5 (a 3WF channel) both had redox readings that were considerably lower than the other channels under their respective treatments. These readings lower treatment averages and thus may be the cause of significant differences observed between sampling techniques.

Microsite variability may account for some of the differences between automated and manual redox potentials (Patrick et al., 1996; Austin and Huddleston, 1999; Vepraskas and Faulkner, 2001; Eshel and Banin, 2002; Mansfeldt, 2003; D'Amore et al., 2004). For example, placement of an electrode near organic matter that is actively undergoing microbial oxidation will result in observation of lower redox potentials (Vepraskas and Faulkner, 2001). Manual readings were taken nearby the automated sensors within a channel but not in the same exact location. Differences in soil properties and drift in calibration may also have contributed to the variability observed in the automated readings. In addition, variability in manual readings may have occurred from a difference in electrode placement since more than one person conducted the field sampling. Even a slight difference in electrode location could create substantial differences in redox potential (Vepraskas and Faulkner, 2001). Installation of additional electrodes, at various locations within the channels might help to reduce variability and improve the predictive ability of our system. Patrick et al. (1996) recommended at least triplicate electrodes at each depth, while Vepraskas and Faulker (2001) recommended 5-10.

Soil pH

Often considered a "master variable" in the soil, pH influences many of the chemical and biological reactions that occur within the soil. For example, changes in soil pH affect the variable surface charges found on soil colloids (Sparks, 2003; Brady and Weil, 2002). In

particular, as soil pH increases from moderately acidic levels, H^+ ions dissociate from colloid OH groups, resulting in negative charges on the colloid surfaces (Brady and Weil, 2002). And the converse is also true: as a soil becomes increasingly acidic, protonation of the surface OH groups takes place, resulting in positive changes on the colloids (Brady and Weil, 2002). The effect of pH on colloid charges impacts CEC and AEC thereby affecting soil nutrient status. The general trend in soil pH with flooding is a shift towards neutrality (Mitsch and Gosselink, 2000), regardless of whether the soil was acidic or alkaline prior to flooding. Reduction reactions that occur under flooded conditions consume H^+ ions, causing the pH to increase in acidic soils; while the production of organic acids can cause the reduction of pH in alkaline soils (Mitsch and Gosselink, 2000). Soil pH in the FTL channels varied with proximity to the limestone covered road that borders the facility to the north; i.e. channels closer to the road have slightly higher pH than the channels closer to the creek. Therefore, we would expect soils in the channels closer to the road to decrease in pH with flooding with a subsequent increase in positive charges on soil colloids and a better retention of anions including NO_3-N. On the other hand, the soil in the channels closer to the creek would experience an increase in pH with flooding and an increase in negative charges on the soil colloids with better retention of cations, such as K^+, Ca^{2+} and Mg^{2+}.

Unfortunately, the pH data recovered from the automated monitoring system was problematic. Anomalies in pH data may be related to three factors: i) dry soil conditions in the control channels, ii) a period of heavy rain midway through the experimental period and iii) damage to the conduits or the cables. Soil pH electrodes are designed to measure pH from soil slurries, not from dry soil. The control channels, which were relatively dry at the beginning of the experiment, were among those that were the most highly variable. In the remaining channels with anomalous data, the problematic readings began during a period of

heavy rain events (Figure 2.8a). It is possible that water leaked into the conduits due to higher than normal water levels in the channels, causing problems with signal transduction. These channels did not recover to expected readings after these rain events; for example, in channel 10, pH readings were negative from the period of the heavy rains until termination of the experiment. Channels with anomalous data are adjacent to each other; specifically channels 6, 7, 8, 9, and 10 all had problematic pH readings. While the sensors in these channels were not connected to a single, common datalogger, some of the cables run through the same conduits (i.e., cables for channels 7 and 8 are in the same conduit and cables for channels 9 and 10 are in the same conduit). There may be problems within these conduits (leakage or rodent damage) that could have contributed to the anomalous data.

Conclusions

This study had three objectives: to characterize the soils of the FTL, to compare the effects of flood duration and flow rate on soil physical and chemical properties in the FTL, and to examine the performance of an automated system for *in situ* monitoring of the changes soil properties affected by flooding. Characterization of FTL soils confirmed anecdotal observations that soil properties differed across the flood channels. Channels closer to the road had higher clay content, as well as higher soil pH, CEC and base saturation than channels closer to the stream. On the other hand, all channels had evidence of past plowing as well as past flooding and therefore, shared a common disturbance history.

Flood treatment (duration and flow rate) did not affect soil TOC, TN or the C:N ratio. Flood treatments also did not differ in their effect on soil NO_3-N or soil NH_4-N. Soil NO_3-N levels decreased with flooding while NH_4-N increased with flooding; both of these trends were regardless of flood treatment differences (i.e., duration and flow rate).

Therefore, the expectation that flooding would reduce soil N was not supported but the expectation that soil NH_4-N would increase relative to soil NO_3-N was upheld. Likewise the expectation that TSP would accumulate in the soil under flood conditions was upheld. The control and 3WF flood treatments had lower levels of TSP than both of the 5-week flood treatments.

Flood treatment did not affect soil temperature, soil volumetric water content, soil pH, or soil dissolved oxygen content. Redox potential decreased with flooding and increased with dry-down. The 5-week flood treatments had the lowest redox potentials but were not different from each other. Redox results were in agreement with expectations; however, soil and flood water dissolved oxygen content were not.

The data from the automated sensors regarding soil redox potential and dissolved oxygen content in particular will be relevant to the other researchers in the FTL as they attempt to explain differences in survival and success of woody hardwoods and herbaceous groundcovers. Redox potentials and dissolved oxygen readings indicate that assumptions made about particular flood treatments may not be valid. In this case, the 5WS treatment was more aerated than the 5WF treatment, an observation that may help explain apparent anomalous results in flood tolerance experiments. The ability to quantify parameters, such as redox potential, dissolved oxygen content and volumetric water content, will provide more information about the actual impact of flooding to the environmental conditions than simply quantifying flood duration and flow rate. Although not its intent, this study also revealed some of the challenges to working in an outdoor laboratory, most notably the difficulty in achieving true control conditions. Soil volumetric water content readings, for example, suggest possible seepage of flood waters through the berms separating adjacent channels resulting in the saturation of soils within the control channels.

The final objective of this study was to examine the performance of an automated system for *in situ* monitoring of the changes soil properties affected by flooding. Automated and manual monitoring of soil redox potential and pH produced significantly different results. In addition, this study revealed potential problems with the automated sensors. However, the convenience of use and ability to capture variation due to factors such as treatment and weather make the automated system preferable. The automated sensors allow for greater sample frequency without disruption to the system, for capturing of variation that would otherwise be missed with periodic manual measurement and for reduced labor needs. The system used in this study was not without some disadvantages and potential cable and/or sensor problems will have to be worked out prior to the next flood cycle. Among the possible flaws were lack of opportunities for periodic calibration, potential degrading of the electrode membranes due to changes in environmental conditions, lack of correlation between automated and manual monitoring and the high initial cost of purchasing and maintaining the system. Results of this study suggest that greater numbers of sensors may be needed in each channel due to the high variability observed in soil characteristics, such as redox potential. In addition, investment in an automated system may only be appropriate at sites were there is a long-term research commitment.

CHAPTER 3

THE EFFECT OF FLOODING AND RESIDUE INCORPORATION ON SOIL CHEMISTRY, GERMINATION AND SEEDLING GROWTH

Introduction

The rate of organic residue decomposition is affected by soil environmental factors as well as residue characteristics. Environmental conditions that promote residue decomposition are those that promote microbial activity. Microorganisms are generally most active with optimal: i) temperatures (20-30 °C), ii) soil oxygen content (i.e. aerobic conditions), iii) moisture (-0.03 to -0.05 MPa), and iv) pH (circumneutral, i.e. pH = 6-7). Residue characteristics including the type and age of the residue, the amount of residue surface area exposed, and the N availability of the residue and the soil, all affect residue decomposition. Of these factors, the current study focuses on the effect of flooded soil conditions and N availability on residue decomposition and nutrient cycling.

Flooding influences decomposition at a site by altering which microorganisms are present and their metabolic processes. With inundation, the soil is cut off from atmospheric O_2 supplies and the O_2 present in the soil is quickly consumed by aerobic microbes. As the soil becomes anaerobic, the soil flora and fauna change. Microorganisms vary in their tolerance to anaerobic environments; some cannot survive without O_2 (obligate aerobes) and some merely tolerate anaerobic conditions (facultative aerobes) while others thrive (obligate anaerobes). Constant inundation will favor anaerobic microbes over other groups, while repeated wetting-drying cycles will favor facultative anaerobic bacteria over obligate bacteria (Baldwin and Mitchell, 2000). The microbes that survive inundation are those that are able to shift from the use of O_2 as a final electron acceptor to the use of alternative electron

acceptors such as NO_3^-, MnO_2 and $Fe(OH)_3$. The longer the soil remains inundated, the more reduced the soil becomes and the potential for anaerobiosis and anaerobic nutrient cycling processes increase. Decomposition, in general, slows due to changes in the microbial populations and the inefficiency of anaerobic decomposition processes. In addition, biota may become N limited due to loss of inorganic-N through volatilization of NH_3 or denitrification (Baldwin and Mitchell, 2000).

The relationship between N cycling and soil moisture has been explored in a number of studies. Schuur and Matson (2001) examined nutrient availability across a precipitation gradient in a montane forest in Hawaii. They determined that soil N availability decreased with increased precipitation and decreased soil redox potential. Likewise, Shelton et al. (2000) observed a correlation between denitrification and % water filled pore space with a considerable loss of inorganic-N occurring from denitrification when water filled pore space exceeds 85%. In an examination of the relationship between depth to water table and soil N cycling in riparian zones located in seven European countries, Hefting et al. (2004) observed that soil moisture controlled the type of N available for wetland plants. When the water table was within -10 cm of the soil surface NH_3 accumulated; however, when the water table was below -30 cm of the soil surface NO_3^- accumulated. Where the water table was between these thresholds, both nitrification and denitrification occurred due to aerobic and anaerobic patches within the soil profile, however denitrification was favored, resulting in reduced N availability. Alternating aerobic and anaerobic conditions can also affect N availability. Reddy and Patrick (1975) examined the effect of cycles of alternating aerobic and anaerobic soil conditions due to flooding on N loss under laboratory conditions. They observed that under alternating conditions as well as under constant anaerobic conditions soil inorganic N was present primarily as NH_4^+; under constant aerobic conditions NO_3^- was the dominant

form of inorganic N. These studies show that soil moisture can affect the amount of N present in the soil, as well as the dominant form of inorganic N.

Residue quality also plays a large role in decomposition processes. Residue C:N ratio is typically considered an important indicator to predicting potential decomposition rates. A C:N ratio of 20 represents a critical threshold (Sylvia et al., 2005); residues with C:N ratios < 20 tend to decompose quickly resulting in N mineralization. On the other hand, residues with C:N ratios > 20 decompose more slowly and result in N immobilization. Other ratios, such as lignin:N, polyphenols:N and (lignin + phenols):N, have also been proposed as indicators of residue decomposability, but with limited support. For example, Taylor et al. (1989) investigated decomposition rates for 8 species of plants (trees, shrubs and herbs) that ranged in lignin content. The C:N ratio was the best predictor of decomposition rate for these species followed by initial N content and finally the lignin:N ratio. They concluded that the C:N ratio was the best predictor when substrates are low in lignin or when a wide range of lignin is considered. When lignin varies widely, lignin will be important in determining the decay rates of some substrates but not of others. Lignin and polyphenolic compounds, with their large complicated structures consisting of numerous aromatic rings and their resistance to enzymatic attack, are considered recalcitrant compounds. Therefore, compounds with higher lignin or polyphenolic content relative to N content should decompose more slowly.

The influence of lignin and polyphenolic compounds may be more relevant for N mineralization than for decomposition rates. Several studies have determined that the lignin:N ratio (Northup et al., 1998) and (lignin + polyphenol):N ratio (Fox et al., 1990; Constantinides and Fowles, 1994; Browaldh, 1997; Mafongoya et al., 1998) are good predictors of net N mineralization. As lignins decompose, phenolic compounds are released

into the soil. The resultant phenolic compounds may, in turn, bind with proteins or amino acids in the soil to from humic polymers that are resistant to decay (Hattenschwiler and Vitousek, 2000). The formation of recalcitrant N-containing polymers effectively reduces the rate of N mineralization and thus the amount of N available to plants. For example, Suominen et al. (2003) determined that a large portion of dissolved organic-N of the forest soil at sites in northern Finland was not readily available to microbes for mineralization due to protein-tannin complexes. Likewise, Browaldh (1997) in a study of green manures from various deciduous tree prunings concluded that nitrates formed during partial nitrification could have formed complexes with phenolic compounds which may have made the nitrates unavailable for both microbes and plants. It is important, however, to consider not only the amount of phenolic compounds present in the system, but also the types of phenolic compounds and their fate within the soil. Soluble polyphenolic compounds have a higher protein binding capacity than those that are insoluble (Mafongoya et al., 1998; Mungai and Motavalli, 2006); therefore, insoluble polyphenolics may have less influence on net N release. In addition, soluble polyphenolic-protein complexes may leach out of the soil, contributing to N loss from the system over and above their influence on N mineralization (Mungai and Motavalli, 2006). Finally, soil properties may affect the relationship between phenolic compounds and N mineralization by influencing the residence time of phenolic compounds in the soil (Inderjit and Mallik, 1997). Abiotic reactions between phenolic compounds and soil particles (clays in particular) result in the sorption of phenolic compounds (Whitehead et al., 1982; Ohno, 2001); these bound compounds would thus have a reduced impact on N mineralization. In addition to soil texture, soil pH may influence the concentration of phenolic compounds in the soil (Whitehead et al., 1982; Inderjit and Mallik, 1997). The establishment of alkaline conditions due to liming, for example, may result in release of

phenolic compounds from the soil into the soil solution (Whitehead et al., 1982). Once in the soil solution, these compounds may be more open to microbial attack (Whitehead et al., 1982; Ohno, 2001), or may be subject to polymerization or transformation into other phenolic compounds with somewhat different properties (Whitehead et al., 1982). Thus, effects of phenolic compounds on soil nutrient conditions depend not only on the type of phenolic compound present, but also on its residence time in the soil. Residence times are affected by sorption of phenolic compounds to soil minerals as well as microbial alteration and microbial mineralization of phenolics (Inderjit and Mallik, 1997).

In addition to their role in soil nutrient cycling, phenolic compounds have long been implicated in allelopathy. In particular, low molecular weight phenolic compounds, such as caffeic, ferulic, *p*-coumaric, *p*-hydroxybenzoic, protocatechuic, sinapic, syringic, and vanillic acids, have been isolated from soils and identified as potential allelopathic agents (Blum et al., 1991). Muscolo et al. (2001) examined several low molecular weight phenolics (i.e., vanillic, *p*-coumaric, *p*-hydroxybenzoic, and protocatechuic acids) separately and in mixtures to determine their effect on germination of *Pinus laricio* seeds. They found that all phenolics bioassayed affected germination, but that effects were variable. In particular, inhibition of germination was greater for mixtures than for individual phenolics; a synergistic interaction between the phenolic compounds likely contributed to the observed effects on germination and enzyme activities. Similarly, Reigosa et al. (1999) also examined the effect of low molecular weight phenolic compounds (i.e., gallic, ferulic, vanillic, *p*-coumaric, and *p*-hydroxybenzoic acids, and *p*-vanillin) and phenolic mixtures on germination and seedling growth of 6 weed species. The phenolic acids affected seedling growth more than germination; however, the effects varied with concentration. The highest concentrations of phenolic acids were inhibitory, while lower concentrations either had no effect or had a

stimulatory effect on germination and seedling growth. Typical soil concentrations were not sufficient to explain the field results. In order for a phenolic compound to act as an allelochemical it must: i) be present in an active form, ii) participate in chemically mediated interactions and iii) be present in sufficient concentrations of the active form so to modify plant or animal behavior (Blum, 2004). Demonstrating allelopathy in the field, therefore, is difficult.

In summary, past research suggests that anaerobic soil conditions due to inundation will result in reduced nutrient availability for riparian plants. Availability of N in particular may decline due to either increases in denitrification and nitrate reduction, or decreases in effective N concentration due to altered residue decomposition. Shifts in microbial metabolism to anaerobic mechanisms under saturated soil conditions results in an overall decrease in decomposition of residues; lignin and polyphenolic decomposition in particular is adversely affected. The accumulation of lignin and phenolics in the soil subsequently leads to a decrease in plant-available N due to the formation of phenolic-N complexes.

Investigations of the decline in productivity of multi-cropped rice systems offer evidence that phenolic compounds accumulate under flooded conditions and that subsequent N deficiencies occur. Declines in yield of continuously cropped, irrigated rice systems have been reported since 1982 (Cassman et al., 1995). Cassman et al. (1995) investigated possible causes for these declines and they found no evidence for zinc, potassium or phosphorous deficiencies. Tissue analysis revealed that levels of these nutrients in rice plants were at or above critical thresholds. In addition, there were no yield responses with additions of these nutrients to the system. Results, however, suggested a decrease in available N despite no changes in total soil N. Other studies have indicated similar changes in soil N (Gaunt et al., 1995; Olk et al., 1996; Schmidt-Rohr et al., 2004).

Work by Olk et al. (1995) and Schmidt-Rohr et al. (2004) lend support to the role of phenolic compounds in the decrease of N availability. Olk et al. (1996) confirmed the accumulation of phenolic compounds in soils with a history of high rice cropping intensity using NMR spectroscopy. They determined that the degree of accumulation was dependent largely on: i) the availability of oxygen, and ii) the amount of rice crop residues that were incorporated. The observed accumulations of phenolic compounds were thought to contribute to abiotic immobilization of N. Schmidt-Rohr et al. (2004), also using NMR spectroscopy, determined that the mobile humic acid fraction of triple-cropped rice soil consisted largely of lignin-derived (i.e. phenolic) residues. When they compared these rice soils with dryland soils, they found that almost 45% of N in rice soil was bonded to aromatic C, while this fraction of total N is < 30% in dryland mobile humic acid fractions.

Objectives and Expectations

The objectives of this study were threefold. The first was to investigate the effects of flooding and plant residue treatments on various soil characteristics, in particular nitrate (NO_3-N), ammonium (NH_4-N) and total soluble polyphenolics (TSP) levels under greenhouse conditions. Flooding was expected to decrease NO_3-N and increase NH_4-N and TSP in soils. The residue with the highest C:N ratio and/or lignin:N ratio was expected to result in an overall decrease in inorganic-N and an increase in TSP accumulated in the soil. The second objective was to determine relationships among various soil variables under flooded conditions. Redox potential was expected to be positively correlated with NO_3-N levels and negatively correlated with NH_4-N and TSP levels. On the other hand, TSP levels are expected to be positively correlated with NH_4-N levels but negatively correlated with NO_3-N levels. The third objective was to investigate how flooding and the resultant soil

chemical conditions influence germination and seedling growth. Soils with the highest levels of TSP at the termination of the flood treatment were expected to be associated with the lowest germination rates.

Methods

Characterization and Selection of Plant Materials

Plant residue materials were selected from several species in each of the following physiognomic groups: trees, grasses and legumes. Stem and leaf material was harvested from the following tree species: bur oak (*Quercus macrocarpa* Michx.), swamp white oak (*Quercus bicolor* Willd.), and a hybrid of northern red oak (*Quercus rubra* L.) and black oak (*Quercus velutina* Lam.). These materials came from a study conducted in the FTL (see Chapter 2) that investigated genetic variation in central hardwood species as reflected by flood tolerance along a hydrological gradient (bottomland to upland). The goal of the initial study was to determine if seed source variation accounts for flood tolerance.

Herbaceous plant material was harvested from plants grown under field conditions at two University of Missouri Farms (Bradford Research & Extension Center and South Farm) both located near Columbia, MO. Herbaceous species were either legumes or grasses and included both introduced and native species. Stem and leaf material was collected from the following legumes: alfalfa (*Medicago sativa* L.), red clover (*Trifolium pratens* L.), white clover (*Trifolium repens* L.), bird's-foot trefoil (*Lotus corniculatus* L.), crownvetch (*Coronilla varia* L.) and false wild indigo (*Amorpha fruticosa* L.); of these, only false wild indigo is native to Missouri. Stem and leaf material was collected from the following grasses: orchardgrass (*Dactylis glomerata* L.), tall fescue (*Festuca arundinacea* Schreb.), smooth brome (*Bromus inermis* Leyss.), reed canary grass (*Phalaris arundinacea* L.), perennial ryegrass (*Lolium perenne* L.), chordgrass

(*Spartina pectinata* Bosc ex Link), switchgrass *(Panicum virgatum* L.), manna grass (*Glyceria striata* Lam.) and eastern gamagrass (*Tripsacum dactyloides* L.). The first 6 grass species listed are introduced and the last 3 are native. As with the tree species, most of these plants are being used in other FTL experiments and in these experiments species were selected either because they are among the most commonly grown forage species in central Missouri or they were considered as having potential for use in floodplain restoration. Only above ground tissue was used; vegetative and reproductive status along with collection site locates was recorded for each sample taken (data not shown).

All plant samples were dried at 20° C in a forced-air oven for 2-3 days and ground to pass a 1 mm sieve. Tissue samples from individuals within a species were combined and then analyzed to determine total organic C (TOC), total N (TN), lignin, and total soluble polyphenolic (TSP) content of each species. All tissue samples were analyzed for lignin content using a procedure that determined acid detergent fiber (ADF)(Appendix D). Total organic carbon and TN of the plant tissues were determined by dry combustion using a TruSpec CN analyzer (LECO; St. Joseph, MI). Total soluble polyphenolics were determined by the Folin-Ciocalteau technique (Constantinides and Fownes, 1994). Plant extracts for this technique were obtained by mixing 0.75 g of ground leaf litter material with 50% methanol for 1 hour in a hot water bath (80° C). Extracts were then filtered (Whatman #1) and tested immediately for TSP. The following procedure was used for the Folin-Ciocalteau test: 1 ml of extract or standard, 20 ml of water, 2.5 ml Folin-Ciocalteau reagent and 10 ml 17% Na_2CO_3 were added to a 50 ml volumetric flask; additional water was added to the 50 ml mark and mixture was shaken. This mixture was incubated for 20 minutes at room temperature to allow for color development. Ferulic acid was used as the standard; absorbance was read at 760 nm using a spectrophotometer.

From all the samples analyzed, three residue types (one tree, one legume and one grass) were selected for incorporation into the soil. Selection was based primarily on levels of lignin and TSP; but the C:N ratio was also considered in the selection process. One goal in the selection process was to have as wide of a range in these variables as possible. In addition, all species selected were native to Missouri and either performed well in FTL trials or are typically found in riparian or floodplain habitats. Swamp white oak (bottomland) leaves were selected to represent the tree category; this residue was the highest of those chosen in lignin (20.54%), TSP (36.95%) and C:N ratio (26). Manna grass was chosen to represent the grass category; this residue was the lowest of those chosen in lignin (5.77%), and C:N ratio (13); it was intermediate in TSP (9.93%). Finally, false wild indigo was chosen to represent the legumes; this residue had an intermediate lignin content (13.08%) and C:N ratio (22) and the lowest TSP content (5.7%) (Table 3.1).

Table 3.1. Plant residue characterization of tree, legume and grass species evaluated for possible incorporation into soil for greenhouse flood experiment. Averages are presented and were used for calculation of ratios; n = 2 for ADF and lignin, n = 3 for TSP, TOC and TN. Note: ADF = acid detergent fiber content, L = lignin, TSP = total soluble polyphenolics, L:N = lignin:N ratio, TSP:N = total soluble polyphenolic:N ratio, (L +TSP):N = (lignin + total soluble polyphenolics):N ratio, RO = red oak, BkO = black oak, BuO = bur oak, SWO = swamp white oak, bot. = bottomland samples, and up. = upland samples. Samples highlighted in bold were chosen for incorporation into the soil.

	ADF %	**L** %	**TSP** μg FA g^{-1} soil	**TOC** %	**TN** %	**C:N**	**L:N**	**TSP: N**	**(L+TSP): N**
TREES									
RO x BkO stems	50.58	14.74	30.27	43.78	0.63	69	23	48	71
RO x BkO leaves	24.58	10.57	28.59	45.38	2.20	21	5	13	18
BuO stems	50.87	16.31	27.48	43.72	0.64	68	25	43	68
BuO leaves	30.44	15.89	40.57	44.72	1.82	25	9	22	31
SWO – bot. - stems	51.54	18.13	29.15	44.02	0.43	103	42	68	111
SWO – bot. – leaves	**35.91**	**20.54**	**36.95**	**44.87**	**1.74**	**26**	**12**	**21**	**33**
SWO – up. – stems	52.04	17.25	48.65	43.62	0.59	74	29	82	111
SWO – up. -- leaves	37.50	20.95	52.27	44.39	1.94	23	11	27	38
Average	41.68	16.79	36.74	44.31	1.23	51	20	41	60
Min.	24.58	10.57	27.48	43.62	0.43	21	5	13	18
Max.	52.04	20.95	52.27	45.38	2.20	103	42	82	111
LEGUMES									
crown vetch	27.27	6.86	7.98	40.59	3.03	13	2	3	5
bird's foot trefoil	31.68	13.64	6.45	34.39	2.20	16	6	3	9
false wild indigo	**46.56**	**13.08**	**5.70**	**45.08**	**2.02**	**22**	**6**	**3**	**9**
white clover	29.23	9.80	10.16	37.86	3.41	11	3	3	6
lespedeza	32.55	7.68	8.29	42.51	2.16	20	4	4	7
alfalfa	31.99	11.91	6.09	35.69	3.74	10	3	2	5
red clover	33.74	8.05	23.49	42.23	2.17	19	4	11	15
Average	33.29	10.14	9.74	39.76	2.67	16	4	4	8
Min.	27.27	6.86	6.09	34.39	2.02	10	2	2	5
Max.	46.56	13.64	23.49	45.08	3.74	22	6	11	15
GRASS SAMPLES									
manna grass	**27.29**	**5.77**	**9.93**	**37.76**	**3.00**	**13**	**2**	**3**	**5**
smooth brome	31.73	7.34	6.69	37.75	2.62	14	3	3	5
perennial rye	30.05	5.50	4.62	39.77	1.96	20	3	2	5
tall fescue	28.28	4.40	6.91	38.14	2.74	14	2	3	4
orchard grass	29.89	4.50	9.58	39.53	2.83	14	2	3	5
prairie cord grass	46.58	8.97	4.95	42.17	0.62	68	14	8	22
reed canary grass	32.60	5.80	5.81	41.24	2.15	19	3	3	5
switch grass	44.13	9.98	6.45	42.23	0.40	106	25	16	41
E. gamma grass	44.47	8.51	7.06	40.87	0.78	52	11	9	20
Average	35.00	6.75	6.89	39.94	1.90	35	7	6	13
Min.	27.29	4.40	4.62	37.75	0.40	13	2	2	4
Max.	46.58	9.98	9.93	42.23	3.00	106	25	16	41

Characterization of Soil

A bulk soil sample was collected from 0-20 cm depth from the Sulphur Creek floodplain adjacent to the FTL. This sample was air dried, ground, and sieved through a 2 mm mesh. Sub-samples were analyzed to determine initial TOC, TN, and inorganic N content. Total organic C and TN were determined by dry combustion using a TruSpec CN analyzer (LECO ; St. Joseph, MI) while inorganic N was determined using the Lachet QuickChem Method 12-107-04-1-B for NO_3-N determination (Appendix A) and the Lachet QuickChem Method 12-107-06-2-A for NH_4-N determination (Appendix B). Samples were sent to the soil characterization lab for further analysis. Results of pre-flood characterizations can be found in Tables 3.2 and 3.3.

Table 3.2. Pre-flood soil chemical characterization. Four random samples were tested to determine pre-flood levels of the following: TOC, TN, C:N ratio, NO_3-N, NH_4-N.

Sample	TOC (%)	TN (%)	C:N ratio	NO_3-N (mg kg^{-1})	NH_4-N (mg kg^{-1})
1	1.33	0.13	10.58	2.76	3.36
2	1.30	0.11	11.83	4.86	3.29
3	1.25	0.11	11.50	4.68	3.90
4	1.36	0.11	11.94	3.28	4.01
Average	1.31	0.11	11.41	3.90	3.64

Table 3.3. Characterization of a bulk soil sample from the Sulphur Creek floodplain used for the greenhouse study.

Textural Class = Silt												
	Total Silt = 55.2%		**Total Sand = 22.7 %**									
Total Clay	**Fine**	**Coarse**	**Very Fine**	**Fine**	**Medium**	**Coarse**	**Very Coarse**	**>Very Fine**				
< 0.002	0.002-0.02	0.02-0.05	0.05-0.10	0.10-0.25	0.25-0.50	0.5-1	1-2	0.10-2				
	% of < 2 mm		----------% of < 2 mm----------									
22.1	26.8	28.5	13.9	8.3	0.35	0.12	0.1	8.8				
Extractable Bases $cmol_c\ kg^{-1}$						**CEC** $cmol_c\ kg^{-1}$		**Base Saturation %**		**pH (1:1)**	
Ca	**Mg**	**Na**	**K**	**Sum Bases**	**Acidity**	**Sum CATS**	**NH_4 OAc**	**Sum**	**NH_4 OAc**	**$CaCl_2$ 0.01 M**	**H_2O**
3.9	0.5	0.1	0.2	4.7	5.3	9.9	7.8	46.7	60	5.8	6.4

Preliminary Experiment

A preliminary experiment was conducted over a nine week period in June-July 2005 to determine residue concentration, flood characteristics (depth of water, drawn down period for intermittent flood treatment, etc) and overall flood duration. Three residue treatments were used: control (no residue added), swamp white oak leaf residue incorporated at 0.2% and swamp white oak leaf residue incorporated at 0.5% along with two flood regimes: control (soil maintained at 60% water-filled pore space) and saturated/stagnant (soil saturated and 5 cm of standing water). At 3, 7, 14, 21, 35, 56 and 63 days after flooding, soil water and soil solid samples were collected. In addition, the following parameters were measured using hand-held meters: soil redox potential (ORP), soil pH, soil temperature and dissolved oxygen (DO) content of the flood water. Soil samples were frozen for subsequent analysis; the final (day 63) sample was analyzed for TOC, TN, inorganic nitrogen (NO_3-N and NH_4-N) and TSP (Folin-Ciocalteau method). To determine the most efficient and effective extraction technique for TSP analysis, two different extraction techniques were

evaluated. Extracts for the 0% and 0.5% residue samples were made by shaking 5.5 g of soil with 17 ml of water for 8 hours, while the extract for the 0.2% residue sample was made by shaking 15 g of soil with 15 ml of water for 24 hours.

The results of the preliminary experiment showed that desired differences in ORP, inorganic N, and TSP levels could be achieved with the selected experimental design. This experiment also resulted in the finalization of the duration of the flood period (8 weeks) and in the selection of a residue incorporation rate (0.5%). Due to limited supplies of plant material, however, the actual residue incorporation rate for the final experiment was 0.4%. The preliminary experiment also revealed the need to limit light penetration into the trays, as the control trays grew moss and the stagnant trays grew algae over the course of the experiment. A companion experiment revealed that a two week dry-down period would be appropriate for the intermittent flood treatment.

Flood Treatments

The three selected flooding treatments replicate natural flood regimes: i) saturated and stagnant (stagnant treatment), ii) saturated and flowing (flowing treatment) and iii) periodic saturated and then drained (intermittent treatment). The control treatment maintained soil at 60% water-filled pore space (WFPS).

The treatment combinations (each flood treatment paired with each plant residue) were established in plastic trays. Trays were clear plastic with white, opaque lids; each measured 22 x 35 cm. Trays were covered with white 13-gallon trash bags to reduce the amount of light entering the tray and thus reduce the probability of algae and moss growth. Approximately 9.2 kg of soil was added to each tray; the soil depth measured approximately 10 cm resulting in each tray having an approximate bulk density of 1.2 g cm^{-3}. Plant residues

(either grass, legume or tree species) were incorporated into the soil by hand at an equivalent rate of 8 Mg ha^{-1} (or 0.4%).

After incorporation of plant residue, the soils were rewetted and flood treatments were imposed. Of the 64 trays, one-fourth were randomly assigned to the control moisture treatment and had soil moisture maintained at 60% WFPS. To establish this condition, 2.5 L of de-ionized water was added to each control tray; these trays were then weighed and the weights were recorded. Control trays were reweighed twice per week; additional de-ionized water was added as needed to maintain the proper WFPS. One-fourth of the trays were randomly assigned the stagnant treatment and another one-forth of the trays was randomly assigned the flowing treatment. Both of these treatments maintained 5 cm of water above the surface of the soil (Cirtain et al., 2004). Flowing water conditions were created by pumping the water through the trays. Each of the flowing trays had its own pump; water re-circulated between the experimental tray, a sediment bucket and a tray containing the pump maintaining an average flow rate of 2.73 L m^{-1} (Figure 3.1). The final one-fourth of the trays was assigned the intermittent treatment. During flooded conditions, 5 cm of water was maintained above the soil level of these trays; during the dry down cycle, surface water was removed, drains in the bottom of the trays were opened and the trays were partially uncovered to facilitate evaporation. These trays were weighed twice each week during the dry down period to determine soil moisture levels; after one week these trays were re-covered. Based on preliminary experiments, the dry down period lasted 2 weeks; therefore the intermittent trays went through 2 flood cycles and 2 dry-down cycles over the course of this experiment. All trays were placed along three benches in a greenhouse (Figure 3.2). Bench 1 housed all of replicate 1 and half of replicate 2, bench 2 housed half of replicate 2 and all of replicate 3 and bench 3 housed all of replicate 4; this arrangement was necessary

due to space limitations but also allowed us to block for temperature differences within the greenhouse if necessary. Soil and ambient temperatures were monitored with HOBO Pro Temp/External Temp dataloggers to determine the consistency and uniformity of temperature conditions in the greenhouse.

Figure 3.1. Flowing flood treatment unit. Water is circulated with a pump between a tray, the experimental tray and a sediment bucket.

Figure 3.2. Greenhouse set-up**.** Experimental trays were arranged on three tables in the greenhouse. Table 1 held all trays from rep 1 and half of rep 2, table 2 held half of rep 2 and all trays of rep 3, and table 3 held all trays of rep 4. Trays with intermittent flood treatment were set on cinder block to accommodate tray drains.

Soil and Water Characterization

To determine how soil variables changed over the course of the experiment, sampling occurred at 3, 7, 14, 21, 35 and 56 days after flooding. Measurements included: soil ORP, soil pH, soil temperature and the DO content of the surface water. Soil ORP was measured with a hand-held meter (Oakton 300; Cole-Parmer, Vernon Hills, IL) equipped with a general purpose, double junction, sealed, OPR electrode (model: 59001-77; Cole-Parmer, Vernon Hills, IL). Soil pH was measured with a hand-held meter (Oakton 300; Cole-Parmer, Vernon Hills, IL) equipped with a soil pH electrode with an Ag/AgCl reference cell (model: 05992-62; Cole-Parmer, Vernon Hills, IL). Dissolved oxygen of the surface water was measured at the water-soil interface using a hand-held meter equipped with a DO probe (Oakton DO 300; Cole-Parmer, Vernon Hills, IL).

Soil water and soil solid samples were taken at the same time that the soil parameters were measured. To avoid any possible edge effect, soil samples were taken from central

locations within the tray; care was taken to avoid the sides and bottom of the tray. A 3.8 cm diameter PVC pipe was used to collect two soil cores from each tray; samples were stored in plastic bags and were frozen for subsequent analysis. All trays except the control trays were equipped with a water sampling tube. This tube consisted of a PVC pipe to which a screen mesh was attached at one end; the screen end was inserted into the soil and the open/exposed end was covered with a cap to prevent contamination and decrease oxygen diffusion into the sample. Prior to sampling, the water in the sampling tube was extracted and the tube was allowed to refill with soil water. Water samples of 10-15 ml were collected with a pipette from these water sampling tubes; the samples were stored in glass scintillation vials and frozen for subsequent analysis.

The soil solids were freeze-dried and then tested for TOC, TN, inorganic N, and TSP. Methods for TOC, TN and inorganic N followed those previously described; TSP content was determined using the Folin-Ciocalteau technique generally following Suominen et al. (2003). Soil extracts for this test were obtained by mixing 15 g soil with 15 ml of distilled water. This mixture was shaken for 8 hours then centrifuged for 30 minutes. The supernatants were then filtered with Whatman #1 filter paper. The Folin-Ciocalteau test was conducted by mixing 2 ml distilled water, 2 ml soil extract, 5 ml Na_2CO_3 and 1 ml Folin-Ciocalteau reagent (Sigma-Aldrich). This mixture was incubated at room temperature for 30 minutes to allow for color development and absorbance was measured at 735 nm using a spectrophotometer. Tannic acid (reagent grade) was used as the standard. Soil water samples were likewise tested for TSP.

Germination Experiment

At the termination of the simulated flood experiments, bulk soil samples were collected and frozen. A germination study was conducted using these soil samples and Virginia wild rye (*Elymus virginicus*) seeds purchased from Missouri Wildflower Nursery (a nursery that uses Missouri ecotypes). The goal of this experiment was to determine germination differences of seeds sown immediately post-flood.

The experiment was established in 9" x 13" (23 cm x 33 cm) aluminum trays. Treatments reflecting the previous simulated flood experiment were assigned to the trays; therefore, there were a total of 64 trays (4 flood regimes (stagnant, flowing, intermittent and control) x 4 plant residue treatments (tree, legume, grass and control) x 4 replications). To control for potential differences in greenhouse conditions, the replicates were each assigned to a location on one of two benches; flood x residue treatments were randomly assigned within each block.

Soils were brought to the greenhouse one day prior to the initiation of the experiment. Soils were placed in trays and allowed to thaw for approximately 24 hours. The following day, the soil was spread as evenly as possible across the bottom of the tray, completely covering the entire bottom surface. Seeds were sown in a grid pattern, with each seed approximately 1 inch from neighboring seeds or the edges of the container resulting in 96 seeds per tray. Trays were moistened as needed with deionized water, i.e., the control and intermittent trays required additional moisture initially, whereas the saturated and flowing trays were moist from the outset. Trays were misted daily to maintain moisture levels. All trays were covered with plastic wrap on the third day to prevent desiccation.

Trays were examined over a 14 day period. Dates of germination were recorded and conditions of seedlings were noted including those that died or developed mold over the

course of the experiment. At the termination of the experiment, ten shoots were randomly chosen from each tray for shoot length (cm) measurements. If a selected seed had not germinated or only had radicle emergence, another seed was randomly selected until 10 seedlings were sampled. In trays with ≤ 10 germinated seeds, all germinated seeds were measured.

Data Analysis

The experimental design was a randomized complete block, split plot in time; main plot effects were flood and residue treatment, while the effect of sampling day and all possible interactions of the day and main plot effects served as the subplot. ANOVA (Proc MIXED) with a repeated statement and compound symmetry covariance structure was used to test the significance of both the soil parameters (i.e., soil ORP, soil pH, soil temperature, and water DO content) as well as the biochemical parameters (i.e., TOC, TN, inorganic N, TSP) across flood and plant residue treatments. Data were reported as least square means; comparisons of least square means were made using PDIFF ($\alpha = 0.05$).

Linear regression was used to determine the relationship between soil variables and biochemical variables. Of particular interest was whether soil ORP could be used as a predictor for inorganic N levels and/or for TSP levels. In addition, Pearson correlation coefficients were used determine if inorganic N levels and TSP levels were correlated.

ANOVA (Proc GLM) was used to determine the effect of flood and residue treatments on the total number of seeds germinated and on seedling growth (shoot length). For both of these analyses, pairwise comparisons (LSD) were employed when significant differences were detected. The effect of soil chemistry (i.e. inorganic N and/or TSP levels at the termination of flood treatments) on seed germination and seedling growth was of

particular interest. Pearson correlation coefficients and linear regression were used to assess these relationships. All statistical measures were performed using SAS 9.1 (SAS Institute Inc 2002-2003).

Results

Preliminary Experiment

Redox levels recorded on day 63 were significantly higher in the control treatments than in the saturated treatments, while pH levels recorded on day 63 were significantly higher in the saturated treatments than in the control treatments (Table 3.4). Changes observed in ORP and pH levels between day 56 and day 63 were minimal, therefore a flood period of 56 days was chosen for the final experiment. Total organic C and TN did not vary with treatment, however, NO_3-N levels were significantly higher and NH_4-N levels were significantly lower under control conditions (Table 3.4). TSP concentrations were significantly higher in the saturated treatments (Table 3.4). Results for inorganic N and TSP were as expected due to the anaerobic conditions created by flooding.

Table 3.4. Results from preliminary simulated flood experiment. Results reflect experimental conditions 63 days after flooding. Note: Res. = residue treatment quantity, Con. = control flood treatment, and Sat. = saturated flood treatment

Flood	**Res.**	**pH**	**ORP** (mV)	**TN** (%)	**NO_3-N** (mg Kg^{-1})	**NH_4-N** (mg Kg^{-1})	**TOC** (%)	**TSP** (µg TA g^{-1} soil)
Con.	0	6.3	584.00	0.14	25.90	3.05	1.41	10.92
Con.	0.2%	6.2	565.50	0.14	41.25	3.70	1.47	3.18
Con.	0.5%	6.4	506.50	0.13	35.65	3.90	1.49	8.00
Sat.	0	7.0	25.85	0.13	0.13	69.45	1.35	20.16
Sat.	0.2%	6.8	13.00	0.14	0.11	68.00	1.42	17.50
Sat.	0.5%	6.9	5.30	0.13	0.08	70.45	1.48	28.72

Simulated Flood Experiment

Soil Redox Potentials

Soil redox potentials indicated that the control treatment remained well oxygenated (Eh > 400 mV) over the course of the experiment, while the flowing and stagnant flood treatments showed a decrease in ORP (Figure 3.3). Although these two flood treatments responded similarly, the flowing flood ORP measures decreased more slowly and remained slightly higher than those of the stagnant flood treatments. Redox potentials for the intermittent flood treatment decreased with flooding and increased with dry down, creating a fluctuating pattern of ORP readings that mirrors the fluctuating pattern of flooding (Figure 3.3). ANOVA results indicated that flood, residue, day, and flood X day interaction all had a significant impact on ORP (Table 3.5). However, there was no significant interaction between residue and flood treatment, between residue treatment and day or between flood treatment, residue treatment and day (Table 3.5).

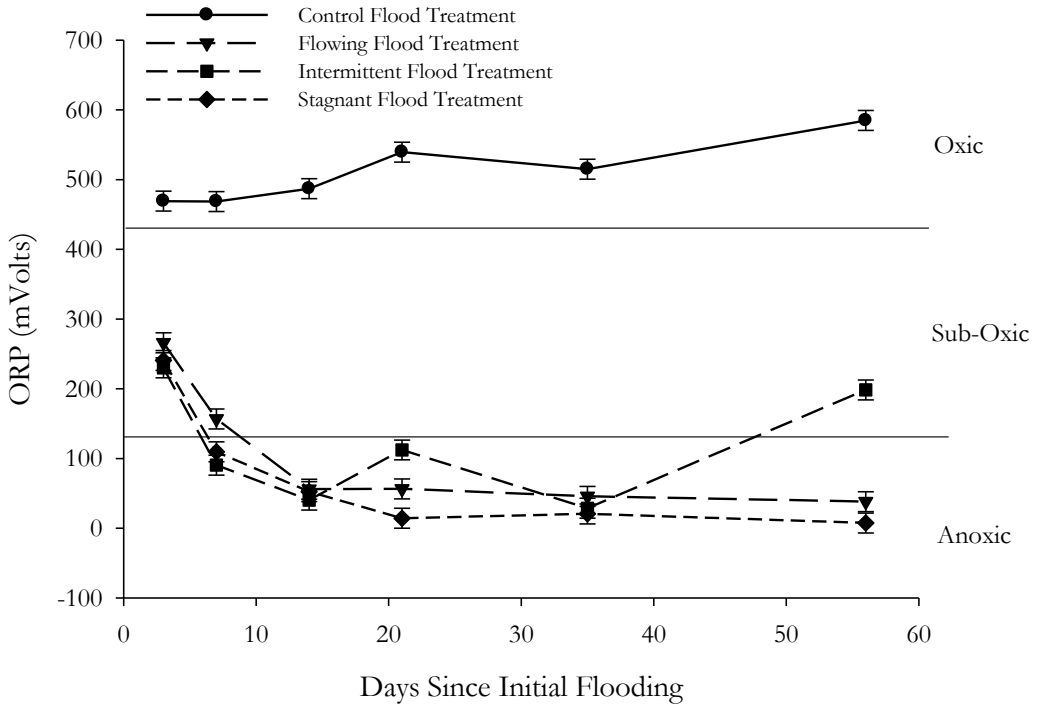

Figure 3.3. Soil redox potentials for simulated flood treatments over time. Data points represent least square means ±1 SE for the flood treatment over all residue treatments. Using pH 7 as reference: oxic or well aerated soils occur at ORP values ≥ 414 mV, suboxic soil conditions occur at ORP values between 414 and 120 mV, and anoxic or anaerobic soil conditions occur at ORP values ≤ 120 mV.

Table 3.5. Analysis of variance results for redox potentials from the simulated flood experiment.

Effect	**F-value**	**P**
Flood	919.62	<0.0001
Residue	3.55	0.0217
Flood X Residue	0.67	0.7307
Day	66.55	<0.0001
Flood X Day	25.30	<0.0001
Residue X Day	1.69	0.0529
Flood X Residue X Day	1.06	0.3778

Soil pH

Soil pH readings differed by flood treatment over the course of the experiment. Average pH readings ranged from 6.0 to 7.7 (Figure 3.4), and significant differences in pH were observed for the main effects of flood and day, as well as for the flood X day interaction (Table 3.6). In general, the control flood treatment had the lowest pH readings; the flowing flood treatment had slightly higher pH readings, followed by the stagnant flood treatment. In general, the intermittent flood treatment had the highest pH readings (Figure 3.4). Readings were slightly elevated on day 35 of the experiment, but decreased or remained the same on day 56.

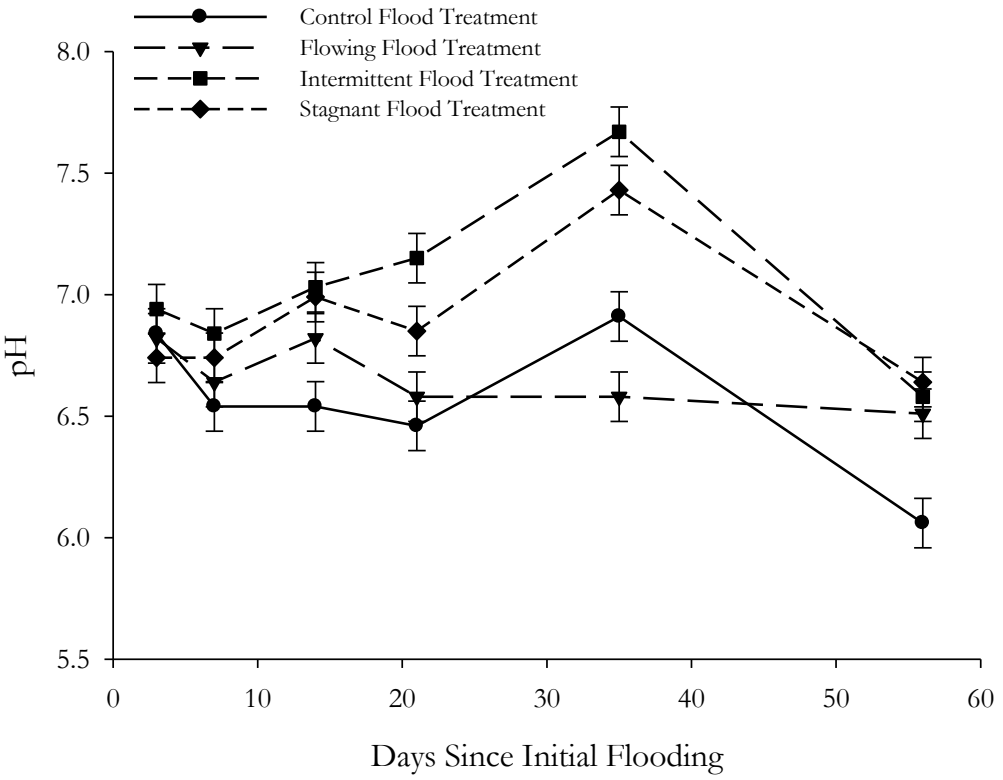

Figure 3.4. Soil pH for flood treatments over time. Data points represent least square means ±1 SE for the flood treatment over all residue treatments.

Table 3.6. Analysis of variance results for pH from the simulated flood experiment.

Effect	F-value	P
Flood	30.09	< 0.0001
Residue	2.16	0.1061
Flood X Residue	1.19	0.3223
Day	20.08	< 0.001
Flood X Day	3.75	< 0.001
Residue X Day	0.75	0.7293
Flood X Residue X Day	0.93	0.6009

Soil Temperature

Soil temperatures were fairly consistent across treatments over the course of the experiment with one notable exception. Soil temperatures were elevated in the control and intermittent flood treatments on day 56 (Figure 3.5). These increases in temperature likely reflect time of day of the readings; on day 56 data for the stagnant and flowing flood treatments were collected before noon, while data for the control and intermittent flood treatments were collected after 2 pm. On all other days, all data collection occurred before noon. ANOVA results indicated that flood, day and the flood X day interaction explained most of the variation in temperature (Table 3.7).

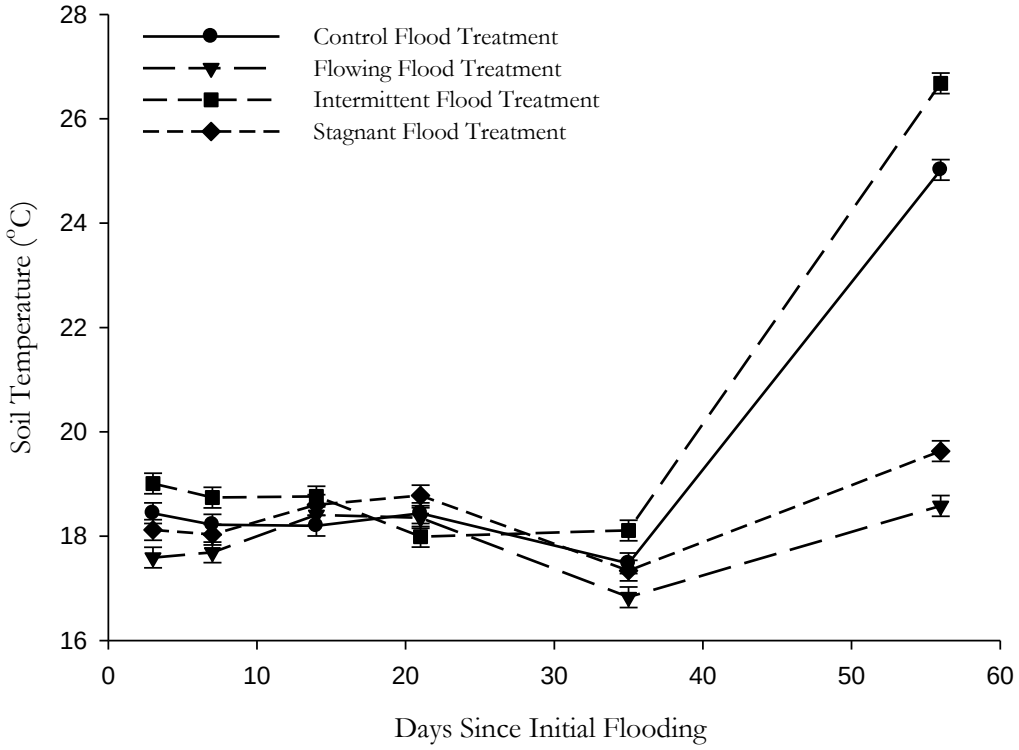

Figure 3.5. Soil temperatures for flood treatments over time. Data points represent least square means ±1 SE for the flood treatment over all residue treatments.

Table 3.7. Analysis of variance results for soil temperature from the simulated flood experiment.

Effect	**F-value**	**P**
Flood	62.57	< 0.0001
Residue	0.14	0.9338
Flood X Residue	0.79	0.6304
Day	570.63	< 0.0001
Flood X Day	106.20	< 0.0001
Residue X Day	0.46	0.9572
Flood X Residue X Day	0.54	0.9929

Dissolved Oxygen

Dissolved oxygen readings were significantly different between the flowing flood and the stagnant flood treatments (Figure 3.6 and Table 3.8). Average DO readings for the flowing flood treatment ranged between 8.4 mg L^{-1} and 11.6 mg L^{-1}, while average DO readings for the stagnant flood treatment ranged between 0.8 mg L^{-1} and 3.4 mg L^{-1} (Figure 3.6). DO readings were also taken for the intermittent flood treatment but were not included in the ANOVA because DO readings could not be taken during the dry-down period. However, the average overall DO readings that were obtained for days 3, 7, 14, and 35 were plotted with the other average overall DO readings (Figure 3.7). On days 3, 7, and 14 the DO levels of the stagnant and intermittent flood treatments were not different, however differences were detected on day 35 (Figure 3.7). The difference on day 35 is likely due to the length of flood.

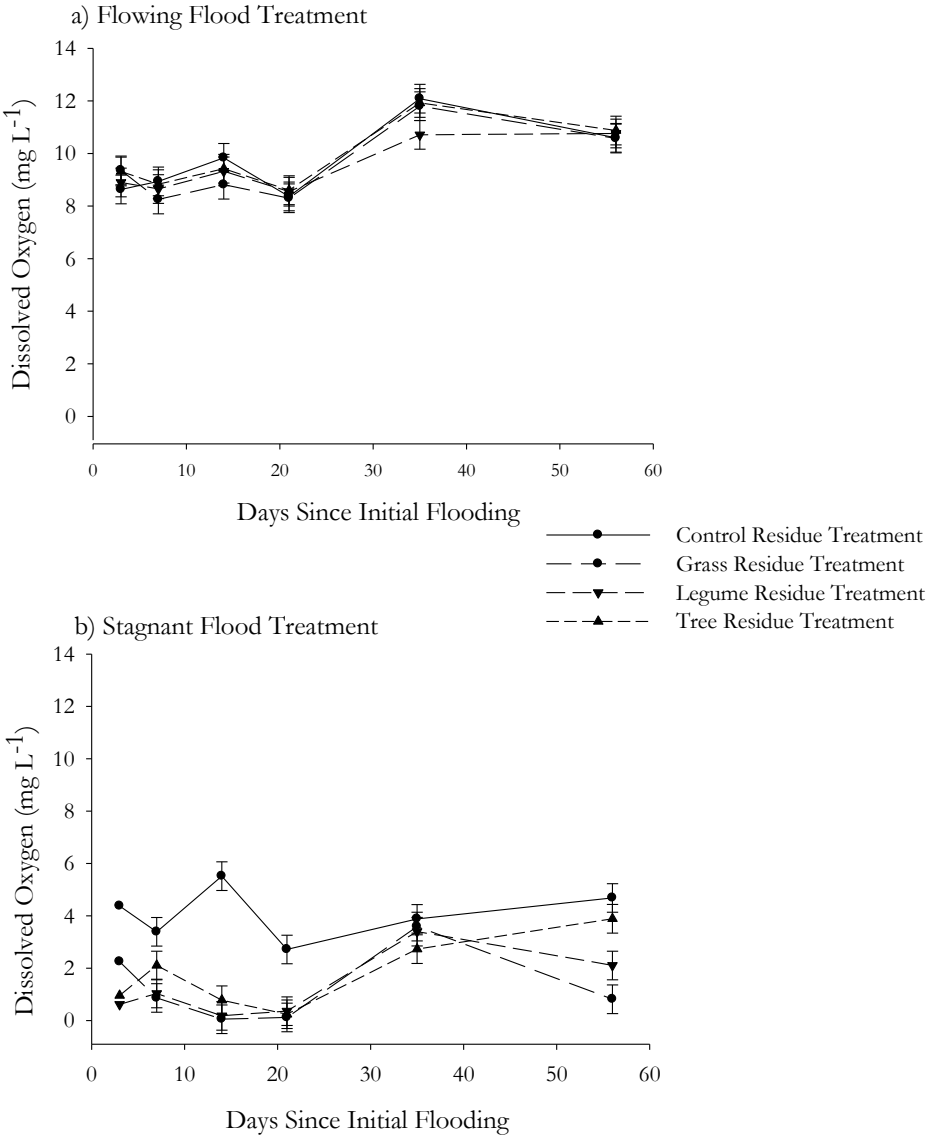

Figure 3.6. Dissolved oxygen content of flood water for a) flowing flood treatment and b) stagnant flood treatment over time. Data points represent least square means ±1 SE for the residue treatment under the selected flood treatment.

Table 3.8. Analysis of variance results for dissolved oxygen from the simulated flood experiment. Note: Comparison of saturated and flowing treatments only.

Effect	F-value	P
Flood	1945.18	< 0.0001
Residue	17.91	< 0.0001
Flood X Residue	13.59	<0.0001
Day	31.38	<0.0001
Flood X Day	1.95	0.0911
Residue X Day	20.9	0.0149
Flood X Residue X Day	1.88	0.0314

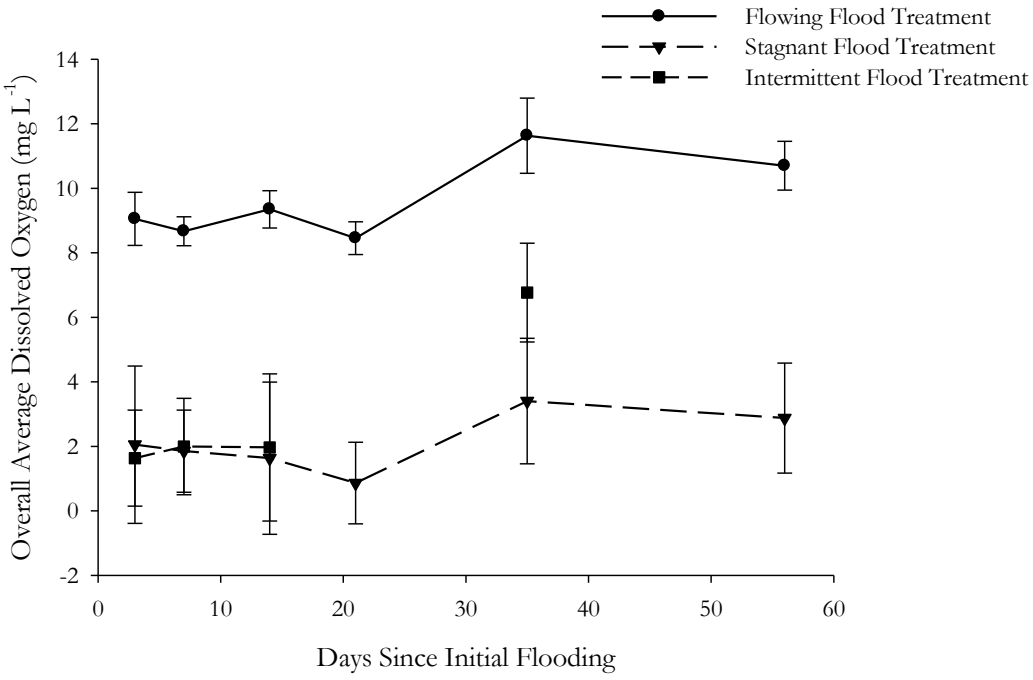

Figure 3.7. Dissolved oxygen content for flood treatments over time. Data points represent least square means ±1 SE for the flood treatment over all residue treatments.

Total Organic Carbon and Total Nitrogen Analysis

Flood treatment did not affect TOC; however, differences were observed among residue treatments over time. The addition of residues resulted in an increase in TOC within the soils; TOC in control trays averaged 1.30-1.35%, while TOC in residue-added trays averaged 1.30-1.68% (Figure 3.8). Early in the experiment (days 3-14), trays with legume and tree residues did not differ notably from each other; however, these treatments had higher TOC than those with grass residue added. Later in the experiment (days 21-56), levels of TOC in legume-residue trays decreased to levels slightly lower than those observed in grass-residue trays. Between days 35-56, TOC decreased in all experimental trays to levels comparable to control trays. ANOVA results revealed significant main effects due to residue and day, as well as a significant residue X day interaction (Table 3.9).

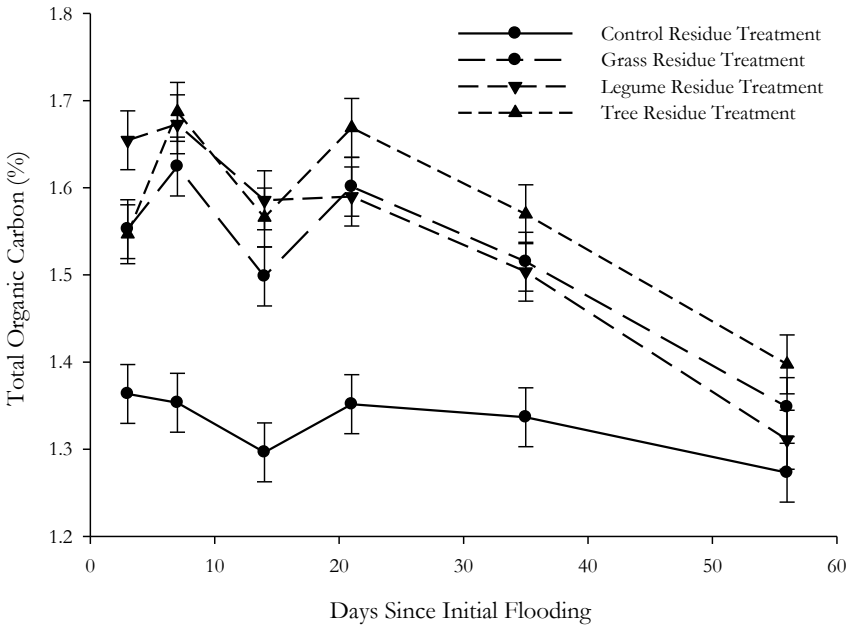

Figure 3.8. TOC for residue treatments over time. Data points represent least square means ±1 SE for the residue treatment under the selected flood treatment.

Table 3.9. Analysis of variance results for total organic carbon from the simulated flood experiment.

Effect	F-value	P
Rep	3.40	0.0255
Flood	0.34	0.7955
Residue	58.40	<0.0001
Flood X Residue	1.60	0.1442
Day	28.10	<0.0001
Flood X Day	1.49	0.1076
Residue X Day	2.39	0.0031
Flood X Residue X Day	0.99	0.4988

The effect of flood or residue treatment on TN is less clear than the effects of these treatments on TOC. ANOVA results indicated a significant main effect of residue treatment (Table 3.10); grass-residue treatment had the highest average TN (0.143%) followed by the legume-residue treatment (0.142%), the tree-residue treatment (0.137%) and finally the control-residue treatment (0.121%). Significant interactions were also observed, including a significant flood X day interaction and a significant residue X day interaction; the flood X residue X day interaction however, was not significant (Table 3.10). All flood treatments (including the control) showed a fluctuating pattern of TN over the course of the experiment (Figure 3.9a); perhaps most notable was the decrease in TN for the intermittent- and stagnant-flood treatments at day 35. The addition of residue, for the most part, resulted in an increase in TN within the soils (Figure 3.9b). Total N levels again appear to fluctuate over the course of the experiment with TN in grass-residue trays becoming similar to tree-residue trays (Figure 3.9b).

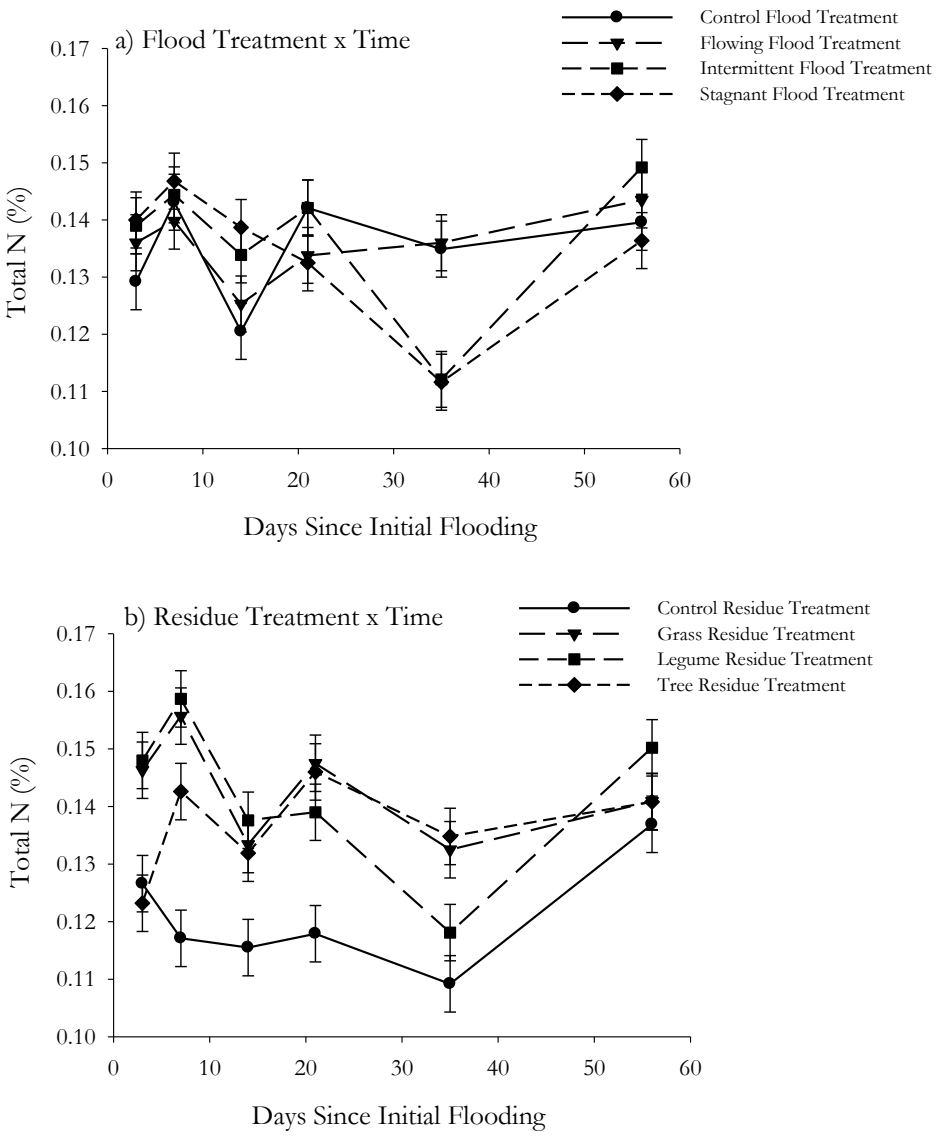

Figure 3.9. Total N content for a) flood treatments over time and b) residue treatments over time. Data points represent least square means ±1 SE.

Table 3.10. Analysis of variance results for total nitrogen from the simulated flood experiment.

Effect	F-value	P
Rep	2.40	0.0806
Flood	0.38	0.7667
Residue	35.40	<0.0001
Flood X Residue	0.70	0.7035
Day	9.06	<0.0001
Flood X Day	2.64	0.0010
Residue X Day	2.74	0.0007
Flood X Residue X Day	0.94	0.5850

For the most part, C:N ratios remained stable over the course of the experiment with one notable exception; C:N ratios decline dramatically between days 35 and 56 (Figure 3.10). The decline observed in TOC (Figure 3.8) accompanied by the increase observed in TN (Figure 3.9) would produce the overall decline in C:N ratios observed at this point in the experiment. ANOVA revealed significant flood X day and residue X day interactions; the main effect of residue treatment was also significant (Table 3.11).

Table 3.11. Analysis of variance results for C:N ratio from the simulated flood experiment.

Effect	F-value	P
Rep	0.66	0.5785
Flood	0.17	0.9192
Residue	10.88	<0.0001
Flood X Residue	1.08	0.3991
Day	26.59	<0.0001
Flood X Day	3.15	<0.0001
Residue X Day	2.24	0.0060
Flood X Residue X Day	0.84	0.7487

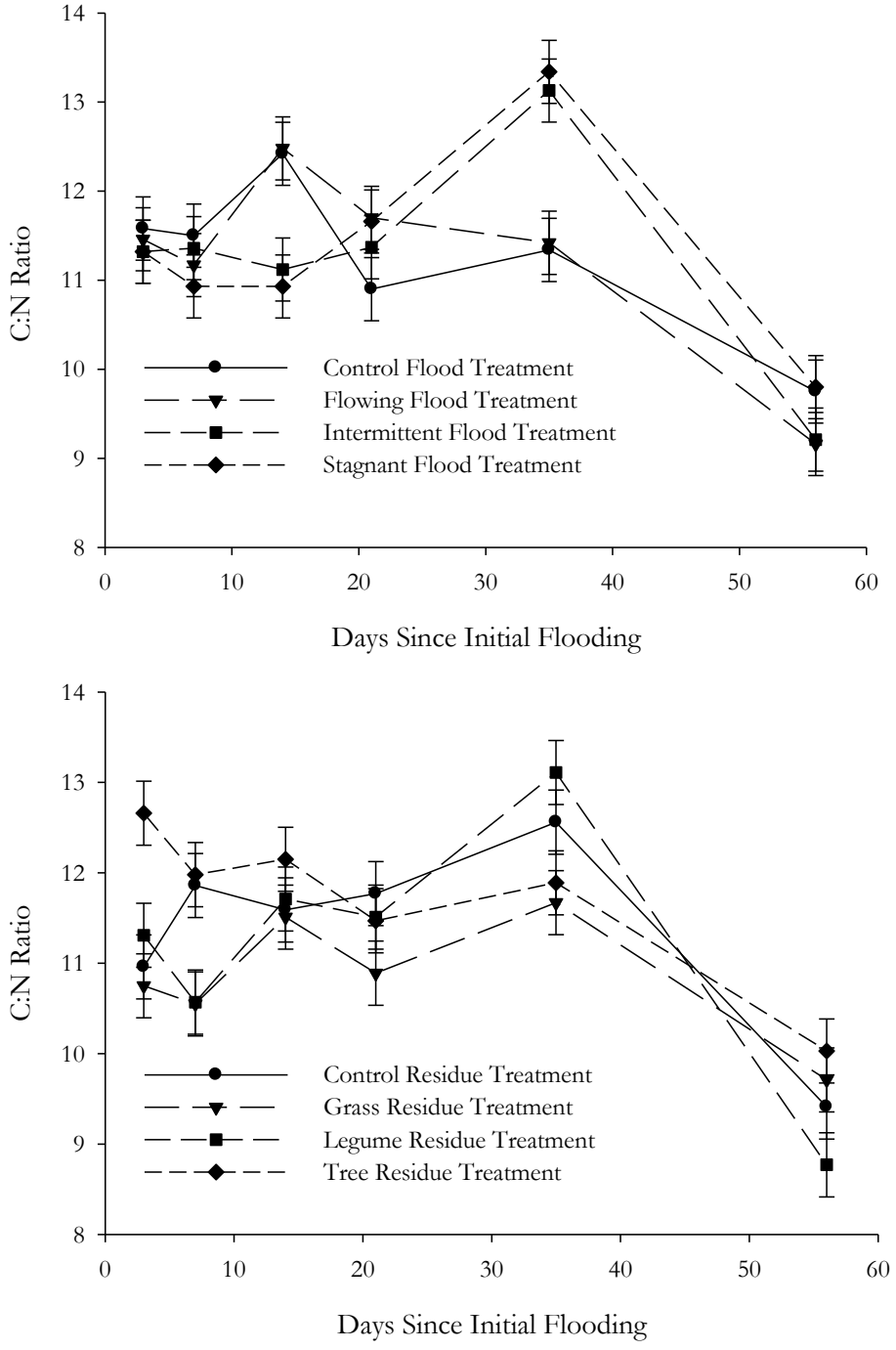

Figure 3.10. C:N ratios for a) flood treatments over time and b) residue treatments over time. Data points represent least square means ±1 SE.

Inorganic Nitrogen Analysis:

All soil samples tested low initially for NO_3-N; the flowing-flood and stagnant-flood-treatments retained these low NO_3-N levels over the course of the experiment (Figure 3.11). Similarly, soils in the intermittent flood treatment remained low in NO_3-N over the first 14 days of the experiment. A slight increase in NO_3-N levels in the intermittent-flood-treatment was detected at day 21; this increase and subsequent decrease corresponded to the first dry-down and re-flooding period. A larger increase in NO_3-N levels was observed in the intermittent-flood-treatment at the termination of the experiment (Figure 3.11). Differences between the two "peaks" of NO_3-N for this treatment were likely due to when the sample was taken in relationship to the amount of dry-down; the first peak is observed for a sample taken one week after dry-down while the second, larger peak was taken two weeks after dry-down. Soils in the control treatment showed relatively steady increases in NO_3-N levels over the course of the experiment with the exception of the grass residue treatment. Three weeks into the experiment, NO_3-N levels in the grass treatment shifted from being equivalent to those found in other treatments to levels higher than all other treatments (Figure 3.11). Nitrate levels in the control/grass treatments remain significantly higher than the other treatments throughout the remainder of the experiment. All ANOVA comparisons were significant (Table 3.12).

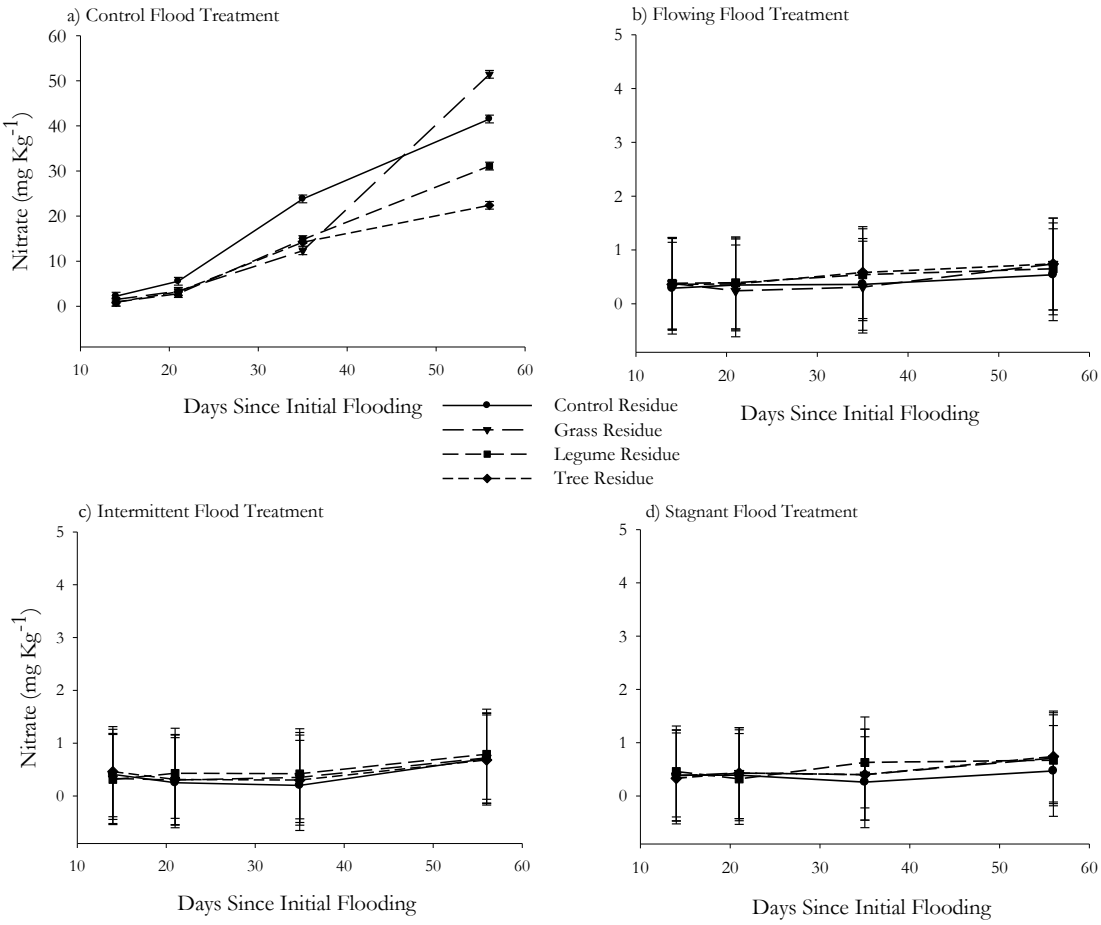

Figure 3.11. Soil NO_3-N for a) control flood treatment over time, b) flowing flood treatment over time, c) intermittent flood treatment over time and d) stagnant flood treatment over time. Data points represent least square means ±1 SE for the residue treatment under the selected flood treatment. Note difference in Y-axis for control treatment.

Table 3.12. Analysis of variance results for soil NO_3-N levels from the simulated flood experiment.

Effect	F-value	P
Flood	1773.46	< 0.0001
Residue	35.70	< 0.0001
Flood X Residue	37.46	< 0.0001
Day	1047.92	<0.0001
Flood X Day	977.19	<0.0001
Residue X Day	37.34	< 0.0001
Flood X Residue X Day	37.92	< 0.0001

Ammonium generally increased with flood duration regardless of flood type (Figure 3.12). Flowing and stagnant flood treatments showed a 3-4-fold increase in NH_4-N over the course of the experiment while the intermittent flood treatment showed a 2-3-fold increase in NH_4-N. Regardless of flood type, the grass residue treatment showed notably higher levels of NH_4-N than the other residue treatments(Figure 3.12). Ammonium levels in the control flood treatments were initially similar to other flood treatments, but decreased over time to near removal of NH_4-N from the soil at time of termination of the experiment (Figure 3.12). As with the NO_3-N levels, all ANOVA comparisons of NH_4-N levels were significant (Table 3.13).

Table 3.13. Analysis of variance results for soil NH_4-N levels from the simulated flood experiment

Effect	F-value	P
Flood	535.05	< 0.0001
Residue	281.20	< 0.0001
Flood X Residue	5.70	< 0.0001
Day	241.37	<0.0001
Flood X Day	126.57	<0.0001
Residue X Day	3.84	< 0.0001
Flood X Residue X Day	3.27	< 0.0001

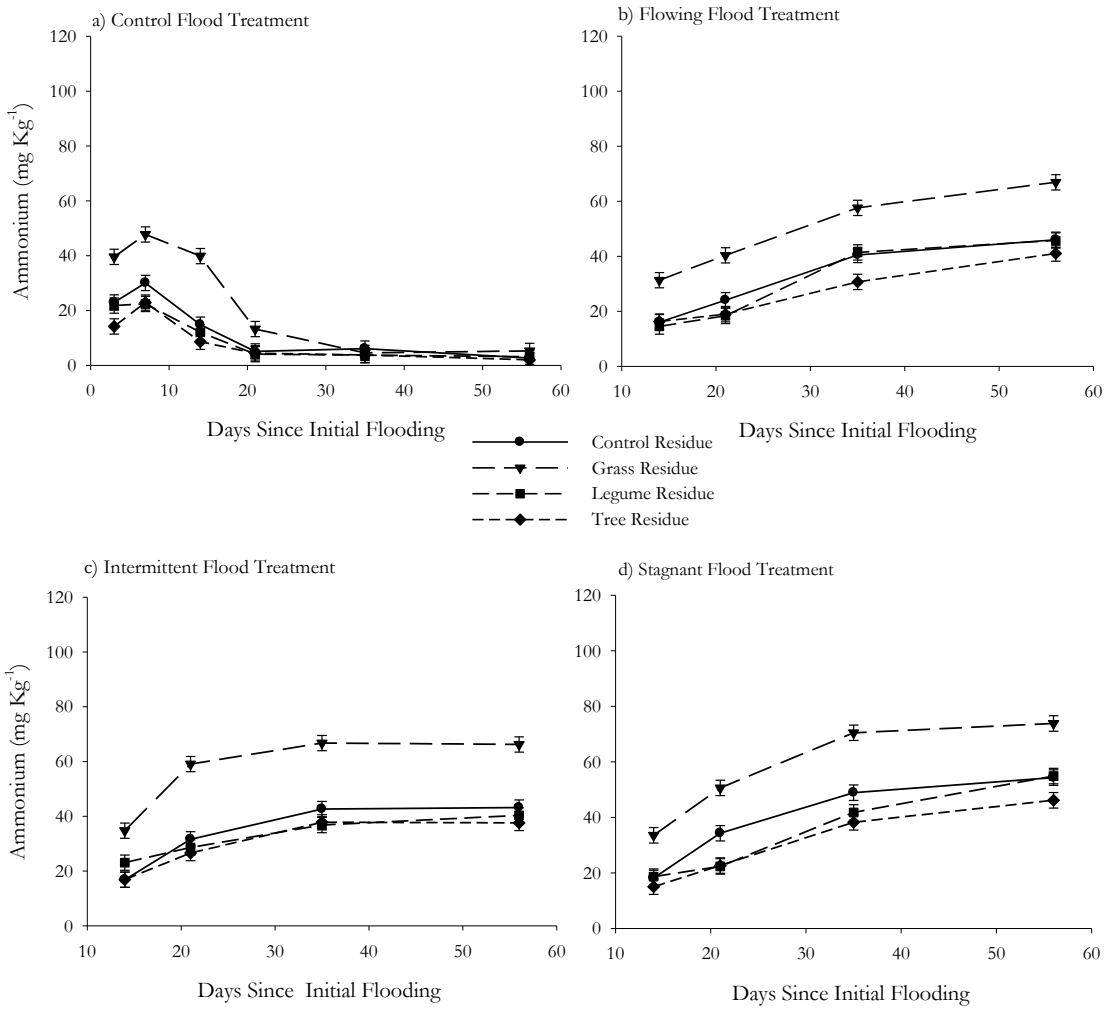

Figure 3.12. Soil NH_4-N for a) control flood treatment over time, b) flowing flood treatment over time, c) intermittent flood treatment over time and d) stagnant flood treatment over time. Data points represent least square means ±1 SE for the residue treatment under the selected flood treatment.

Total Soluble Polyphenolic Analysis

Total soluble polyphenolic concentrations in the soil initially increased with flood duration but decreased by the end of the eight week flood period (Figure 3.13a). Little difference between flood treatments was observed over the first three weeks of flooding (Table 3.13a). However, at the final two data collections (days 35 and 56), the intermittent treatment had significantly lower levels of TSP. Polyphenolic concentrations in the control flood treatment did not change over time (Figure 3.13a). Residue treatments showed a similar pattern of effect on soil TSP concentration. TSP concentration increased initially but declined between days 35 and 56 (Figure 3.13b). This pattern was observed regardless of residue type; however the soil TSP concentration was lower under the control residue treatment as compared to other residue treatments. ANOVA results revealed flood X residue, flood X day and residue X day interactions (Table 3.14).

Table 3.14. Analysis of variance results for soil TSP concentrations from the simulated flood experiment.

Effect	**F-value**	**P**
Flood	199.96	< 0.0001
Residue	54.73	< 0.0001
Flood X Residue	3.05	0.0057
Day	54.61	<0.0001
Flood X Day	12.29	<0.0001
Residue X Day	2.11	< 0.0001
Flood X Residue X Day	1.38	0.0674

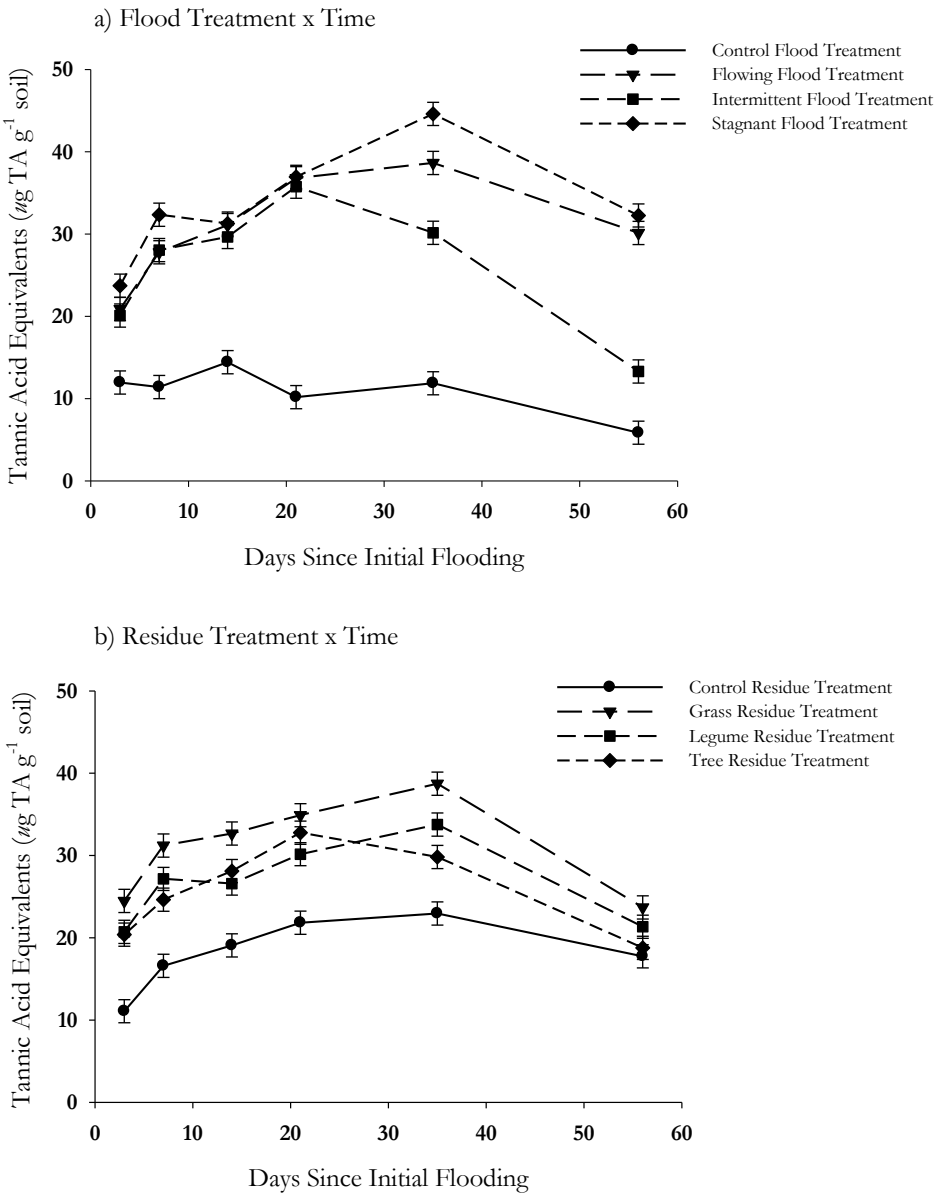

Figure 3.13. Soil TSP concentrations for a) flood treatments over time and b) residue treatments over time. Data points represent least square means ±1 SE.

Residue type affected TSP concentrations of the water samples (Figure 3.14a). The grass residue treatment resulted in significantly higher water TSP concentrations than other residue treatments; water TSP levels for the legume residue were slightly higher than those observed for tree residue, while TSP levels remained consistently low under the control residue. Water TSP levels under the various residue treatments were shown to decrease between days 35 and 56. Residue effects were shown to be significant; however no flood X residue or residue X day interaction was observed (Table 3.15). Patterns in water TSP concentrations due to flood treatment are more difficult to discern. First, no water samples were available for the control flood treatment or for the intermittent flood treatment under dry-down conditions. Therefore, only the flowing flood and stagnant flood treatments could be compared. The water TSP concentrations under the flowing treatment fluctuated around 35 ppm, while those in the stagnant flood treatment increased over the course of the experiment to a peak of 49 ppm at day 35 (Figure 3.14b). As observed previously, the water TSP concentrations under both flood treatments decreased between days 35 and 56. Effects of flood were not found to be significant; however a significant flood X day interaction was observed (Table 3.15).

Table 3.15. Analysis of variance results for water TSP concentrations from the simulated flood experiment.

Effect	**F-value**	**P**
Flood	1.04	0.3196
Residue	22.97	< 0.0001
Flood X Residue	0.81	0.5028
Day	7.39	<0.0001
Flood X Day	2.45	0.0378
Residue X Day	1.17	0.3064
Flood X Residue X Day	1.32	0.2028

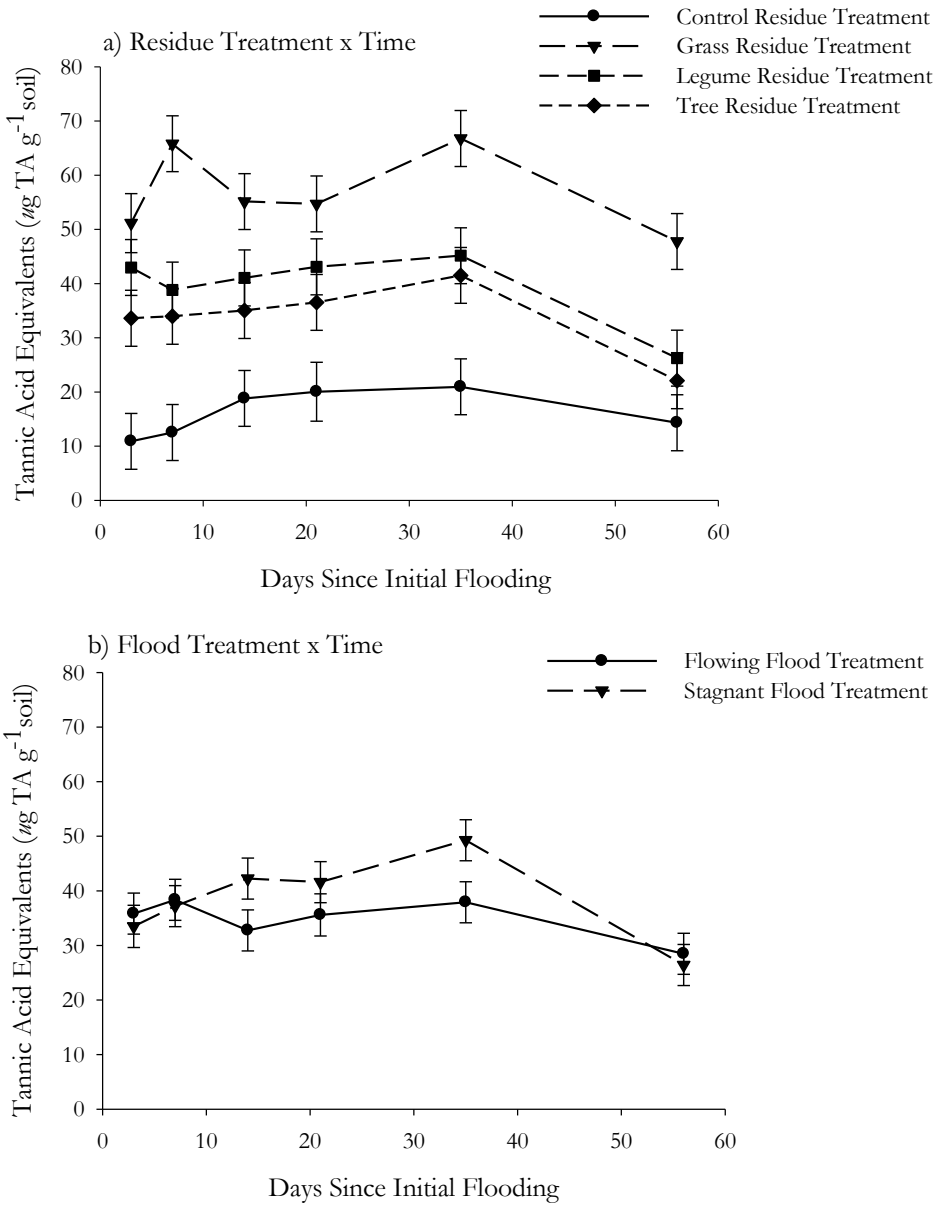

Figure 3.14. Water TSP concentrations for a) residue treatments over time and b) flood treatments over time. Data points represent least square means±1 SE.

Correlation and Regression Analysis

Soil ORP was positively correlated with NO_3-N and negatively correlated with NH_4-N and soil TSP (Table 3.16). Soil ORP was a good predictor of inorganic-N levels as well as soil TSP concentrations. Linear regression analysis revealed that soil ORP explained 48% of the variation observed in NO_3-N levels (Figure 3.15), 50% of the variation observed in NH_4-N levels (Figure 3.16) and 55% of the variation observed in soil TSP concentrations (Figure 3.17); all of these were significant. However, scatter plots of the data (Figures 3.15-3.17) suggest that a non-linear regression model may better explain these relationships. Soil ORP was not a good predictor of water TSP (Table 3.16). Linear regression analysis revealed that soil ORP explained only 1% of the variation in water TSP levels (data not shown).

Table 3.16: Correlations between select physical and chemical variables from the greenhouse simulated flood experiment. The first number for each comparison represents the Pearson correlation coefficients (r) and the second number represents the P value for the comparison. Significant relationships are highlighted in bold.

	ORP	**TOC**	**TN**	**C:N Ratio**	**NO_3-N**	**NH_4-N**	**Soil TSP**	**Water TSP**
ORP	**1.00**	-0.03	0.03	-0.06	**0.70**	**-0.71**	**-0.74**	-0.12
		0.55	0.61	0.27	**<0.0001**	**<0.0001**	**<0.0001**	0.07
TOC		**1.00**	**0.55**	**0.11**	**-0.11**	**-0.15**	**0.24**	**0.32**
			<0.0001	**0.04**	**0.03**	**0.004**	**<0.0001**	**<0.0001**
TN			**1.00**	**-0.76**	0.02	-0.06	0.05	**0.18**
				<0.0001	0.74	0.26	0.288	**0.004**
C:N				**1.00**	**-0.11**	-0.03	**0.13**	0.04
					0.03	0.49	**0.01**	0.57
NO_3-N					**1.00**	**-0.54**	**-0.51**	**0.16**
						<0.0001	**<0.0001**	**0.01**
NH_4-N						**1.00**	**0.66**	**0.23**
							<0.0001	**0.0002**
Soil TSP							**1.00**	**0.48**
								<0.0001
Water TSP								**1.00**

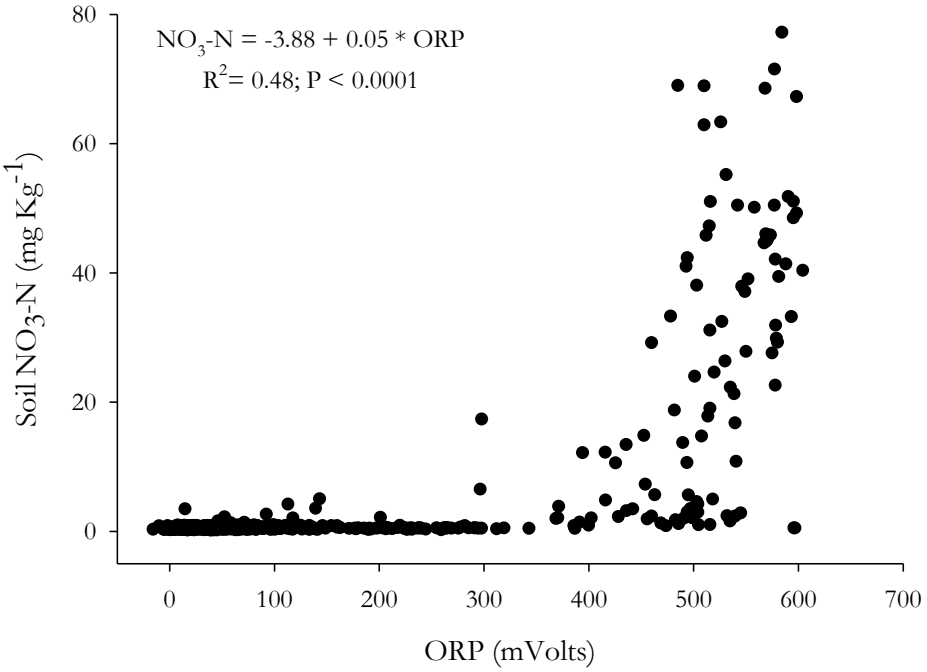

Figure 3.15. Relationship between soil ORP and NO_3-N levels.

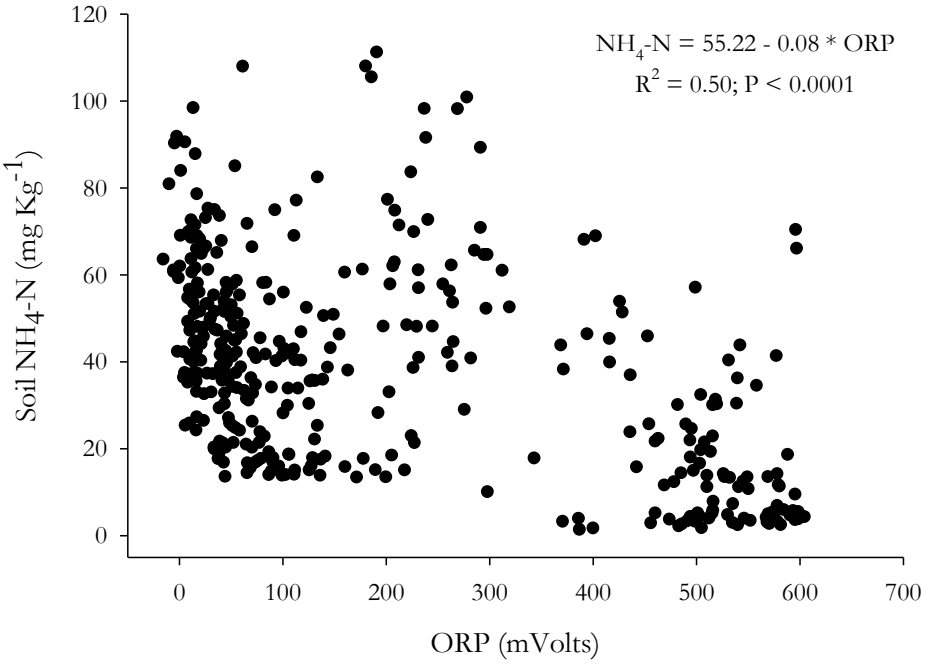

Figure 3.16. Relationship between soil ORP and NH_4-N levels.

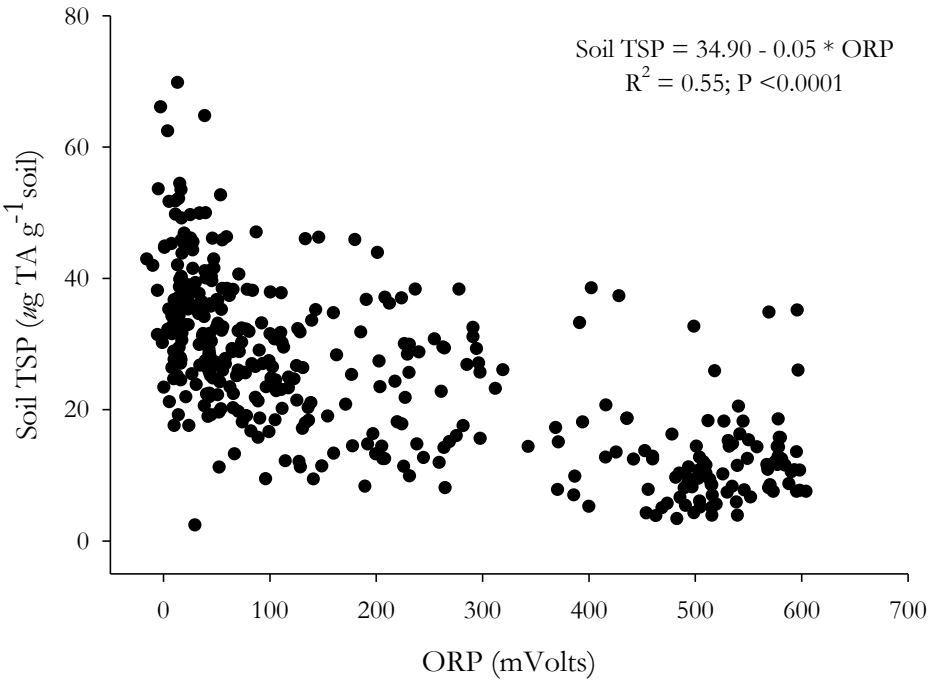

Figure 3.17. Relationship between soil ORP and soil TSP levels.

Total organic C and TN, as well as C:N ratio were not highly correlated with soil or water TSP (Table 3.16). Total organic C was positively correlated with both TSP parameters, whereas TN had a positive correlation only with water TSP concentrations. The C:N ratio was positively correlated with soil TSP. Linear regression analysis showed that TOC explained about 6% of the variation observed in soil TSP concentrations and about 10% of the variation observed in water TSP concentrations. Total N explained approximately 3% of the variation observed in water TSP levels and the C:N ratio explained approximately 2% of the variation observed in soil TSP concentrations.

Inorganic nitrogen levels were shown to be correlated with soil TSP concentrations. Nitrate was negatively correlated with soil TSP levels; while NH_4-N was positively correlated

with soil TSP levels (Table 3.16). As with ORP, neither of these measures showed a strong correlation with observed water TSP levels (Table 3.16). Since the relationship between NH_4-N and soil TSP was the stronger of the two comparisons, a linear regression analysis was conducted for NH_4-N and soil TSP. This analysis revealed that NH_4-N levels explained approximately 43% of the variation seen in soil TSP concentrations (Figure 3.18). When a multiple linear regression analysis was conducted using both ORP and NH_4-N levels as predictor variables for soil TSP concentration, it was shown that these two parameters together explained about 58% of the variation observed in soil TSP levels (regression equation: soil TSP = 26.86 – 0.04 * ORP + 0.14 * NH_4-N; $R^2 = 0.58$; $P < 0.0001$). Thus by the addition of a second parameter, an additional 3% of the variation in soil TSP concentrations could be explained. Such an increase is too weak to be meaningful; thus, ORP alone is the best predictor of soil TSP.

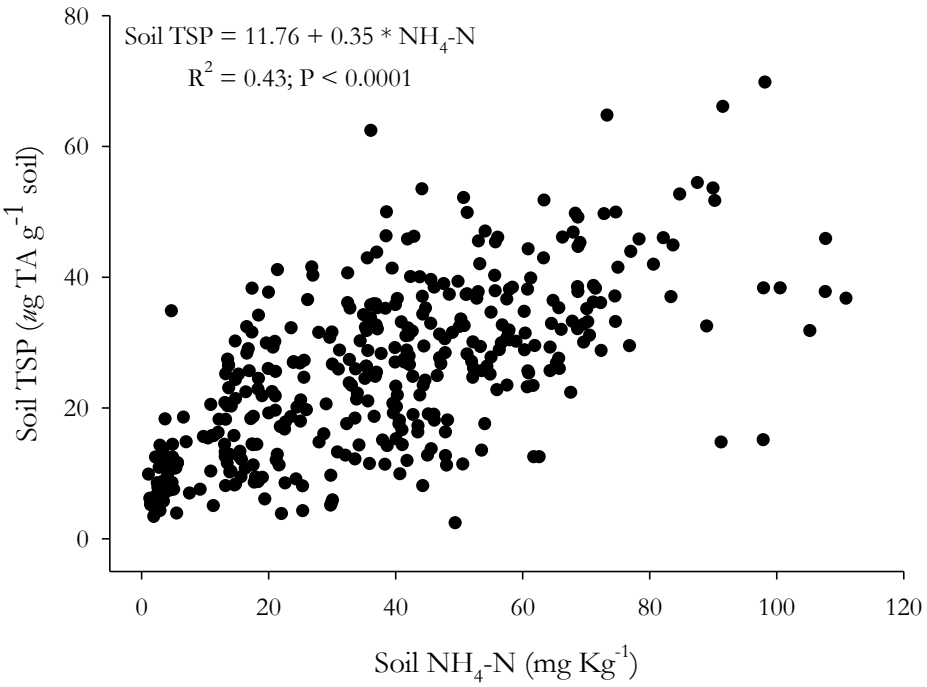

Figure 3.18. Relationship between soil NH_4-H and soil TSP levels.

Germination Experiment

Total Germination

Five days after the experiment was initiated, germination occurred in a small number (9/64) of trays. In general, germination rates increased rapidly over the subsequent 5-6 days and then leveled off over the final 4-5 days of the experiment. When pooled across residue types, germination rates ranked as follows: intermittent flood > control > flowing flood > stagnant flood (Table 3.17a). On the other hand, when germination was pooled over flood type, trends were less clear with % germination ranging from a low of 52.4% (grass residue) to a high of 67.1% (control residue); the legume residue and the tree residue had nearly equal % germination (Table 3.17b). These trends were also evident when flood and residue

treatments were considered together. The intermittent flood treatments had 3 of the 4 highest rates of germination, while the grass residue treatments were consistently lower than other flood x residue combinations (Table 3.17c). ANOVA revealed that flood treatment was the only significant factor influencing germination ($F = 4.39$; $P < 0.01$); neither the residue treatment nor the interaction of residue and flood influenced germination and no differences were detected between replicates. Mean separation techniques (LSD) showed that the intermittent flood treatment resulted in significantly greater germination than the flowing flood treatment or the stagnant flood treatment (Table 3.17a).

Seedling Length

Flood treatment was the only factor that contributed significantly to seedling length ($F = 8.26$, $P < 0.001$) (Table 3.18 a). Shoot lengths were greatest from the intermittent flood treatments. No differences in shoot length were detected between the control, flowing, and stagnant treatments (Table 3.18 a & c). No differences in shoot length were attributed to residue treatments (Table 3.18 b & c).

Table 3.17. Total and average germination by a) flood treatment, b) residue treatment, and c) flood X residue treatment combinations. Total germination is expressed as number of seeds germinated over a 14 day period and as % germinated of total seeds sown for that treatment. Means with the same letter are not significantly different at $\alpha = 0.05$.

Treatment		Total Germination	Average Germination	Standard Deviation
a) Flood	Control	988 (64.3%)	61.75 **AB**	22.67
	Intermittent	1126 (73.3%)	70.38 **A**	14.34
	Flowing	833 (54.2%)	52.06 **B**	17.94
	Stagnant	774 (54.4%)	48.38 **B**	18.34
b) Residue	Control	1030 (67.1%)	64.38	15.59
	Grass	805 (52.4%)	50.31	26.40
	Legume	957 (62.3%)	59.81	18.41
	Tree	929 (60.5%)	58.06	17.43
c) Flood X Residue Combinations				
Control	Control	257 (66.9%)	64.25	21.19
Control	Grass	226 (58.9%)	56.50	31.93
Control	Legume	271 (70.6%)	67.75	27.27
Control	Tree	234 (60.9%)	58.50	15.80
Intermittent	Control	309 (80.5%)	77.25	5.91
Intermittent	Grass	251 (65.4%)	62.75	22.84
Intermittent	Legume	272 (70.8%	68.00	14.12
Intermittent	Tree	294 (76.6%)	73.50	10.54
Flowing	Control	216 (56.3%)	54.00	17.61
Flowing	Grass	192 (50.0%)	48.00	29.74
Flowing	Legume	236 (61.5%)	59.00	12.11
Flowing	Tree	189 (49.2%)	47.25	12.09
Stagnant	Control	248 (64.6%)	62.00	6.98
Stagnant	Grass	136 (35.4%)	34.00	20.94
Stagnant	Legume	178 (46.4%)	44.50	10.85
Stagnant	Tree	212 (55.2%)	53.00	22.46

Table 3.18. Shoot length of grass seedlings by a) flood treatment, b) residue treatment, and c) flood X residue treatment combinations. Means with the same letter are not significantly different at $\alpha = 0.05$.

Treatment		Average Shoot Length (cm)	Standard Deviation
a) Flood	Control	5.05 **B**	2.99
	Intermittent	6.82 **A**	7.19
	Flowing	3.72 **B**	2.17
	Stagnant	3.68 **B**	2.24
b) Residue	Control	5.50	7.24
	Grass	4.32	3.05
	Legume	4.75	2.68
	Tree	4.71	2.70
c) Flood X Residue Combinations			
Control	Control	5.21	3.29
Control	Grass	5.33	3.41
Control	Legume	5.08	2.99
Control	Tree	4.58	2.20
Intermittent	Control	8.21	13.57
Intermittent	Grass	5.44	2.77
Intermittent	Legume	6.29	2.84
Intermittent	Tree	7.33	2.55
Flowing	Control	4.10	1.91
Flowing	Grass	3.37	2.78
Flowing	Legume	3.42	1.87
Flowing	Tree	3.99	1.97
Stagnant	Control	4.49	2.16
Stagnant	Grass	3.13	2.48
Stagnant	Legume	4.19	2.03
Stagnant	Tree	2.92	1.93

Correlation and Regression Analysis:

The relationship between soil chemistry variables (i.e., TOC, TN, C:N, NO_3-N, NH_4-N and TSP) and seedling variables (i.e., total germination and shoot length) were determined through correlation and regression analysis. Soil characteristics determined at the termination of the simulated flood experiment were used for these analyses. Total germination (expressed as %) was transformed ($p' = \arcsin \sqrt{p}$) prior to these analyses.

Total germination and shoot length were positively correlated ($r = 0.71$, $P < 0.0001$). Total germination was negatively correlated with NH_4-N levels and with soil TSP levels; both of these correlations were weak, but significant (Table 3.21). However, NO_3-N levels and total germination were not correlated. Simple linear regression analysis showed that soil TSP levels explained about 17% of the variation in total germination (Figure 3.19) while NH_4-N levels explained only 9%.

Shoot length was negatively correlated with NH_4-N levels and with soil TSP levels. Correlations were similar to those observed between these variables and total germination; again correlations were weak but significant (Table 3.21). Similar to total germination results, shoot length was not correlated with NO_3-N levels. Simple linear regression analysis revealed that soil TSP levels explained about 18% of the variation in shoot length (Figure 3.20), while NH_4-N levels explained about 7% of the variation.

Table 3.19. Correlation between chemical variables at the termination of the greenhouse simulated flood experiment with total germination (i.e., number of seeds germinated) and shoot length of grass seedlings. The first number for each comparison represents the Pearson correlation coefficients (r) and the second number represents the P value for the comparison. Significant relationships are highlighted in bold.

	TN	TOC	C:N Ratio	NO_3-N	NH_4-N	Soil TSP
Total	0.05	-0.10	-0.12	0.11	**-0.30**	**-0.41**
Germination	0.72	0.42	0.34	0.38	**0.02**	**0.0008**
Shoot	0.07	-0.10	-0.13	0.10	**-0.27**	**-0.43**
Length	0.60	0.43	0.31	0.42	**0.03**	**0.0004**

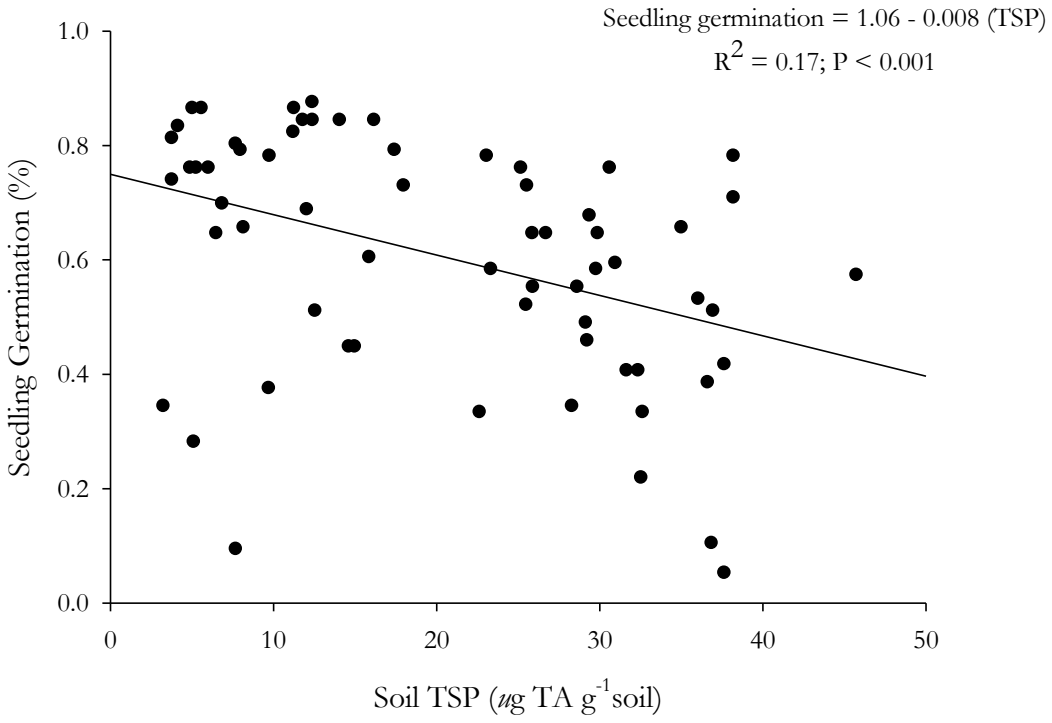

Figure 3.19. Relationship between soil TSP and seedling germination.

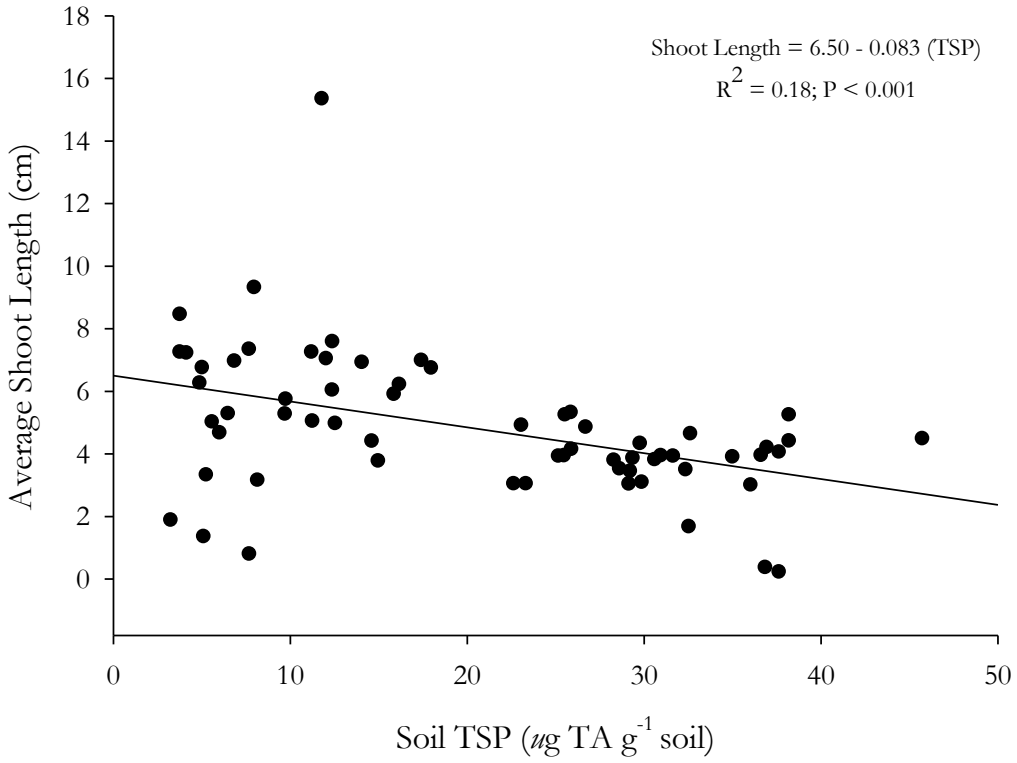

Figure 3.20. Relationship between soil TSP and average shoot length.

Discussion

Soil Oxygen Status

The monitoring of soil redox potential (ORP) as well as dissolved oxygen (DO) levels at the soil-water interface allowed determination of soil oxygen status. Redox reactions can be represented by the following half-cell reduction equation: $Ox + mH^+ + ne^- \rightarrow Red$, where Ox is the oxidized component or electron acceptor, Red is the reduced component or the electron donor, *m* is the number of hydrogen ions involved in the reaction and *n* is the number of electrons involved in the reaction (Patrick et al., 1996). In general, ORP increases with increasing activity of the oxidized component and decreases with

increasing activity of the reduced component; however ORP is pH dependent and will also increase with an increase in H^+ activity (Patrick et al., 1996). Using a benchmark of pH = 7, oxic or well-aerated soil conditions occur at ORP > 414 mV, and anoxic or anaerobic soil conditions occur at ORP < 120 mV (Sparks, 2003). Systems with ORP levels between these two thresholds are considered sub-oxic, indicating the presence of some oxygen as well as reduced forms of some inorganic compounds such as NO_3^-, MnO_2 and $Fe(OH)_3$.

Redox potential and DO results revealed that flood regimes (stagnant, flowing or intermittent) affected soil oxygen status. While ORP readings for the control treatment stayed above the 414 mV threshold indicating well aerated soil conditions, ORP readings for all flood treatments decreased between days 3-14. The flowing and stagnant flood treatments do not show further reductions in ORP after day 21 and ORP readings for the flowing treatment remain slightly above (but not necessarily different than) ORP readings for the stagnant flood. Dissolved oxygen readings for the flowing treatment were significantly higher than those for the stagnant flood. Redox potentials for the intermittent flood treatment showed a fluctuating pattern, with ORP increasing during periods of dry-down and decreasing upon subsequent re-flooding. Initially, DO readings for the intermittent flood treatment were not different from those recorded for the stagnant treatment. However, dry-down and re-flooding resulted in intermediate DO levels for the intermittent treatment at day 35; DO levels for the intermittent flood treatment were lower than those for the flowing flood but higher than those for the stagnant flood. Due to sensor limitations, DO readings were not available for the intermittent flood treatment during dry-down or for the control treatment. Redox potential, therefore, was the sole indicator of soil oxygen status for subsequent analyses.

The patterns of redox potential response to different flood treatments in this study were similar to those described in other systems. For example, a number of studies have demonstrated a decrease in redox potential with increased precipitation (Austin and Huddleston, 1999; Schuur and Matson, 2001). Likewise, Austin and Huddleston (1999) and D'Amore et al. (2004) both described a rapid decline in redox potentials with saturation and a subsequent rise in redox potentials with drainage of the soil. In addition, D'Amore et al. (2004) and Mansfeldt (2003) indicated that lower redox potentials were associated with longer periods of saturation. In the case of the current study, lower redox potentials were associated with flowing and stagnant flood conditions than with intermittent flood conditions. The maximum period of saturation for the intermittent treatment was 2 weeks whereas flowing and saturated treatments were both inundated for 8 weeks. The primary difference between the flowing and saturated treatments was the circulation of the flood water under the flowing treatment. The recirculation of the flood water resulted in significantly higher DO readings for the flowing flood and contributed to the slight difference in ORP readings between the flowing and saturated flood treatments. Oxygen diffusion into the soil from the floodwater is possible; however, diffusion of a gas through water is about 10,000 times slower than diffusion of a gas through air. Therefore, under inundated conditions, oxygen diffusion will be slow at best and will only help maintain aerated conditions at the surface. DO readings were taken at the soil-water interface, while ORP readings and soil samples were collected from the middle of the tray. The effect of flowing water on soil oxygen and subsequently N status would have been limited to the upper 2-5 cm of the soil and thus may have been missed due to collection protocols.

Soil Chemistry

Neither flood nor residue treatment influenced soil TOC or TN. The addition of residue resulted in significantly higher TOC and TN than the control treatment, but there were no differences due to residue type. Differences in NH_4-N and NO_3-N concentrations due to residue type were not observed, with one exception. The grass residue treatment resulted in higher NH_4-N concentrations than the other residue treatments under flooded conditions and in higher NO_3-N concentrations than other residue treatments under control conditions. Initial residue qualities (Table 3.1) may explain higher inorganic-N levels under the grass residue treatment. Residues with C:N ratios < 20, such as the grass residue used in this study, tend to decompose quickly resulting in net N mineralization. On the other hand, residues with C:N ratios > 20, such as the tree and legume residues used in this study, decompose more slowly and result in net N immobilization (Sylvia et al., 2005). Therefore, it was expected that the grass residue treatment would result in N mineralization and that the tree residue treatment would result in N immobilization; since the C:N ratio for the legume treatment was close to the threshold value, either mineralization or immobilization could have occurred depending on other limiting factors. Results seem to support these expectations; grass residues resulted in N mineralization with the form of inorganic-N released dependant on flood conditions (NO_3-N under aerobic conditions and NH_4-N under anaerobic conditions), while legume and tree residues resulted in N immobilization.

Studies have shown that soil inundation contributes to a decline in soil N availability including considerable losses of NO_3^- (Lockaby et al., 1996; Shelton et al., 2000; Schuur and Matson, 2001). Nitrate is a mobile form of N which can be leached from the system under flooded conditions. Since the saturated flood treatments in this experiment resembled a closed system, NO_3-N losses due to leaching were not expected. However, leaching could

have occurred in the flowing and intermittent flood treatments. Changes in TN with flood treatment were not observed (thus no losses in N due to volatilization or leaching of NO_3-N), but the relative contributions to TN by NO_3-N and NH_4-N varied by flood treatment. Under control conditions, NO_3-N increased and NH_4-N decreased. Soils in the control trays were aerobic and nitrification is the dominate process in well-aerated soils. Stagnant and flowing treatments were not different. In both cases NH_4-N increased whereas concentrations of NO_3-N remained low. Finally under intermittent flood conditions, NO_3-N was generally low, but increased toward the end of the experimental period when these trays were under dry-down conditions; increases that had been occurring in NH_4-N leveled off during this terminal dry-down period. As discussed in Chapter 2 and in the introduction to this chapter, a shift in the type of inorganic-N found in the soil with inundation has been reported in other studies (Reddy and Patrick, 1975; Hefting et al., 2004).

Investigations in rice systems have shown an increase in phenolic compounds with intensified cropping (Olk et al., 1996; Olofsdotter et al., 2002) or with incorporation of plant residue materials (Tsutsuki and Ponnamperuma, 1987). In the current study, soil TSP concentrations under flood treatments increased over the first 35 days of the study period, but decreased between days 35 and 56. Flood treatment generally did not affect TSP concentrations except that soil TSP declined more sharply between days 35-56 under the intermittent flood treatment than under other flood treatments. The grass residue resulted in slightly higher soil TSP concentrations than other residue additions, and the addition of residue resulted in higher soil TSP concentrations than control conditions.

Phenolic compounds are considered recalcitrant compounds; the presence of one or more aromatic rings within their structure makes them more resistant to enzymatic attack by microorganisms (i.e., not all microbes produce the enzymes necessary to cleave aromatic

rings). When a residue is added to the soil environment, simpler substrates, such as simple sugars and free amino acids, are readily used by the soil microbes. As decomposition progresses, these compounds are consumed and more complex substrates, such as hemicellulose, cellulose and chitin, are attacked. Eventually, lignin and other polyphenolic compounds are the primary substrates available and autochthonous microflora colonize these recalcitrant materials and begin their decomposition. Therefore, residue decomposition involves a succession of microbial organisms that moves toward organisms that are able to metabolize substrates of increasing chemical complexity (Sylvia et al., 2005). The decline in soil TSP concentrations between days 35-56 may be a result of this microbial/ decomposition succession. Early on, TSP accumulates due to preference for other substrates by microbes but also due to transformation reactions that are occurring within the soil. Phenolic compounds liberated by the decomposition of lignin may re-polymerize to form polyphenolic compounds that constitute soil organic matter or humus (Sylvia et al., 2005). Once the simple substrates are consumed, microbes turn to recalcitrant compounds, resulting in a decline in soil TSP. In the intermittent treatment, the last 2 weeks of the experiment were a dry-down period. As the soil begins to dry out, water-filled pore space is reduced and O_2 reenters the soil. With this influx of O_2 aerobic respiration is resumed, decomposition rates increase and TSP levels decline at a faster rate than in soils that remain inundated.

Relationship between Soil Oxygen Status and Soil Chemistry

In summary, inundation resulted in anoxic soil conditions and affected the form of inorganic-N present in the soil. In the control treatment NO_3-N increased whereas under flowing and stagnant flood conditions NH_4-N concentrations increased. Intermittent flood

conditions resulted in fluctuations in soil oxygen and inorganic-N; oxygen levels and NO_3-N concentration decreased with flooding but increased under dry-down while NH_4-N concentrations increased with flooding and decreased under dry-down. Inundation also resulted in the accumulation of TSP to a point; between 35-56 days of flooding, TSP levels began to decline with greatest declines occurring under dry-down conditions. While flooding seems to be the primary driver of the changes observed, residue treatment also had an effect on inorganic-N and TSP levels. In particular, grass residues (rather than legume or tree residues) resulted in the highest inorganic-N concentrations (NO_3-N under control conditions and NH_4-N under flooded conditions) as well as the highest TSP levels. These results are attributed to the low C:N ratio for the grass residue. With a C:N ratio of 13, high rates of decomposition and N mineralization are expected.

Although it may be important to know precise concentrations of inorganic-N or TSP in the soil, this is not always practical. Redox potential is a useful tool for elucidating soil oxygen status, but monitoring it can be complicated and expensive (see Chapter 2). Results show that ORP is positively correlated with NO_3-N and negatively correlated with NH_4-N and TSP levels. These correlations may allow for the collection of one variable (e.g. ORP) and the estimation of another. ORP in particular was shown to be a good predictor of soil NO_3-N, NH_4-N and TSP levels. Similarly, NH_4-N predicted TSP. The ability of inorganic-N levels to predict ORP was not tested; however the coupling of NH_4-N concentrations, TSP levels and other soil properties such as soil color may provide an adequate picture of soil oxygen levels (see Chapters 2 and 5).

Seedling Germination and Growth

Allelopathy has been defined as: "any direct or indirect harmful or beneficial effect by one plant (including microorganisms) on another through the production of chemical compounds that escape into the environment" (Rizvi et al., 1992). Chemicals typically referred to as "secondary metabolites" (i.e. compounds produced from metabolic pathways that are offshoots of primary metabolic pathways) are often implicated in allelopathic reactions; simple and complex phenolic compounds are among this long list of compounds. Allelochemicals may have a direct or indirect mode of action. Direct modes of action affect various aspects of plant growth and metabolism while indirect modes include effects on plants via altered soil properties, such as nutritional status of soil or altered microbial populations (Rizvi et al., 1992). The phenolic compounds that accumulate due to soil inundation may therefore be considered allelopathic due to their ability to limit nutrient availability (Northup et al., 1998). Polyphenolic compounds are known to bind to N, rendering it unavailable for microbial conversion or plant uptake (Gaunt et al., 1995; Inderjit and Mallik, 1997; Kruse et al., 2004; Schmidt-Rohr et al., 2004). This decrease in plant available N may subsequently reduce net primary productivity as seen in rice studies (Cassman et al., 1995; Schmidt-Rohr et al. 2004).

The post-flood germination study conducted here provides some evidence for indirect allelopathic actions by phenolic compounds. Although the use of bulk soil samples in place of more traditional germination assay methods (i.e. filter paper lined Petri dishes with applications of known concentrations of specific compounds) results in increased uncertainty in the results (i.e. greater variability and thus reduced predictability) some effects were observed. For example, the highest germination rates and the longest shoot growth measurements occurred on soils from the intermittent flood treatment. On the other hand,

the lowest germination rates were associated with soils from the stagnant flood treatments; low germination was also associated with the grass residue treatment. Finally, germination and shoot length were negatively correlated with NH_4-N and TSP. TSP was the better predictor of these measures; however, predictability was low.

Increased germination and seedling growth under intermittent flood treatments may have to do with a combination of soil moisture levels and soil chemistry. At the initiation of the germination experiment, soils from the saturated and flowing flood treatments were still saturated with water, while those from the intermittent and control treatments had less water-filled pore space and thus were better aerated. Soils from the intermittent flood treatment had a mixture of NH_4-N and NO_3-N available while other treatments had a dominance of one form or the other. The control treatment had high levels of NO_3-N but negligible amounts of NH_4-N; on the other hand, the flowing and stagnant treatments had high levels of NH_4-N and very low levels of NO_3-N. While total N did not vary between flood treatments and thus TN was not more limiting under stagnant or flowing flood treatments, perhaps there is some advantage to plants when soil inorganic-N consists of both NH_4-N and NO_3-N (Brady and Weil, 2002). The intermittent flood treatment had lower TSP than the stagnant and flowing flood treatments at the termination of the experiment. With lower TSP, there is a possibility that less phenolic-N binding is occurring and therefore, the inorganic-N that is present is more available for plants.

A similar argument may be made to explain the low germination observed under grass residue treatments. The grass residue treatment resulted in higher levels of NH_4-N under flooded conditions (stagnant, flowing or intermittent) than other residue treatments. However the grass residue also resulted in the highest levels of TSP when compared with other residue treatments. It is possible then, that the accumulation of TSP under the grass

treatment lead to phenolic-N binding, rendering the N that was mineralized unavailable to the germinating seeds.

Conclusions

Under the controlled conditions of the greenhouse, the effect of flood and residue type on soil inorganic-N and TSP levels were examined. Flood treatment had a greater effect than residue treatment on soil oxygen status. Stagnant flood treatments had the lowest ORP and DO readings, followed by flowing and intermittent flood treatments. The incorporation of residue increased TOC and TN over the control but individual residue treatments did not differ and no differences in TOC and TN were observed due to flood treatment. While TN did not vary with flood treatment, the form of inorganic-N present did. Stagnant and flowing flood treatments resulted in a dominance of NH_4-N, while the control treatment had a dominance of NO_3-N; the intermittent treatment had a mixture of NH_4-N and NO_3-N. Redox potential was a good predictor of NO_3-N, NH_4-N and TSP. TSP accumulated due to soil inundation; however toward the termination of the experiment, TSP levels were declining. Grass residue incorporation resulted in more N mineralization and greater accumulation of TSP than tree or legume residue. The follow-up seed germination experiment seems to show an effect of soil TSP levels on germination. Treatments, such as the stagnant flood and the grass residue, resulted in greater soil TSP levels had the least germination. These results are in agreement with expectations stated at the beginning of the experiment.

Results of the current study are similar to those observed for rice systems but may not be applicable to floodplain systems. The simulated floods in the greenhouse were generally closed systems under careful control. Floodplains are open systems where water,

sediments, and nutrients are routinely exchanged between the stream and floodplain. In addition, greater variability in soil properties will be encountered across the floodplain. While phenolic compounds may accumulate in floodplain soils under flood conditions, their effect on seedling germination and plant growth may be minimal. The nature of the phenolic compound (i.e. soluble vs insoluble) will determine its ability to bind N and thus its ability to affect plant nutrition. The effect of phenolic compounds will also depend on their residence time in the soil. Phenolic compounds that are bound to soil particles will have reduced N-binding ability. Likewise, phenolic compounds that are attacked and/or altered by microbial organisms will have reduced N-binding ability. Phenolic compounds that are unable to bind N, will have less indirect effects on plant productivity. Further work to identify specific phenolic compounds formed under these systems (i.e. flood and residue combinations) and their effect on plant productivity is needed.

CHAPTER 4

EFFECTS OF FLOODING ON SOIL MICROBIAL POPULATIONS

Introduction

A disturbance such as flooding affects both above- and below-ground ecosystem processes. Although often ignored, changes in below-ground environments following flooding are no less important than those that occur above-ground. Similar to their above-ground counterparts, soil microorganisms are sensitive to disturbance (Kennedy 1999). Cropping, tillage and other land management practices can have significant effects on soil microbial community characteristics (Buyer and Drinkwater, 1997; Suzuki et al., 2005; Fraterrigo et al., 2006). Conventional and organic farming systems may support different soil microbial communities (Buyer and Drinkwater, 1997) and the application of chemical fertilizers may alter the bacterial:fungal ratio of a given community (Suzuki et al., 2005). The effects of intensive agriculture on soil microbial communities may persist long after the disturbance has ceased. For example, in a comparison study of southern Appalachian forest stands, Fraterrigo et al. (2006) found that previously farmed stands had a high abundance of Gram-negative bacterial markers and a low abundance of fungal indicators, while logged and reference stands had high levels of fungal markers and Gram-positive bacteria markers. Shifts in soil microbial community structure are expected when anaerobic conditions develop from flooding (Kennedy, 1999; Elhottova et al., 2002; Mentzer et al., 2006). These changes may subsequently affect above-ground components of the ecosystem due to the critical roles that bacteria and fungi play in decomposition and nutrient cycling. Microbial diversity as a whole can directly influence plant productivity and diversity by influencing

plant growth and development, plant competition and nutrient and water uptake (Suzuki et al., 2005).

Any attempt to understand how a disturbance affects the characteristics of the soil microbial community however, must address the challenge of quantifying that community. Most soil microorganisms cannot be cultured and not more than 1% of total microbes in the soil community have been identified (Buyer and Drinkwater, 1997; Buyer et al., 2002; Dierksen et al., 2002; Suzuki et al., 2005). Species isolation is difficult and time-consuming, often resulting in the processing of a limited number of samples (Buyer and Drinkwater, 1997) and even then, many species of bacteria are not detected by isolation plating (Buyer et al., 2002). The result of this selective culturing is often a severe underestimate of the actual diversity of the soil microbial community (Cavigelli et al., 1995). Phospholipid fatty acid (PLFA) analysis uses membrane phospholipids for microbial community characterization and represents one method that overcomes the problem of selective culturing (Buyer and Drinkwater, 1997; Ibekwe and Kennedy, 1998). PLFA has been shown to be a powerful tool for structural analysis of microbial communities (Buyer and Drinkwater, 1997).

PLFA analysis creates a profile or fingerprint of the soil microbial community. The primary assumptions of PLFA analysis are: i) phospholipids make up a relatively constant proportion of the cell biomass and ii) fatty acid variation among taxonomic groups results in markers which can be used to interpret community-level profiles (Ibekwe and Kennedy, 1998). PLFA analysis can determine relative quantity of basic species groups (Kennedy, 1999) such as "Gram-negative bacteria" or "aerobic bacteria". PLFA analysis does not provide species or strain information (Kennedy, 1999) but offers insight into changes in microbial groups (Ramsey et al., 2006). PLFA analysis of overall soil communities cannot be used to detect differences in species diversity, detect changes in the abundance of individual

species or attribute differences in function to differences in the abundance of any one fatty acid or organism (Carney and Matson, 2005). However, PLFA has a number of advantages over other methods, perhaps the most important of which is that it does not require growth in culture (Buyer and Drinkwater, 1997; Nannipieri et al., 2003). PLFA analysis is selective for the living component of the soil microbial community. Other methods, such as fatty acid methyl ester (FAME) analysis, result in a profile that includes living cells, dead cells, humic materials and exudates from plants and roots (Ibekwe and Kennedy, 1999). Through the isolation of the phospholipids from cell membranes which are believed to be rapidly scavenged from non-living cells, PLFA analysis focuses on the living component and thus on the active fraction of the soil (Bossio and Scow, 1998; Ibekwe and Kennedy, 1998; Kennedy, 1999; Nannipieri et al., 2003). In a comparison of techniques including community level physiological profiling (CLPP) and various PCR-based methods, Ramsey et al. (2006) concluded that PLFA was the most powerful of the methods tested in terms of demonstrating changes in microbial community structure.

PLFA analysis can show shifts in species groups (i.e. changes in microbial community structure) due to environmental changes resulting from disturbance or land management practices. Additional analyses are necessary, however, to demonstrate how microbial community function changes due to environmental conditions. Because of the role of both the soil microbial community and soil enzymes in nutrient cycling (Bandick and Dick, 1999; Parham and Deng, 2000; Michel and Matzner, 2003; Acosta-Martinez et al., 2007), and because the majority of soil enzymes are of microbial origin (Bandick and Dick, 1999; Parham and Deng, 2000), enzyme assays can be utilized for the assessment of soil microbial community function. Indeed, strong relationships have been found between microbial processes and community enzyme activity (Burns and Ryder, 2001).

Enzyme assays can be used to assess different nutrient cycling processes. For example, β-glucosaminidase activity has been used as an index of N mineralization in soils (Parham and Deng, 2000; Ekenler and Tabatabai, 2004; Acosta-Martinez et al., 2007) and β-glucosidase has been used as an index of C mineralization in soils (Michel and Matzner, 2003; Gil-Sotres et al., 2005; Acosta-Martinez et al., 2007). β-Glucosidase is involved in the last limiting step of cellulose degradation (Acosta-Martinez et al., 2007). Specifically, β-glucosidase hydrolyzes the disaccharide cellobiose to glucose, a major C source for microorganisms (Eivazi and Tabatabai, 1988; Michel and Matzner, 2003). β-Glucosaminidase participates in conversion of chitin into amino sugars (Parham and Deng, 2000; Acosta-Martinez et al., 2007). Specifically, this enzyme cleaves *N*-acetyl-β-D-glucosamine residues from the terminal non-reducing ends of chitooligosaccharides (Parham and Deng, 2000; Acosta-Martinez et al., 2007). Because chitin is an important transient pool of organic C and N in the soil, β-glucosaminidase may play an important role in both C and N cycling in soil (Parham and Deng, 2000).

Soil enzyme assays have shown changes in microbial functioning due to past management practices and disturbances. For example, Bandick and Dick (1999) showed differences in soil enzyme activities between grasslands and cultivated fields. Their work also revealed differences in soil enzyme activities in cultivated fields under various cover crops or in cultivated fields with and without organic residue additions. Differences in soil enzyme activities in soils under pasture, agriculture and forested conditions have also been observed (Acosta-Martinez et al., 2007). Enzyme activity has also been shown to be affected by disturbances such as waterlogging (Pulford and Tabatabai, 1988), and fluctuating water levels (Corstanje and Reddy, 2004; Mentzer et al., 2006). Other environmental disturbances, such as N deposition, may also affect soil enzyme activity. For example, N deposition in

Norway spruce forests in Germany resulted in decreased β-glucosidase activity (Michel and Matzner, 2003). Despite the evidence that soil microbial function may be altered by management practices and disturbance, others caution that process level measurements may be insensitive to community level changes due to redundancy of these functions within an ecosystem (Kennedy, 1999; O'Donnell et al., 2005).

Objectives and Expectations

The primary objective of this experiment was to determine the effect of various flood treatments on soil microbial community structure and function. Microbial community structure will be assessed using PLFA analysis while enzyme assays will be used to asses microbial community function. Microbial community structure is expected to decrease in complexity with increased flood duration. In particular, stagnant flood conditions should result in less diverse microbial populations, while intermittent or fluctuating flood conditions which create both aerobic and anaerobic soil conditions are expected to increase microbial diversity due to increased habitat diversity. Changes in diversity will be recognized through changes in microbial groups detected or through changes in the levels of response by detected groups. Fungal groups are expected to decline; this decline will be indicated by reduced response of the fungal marker as well as by an increased bacterial:fungal ratio. Changes in microbial community structure with inundation should be reflected in changes in enzyme activity. In other words, enzyme activity is expected to decrease with increased flood duration. However, fluctuating flood conditions are expected to result in enhanced enzyme activity.

Methods

Soil Samples

Two sets of soil samples were examined. The first set included soil samples collected at the termination of the simulated flood experiment (See Chapter 3). The second set of soil samples included the pre- and post-flood samples from the FTL (See Chapter 2). Soils collected from the floodplain adjacent to the FTL serve as pre-experiment reference samples for the simulated flood experiment. All soil samples were analyzed by dry combustion for total N (TN) and total organic C (TOC) using a LECO TruSpec CN analyzer; C:N ratios were calculated from these measurements.

Microbial Community Structure

Microbial community structure was assessed using PLFA analysis. This analysis allowed for quantification of groups of soil microorganisms, determination of overall microbial biomass, calculation of a bacterial to fungal (B:F) ratio and determination of stress indicators. The PLFA procedure generally follows Ibekwe and Kennedy (1998) and Petersen and Klug (1994) with some modifications. This procedure involves three phases: i) extraction, ii) separation and extraction of phospholipids using columns, and iii) analysis of PLFAs.

Soil was extracted by mixing 2 g of freeze-dried soil with 1.8 ml phosphate buffer (50 mM, pH 7.4) and 7.5 ml methanol:dichloromethane (2:1). Samples were extracted for 2 hours on a Whirly mixer. Next, 2.5 ml of dichloromethane and 10 ml of supersaturated NaBr were added to the samples which were returned to the Whirley mixer for overnight extraction (i.e. minimum of 12 hours). Samples were centrifuged at 3000 rpm for 5 minutes. The organic (surface) layer was transferred to 10 ml Pyrex tubes with Teflon-lined caps and

allowed to evaporate completely under a stream of nitrogen. Sample extracts were made by eluting the dried lipid extracts with 300 µl chloroform.

Phospholipids were separated by solid phase extraction. Twelve columns were mounted on a vacuum manifold and connected to a pump via a side arm flask for collection of solvents. Conditioning of the columns was necessary for the selective retention of the phospholipids. This was achieved through sequential addition of 3 ml hexane, 1.5 ml hexane/chloroform (1:1) and 100 µl chloroform; a slight vacuum (1-2 in. Hg.) was applied to the columns after the addition of each solvent. Once the columns were conditioned, a standard solution of known phospholipid composition was added to the columns along with the sample extract. After the addition of sample extracts, the columns were rinsed through the sequential addition of 1.5 ml chloroform/2-propanol (1:1) and 1.5 ml 2% acetic acid in diethyl ether. Again vacuum was applied after each solvent addition. Finally, phospholipids were eluted from the columns with 2 ml methanol, and evaporated under nitrogen in preparation for extraction of the PLFAs. The dried methanol extracts were dissolved in 1 ml methanol:toluene (1:1) and 1 ml 0.2 M KOH in methanol. This mixture was vortexed briefly, and then heated for 15 minutes at 37°C (water bath). Next, 2 ml hexane, 0.3 ml 1 M acetic acid and 2 ml demineralized water were added sequentially. Solutions were vortexed thoroughly and centrifuged at 3000 rpm for 5 minutes. The organic (surface) phase was transferred to a test tube, 2 ml hexane was added and the extraction was repeated. The combined organic phase was evaporated to dryness under nitrogen and then redissolved in 75 µl hexane:MTBE (1:1) and transferred to a gas chromatograph (GC) vial with a low volume insert.

Fatty acids were analyzed by gas chromatography (GC) using an automated procedure developed by Microbial I.D. Inc, Newark, DE (Sherlock Microbial Identification

System, 1996) was used for this analysis. The GC (Agilent Technologies 6890N) was equipped with a flame ionization detector, an automatic sampler and an integrator. The column was an Ultra 2 nonpolar fused silica capillary column (25 m by 0.2 mm by 0.33 mm) (Hewlett-Packard). Flow rates of N_2 (make-up gas), H_2 (carrier gas), and dry air (used to support the flame) were 30, 30/55/5 and 400 ml min^{-1}, respectively. The temperature program ramps from 170°C to 300°C at 5°C per min. The temperature then is held at 300°C for 12 minutes to allow cleaning of the column. Total run-time was 38 minutes.

Each sample peak was compared against a database of known microbial fingerprints. Peaks that correspond to carbon chain lengths of 12-20 carbons are generally associated with microorganisms (Table 4.1). Fatty acids are designated by the number of carbon atoms, followed by a colon, the number of double bonds and then by the position of the first double bond from the methyl (ω) end of the molecule; *cis* and *trans* isomers are indicated by c or t(Ibekwe and Kennedy, 1998). For example the PLFA 16:1 ω7c represents the *cis* isomer of a 16 carbon fatty acid molecule with 1 double bound at the 7^{th} carbon from the methyl end of the molecule. Branched chain fatty acids are indicated by the prefixes 'i' and 'a' for *iso* and *anteiso* branching respectively. The prefix 'cy' designates cyclopropane fatty acid (Ibekwe and Kennedy, 1998).

Table 4.1. Fatty acid markers and the associated categories of organism used in this study.

Fatty Acids	**Marker 1** ***Organisms***	**Marker 2** ***B:F ratio***	**Marker 3** ***Stress***	**Marker 4** ***Monounsaturated Fatty Acids***
10:0B	Gram negative	Bacteria		
12:0b	Gram negative	Bacteria		
12c alcohol	Gram negative	Bacteria		
14:0			strs31	mono
14:0i		Bacteria		
15:00	Gram positive	Bacteria	strs31	mono
15:00 all	Gram positive	Bacteria		
15:0a	Gram positive	Bacteria		
15:0i	Gram positive	Bacteria		
15:1cy	Anaerobe	Bacteria		
16:0		Bacteria ?	strs31	mono
16:0Me 10	Gram positive /Actinomycete	Bacteria		
16:1ω11c			strs32	
16:1ω5	Mycorrhizae	Fungi		
16:1ω5c			strs32	
16:1ω7	Aerobe	Bacteria		
16:1ω7c			strs12	
16:1ω7c			strs32	
16:1ω7t	Aerobe	Bacteria		
16:1ω9c			strs32	
16c alcohol	Gram positive	Bacteria		
17:0			strs31	mono
17:0a	Gram positive	Bacteria		
17:0cyc	Gram negative /Anaerobic	Bacteria	strs11	
17:0i	Gram positive	Bacteria		
17:0me10	Actinomycete	Bacteria		
17:1ω5c			strs32	
17:1ω6	Sulfate	Bacteria		
17:1ω7c			strs32	
17:1ω7i	Sulfate	Bacteria		
17:1ω8c			strs32	
17:1ω9c			strs32	
18:0			strs31	mono
18:1ω7	Aerobe	Bacteria		
18:1ω7c	Gram negative	Bacteria	strs22	
18:1ω9	Fungi	Fungi		
18:1ω9c	Fungi	Fungi		

Table 4.1 Cont.

Fatty Acids	**Marker 1** ***Organisms***	**Marker 2** ***B:F ratio***	**Marker 3** ***Stress***	**Marker 4** ***Monounsaturated Fatty Acids***
18:2ω6,9	Mycorrhizae	Fungi		
18:3ω3	Fungi	Fungi		
18:3ω6	Fungi	Fungi		
18:3ω6c	Fungi	Fungi		
19:0			strs31	mono
19:0cyc	Gram negative /Anaerobic	Bacteria	strs21	
20:0			strs31	mono
20:3ω6	Protozoan	Protozoan		
20:4ω6	Protozoan	Protozoan		

Peak chromatographic responses were translated into mol responses for each microbial group. To calculate mol response, the response for each chain length was divided by the appropriate molecular weight. Mol responses from each sample were summed to determine total mol response and mol percents were calculated from these measures. Mol percent and the biomarkers representing both bacterial and fungal components were summed individually and bacterial:fungal (B:F) ratios were calculated for each sample. Biomass was calculated from mol response readings using the relationship determined by Bailey et al. (2002). The mol response for each sample was summed and then multiplied by an extraction efficiency factor (based on internal standards added to each run); the resultant response was then entered into the following equation: biomass = 2.4 (response) + 46.2.

In addition to the parameters described above, stress indicators and monounsaturated fatty acids (MFA) were evaluated. Monounsaturated fatty acids are a general indicator of aerobic conditions and of high substrate availability (Bossio and Scow, 1998) therefore, a decrease in MFA would indicate more stressful conditions. Stress

indicators were calculated based on the ratios of the cyclopropyl fatty acids to monoenoic precursors and the total saturated to total monounsaturated fatty acids (Kieft et al., 1997; Bossio and Scow, 1998; Fierer et al., 2003). Specific peaks used to calculate the cyclopropyl fatty acids to monoenoic precursor ratios were cy17:0 to 16:1ω7c and cy19:0 to 18:1ω7c (Table 4.1). A change in these ratios are thought to indicate a change in the physiological state of some bacteria, and increases in cyclopropyl fatty acids have been observed under conditions of low C, low O_2, high acidity, and high temperature (Bossio and Scow, 1998). The ratio of total saturated to total monounsaturated fatty acids is based on the fatty acid designations in Table 4.1 (Marker 3; which is the ratio of the sum of strs31 markers to the sum of strs32 markers).

Microbial Activity

Microbial activity was assessed through N- and C-utilization patterns determined by enzyme assays. Nitrogen utilization was assessed by determination of β-glucosaminidase activity following the methodology outlined in Parham and Deng (2000). One gram of soil in 4.0 ml 0.1M acetate buffer (pH 5.5) and 1.0 ml 10mM p-nitrophenyl-N-aceetyl-β-D-glucosaminide (PNNAG) solution was incubated at 37°C for one hour. Following incubation, 1.0 ml of 0.5M $CaCl_2$ and 4.0 ml of 0.5M NaOH were added to the sample solution to stop the reaction. Samples were filtered (Whatman #2) and then measured at 405 nm with a spectrophotometer. Carbon utilization was assessed by determination of β-glucosidase activity following the methodology outlined in Tabatabai (1994). One gram of soil in 0.25 ml toluene, 4.0 ml MUB (pH 6.0) and 1.0 ml 10mM p-nitrophenyl-β-glucoside (PHG) solution was incubated at 37°C for 1 hour. Following incubation, 1.0 ml of 0.5 M

$CaCl_2$ and 4.0 ml of 0.1 THAM buffer (pH 12) were added to stop the reaction. Samples were filtered (Whatman #2) and then measured at 410 nm with a spectrophotometer.

Data Analysis

Principle component analysis (PCA) was used as exploratory data analysis to reduce the dimensionality in the data and to examine the associations in the microbial populations (Tabachnick and Fidell, 2001). For soil samples from the simulated flood experiment, two-way ANOVA (Proc GLM) was used to test the effects of flood and residue treatments on: i) PLFA PCA axes 1 and 2, ii) B:F ratio and microbial total biomass, iii) responses due to various microbial markers, iv) stress indicators and MFA, and v) C- and N-utilization. Pairwise comparisons (LSD) were employed when significant differences were detected. For soil samples from the FTL, one-way ANOVA (Proc GLM) was used to analyze the effects of sampling date, sample depth and flood treatment on: i) PLFA PCA axes 1 and 2, ii) B:F ratio and microbial total biomass, iii) responses due to various microbial markers, iv) stress indicators and MFA, and v) C- and N-utilization. For the flood treatment analyses, each sample date (pre- and post-flood) and depth (upper (0-10 cm) and lower (10-20 cm)) combination was tested separately. Pairwise comparisons (LSD) were employed when significant differences were detected. In addition, T-tests were used to compare the pre- and post-flood samples for changes in these soil microbial characteristics. Pearson correlation coefficients were calculated to determine the relationships between i) soil chemical characteristics (TOC, TN, and C:N ratio) and soil microbial community characteristics (PLFA's and β-glucosidase and β-glucosaminidase activities) and between ii) β-glucosidase and β-glucosaminidase activities and the responses due to various microbial markers. All analyses were conducted using SAS 9.1 Statistical Software (SAS Institute Inc 2002-2003).

Results

Microbial Community Structure

Simulated Flood Experiment

Principal component analysis of PLFAs revealed flood treatment effects but not residue effects on the soil microbial community (Figure 4.1)(residue effects not shown). PC1 accounted for 53% of the variance and PC2 was responsible for explaining 18% of the data variance. Soil samples separated into four clusters that aligned with flood treatments. Aerobic flood treatments (i.e. control and intermittent flood treatments) and anaerobic flood treatments (i.e. flowing and stagnant flood treatments) were separated along the PC1 axis. Eigenvectors for the analysis included 14:0me2, Phytanic acid, and 18:3ω6c for the separation to the left lower quadrat and 19:0cyclo c11-12, to the upper left. The peak 15:0i was responsible for the separation to the lower right quadrat and 17:0cyc, 18:1ω9t, 16:1ω7c, and 16:0 for the upper right quadrat. These peaks corresponded to fungal components responsible for separation to the lower left of the graph and bacterial components for separation to the upper and lower right.

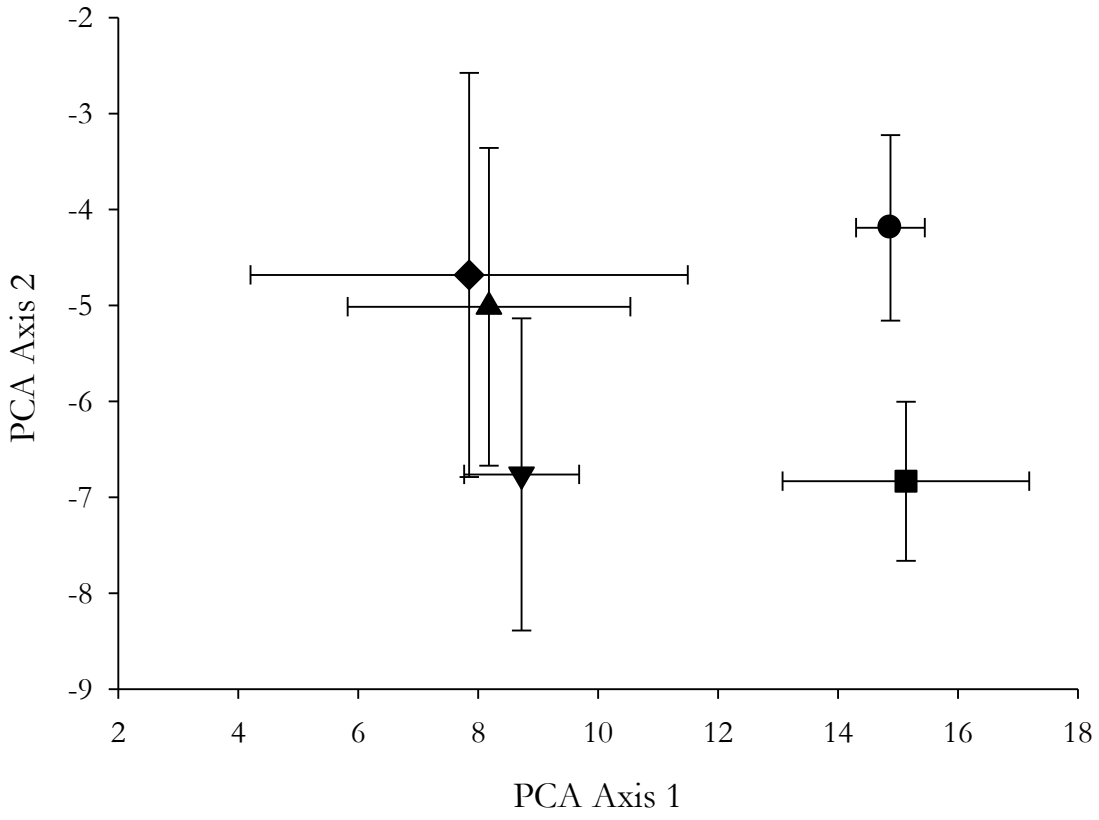

Figure 4.1. Principal component analysis of phospholipid fatty acids from the simulated flood experiment greenhouse experiment. PC1 explained 53% of the variation and PC2 explained 18% of the variation. Means are represented by symbols: triangle = pre-flood reference sample, circle = control flood treatment, square = intermittent flood treatment, inverted triangle = flowing flood treatment and diamond = stagnant flood treatment; lines drawn from each symbol represent ± 1 standard deviation along PC1 and PC2 axes.

ANOVA of PC1 revealed a significant flood effect and a flood x residue interaction ($F = 68.98$, $P < 0.0001$ and $F = 3.35$, $P < 0.01$ respectively); a significant effect due to residue treatment was not observed for this axis ($F = 0.60$, $P = 0.62$). LSD analysis for PC1 revealed that the intermittent and control treatments were significantly different from the flowing and stagnant treatments. ANOVA of PC2 revealed a significant flood effect and a significant residue effect ($F = 22.52$, $P < 0.0001$ and $F = 10.94$, $P < 0.0001$ respectively), but

no interaction ($F = 1.78$, $p = 0.10$). Control and stagnant flood treatments were significantly different from flowing and intermit flood treatments on PC2; likewise, the control residue treatment was different from other residue treatments.

Flood treatment had a greater effect on the soil microbial community structure than residue treatment or the flood X residue interaction (Table 4.2). Total microbial biomass and the mol percent of response due to aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria and mycorrhizal fungi all varied with flood treatment (Figures 4.2 & 4.3). Furthermore, flood treatment affected the B:F ratio as well as the stress indicators and the sum of MFA peaks (Figures 4.4 & 4.5). The B:F ratio and the Gram-negative bacteria marker were also affected by residue treatment (Table 2). A significant flood X residue interaction was observed for the B:F ratio and for the Gram-positive bacteria marker. The mol percent of response attributed to anaerobic bacteria did not vary with flood or residue treatment.

Table 4.2. ANOVA results for PLFA analysis of soil samples from the simulated flood experiment. For each microbial component, numbers in the first row are the F value; numbers in the second row are the P value. Significant results are highlighted in bold.

Microbial Component	Flood	Residue	Flood x Residue
Microbial Biomass	**17.83**	0.80	1.55
	<0.0001	0.50	0.16
Aerobic Bacteria	**27.61**	1.64	1.57
	<0.0001	0.19	0.15
Anaerobic Bacteria	0.55	1.90	1.49
	0.65	0.14	0.18
Gram-Negative Bacteria	**60.96**	**3.23**	1.06
	<0.0001	**0.03**	0.41
Gram-Positive Bacteria	**141.02**	0.40	**2.22**
	<0.0001	0.75	**0.04**
Mycorrhizal Fungi	**16.65**	0.28	0.40
	<0.0001	0.84	0.93
B:F Ratio	**59.02**	**5.65**	**7.32**
	<0.0001	**0.0021**	**<0.0001**
Stress Indicators	**2.95**	0.20	1.27
	0.04	0.90	0.28
Monounsaturated	**8.58**	0.35	0.72
Fatty Acids	**0.0001**	0.79	0.69

Biomass was greatest in the intermittent flood treatment and lowest in the stagnant flood treatment (Figure 4.2). Total biomass for the flowing treatment was intermediate and did not differ significantly from the intermittent or the control treatments. A similar pattern was observed for the aerobic bacteria marker. The intermittent flood treatment showed the greatest response, while the stagnant flood treatment showed the least (Figure 4.3). The flowing and control flood treatments were not different in terms of mol percent response for aerobic bacteria. Therefore, the flood treatments that were the most aerobic (i.e. control and intermittent flood) had the greatest amount of aerobic bacteria present within the soil community.

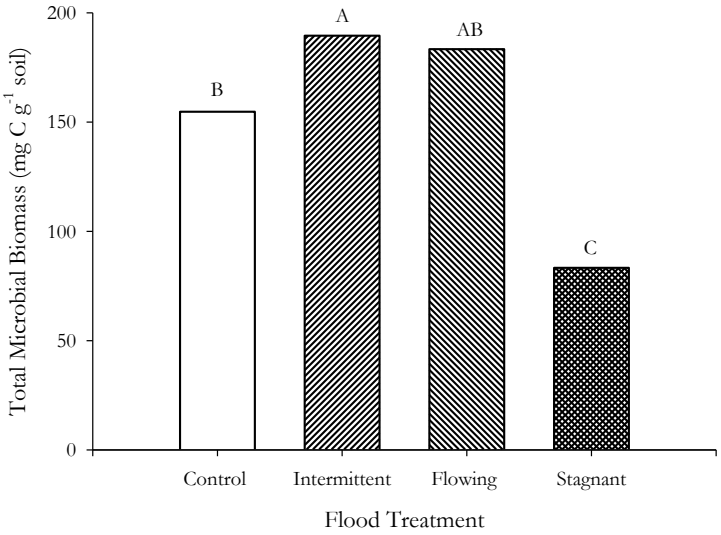

Figure 4.2. Total microbial biomass by flood treatment for simulated flood experiment. Columns with the same letter are not significantly different.

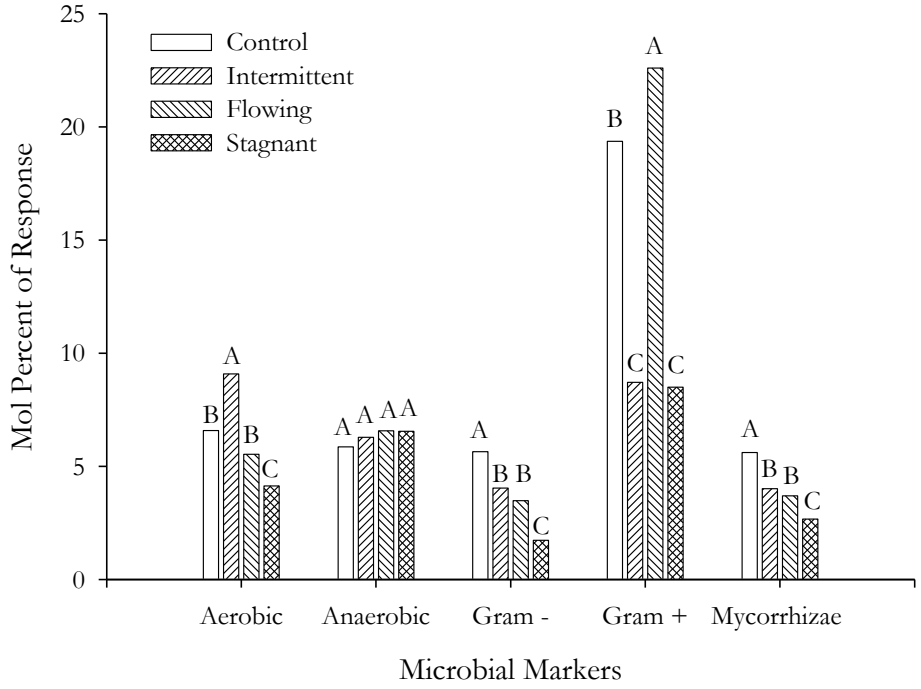

Figure 4.3. Mol percent response of various microbial markers to flood treatment in simulated flood experiment. Bars within the same microbial marker group with the same letter are not significantly different.

Gram-negative bacteria and mycorrhizal fungi responded similarly to flood treatment (Figure 4.3), with the control treatment having the greatest response, and the stagnant treatment having the lowest response. The intermittent and flowing flood treatments did not differ; these flood treatments resulted in an intermediate response for these microbial markers. Gram-negative and Gram-positive bacteria as well as mycorrhizal fungi were negatively affected by anaerobic conditions brought about by stagnant flood waters. Gram-positive bacteria were most prevalent under flowing flood and control conditions and least prevalent under intermittent and stagnant flood conditions (Figure 4.3). Gram-negative bacteria differed with residue treatment (data not shown). The greatest response for Gram-negative bacteria was observed with grass residue; the other residue treatments showed less response.

Flood and residue treatments as well as a flood X residue interaction significantly affected the B:F ratio (Table 4.2). The B:F ratio was significantly greater under control treatments than in the other treatments (Figure 4.4). With regards to the main effect of flooding, the flowing flood had the highest B:F ratio. Bacterial:fungal ratios for intermittent and stagnant floods were significantly less than the flowing flood but were not different from each other (Figure 4.4). With regards to the main effect of residue, the tree, legume and grass residue treatments were not different from each other. Bacterial:fungal ratios for the control flood treatments were 6-9 times higher than those for the flowing and stagnant flood treatments and 4-6 times higher than those for the intermittent flood treatment (Figure 4.4). An exception is the flowing flood/control residue combination which was not significantly different than the control flood samples (Figure 4.4).

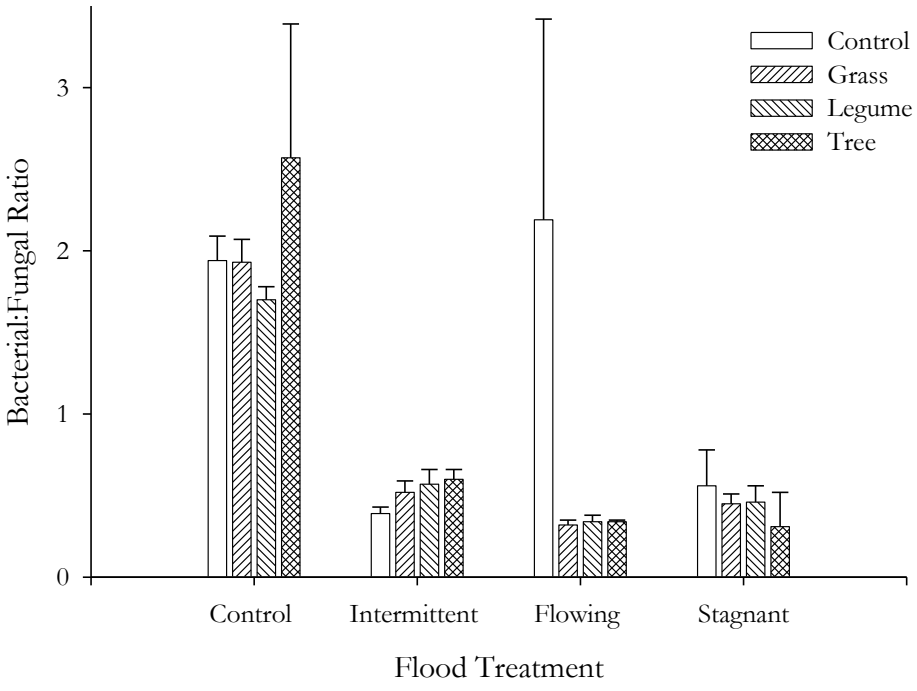

Figure 4.4. Bacteria:Fungal ratio by flood and residue treatment for soil samples from the simulated flood experiment. Error bars represent ±1 standard deviation.

Variation in stress indicators and in MFA was due primarily to flood treatment; there were no differences due to residue treatment or flood X residue interaction (Table 4.2). Stress indicators were highest in the stagnant and flowing treatments (i.e. under anaerobic conditions) and lowest in the control and intermittent flood conditions (Figure 4.5a). Conversely, MFA were highest in the intermittent and control treatments and lowest under stagnant flood conditions (Figure 4.5b).

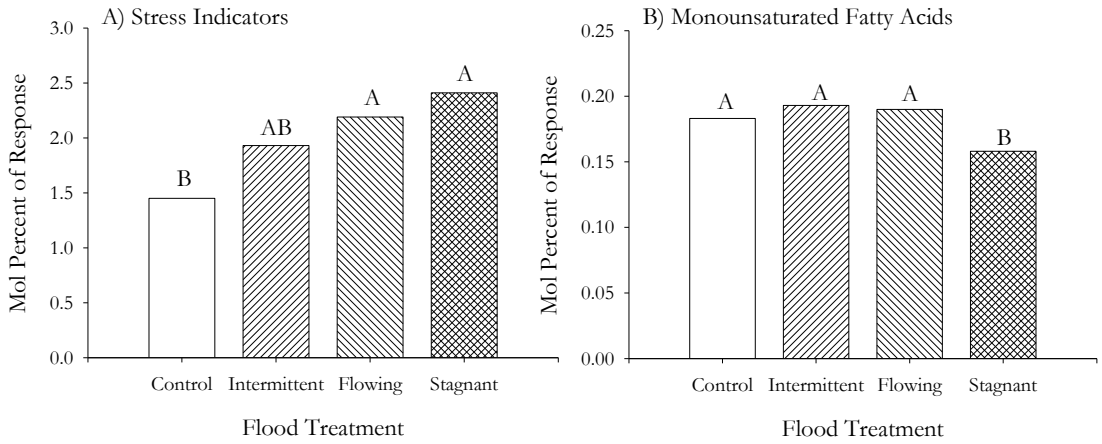

Figure 4.5. Response of stress indicators and monounsaturated fatty acids to simulated flood treatments in the greenhouse. Bars with the same letter are not significantly different.

Flood Tolerance Laboratory

PCA analysis of PLFA from FTL soils indicated that most of the variation was attributed to sampling depth (upper or lower) and date (pre- or post-flooding) but not to flood treatment (Figure 4.6)(flood treatments not shown). Principal components 1 and 2 explained 53% of the variance in the data with PC1 accounting for 34% and PC2 responsible for 19% of the overall variance. ANOVA results indicated significant differences due to sampling date along both axes ($F = 4.60$, $P = 0.04$ for PC1 and $F = 4.24$, $P = 0.05$ for PC2) and sampling depth along PC2 ($F = 11.23$, $P < 0.002$). Eigenvectors for the analysis included iso 17:1g, 15:0i and 17:1anteiso responsible for the separation to the right portion of the graph. Microbial community changes in Gram-positive bacteria occurred between the pre-flood conditions verses the post-flood with Gram-positive bacteria being higher in the post-flood samples.

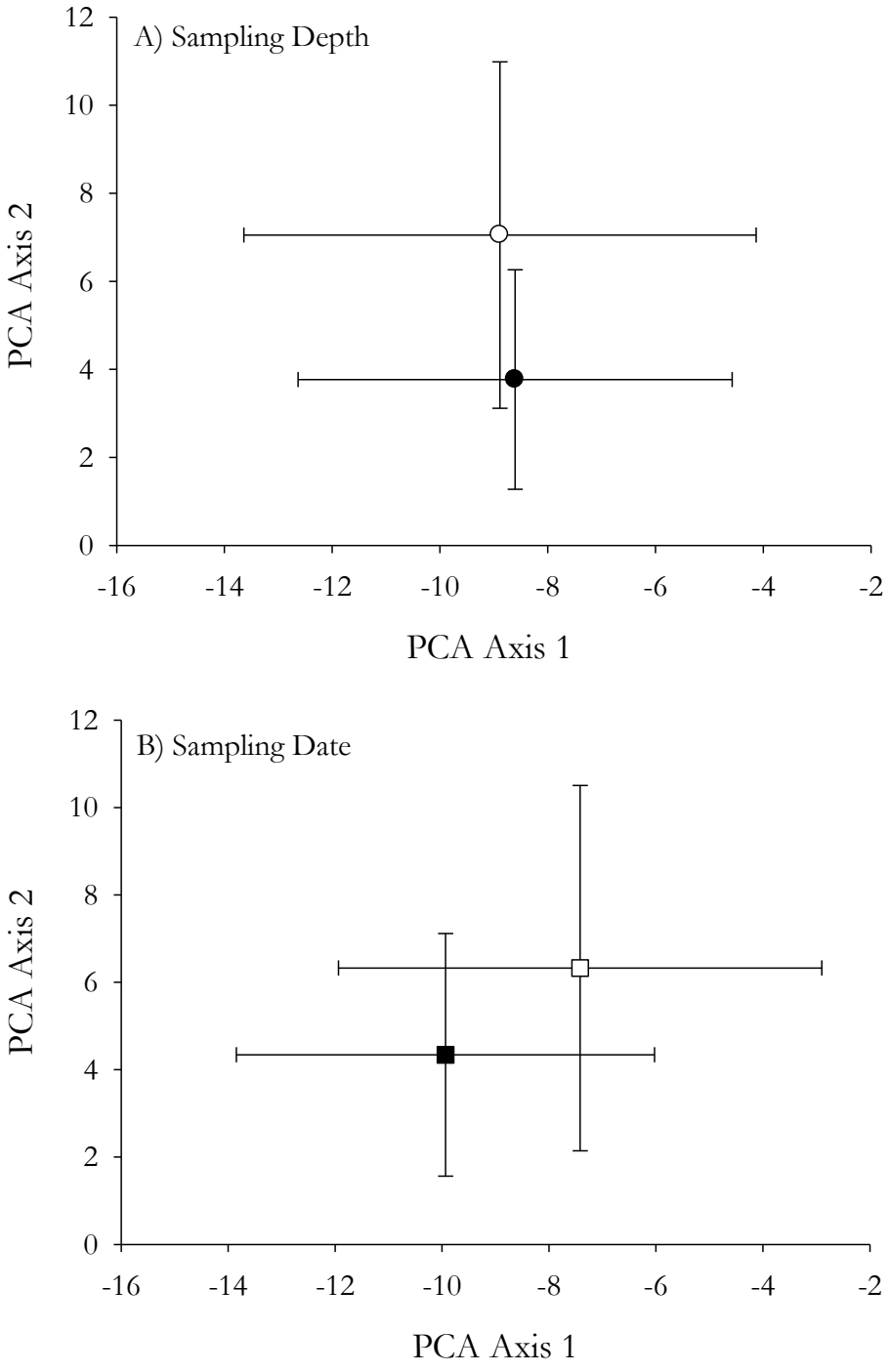

Figure 4.6. Principal component analysis of phospholipid fatty acids from FTL soil samples based on differences due to sampling depth (A) (closed circles = upper sampling depth (0-10 cm), open circles = lower sampling depth (10-20 cm)) and due to sampling date (B) (closed squares = pre-flood sampling (May 2004); open squares = post-flood sampling (July 2005)). Symbols represent means; lines represent ± 1 standard deviation.

ANOVA revealed that microbial community structure was largely unaffected by sampling date, sampling depth or flood treatment (i.e. flood duration and condition of flood water) (Table 4.3). The B:F ratio for the pre-flood sampling was significantly greater than the B:F ratio for the post-flood sampling (Table 4.4). Likewise, the stress indicators and MFA increased significantly between pre- and post-flood samplings (Table 4.4). However, sampling date had no significant effect on total biomass or on the mol percent response of any of the following microbial groups: aerobic, Gram-negative, and Gram-positive bacteria, and mycorrhizal fungi (Table 4.4). Similarly, sampling depth had no significant effect on microbial biomass, the B:F ratio, the stress indicators or the response of Gram-negative bacteria, Gram-positive bacteria or mycorrhizal fungi (Table 4.4). Generally, microbial community response increased with sampling date, and decreased with sample depth. A significantly lower response for aerobic bacteria was detected at the lower sampling depth (Table 4.3).

Table 4.3. ANOVA results for PLFA analysis of soil samples from the FTL. For each microbial component, numbers in the first row are the F value; numbers in the second row are the P value. Significant results are highlighted in bold.

Microbial Component	Flood Treatment	Sampling Depth	Sampling Date
Microbial Biomass	0.64	0.71	0.43
	0.60	0.40	0.51
Aerobic Bacteria	0.41	**5.79**	0.08
	0.75	**0.02**	0.77
Gram-Negative Bacteria	**3.24**	0.42	1.81
	0.03	0.52	0.18
Gram-Positive Bacteria	0.59	1.99	0.06
	0.63	0.17	0.80
Mycorrhizal Fungi	0.32	0.48	1.26
	0.81	0.49	0.27
B:F Ratio	0.10	3.24	**6.43**
	0.96	0.08	**0.02**
Stress Indicators	0.24	3.35	**5.80**
	0.87	0.07	**0.02**
Monounsaturated	0.31	0.64	**13.88**
Fatty Acids	0.82	0.43	**< 0.001**

The soil microbial community was also largely not affected by flood treatment (Tables 4.3 & 4.4). No significant differences due to flood duration and condition of flood water were observed for the B:F ratio, total biomass, the stress indicators, the sum of MFA, or the mol percent response of aerobic bacteria, Gram-positive bacteria, or mycorrhizal fungi. Flood treatment affected the mol percent response of the Gram-negative bacteria marker. The 5WF treatment had the greatest response for this bacteria group, followed by the control, the 5WS and finally the 3WF treatment. The 5WF and control flood samples were not different from each other, nor were the control, 5WS and 3WF samples.

Table 4.4. Mean values for PLFA analysis of soil samples from the FTL. BIO = microbial biomass, AER = aerobic bacteria, GNB = Gram-negative bacteria, GPB = Gram-positive bacteria, MYC = mycorrhizal fungi, B:F = bacterial to fungal ratio, STR = stress indicators and MFA = monounsaturated fatty acids, , 3WF = 3-week-flowing flood, 5WF = 5-week-flowing flood, 5WS = 5-week-stagnant flood, upper = 0-10 cm, lower = 10-20 cm, pre = pre-flood sample, post = post-flood sample.

		BIO	**AER**	**GNB**	**GPB**	**MYC**	**B:F**	**STR**	**MFA**
		mg C g^{-1} soil	---------- mol percent ----------						
Upper Pre	**Con.**	164.18	71.00	102.33	208.01	137.17	1.73	1.91	0.16
	3WF	145.14	65.96	95.62	154.97	71.03	1.95	1.89	0.17
	5WF	132.65	59.88	97.91	177.99	63.81	1.90	2.30	0.17
	5WS	131.97	61.94	86.03	206.08	64.89	1.92	1.94	0.17
Upper Post	**Con.**	118.88	49.85	78.51	170.90	68.16	2.04	2.16	0.17
	3WF	114.65	52.39	78.13	164.76	59.26	1.88	2.18	0.20
	5WF	165.11	80.57	91.75	238.32	89.74	1.92	2.02	0.19
	5WS	158.75	80.00	85.54	220.75	99.60	1.84	1.94	0.18
Lower Pre	**Con.**	132.32	54.10	101.94	178.39	62.98	2.60	2.16	0.17
	3WF	117.55	46.04	77.91	154.97	44.94	2.82	2.10	0.16
	5WF	114.38	48.28	97.91	156.67	60.61	2.36	1.80	0.15
	5WS	105.23	47.10	77.13	157.67	45.70	2.69	1.96	0.16
Lower Post	**Con.**	145.81	53.06	83.58	181.39	83.37	1.71	2.45	0.19
	3WF	142.74	53.54	56.70	164.76	130.82	1.34	2.85	0.19
	5WF	183.21	66.94	91.75	230.96	82.47	2.30	2.46	0.19
	5WS	91.63	33.17	63.06	108.79	64.05	1.73	2.51	0.17

Analysis of the FTL samples using t-tests also revealed few differences due to sampling depth and flood treatment over time (Table 4.5). In general, the B:F ratio decreased with flooding. The B:F ratio decreased significantly in the lower 3WF samples. Significant differences were also detected when all lower samples were pooled and when all samples were considered together. In most cases, stress indicators and MFA increased with flooding. Stress indicators increased significantly in the lower 5WS samples; significant increases were also observed when all lower samples were pooled and when all samples were considered together. Monounsaturated fatty acids increased significantly when 5WF samples were pooled. Significant increases in MFA were also observed when all lower samples were pooled, when all upper samples were pooled and when all samples were considered together.

Changes in total biomass and in mol percent responses due to microbial groups were more variable (i.e., in some cases increases were observed, while decreases were observed in other cases) with no overall trends evident. The lower, 5WS sample revealed significant decreases in aerobic bacteria and Gram-negative bacteria markers. Gram-negative bacteria markers decreased significantly in the upper, 3WF sample and when the control samples were pooled. Mycorrhizal fungi decreased in the lower 5WF sample and in the pooled 5WF samples.

Table 4.5. T-test results for soil microbial community characteristics for FTL soil samples. Positive t-values indicate an increase in the variable over time, while negative t-values indicate a decrease in the variable overtime. Significant t-values ($P < 0.05$) are highlighted in bold. BIO = microbial biomass, AER = aerobic bacteria, GNB = Gram-negative bacteria, GPB = Gram-positive bacteria, MYC = mycorrhizal fungi, B:F = bacterial to fungal ratio, STR = stress indicators and MFA = monounsaturated fatty acids. 3WF = 3-week-flowing flood, 5WF = 5-week-flowing flood, 5WS = 5-week-stagnant flood, upper = 0-10 cm, lower = 10-20 cm.

		BIO	**AER**	**GNB**	**GPB**	**MYC**	**B:F**	**STR**	**MFA**
Control	**Upper**	-2.12	-3.52	-2.21	-1.93	-1.18	1.21	2.13	0.60
	Lower	0.54	-0.23	-1.58	0.12	1.65	-2.21	0.72	1.40
	Both	-0.81	-1.97	**-2.94**	-1.02	-0.73	-0.87	1.44	1.54
3WF	**Upper**	-0.94	-1.09	**-5.17**	-0.92	-0.86	-0.29	1.39	2.94
	Lower	0.85	0.62	-0.93	0.31	1.12	**-5.65**	1.42	1.13
	Both	-0.11	-0.34	-1.88	-0.53	0.90	**-2.19**	1.89	2.44
5WF	**Upper**	1.05	1.07	-0.51	1.15	1.57	0.06	-0.74	3.20
	Lower	1.86	3.30	1.23	1.83	**9.61**	-0.34	2.85	4.26
	Both	2.19	2.18	0.76	2.26	**3.19**	-0.12	0.66	**4.68**
5WS	**Upper**	0.82	0.74	-0.03	0.17	2.22	-0.47	0.03	0.87
	Lower	-3.73	**-4.37**	**-8.36**	-2.10	1.08	-2.37	**4.71**	0.41
	Both	0.38	0.16	-0.84	-0.41	2.42	-1.87	1.79	0.98
All	**Upper**	-0.25	0.11	-2.03	0.03	-0.27	0.34	0.54	**3.28**
All	**Lower**	1.61	0.59	-0.76	0.51	1.93	**-4.09**	**3.49**	**2.93**
All	**Both**	0.87	0.38	-1.77	0.32	1.16	**-2.71**	**2.81**	**4.28**

Correlations of Microbial Diversity with Soil C and N

For the samples from the simulated flood experiment, soil community structure was largely unrelated to soil C and N (Table 4.6). The only significant relationships were positive correlations between TN and the aerobic bacteria marker and between TN and MFA; the C:N ratio and MFA were negatively correlated (Table 4.6). On the other hand, a number of correlations were observed for the FTL samples (Table 4.7). Biomass, along with the mol percent responses of aerobic bacteria, Gram-negative bacteria, and Gram-positive bacteria were all positively correlated with both TOC and TN. Stress indicators were negatively correlated with TOC, TN and the C:N ratio. No correlations with TOC, TN or C:N ratio were observed for the B:F ratio, monounsaturated fatty acids, or the mol percent response of the mycorrhizal fungi. The microbial markers were not correlated with the C:N ratio.

Table 4.6. Pearson correlation coefficients (r) and P-values of soil microbial community components with TN, TOC and C:N ratio for the simulated flood experiment. Significant correlations are highlighted in bold.

Microbial Component	TN	TOC	C:N Ratio
Microbial Biomass	0.23	-0.06	-0.22
	0.06	0.62	0.07
Aerobic Bacteria	**0.39**	0.08	-0.22
	<0.001	0.50	0.07
Anaerobic Bacteria	-0.12	-0.06	0.05
	0.33	0.65	0.71
Gram-Negative Bacteria	0.10	0.16	0.05
	0.41	0.19	0.71
Gram-Positive Bacteria	0.12	-0.04	-0.11
	0.34	0.76	0.37
Mycorrhizal Fungi	0.05	0.08	0.04
	0.67	0.52	0.72
B:F Ratio	0.004	0.06	-0.21
	0.98	0.61	0.08
Stress Indicators	0.09	0.04	-0.05
	0.45	0.75	0.68
Monounsaturated	**0.39**	0.07	**-0.25**
Fatty Acids	**0.001**	0.58	**0.04**

Table 4.7. Pearson correlation coefficients (r) and P-values of soil microbial community components with TN, TOC and C:N ratio for the FTL experiment. Significant correlations are in bold.

Microbial Component	TN	TOC	C:N Ratio
Microbial Biomass	**0.40**	**0.35**	-0.06
	0.005	**0.01**	0.68
Aerobic Bacteria	**0.53**	**0.51**	0.06
	0.0001	**0.0002**	0.68
Gram-Negative Bacteria	**0.41**	**0.39**	0.03
	0.003	**0.005**	0.82
Gram-Positive Bacteria	**0.41**	**0.40**	0.02
	0.003	**0.005**	0.91
Mycorrhizal Fungi	0.27	0.20	-0.15
	0.06	0.16	0.30
B:F Ratio	-0.04	0.03	0.21
	0.81	0.83	0.15
Stress Indicators	**-0.29**	**-0.39**	**-0.37**
	0.046	**0.006**	**0.01**
Monounsaturated	-0.09	-0.14	-0.19
Fatty Acids	0.54	0.33	0.20

Microbial Activity

Simulated Flood Experiment

Carbon utilization by the soil microbial community varied with both flood and residue treatments (Figure 4.7). Significant effects due to flood treatment were observed ($F = 50.41$, $P < 0.0001$) (Table 4.8). Control and intermittent flood treatments were not different; however, these treatments had significantly higher β-glucosidase activity than the flowing and stagnant floods. Samples from the flowing flood had significantly greater β-glucosidase activity than those from the stagnant flood. Significant effects due to residue treatment were also observed ($F = 6.66$, $P < 0.001$) (Table 4.9). β-Glucosidase activity ranked as follows: grass residue > legume residue > tree residue > control. Samples from

grass residue treatment had significantly higher β-glucosidase activity than those from the tree residue or the control. Samples from the legume residue and tree residue treatments were not different but both had greater activity than the control samples. A flood X residue interaction was not observed (F = 0.75, P = 0.66).

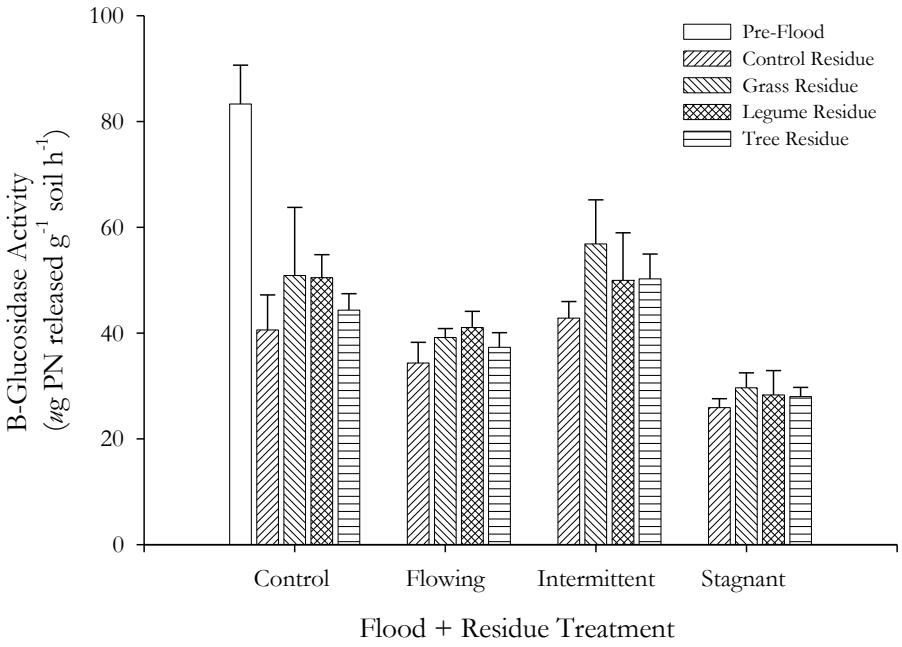

Figure 4.7. Carbon utilization of soil microbial organisms as measured by β-glucosidase activity by flood and residue treatment for the greenhouse simulated flood experiment. The open bar on the far-left of the control samples represents C-utilization in a pre-flood soil that was not manipulated. Error bars represent ± 1 standard deviation from the mean.

Table 4.8. Average β-glucosidase activity for soil samples from simulated flood experiment by flood treatment. Means with the same letter are not significantly different (α = 0.05).

Flood Treatment	**Mean (µg PN released g^{-1} soil h^{-1})**
Intermittent	49.96 A
Control	46.57 A
Flowing	37.95 B
Stagnant	27.96 C

Table 4.9. Average β-glucosidase activity for soil samples from simulated flood experiment by residue treatment. Means with at least one letter in common are not significantly different (α = 0.05).

Residue Treatment	**Mean (μg PN released g^{-1} soil h^{-1})**
Grass	44.13 A
Legume	42.45 AB
Tree	39.96 B
Control	35.91 C

Nitrogen utilization by this soil microbial community varied with flood ($F = 155.43$, $P < 0.0001$) and residue treatments ($F = 6.40$, $P = 0.001$) (Figure 4.8). A significant flood X residue interaction was also observed ($F = 6.57$, $P < 0.0001$). With regards to the main effect of flood treatment, β-glucosaminidase activity ranked as follows: intermittent flood treatment > control treatment > stagnant flood treatment > flowing flood treatment (Table 4.10). With regards to the main effect of residue treatment, samples from grass, legume, and tree residue treatments were not different but all residue treatments had greater β-glucosaminidase than the control samples (Table 4.11).

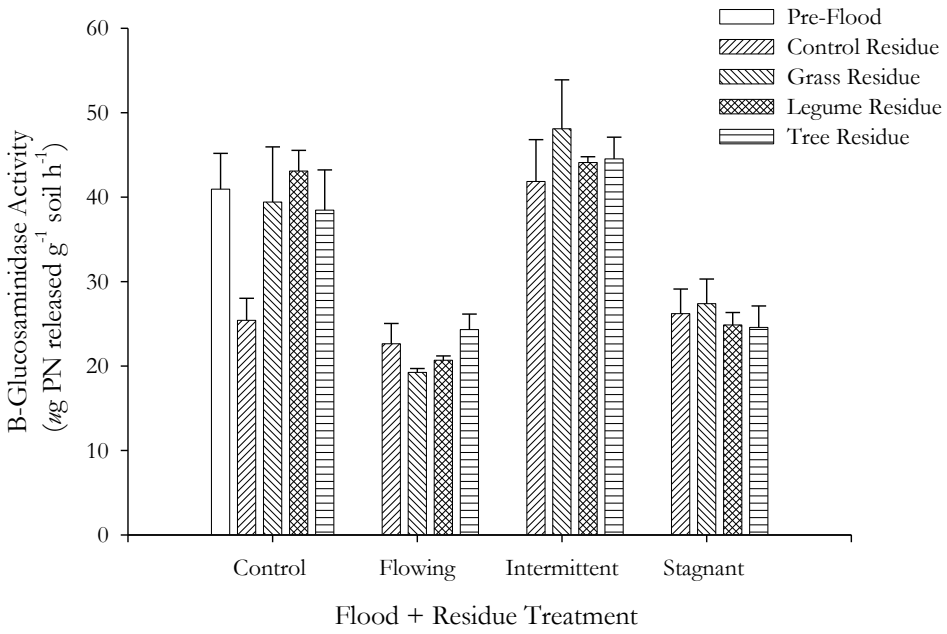

Figure 4.8. Nitrogen utilization of soil microbial organisms as measured by β-glucosaminidase activity by flood treatment and residue treatment for the greenhouse simulated flood experiment. The open bar on the far-left of the control samples represents N-utilization in a pre-flood soil that was not manipulated. Error bars represent ± 1 standard deviation from the mean.

Table 4.10. Average β-glucosaminidase activity for soil samples from simulated flood experiment by flood treatment. Means with the same letter are not significantly different (α = 0.05).

Flood Treatment	**Mean (µg PN released g^{-1} soil h^{-1})**
Intermittent	44.65 A
Control	36.60 B
Stagnant	25.76 C
Flowing	21.73 D

Table 4.11. Average β-glucosaminidase activity for soil samples from simulated flood experiment by residue treatment. Means with the same letter are not significantly different (α = 0.05).

Residue Treatment	Mean (μg PN released g^{-1} soil h^{-1})
Grass	33.54 A
Legume	33.19 A
Tree	32.97 A
Control	29.03 B

Flood Tolerance Laboratory

Neither carbon nor nitrogen utilization by the microbial population were affected by flood type (duration or condition (i.e. stagnant vs flowing)) (Tables 4.12 and 4.13). However, pre- vs post-flood comparisons indicated changes in C-utilization over time (Table 4.14, Figure 4.9). On the whole, C-utilization decreased with flooding. Specifically, decreases in β-glucosidase activity were observed at the upper sampling depth (0-10 cm) of the control and the 5WF treatments and in the lower sampling depth (10-20 cm) of the control treatment. When upper and lower samples were pooled, decreases in β-glucosidase activity were observed for the control and both of the flowing treatments; no changes were observed in the 5WS treatment. On the other hand, pre- vs post-flood comparisons revealed few changes in N-utilization (Table 4.14, Figure 4.10). The 5WF treatment had decreased β-glucosaminidase activity at the lower sampling depth (10-20 cm).

Table 4.12. β-glucosidase activity for FTL soil samples. Average and standard deviation for C-utilization for each sample date and depth for each treatment. Overall averages summarize all sample dates and depths for a particular flood treatment. 3WF = 3-week-flowing flood, 5WF = 5-week-flowing flood, 5WS = 5-week-stagnant flood.

	Pre-Flood 0-10 cm		**Pre-flood 10-20 cm**		**Post-Flood 0-10 cm**		**Post-Flood 10-20 cm**		**Overall Average**	
	Mean (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**
3WF	106.52	27.34	57.82	15.58	59.37	9.74	47.48	6.98	67.80	27.83
5WF	88.95	10.41	70.74	6.81	64.15	21.23	52.39	10.64	69.06	17.94
5WS	95.41	24.25	141.8	107.9	61.82	24.37	43.48	25.28	85.63	63.06
Control	119.32	22.28	66.86	14.21	62.21	18.03	49.42	10.06	74.45	31.32

Table 4.13. β-glucosaminidase activity for FTL soil samples. Average and standard deviation for N-utilization for each sample date and depth for each treatment. Overall averages summarize all sample dates and depths for a particular flood treatment. 3WF = 3-week-flowing flood, 5WF = 5-week-flowing flood, 5WS = 5-week-stagnant flood.

	Pre-Flood 0-10 cm		**Pre-flood 10-20 cm**		**Post-Flood 0-10 cm**		**Post-Flood 10-20 cm**		**Overall Average**	
	Mean (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**	**Mean** (μg PN g^{-1} soil h^{-1})	**Std.**
3WF	40.11	5.96	30.76	6.29	42.33	5.94	33.00	8.86	36.55	7.71
5WF	41.43	7.23	36.86	6.10	37.65	7.47	35.24	2.88	39.80	5.80
5WS	39.10	8.49	33.15	4.76	40.46	11.58	33.12	4.74	36.46	7.61
Control	44.94	13.56	40.11	12.46	34.37	6.59	35.54	11.18	38.74	10.55

Table 4.14. T-test results for β-glucosidase and β-glucosaminidase activity for FTL soil samples. Positive t-values indicate an increase in the variable over time, while negative t-values indicate a decrease in the variable overtime. Significant t-values ($P < 0.05$) are highlighted in bold. 3WF = 3-week-flowing flood, 5WF = 5-week-flowing flood, 5WS = 5-week-stagnant flood, upper = 0-10 cm, lower = 10-20 cm.

		β-Glucosidase		β-Glucosaminidase	
		t-value	**P**	**t-value**	**p**
Control	**Upper**	**-6.35**	**0.02**	-1.09	0.39
	Lower	**-4.41**	**0.05**	-1.62	0.25
	Both	**-3.77**	**0.01**	-2.04	0.10
3WF	**Upper**	-2.58	0.12	1.41	0.29
	Lower	-0.87	0.48	1.18	0.36
	Both	-2.25	0.07	2.02	0.10
5WF	**Upper**	-3.94	0.06	-0.38	0.74
	Lower	-1.98	0.19	-3.06	0.09
	Both	**-4.13**	**0.01**	-1.33	0.24
5WS	**Upper**	-1.66	0.24	-0.01	0.39
	Lower	-1.45	0.28	0.43	0.71
	Both	-1.89	0.12	0.40	0.71
All	**Upper**	**-5.57**	**0.002**	-0.64	0.54
All	**Lower**	-1.96	0.08	-1.21	0.25
All	**Both**	**-3.96**	**0.001**	-1.37	0.18

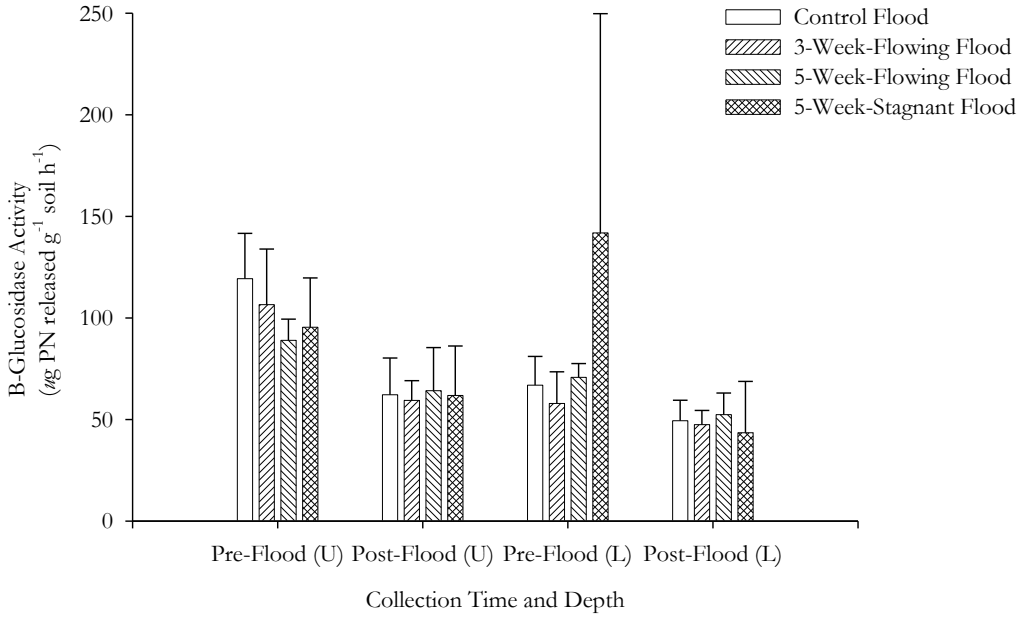

Figure 4.9. Changes in β-glucosidase with flooding for soils from the FTL. Pre-flood samples = May 2004; post-flood samples = July 2005. Upper (U) samples = 0-10 cm; lower (L) samples = 10-20 cm. Each sample is a composite of 10 cores taken within the sampling zone closest to the automated sensors. Bars represent averages from three channels for each of the flood treatments; error bars represent ± 1 standard deviation.

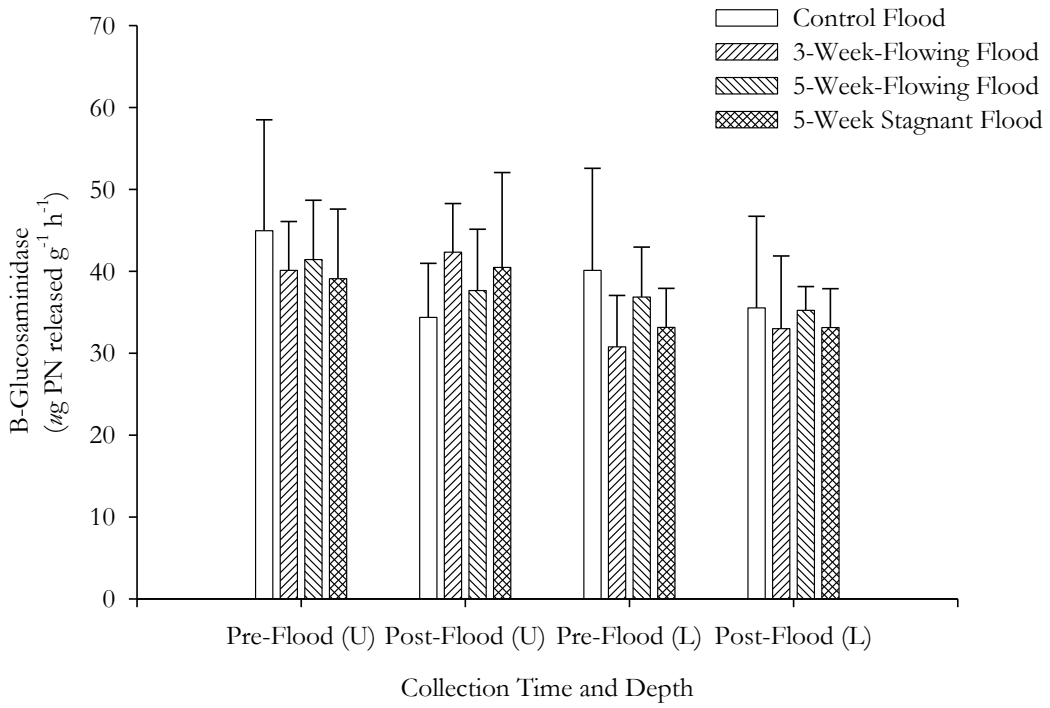

Figure 4.10. Changes in β-glucosaminidase with flooding for soils from the FTL. Pre-flood samples = May 2004; post-flood samples= July 2005. Upper (U) samples = 0-10 cm; lower (L) samples = 10-20 cm. Each sample is a composite of 10 cores taken within the sampling zone closest to the automated sensors. Bars represent averages from three channels for each of the flood treatments; error bars represent ± 1 standard deviation.

Correlations of Enzyme Activity with Soil C and N

For soil samples from the simulated flood experiment, no correlations between C-utilization or N-utilization and TOC, TN or C:N ratio were observed (Table 4.15). However, β-glucosidase and β-glucosaminidase activities were significantly positively correlated ($r = 0.65$; $p < 0.0001$). For soil samples from the FTL, C and N-utilization were correlated with select soil chemical parameters (Table 4.15). β-Glucosidase activity was positively correlated with TOC and with C:N ratio but was not correlated with TN. β-Glucosaminidase activity was positively correlated with TN and TOC but was not correlated with the C:N ratio. β-

Glucosidase and β-glucosaminidase were also weakly but significantly correlated ($r = 0.37$; $p < 0.01$).

Table 4.15. Pearson correlation coefficients between enzyme activity and soil C and N for soils from the simulated flood experiment and for soils from the FTL experiment. Significant relationships are highlighted in bold.

	Simulated Flood Experiment				**FTL Experiment**			
	C-utilization		**N-utilization**		**C-utilization**		**N-utilization**	
	r	**P**	**r**	**p**	**r**	**p**	**r**	**p**
TN	-0.13	0.30	0.11	0.37	0.27	0.07	**0.79**	**< 0.0001**
TOC	0.10	0.41	0.17	0.16	**0.37**	**0.01**	**0.76**	**< 0.0001**
C:N ratio	0.19	0.13	0.05	0.66	**0.31**	**0.03**	0.07	0.64

Correlation of Enzyme Activity with Microbial Community Structure

For soil samples from the simulated flood experiment, soil microbial community structure is more closely correlated with N-utilization patterns (i.e. β-glucosaminidase activity) than with C-utilization patterns (i.e. β-glucosidase activity) (Table 4.16). Mol percent response of aerobic bacteria, Gram-negative bacteria, and mycorrhizal fungi were all positively correlated with N-utilization while the response of Gram-positive bacteria and the stress indicators were negatively correlated with N-utilization. Mycorrhizal fungi was the only microbial group correlated with C-utilization for this set of soil samples. Both the stress indicators and MFA were negatively correlated with C-utilization, while the B:F ratio was not correlated with either N-utilization or C-utilization. The stress indicators and MFA were positively correlated with each other ($r = 0.26$, $P = 0.03$).

Table 4.16. Pearson correlation coefficients (r) and P-values of soil microbial community structure with N- and C-utilization rates for simulated flood experiment. Significant correlations are highlighted in bold.

Microbial Component	N-Utilization	C-Utilization
Microbial Biomass	**0.25**	0.09
	0.04	0.47
Aerobic Bacteria	**0.48**	0.18
	<0.0001	0.15
Anaerobic Bacteria	-0.08	0.18
	0.52	0.15
Gram-Negative Bacteria	**0.33**	0.20
	< 0.01	0.11
Gram-Positive Bacteria	**-0.40**	-0.10
	<0.001	0.43
Mycorrhizal Fungi	**0.34**	**0.25**
	0.004	**0.04**
B:F Ratio	0.10	0.07
	0.43	0.55
Stress Indicators	**-0.25**	**-0.31**
	0.04	**0.01**
Monounsaturated	-0.03	**-0.27**
Fatty Acids	0.84	**0.03**

For soil samples from the FTL, N-utilization patterns were more important than C-utilization patterns with regards to microbial community structure (Table 4.17). Mol percent response of aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria, and mycorrhizal fungi were all positively correlated with N-utilization (i.e. β-glucosaminidase activity). The stress indicators were negatively correlated with N-utilization, while MFA and the B:F ratio were not correlated with N-utilization. C-utilization (i.e. β-glucosidase activity) was positively correlated with the mol percent response of Gram-positive bacteria only. As with samples from the simulated flood experiment, the stress indicators and MFA were positively correlated with each other ($r = 0.57$, $P < 0.0001$) for the FTL samples.

Table 4.17. Pearson correlation coefficients (r) and P-values of soil microbial community structure with N- and C-utilization rates for FTL experiment. Significant correlations are highlighted in bold.

Microbial Component	N-Utilization	C-Utilization
Microbial Biomass	**0.46**	0.18
	0.001	0.22
Aerobic Bacteria	**0.53**	0.25
	0.0001	0.08
Gram Negative Bacteria	**0.39**	0.28
	< 0.01	0.06
Gram Positive Bacteria	**0.47**	**0.29**
	<0.001	**0.04**
Mycorrhizal Fungi	**0.43**	0.10
	< 0.01	0.48
B:F Ratio	-0.07	0.16
	0.62	0.28
Stress Indicators	**-0.30**	-0.25
	0.04	0.09
Monounsaturated	-0.23	-0.26
Fatty Acids	0.12	0.08

Discussion

Microbial Community Structure

The microbial communities of the soils (greenhouse and FTL) varied in composition and in response to flood disturbances. Samples collected from the greenhouse simulated flood experiment showed a change in community structure with flooding; however, samples from the FTL showed a change in community structure with sampling time and depth, not with flood treatment. Similarly, Ibekewe and Kennedy (1998 and 1999) described differences between greenhouse and field soil samples. Their first study was specifically designed to test microbial community structure under field and greenhouse conditions, and revealed higher % PLFA composition and higher microbial biomass in field soils rather than greenhouse soils. In a subsequent study of two agricultural soils, Ibekewe and Kennedy

(1999) again found evidence for an environmental (i.e. greenhouse vs field) influence. In this case, field samples were found to be more highly variable in fatty acid concentrations than the greenhouse samples. In the current study, different groups of microbial markers were found in the two soil samples. The anaerobic bacterial group was detected in the greenhouse samples but not in the FTL samples. Protozoa were present in 19% of the FTL samples but were absent from greenhouse samples. In addition, the mol percent responses of the microbial groups were much higher in the FTL samples than in the simulated flood samples. For example, the average mol percent response for aerobic bacteria under controlled conditions in the FTL was nearly 10 times higher than that observed in the greenhouse. These results are therefore similar to Ibekewe and Kennedy (1998 and 1999), where environmental (greenhouse vs field) differences were observed and field conditions supported greater microbial biomass than the greenhouse.

The simulated flood experiment revealed changes in microbial community structure with flooding but not with residue addition. General trends included greater aerobic bacteria response under more aerobic conditions and likewise greater anaerobic bacteria response under more anaerobic conditions. Under stagnant flood conditions microbial biomass was lowest as was the response of aerobic bacteria, Gram-negative bacteria, and mycorrhizal fungi. These responses were observed regardless of the type of residue addition (i.e. grass, legume or tree). Other studies have shown similar responses (Drenovsky et al., 2004; Mentzer et al., 2006). Mentzer et al. (2006) found that prolonged flooding had a greater effect than nutrient loading in that flooding altered both compositional and functional aspects of microbial community. Specifically, flooding greatly reduced the response of mycorrhizal fungi while at the same time greatly increased the responses of Gram-negative anaerobic bacteria and Gram-positive bacteria (Mentzer et al., 2006). Drenovsky et al.,

(2004) also observed a decrease in fungal biomarkers but not bacterial biomarkers with increased soil water content. Furthermore, Bossio and Scow (1998) observed a decrease in fungal and aerobic indicators and an increase in Gram-positive indicators with flooding; however, they also observed changes in microbial indicators with straw incorporation. The decreased importance of fungi under flooded conditions observed in the current study and other studies (Bossio and Scow, 1998; Drenovsky et al., 2004; Mentzer et al., 2006) is consistent with the hypothesis that fungi are less prevalent in inundated soils.

Changes in microbial community structure imply changes in decomposition and nutrient cycling processes. Residue decomposition involves a succession of microbial organisms that moves toward organisms that are able to metabolize substrates of increasing chemical complexity (Sylvia et al., 2005). Actinomycetes and fungi are the primary decomposers of complex nutrients such as lignin and humus. Decreases in the fungal component due to flooding, therefore, would contribute to the accumulation of these phenolic-based compounds in the soil. Fungi are the dominant saprophytic heterotrophs in many forest soils, especially acidic soils, as fungi are more tolerant of acid conditions than other soil microbes (Kimmins, 2004). The implications of inundation of forest soils are thus greater than those for grassland or agricultural soils.

Analysis of the FTL soil samples revealed the importance of sampling date and depth. General decreases in biomass and reduced response of microbial markers were observed with increased sampling depth. Bacteria are typically most numerous in surface layers that are rich in organic material or in the rhizosphere where plant roots release sugars, amino acids and other organic compounds (Sylvia et al., 2005). In addition, mycorrhizae are associated with plant roots concentrated in the upper soil layers. A soil profile analysis conducted by Fierer et al. (2003) demonstrated the changes in soil microbial community

structure with depth. An overall decline in microbial diversity was detected in this study along with declines in individual PLFA markers, but not all groups responded consistently. Gram-negative bacteria, fungi and protozoa all declined with depth; on the other hand, Gram-positive bacteria and actinomycetes tended to increase in proportion with depth (Fierer et al., 2003). Others have also noted a decline in microbial biomass and changes in PLFA markers with depth (Fritze et al., 2000; Peacock et al., 2001). In both of these cases, microbial biomass was greatest at the surface. For example, Peacock et al. (2001) observed that microbial biomass was twice as great in the surface layers (0-5 cm) than at 5-10 cm or 10-15 cm. In each of these examples, the authors relate the declines in microbial community diversity and biomass with depth to changes in soil nutrient status with depth (Fritze et al., 2000; Peacock et al., 2001; Fierer et al., 2003). In the current study, an increase in depth of 10 cm (0-10 cm vs. 10-20 cm sampling depth) resulted in a 9% decrease in microbial biomass as well as a 23% decrease in aerobic markers, a 5% decrease in Gram-negative markers, a 15% decrease in Gram-positive markers and a 12% decrease in mychorrizal fungi markers. The same increase in depth resulted in a 12-13% decrease in TOC and a 9-10% decrease in TN. Carbon inputs are thought to decrease not just in availability but also in quality with depth (Fierer et al., 2003). Carbon enters the soil profile primarily through leaf litter or plant residues on the surface or via root exudates in the upper soil horizons. As litter and residues are broken down first by macro- and micro-fauna and later by bacteria and fungi, carbon is transferred through the soil profile. Some microbial groups prefer the more readily available forms of carbon, such as sugars and amino acids, while other groups prefer the more recalcitrant compounds, such as lignin and cellulose (Sylvia et al., 2005). Thus, the more labile carbon products are removed first, while the more recalcitrant carbon products are passed farther through the soil profile.

Changes in microbial community structure due to sampling date were more variable than the general decline in microbial biomass and diversity observed with increased sampling depth. The B:F ratio and the response of Gram-negative bacteria decreased while biomass and the responses of aerobic bacteria, Gram-positive bacteria and mycorrhizal fungi increased over time. These differences may have more to do with seasonal influences than with the flood treatments employed; flood duration and condition (stagnant vs. flowing) did not result in significant changes in the microbial community in the FTL. A seasonal influence might be explained by the actual sampling dates. The pre-flood samples were collected in May 2004 while the post-flood samples were collected in July 2005 (i.e. immediately after the 2005 flood treatments that occurred from May-June 2005). Undoubtedly a number of changes in the soil microbial community structure occurred in the year between when the initial soil samples were collected and when the current flood treatments were imposed. Thus the pre-flood samples may not be appropriate reference samples. Despite this, a seasonal influence is still possible and likely. A number of other studies have demonstrated changes in soil microbial community structure related to temporal or seasonal influences (Buyer and Drinkwater, 1997; Bossio et al., 1998; Bossio and Scow, 1998; Acosta-Martinez et al., 1999; Bardgett et al., 1999; Petersen et al., 2002; Mentzer et al., 2006). In some of these cases treatment effects were observed (for example, changes in hydrologic regime resulting in changes in soil microbial community structure or changes in soil microbial community with management), but each also note a temporal influence that appeared to interact with the treatment effect (Buyer and Drinkwater, 1997; Bossio et al., 1998; Bossio and Scow, 1998; Mentzer et al., 2006). Petersen et al. (2002) on the other hand, concluded that microbial community structure was modified by growing season

environmental conditions and that changes were due to long-term processes that occur over the time scale of months.

Differences in microbial community response to flooding in terms of the stress indicators and the sum of monounsaturated fatty acids were observed for the two experiments (greenhouse vs. FTL). The stress indicators increased with increased anaerobic conditions due to flooding in the greenhouse but not in the FTL. In the greenhouse, the stagnant and flowing flood conditions resulted in the highest stress indicators, while intermittent flood and control conditions resulted in the lowest stress indicators. On the other hand, increases in the stress indicators in the FTL samples were associated with sample timing and sample depth. The stress indicators were significantly higher in post-flood than in pre-flood FTL samples, and these indicators increased significantly with increased sampling depth in the FTL. The stress indicators include the ratio of the relative abundance of cyclopropyl fatty acids to their monoenoic precursors; the abundance of cyclopropyl fatty acids is widely used as an indicator of anaerobic conditions (Bossio and Scow, 1998). While the current study revealed increases in stress indicators with flooding in the greenhouse, other studies have failed to show such a relationship. Bossio and Scow (1998), for example, found no changes in cyclopropyl fatty acids due to flooding. Meanwhile, increases in cyclopropyl fatty acids observed by Fierer et al. (2003) were associated with increased soil depth (as was observed in the FTL). Fierer et al. (2003) speculated that deeper soil horizons have more severe resource limitations than surface soil horizons resulting in shifts in microbial membrane fatty acids.

While the response of the stress indicators was similar to patterns observed for the other microbial markers (i.e. affected by flood treatment in the greenhouse and by sampling date and depth in the FTL), the response of MFA is less clear. Monounsaturated fatty acids

are strongly related to higher substrate availability (Bossio and Scow, 1998), therefore, this measure should decrease under more stressful conditions. In the simulated flood experiment, the stagnant flood treatment resulted in significantly lower MFA levels than the other flood treatments; the other flood treatments were not different from each other or from the control treatment for this indicator. However, no differences (due to flood treatment, sampling depth or sampling date) in MFA were observed in the FTL samples. Bossio and Scow (1998) likewise found no differences in this indicator due to flood treatment or residue application; they cite detection limits as a hindrance to the use of this indicator. Other studies have shown that Gram-positive and Gram-negative bacteria respond to stress differently (Kieft et al., 1997). For example, Gram-negative bacteria typically show increases in saturated to unsaturated fatty acid ratios under stressful conditions, while Gram-positive bacteria seem to alter their membrane lipids little if at all (Kieft et al., 1997). Both soils in this study (greenhouse and FTL) had higher levels of Gram-positive bacteria than Gram-negative bacteria. If Gram-positive bacteria truly do not change their membrane structure in response to stressful conditions such as flooding, then their relative abundance may mask the shifts in fatty acids that occur within other groups, resulting in no apparent changes in monounsaturated fatty acid levels.

Microbial Activity

Limited research has been conducted on the effects of soil inundation on enzyme activity. Pulford and Tabatabai (1988) observed that the response of β-glucosidase to changes in redox potential varied with soil type and that when results obtained with each soil type were pooled, correlations were not significant. Their results also showed different responses in β-glucosidase to waterlogging based on the initial activity of β-glucosidase.

When initial β-glucosidase was low (43-130 μg *p*-nitrophenol released g^{-1} soil h^{-1}) activity increased following waterlogging, however when initial activity was high (159-283 μg *p*-nitrophenol released g^{-1} soil h^{-1}) waterlogging decreased activity (Pulford and Tabatabai, 1988). The results presented here are contrary to Pulford and Tabatabai's (1988) observations. For the soils from the simulated flood experiment, comparisons can be made with the reference samples. The β-glucosidase activity level of the reference sample was 80 μg *p*-nitrophenol released g^{-1} soil, which would suggest, according to Pulford and Tabatabai's (1988) observations that inundation should result in an increase in C-utilization for this system. However, this was not the case. Soils from the simulated flood experiment showed an overall decrease in C-utilization, with the flowing and stagnant treatments having significantly less activity then the control and intermittent flood treatments. Likewise, for soils from the FTL, with the exception of one observation, β-glucosidase levels decreased with inundation regardless of initial activity levels.

Regardless of system (simulated flood experiment or FTL), β-glucosaminidase activity was generally low. If Pulford and Tabatabai's (1988) results also apply to β-glucosaminidase activity, then increased N-utilization would be expected under both of these systems. In the simulated flood experiment, β-glucosaminidase activity decreased with flooding with significantly less activity observed under stagnant and flowing flood treatments. And 58% of the samples from the FTL showed a decrease in activity with inundation.

Differences in soil type may account for some of the variation observed within and between the two systems of this study. Soil characteristics are not consistent across the FTL facility, with particular differences in soil texture, pH, CEC and base saturation. Soils for the simulated flood experiment were collected from an area adjacent to the FTL facility.

However, due to collection location, soil for this experiment would be expected to be similar to that of some of the FTL channels but not all. In addition, within system variation for the FTL soils may be due to collection dates; pre-flood samples for this study were collected in May 2004 and post-flood samples were collected in July 2005. The pre-flood samples may not represent accurate reference points, as the soil ecosystem undoubtedly underwent a number of changes between May 2004 and May 2005.

Soil type may also account for differences between these systems and that of Pulford and Tabatabai (1988). Soils from mid-Missouri would be expected to differ from those found in Iowa. A cursory comparison between the samples used by Pulford and Tabatabai (1988) and the samples used in this study reveal that organic C content is generally lower and clay content is generally higher in the samples collected from mid-Missouri. Different activity levels have been reported for different soil types (Eivazi and Tabatabai, 1988; Pulford and Tabatabai, 1988).

Experimental conditions (i.e., laboratory vs. field conditions) may also have contributed to differences between Pulford and Tabatabia's (1988) results and those obtained from the FTL experiment. Soils in the FTL were flooded in place (i.e., in the field) and then brought into the lab for analysis. Pulford and Tabatabia's (1988) study was conducted with soil samples that were collected from various sites and then brought into the laboratory; waterlogging occurred with small samples in 50 ml plastic centrifuge tubes. Nevertheless, other investigations in field settings also show results that are contrary to those of the FTL study. Burns and Ryder (2001) investigated soils along river floodplains and found that enzyme activity peaked 1-7 days after inundation. In a study of a simulated wet prairie, Mentzer et al. (2006) showed higher enzyme activity under the constant flood treatment than under other flood treatments (intermittent flood and early season flood).

However, they noted that enzyme activities also increased under higher nutrient addition treatments. In both of these cases, the systems of interest experienced nutrient additions, while in the case of the FTL the system is closed and nutrient additions do not occur. Enzyme activity, in general, decreased with flooding in the FTL. Since enzyme activity is known to be correlated with TOC, for example, the increases in activities observed for these other studies may be more due to nutrient flux than due to specific flood treatments. Further investigations may help clarify the role of inundation and waterlogging on soil enzyme activity.

Correlations – Soil Chemistry, Microbial Community Structure and Activity

Glucosidase enzymes are involved in carbohydrate degradation in soils and are thought to provide an important energy source for microorganisms (Eivazi and Tabatabai, 1988; Eivazi and Tabatabai, 1990; Tabatabai, 1994; Michel and Matzner, 2003). It is no surprise then that the β-glucosidase and β-glucosaminidase activities observed in this study (FTL soil samples only) were correlated with TOC. Others have noted the correlation between TOC and β-Glucosidase (Eivazi and Tabatabai, 1990; Bandick and Dick, 1999). β-Glucosaminidase activity is thought to play a role in both C and N cycling in the soil (Parham and Deng, 2000; Ekenler and Tabatabai, 2004). This dual role may explain why β-glucosaminidase activity was correlated with both TN and TOC in this study.

The microbial communities of the two soil samples differed in their relationship with soil chemistry variables. Microbial markers from the simulated flood experiment were generally not correlated with TOC, TN or C:N ratio. However, microbial biomass, and the mol percent responses of aerobic bacteria, Gram-negative bacteria and Gram-positive bacteria from the FTL soils were all positively correlated with TOC and TN. It is difficult to

know if these findings are consistent with others using PLFA analyses. In many cases the chemical characterization of the soils are given; however, study results are expressed in terms of treatment effects and direct relationships between PLFA results and soil chemistry measures are not given (i.e. direct correlations between soil N and microbial community structure are not made). A few studies have examined the direct relationships between PLFA's and soil chemistry, although these studies primarily examine changes in soil N and C in terms of fertilizer treatments (Acosta-Martinez et al., 1999; Bardgett et al., 1999; Clegg et al., 2003; Marschner et al., 2003). Marschner et al. (2003) observed that microbial community structure was not affected by soil TN; however, bacterial biomass was weakly correlated with TN and bacteria and eukaryotic community structures were correlated with C:N ratio. Bardgett et al. (2001) observed that total PLFA measures were negatively related to C:N ratio and B:F ratios were negatively related to N, but that PLFA evenness measures were positively related to TC and C:N. On the other hand, Clegg et al. (2003) found no relationship between PLFA community structure and TC or TN. Bardgett et al. (1999) concluded that PLFA patterns due to mineral-N availability were inconsistent. Further studies to clarify the relationships between soil chemistry measures and soil microbial community analyses are needed.

Despite the apparent lack of relationship between soil TN and microbial community structure, correlations between soil community structure and microbial activity measures point to N as an influencing factor. In both the simulated flood and FTL experiments, biomass and microbial marker responses were more often correlated with N-utilization than with C-utilization. Overall, N-utilization was correlated with biomass, and the response of aerobic bacteria, Gram-negative bacteria, Gram-positive bacteria and mycorrhizal fungi; however, the response of Gram-positive bacteria was positively correlated with N-utilization

for the FTL study, and negatively correlated for the simulated flood study. Carbon-utilization was correlated only with the response of mycorrhizal fungi for the simulated flood study and only with the response of Gram-positive bacteria for the FTL investigation. Correlations with N-utilization may reflect that soil microbes, similar to the above-ground counterparts, are often N limited (Marschner et al. 2003) so an increase in N-utilization should be reflected in increases in various microbial markers, such as the response of aerobic bacteria or mycorrhizal fungi. Caution should be applied to such generalizations. Functional redundancy within the soil microbial community is cited by a number of authors (Kennedy, 1999; Marschner et al., 2003; O'Donnell et al., 2005). If functional redundancy is the norm in soil microbial communities, then a single function is carried out by a range of organisms and thus changes in structure do not necessarily relate to changes in function. Furthermore, evaluations of enzymatic activity describe only the potential activity of a given enzyme (Marschner et al., 2003). Enzymes such as those examined in this study are exoenzymes which are excreted into the soil and likely accumulate. Therefore, a measure of enzyme activity may overestimate microbial activity.

Conclusions

The primary objective of this experiment was to determine the effect of various flood treatments on soil microbial community structure and function. Responses to flood treatments varied with environmental conditions (i.e., greenhouse vs field), soil type, sampling depth, and sampling date. Therefore, this study illustrates the heterogeneous nature of the soil ecosystem and questionable the use of non-*in situ* studies to evaluate biological soil properties. The microbial communities responded to flood treatment under

simulated conditions in the greenhouse, but not in the field. Flood treatments in the greenhouse study did not affect which microbial groups representation (i.e., the same groups, aerobic bacteria, Gram-negative bacteria, etc were present regardless of flood treatment) but did influence the response of the groups present. For example, stagnant flood conditions in the greenhouse resulted in the decrease of microbial biomass as well as decreases in the responses of aerobic bacteria, Gram-negative bacteria Gram-positive bacteria and mycorrhizal fungi. Fluctuations in environmental conditions brought on by the intermittent flood treatment were expected to alter the soil microbial composition; however, this was not the case. The intermittent flood treatment resulted in the greatest microbial biomass but the responses of the various microbial markers under the intermittent flood treatment were not different from those under the non-flood control and/or flowing flood treatments. A general decline in fungal groups with inundation was expected. However, results were ambiguous; while response of the fungal group decreased with flooding, the bacterial:fungal ratio did not increase as expected. Effects of flood treatments in the field were masked by effects due to sampling depth and date. General decreases in microbial biomass and microbial marker response with depth were observed. Changes in pre- and post-flood samples were more likely due to seasonal influences (spring vs summer) than due to specific flood treatments.

Changes in microbial community structure with inundation should be reflected in changes in enzyme activity. As observed with the community structure analyses, responses of enzyme activity to flood treatments varied with environmental conditions (i.e., greenhouse vs. field). Responses to flood treatments were observed for microbial community function under simulated flood conditions in the greenhouse but not in the field. Under greenhouse conditions, both C- and N-utilization were greater under control and intermittent flood

treatments than under flowing and stagnant flood treatments. Under field (i.e., FTL) conditions, enzyme activity was not affected by flood treatment (i.e., duration or condition of flood water). Carbon-utilization decreased over time in the FTL, but N-utilization remained unchanged. In both the greenhouse and field experiments, the soil microbial community structure was more closely related to N-utilization than to C-utilization, suggesting that N is limiting for these microbial communities.

Few investigations into the effects of flooding on the soil microbial community structure have been conducted. This study revealed that anaerobic soil conditions due to stagnant floods resulted in reduced total microbial biomass, reduced response of microbial groups, and increased stress indicators within the microbial community. Of particular note is the effect of stagnant flood conditions on mycorrhizal fungi. Fungi are important decomposers of recalcitrant C compounds, such as lignin, within the soil. Therefore, a decline in fungal response may affect nutrient availability for plants. In addition, many plants rely on mycorrhizae for enhanced nutrient and water absorption; therefore, loss of mycorrhizal associations may have implications for plant establishment and survival.

CHAPTER 5

THE INFLUENCE OF MICROTOPOGRAPHY ON SOIL CHEMISTRY AND UNDERSTORY RIPARIAN VEGETATION

Introduction

Flooding as a disturbance affects vegetation possibly by indirect effects on soil. If wetland preservation and restoration efforts efforts are to be successful, understanding abiotic influences, such as topography and soil characteristics, on vegetational patterns is essential. Flood frequency, intensity and duration will vary according to stream order and watershed size. Within a watershed, position on the landscape is important; as distance from river increases, particularly when moving from bottomlands to upslope positions, the frequency, intensity and duration of flooding decreases. Differences in soil and vegetional characteristics would be expected between these bottom- and up-land slope positions.

A number of studies of riparian systems indicate plant species distributions correlate with soil morphological and microtopographical variations (Beatty, 1984; Nilsson et al., 1994; Ohmann and Spies, 1998; Grell et al., 2005; Holmes et al., 2005; Lyon and Gross, 2005). Beatty (1984) found that forest floor microrelief could explain distributions of the most common herbs and woody seedlings in maple-beech forests of eastern New York. Grell et al. (2005) concluded that differences in vegetation resulted primarily from subtle elevational variation within old-growth *Pinus taeda* forests of southern Arkansas. Holmes et al. (2005) observed differences in ground-flora composition between floodplain and upland landforms in north-central Ohio. However, Becker (1999) found that environmental variables affecting vegetation distribution differed depending on the creek of interest. Ozark streams of lower order (1st and 2nd order) in this study were influenced by bedrock, parent

material, pH of the A horizon and waterway position, while higher order Ozark streams (3rd – 5th) were influenced by landform features, pH of the A horizon and soil texture characteristics (Becker, 1999). In addition, several studies found that species associations shift along key environmental gradients but different forest vegetation layers do not necessarily shift in concert. Site heterogeneity was a likely contributing factor to this differential response (Sagers and Lyon, 1997; Lyon and Sagers, 1998; Lyon and Gross, 2005). Collectively these results illustrate the need to study multiple watersheds within a region and the need to include multiple layers and especially herbaceous species in all characterizations of riparian forests.

The species-level response to disturbance is influenced by life history traits. Properties of plant species, such as seed dispersal mechanisms and requirements for germination, ability to survive translocation, ability to compete, and ability to survive abrasive effects of specific flow velocity and sediment and transport regimes, play a role (Gurnell, 1997). Species that are able to complete their life cycles rapidly may be well suited for persistence in the floodplain where disturbance is frequent and conditions are uncertain (Adams and Anderson, 1980; Menges and Waller, 1983; Sagers and Lyon, 1997; Holmes et al., 2005). Menges and Waller's (1983) study of five floodplains in southern Wisconsin revealed that certain life history traits were correlated with specific landscape positions. For example, at higher elevations within the floodplain, many species were light generalists, while at lower elevations there were few important light generalists (Menges and Waller, 1983). In addition, annuals were only important at lower elevations, while perennial forbs dominated at higher elevations; these perennials showed increased height with increased elevation. Menges and Waller (1983) summarized their findings by categorizing floodplain (low elevation) herbs as either stress-tolerant perennials or small ruderal (annual) forbs; at mid-

elevations, light specialists in the form of perennial forbs and annuals dominate, while at higher elevations, tall, fast-growing competitive plants dominate. Similarly, in their study of an old-growth headwater forest in north-central Ohio, Holmes et al. (2005) found the floodplains (low elevations) dominated by graminoids and annual forbs and the uplands dominated by woody tree seedlings and woody vines. Adams and Anderson (1980) determined that tree diversity was greatest on mesic sites or intermediate sites; diversity decreased in either drier or wetter habitats. This pattern was explained to be the result of conditions on xeric and lowland sites being unfavorable for invasion by many species adapted to mesic conditions. Such findings suggest a need to identify plant characteristics and functional attributes outside the usual classification based on successional status or adaptation to light, stress or competition.

Both the trends in species association and soil characteristics are related to the disturbance caused by flooding. Potentially significant soil gradients may exist between the floodplain and upland sites. For instance, floodplain forests have been found to have a higher percentage of organic matter, percent clay, percent sand and a higher pH than upland soils; upland soils had higher percentages of silt (Holmes et al., 2005). Environmental gradients likewise contribute to plant species distributions. Species persisting in the floodplains must be adapted to inundation and habitat disturbance caused by floods while upland species must be adapted to moisture stress in the form of increasing moisture deficits with elevation (Holmes et al., 2005). Gurnell (1997) concluded that distributions of plants in floodplains reflect sensitivity to degree and frequency of waterlogging, the energy and frequency of flooding, soil and river water quality, the size and organic content of river corridor sediments and rate of sedimentation.

Objectives and Expectations

The overall goal of this research is to determine if riparian plant community composition is related to soil chemistry, and if this relationship occurs, determine if it is mediated via flooding. Specific objectives include determining: i) how soil chemical characteristics such as, inorganic-N and total soluble polyphenolics, change with microtopography and flood frequency, ii) how understory vegetation (herbaceous and shrub layers) changes with microtopography, flood frequency and soil characteristics, and iii) overstory differences between watersheds in the Central Dissected Till Plains of northwest Missouri. Distance from river will serve as a proxy for flood frequency in this study; it is expected that with decreased flood frequency (i.e. increased distance from river) the following trends will be observed: nitrate levels will increase, and ammonium levels will decrease; total soluble polyphenolics will decrease; and herbaceous and shrub vegetation richness and diversity will increase. Concave features in the landscape are expected to collect and temporarily retain water; therefore, these landscape positions should have higher ammonium and total soluble polyphenolic levels and lower nitrate levels and understory diversity and richness than convex landscape positions. Understory richness and diversity should correlate with landscape positions that have higher total N but lower total soluble polyphenolics levels than neighboring landscape positions.

Methods

Study Sites

This study was conducted in Central Dissected Till Plains of Missouri; this ecoregion covers almost all of Missouri north of the Missouri river (Nigh and Schroeder, 2002). Floodplains along three streams in northwest section of this ecoregion were selected: Locust

Creek (Pershing State Park), Yellow Creek (Yellow Creek Conservation Area), and Thompson River (Crowder State Park) (Figure 5.1); all are 2nd order streams and tributaries of the Grand River. Two of the streams, Locust Creek and Thompson River, have gage stations nearby that are continuously monitored by the USGS (http://waterdata.usgs.gov/mo/nwis/rt). For each of these sites, gage height and discharge data were available for the sampling period. Discharge data for Locust Creek and Thompson River reveal "flashy" systems; both of these streams display a relatively low, base-line discharge rate punctuated by brief periods of rather high discharge (Figure 5.2). Thompson River has a drainage area of approximately 1,720 square miles, while Locust Creek has a drainage area of approximately 550 square miles. Correspondingly, the base-line discharge of Thompson River is about 3-4 times that of Locust Creek (Figure 5.2). No gage data were available for Yellow Creek and discharge measurements were not collected for this stream.

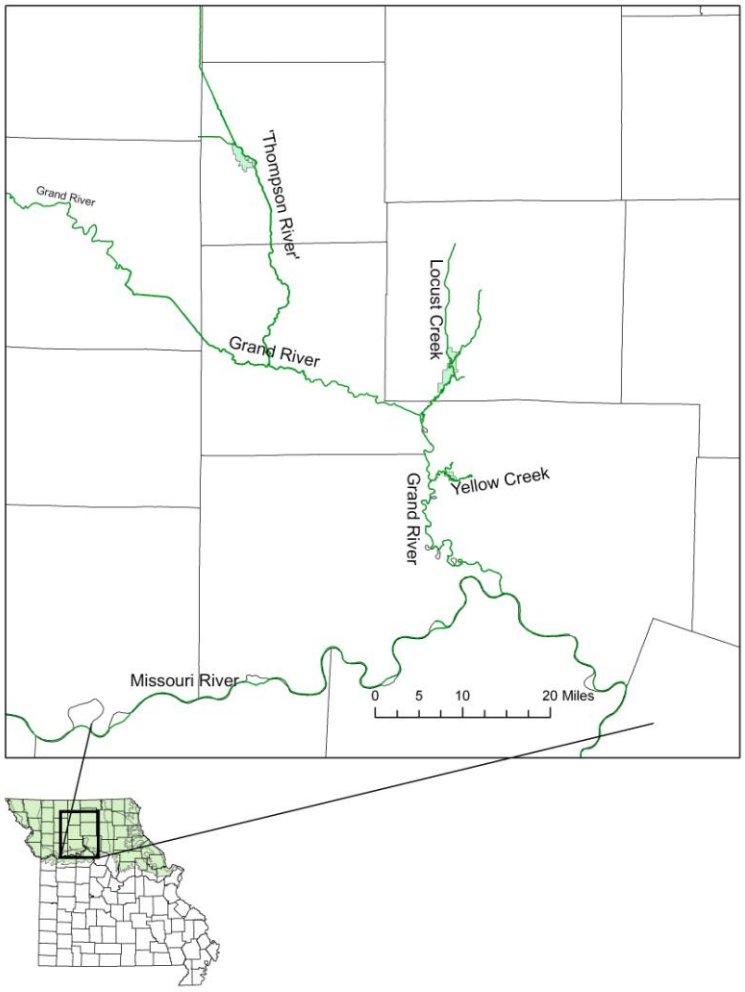

Figure 5.1. Floodplain study sites. Study sites were located within the Central Dissected Till Plains ecoregion in Missouri (shaded portion of state map). Floodplains along three creeks in northwest Missouri were investigated: Locust Creek in Pershing State Park, Yellow Creek in Yellow Creek Conservation Area and Thompson River in Crowder State Park. All are tributaries of the Grand River.

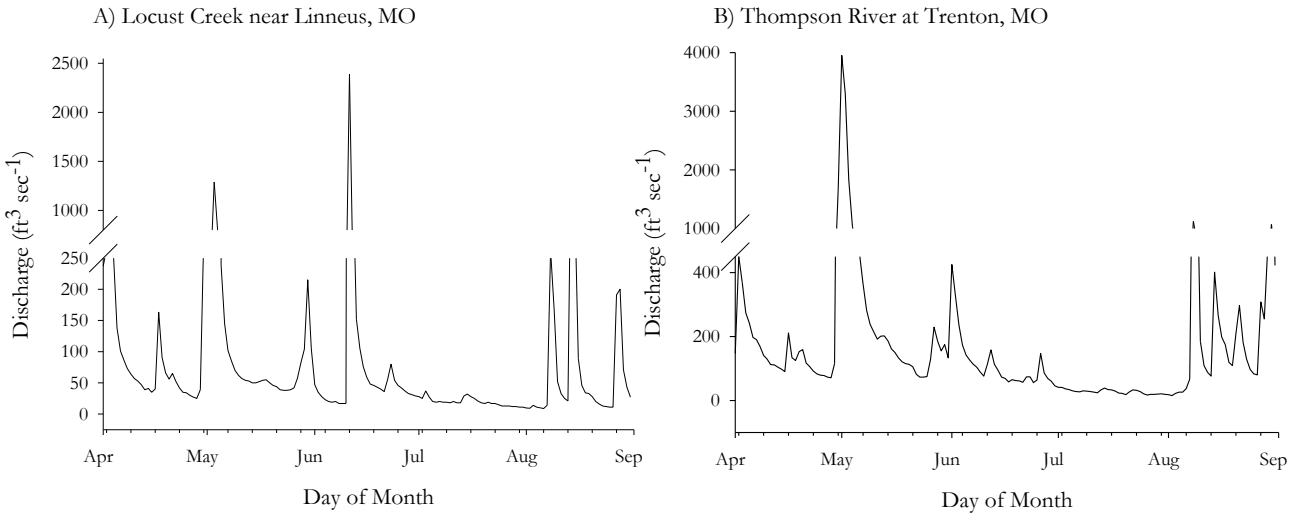

Figure 5.2. Stream discharge data April-September 2006 for a) Locust Creek at Linneus, MO (39°53'45.4"N, 93°14'11.5"W) and b) Thompson River at Trenton, MO (40°04'09.5"N, 93°38'16.9" W) collected from USGS database (http://waterdata.usgs.gov/mo/nwis/rt).

Sampling Plots

At each stream, two plots, 100 m apart (the second down stream from the first), were established. Plots were 30 m x 30 m and consisted of a grid of transect lines: 4 lines oriented parallel to the stream and 4 lines oriented perpendicular to the stream. Transect lines parallel to the stream began at 20 m from the creek edge and were 10 m apart. Transect lines perpendicular to the stream were likewise 10 m apart. At the intersections of the transect lines, a 1 m^2 understory sampling quadrat was established for a total of 16 quadrats per plot (Figure 5.3).

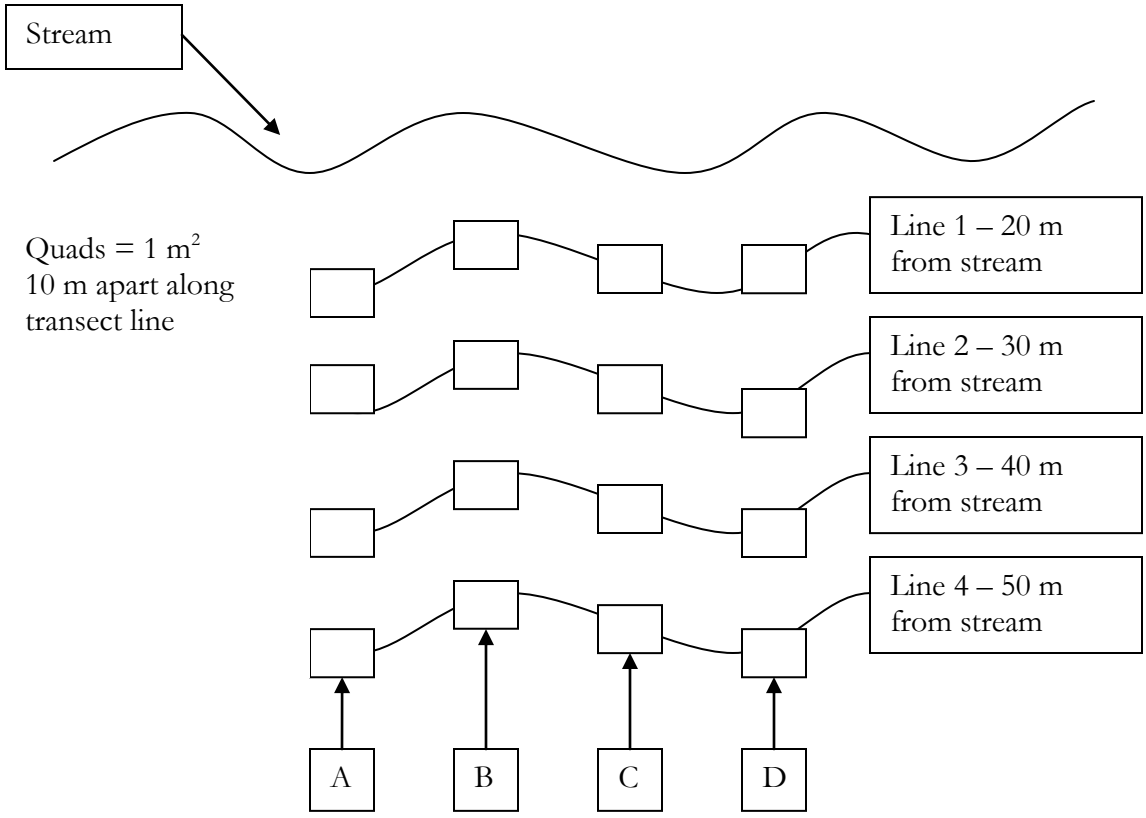

Figure 5.3. Diagram of sampling grid. Two such plots (30 x 30 m) were established at each site.

Floodplain Characteristics

Topographical maps obtained for sites revealed little relief in the areas chosen for this study. In order to determine site micro-relief, standard survey equipment was used to measure changes in ground-level (i.e. elevation) as compared to a reference location (reference height = 100 cm). Elevation measurements were taken at the center of each quadrat, at locations mid-way between quadrats and at locations 5 m outside the quadrats (i.e. around the perimeter of the study plot), for a total of 81 measurements per plot. ArcGIS was used to determine planform, flow accumulation (FA) and slope for each quadrat.

Planform is a measure of the change in slope perpendicular to the slope direction. A negative planform value indicates that the surface is concave at that cell, while a positive value indicates that the surface is convex at that cell (Gallant and Wilson, 2000). Since planform describes relative convexity and concavity (Gallant and Wilson, 2000) it could be used to describe areas of water accumulation in a landscape. Flow accumulation is a proxy measurement of the amount (i.e. volume) of water flowing into (or collected by) the quadrat; the higher the value, the greater the water flow into the cell (Gallant and Wilson, 2000). Flow accumulation for a quadrat is determined by the number of adjacent quadrats flowing into it (Sharma et al., 2006). Slope (in degrees) is a measure of the change of elevation between each quadrat and its neighbors (Sharma et al., 2006). Slope affects the velocity of water movement and thus would have affect the transport of sediment and dissolved substances. Together these measures indicate places areas in floodplains where water might be expected to accumulate and thus have an impact on soil nutrient cycling and vegetative growth.

Soil Sampling

Soil core samples were collected in July, 2006 to determine how the physical characteristics (i.e., color, texture and redoximorphic features) of the soil related to flood frequency. A push-probe was used to collect a soil column (up to 30 cm deep) from each transect line parallel to the river. Field determinations of soil color and presence of redoximorphic features (mostly soil mottles) were made using Munsell color charts; and the ribbon technique was employed to determine soil textures.

Composite surface soil samples were collected from each quadrat in early June, 2006 to determine how soil chemistry related to flood frequency. The top 20 cm of soil was collected from the center and the four corners of each 1 m^2 quadrat with a push-probe; these

samples were mixed together to create a single sample per quadrat. Samples were placed in a cooler in the field and then frozen upon return. Samples were later freeze dried, ground and passed through a 2 mm sieve. The following parameters were measured: nitrate (NO_3-N), ammonium (NH_4-N), total N (TN), total organic C (TOC), C:N ratio, and total soluble polyphenolics (TSP). Soil inorganic N content was determined using the Lachet QuickChem Method 12-107-04-1-B for NO_3-N determination (Appendix A) and the Lachet QuickChem Method 12-107-06-2-A for NH_4-N determination (Appendix B). Total organic C and TN were determined by dry combustion using a TruSpec CN analyzer (LECO; St. Joseph, MI); the C:N ratio for each sample was calculated from these determinations. Total soluble polyphenolic content was determined using the Folin-Ciocalteau technique (Suominen et al. 2003). Soil extracts for this test were obtained by mixing 15 g soil with 15 ml of distilled water. This mixture was shaken for 8 hours then centrifuged for 30 minutes; extracts were then filtered with Whatman #1 filter paper. The Folin-Ciocalteau test was conducted by mixing 2 ml distilled water, 2 ml soil extract, 5 ml Na_2CO_3 and 1 ml Folin-Ciocalteau reagent (Sigma-Aldrich). This mixture was incubated for 30 minutes to allow for color development and absorbance was read at 735 nm using a spectrophotometer. Tannic Acid (reagent grade) was used as the standard.

Vegetation Sampling

Vegetation sampling occurred in July, 2006; a mid-season date was chosen to allow the capture of the maximum understory diversity with a single sampling event. The 1 m^2 quadrats were used to sample herbaceous vegetation. Any herbaceous vegetation that was established within the quadrat was identified to species where possible and percent cover was estimated. Any woody vegetation < 1 m in height was included; however instead of

percent cover, the number of stems of each species within the quadrat was counted. Estimates of herbaceous percent cover and woody stem counts were used to determine plot species richness, diversity (Shannon Diversity Index: $H' = -\sum p_i \log_e p_i$, where p_i is the proportion of the *i*th species in the total sample) and evenness ($J' = H'/ \log_e S$, where S is the number of species present).

Two belt transects were established within each plot to further characterize the shrub layer. These transects corresponded to the parallel transect lines at 30 m and 50 m from the stream. These transect lines were traversed and any shrubs or understory trees (dbh < 5 cm) that were rooted within 1 m of the line were identified to species and tallied. To characterize the overstory, the entire 30 x 30 m plot was surveyed. Individuals were identified to species; dbh and crown class were also recorded. Basal area, relative density, relative dominance, and importance values were calculated for each species. Species richness was determined for each plot; diversity and evenness were calculated (as above) using relative density and relative dominance measures.

Data Analysis

ANOVA (Proc GLM) was used to determine if soil chemical variables (i.e., NO_3-N, NH_4-N, TN, TC, C:N ratio and TSP) varied with distance from the stream. Likewise, ANOVA was used to determine if increasing distance from the stream influenced understory vegetation characters (i.e., % herbaceous cover or number of woody stems, species richness, diversity, and evenness). In addition, ANOVA was used to determine if soil chemical variables and understory vegetation characters differed between plots at the same stream or among streams. Herbaceous % cover (arcsine square root) as well as NO_3-N and NH_4-N data were transformed ($\log_{10}$) to meet assumptions of normality. ANOVA was also used to

determine if overstory species composition (i.e. importance values) varied between plots at the same stream or between different streams. Data were reported as least square means; comparisons of least square means were made using PDIFF ($\alpha = 0.05$).

Pearson correlation coefficients (Proc CORR) were examined between the soil chemical variables, the understory vegetation characteristics and the topographical characteristics (planform, FA, slope and elevation). Regression analysis (Proc REG) was used to determine if any of the soil chemical variables explained the variation observed in understory vegetation characteristics. Likewise, regression analysis was used to determine if topographical characteristics explained the variation in soil chemical variables or in understory vegetation characteristics. All statistical analyses were performed using SAS 9.1 (SAS Institute Inc 2002-2003).

To identify patterns in the understory species, chemical, and microtopographical data, multivariate statistical analyses were used. Detrended Correspondence Analysis (DCA) was used to examine the patterns in understory species, soil chemicals and microtopography across the sites/plots. For the understory analysis, herbaceous and woody species observed in the 1 m^2 sampling quadrats were analyzed separately due to different data collection techniques (i.e. herbaceous species cover vs number of woody stems). Rare species tend to distort DCA results because they tend to generate high χ^2 values (Shaw, 2003). Therefore, species that were observed in < 5% of the sampling quadrats were removed prior to analysis, and rare species that remained in the data set were down-weighted in proportion to their frequency during the analysis (McCune and Mefford, 1999; Shaw, 2003). Negative values cannot be included in DCA matrices; therefore, untransformed data from the soil chemical and microtopographical data sets were used for these analyses and the planform measure was eliminated. To evaluate the effectiveness of the ordinations, a post-hoc coefficient of

determination between relative Euclidean distance in the unreduced species space and Euclidean distance in the ordination space was performed (McCune and Mefford, 1999).

To examine the relationships between understory species and soil chemicals or site microtopography, Canonical Correspondence Analysis (CCA) was used. CCA, like DCA, is sensitive to rare species (Shaw 2003); therefore, species that were observed in <5% of the sampling quadrats were removed prior to analysis. Understory species were analyzed separately (i.e. herbaceous species vs woody species) and were entered as the main matrix with either soil chemistry or site microtopography was entered as the second matrix. Log transformed data (soil chemistry: NO_3-N, NH_4-N and site microtopography: FA and slope) were used for these analyses; planform was included in the site microtopography dataset. CCA was also used to explore the relationship between soil chemicals and site microtopography; in this case, soil chemical data was entered as the main matrix and site microtopography was entered as the second matrix. All multivariate statistics were preformed using PC-ORD Version 5 (MjM Software, 2006)

Results

Physical Characterization of the Sites

Yellow Creek

A distinguishing feature of the Yellow Creek site, relative to the other sites, was the high turbidity of the water. Floodplain soil texture was primarily silty clay; and soil colors were mostly dark gray (2.5 Y 4/1) or dark grayish brown (2.5 Y 3/1) with little change in texture or color with depth. Mottles were infrequent but when they occurred they were mostly brown (10YR 5/3), yellowish brown (10 YR 5/4), dark brown (10 YR 4/3) or yellow/olive brown (2.5 Y 5/3). The observed low chroma values would indicate that this

site had a wet regime (due to flooding from the creek and/or the influence of a high water table) with the soils maintaining anaerobic/reduced conditions. While dry periods are likely to occur, the fine texture of soils would allow for capillary rise of water, maintaining high level of soil moisture. A visit to the site in early 2007 following a period of heavy rains revealed several areas of ponding within the floodplain; these areas were drained one week later when the site was revisited.

Topographical evaluation of the Yellow Creek plots revealed a flat terrain. At plot 1, the elevation gradually decreased as distance from the river increased; the change in elevation across the plot was a total of 0.40 cm (Figure 5.4A). Change in elevation at plot 2 was more subtle, with a difference of only 0.15 cm across the plot; general trends were less discernable however transects closer to the river were lower (Figure 5.4B).

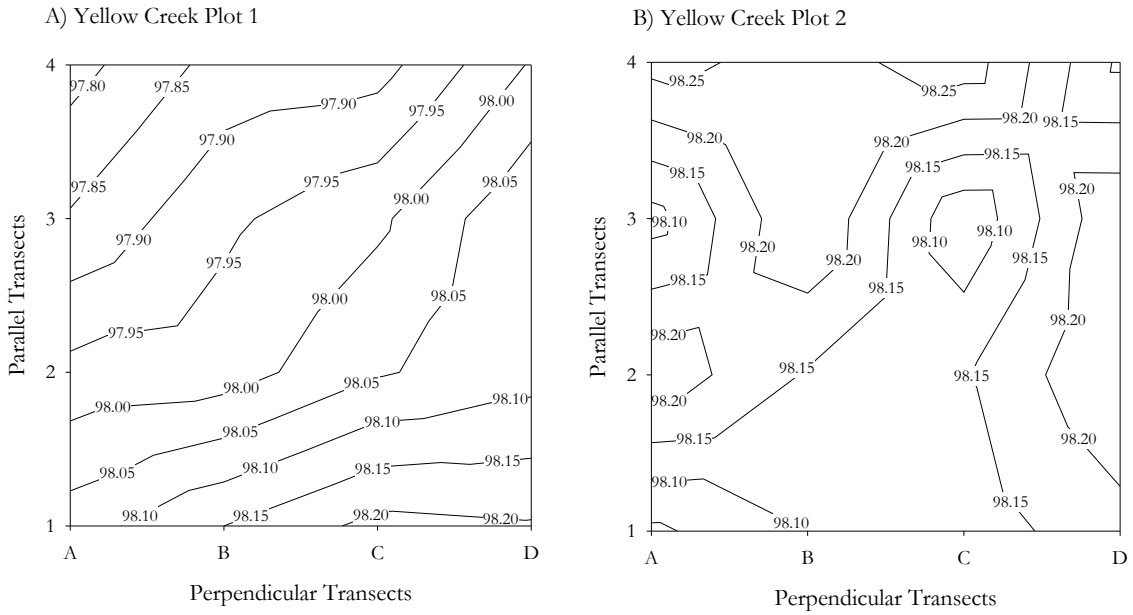

Figure 5.4. Elevation (cm) maps of Yellow Creek Plots (30 m x 30 m): A) Yellow Creek Plot 1, B) Yellow Creek Plot 2. Elevations for each quadrat were taken with standard survey equipment using a reference point of 100 cm. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank).

Planform analysis for plot 1 revealed concave areas associated with the 30 m parallel transect line (quadrats B2, C2, and D2) and parts of the 50 m parallel transect line, in particular near quadrat A4 and D4 (Figure 5.5A). Planform features for plot 2 closely mimicked elevation changes. In plot 2, the 20 m parallel transect showed a concave planform; this area of concavity extended diagonally across the plot and corresponded to areas designated as having lower elevations such as quadrats C2 and C3 (Figures 5.4B & 5.5B). One small area, near quadrat A2 showed a convex character.

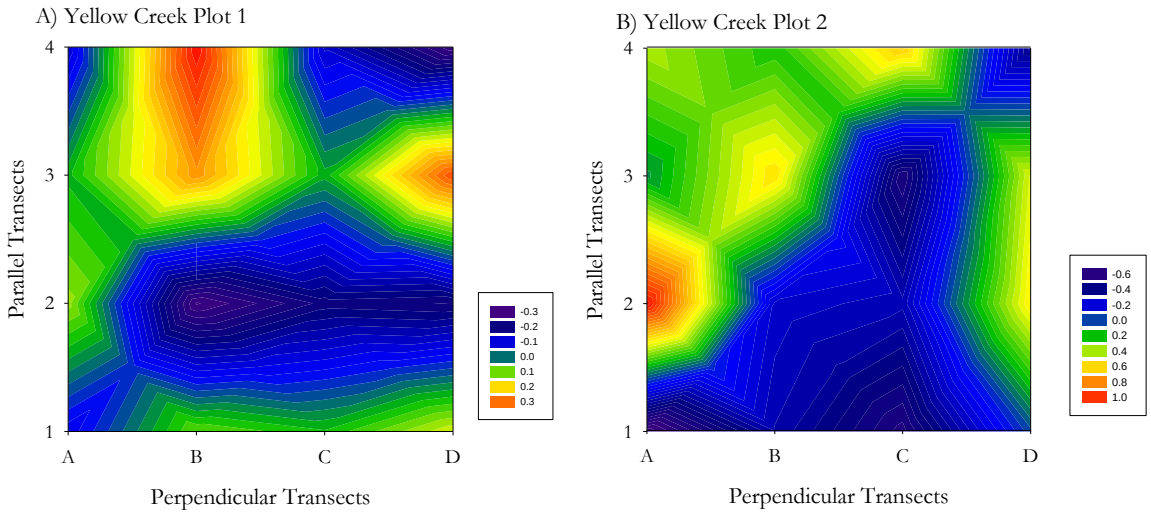

Figure 5.5. Planform maps of Yellow Creek Plots (30 m x 30 m): A) Yellow Creek Plot 1, B) Yellow Creek Plot 2. Elevations from each quadrat, from mid-points between quadrats and from points 5 m outside the plot were used for planform calculations. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank). Note different scales for each plot; colors in violet and blue end of spectrum represent depressions or concave parts of the landscape, while colors in the yellow-red range indicate convex parts of the landscape.

In plot 1, the greatest FA was associated with quadrat A4 (FA = 169.0). Other quadrats with high FA included B3 (FA = 41.8), B2 (FA = 28.0), and C2 (FA = 24.9). Slope was minimal across the plot with the highest slopes observed for quadrats B3 and C1 (slope = 0.023° for both). In plot 2, FA varied little across the landscape. Quadrats C2 and D4 each had an FA of 10.0; all other quadrats had an FA = 5.0. Again slope was minimal with the highest slopes recorded in quadrats A4, C4 and D4 (slope = 0.04° for each)

Locust Creek

Locust Creek appeared to be a faster moving, higher energy stream, prone to more fluxes and overflow; the water of Locust Creek was clear and a sandy matrix at the channel bottom was evident. Soil textures at these plots ranged from silt loam to silty clay loam. Soil colors were mostly dark brown (10YR 3/3 and 10 YR 3/4), brown/dark brown (10YR 4/3) and dark yellowish brown (10YR 4/4). Mottles were infrequent but when they occurred they were mostly grayish brown (10YR 5/2). The soils in these plots were well stratified with fine and very fine sands. As distance from stream increased, soil texture very generally "fined", i.e., silt and clay content increased, with distance from stream. The soils appeared better drained than those at Yellow Creek with little evidence of standing water. The water table could be close to stream level; however, soils were less hydric than those at Yellow Creek as indicated by higher chroma values.

As with the Yellow Creek plots, elevation differences across the floodplains were minimal for the Locust Creek sites. Locust Creek plot 1 showed a similar pattern to Yellow Creek plot 1, with elevation gradually decreasing diagonally across the plot from a "high" at quadrat D1 to a "low" at quadrat A4 (Figure 5.6A). Similarly, Locust Creek plot 2 had highest elevation at the 20 m parallel transect line, and a gradual decrease in elevation with increased distance from the river (Figure 5.6B). The total elevation gradients for plot 1 and 2 were 0.3 cm and 0.2 cm respectively.

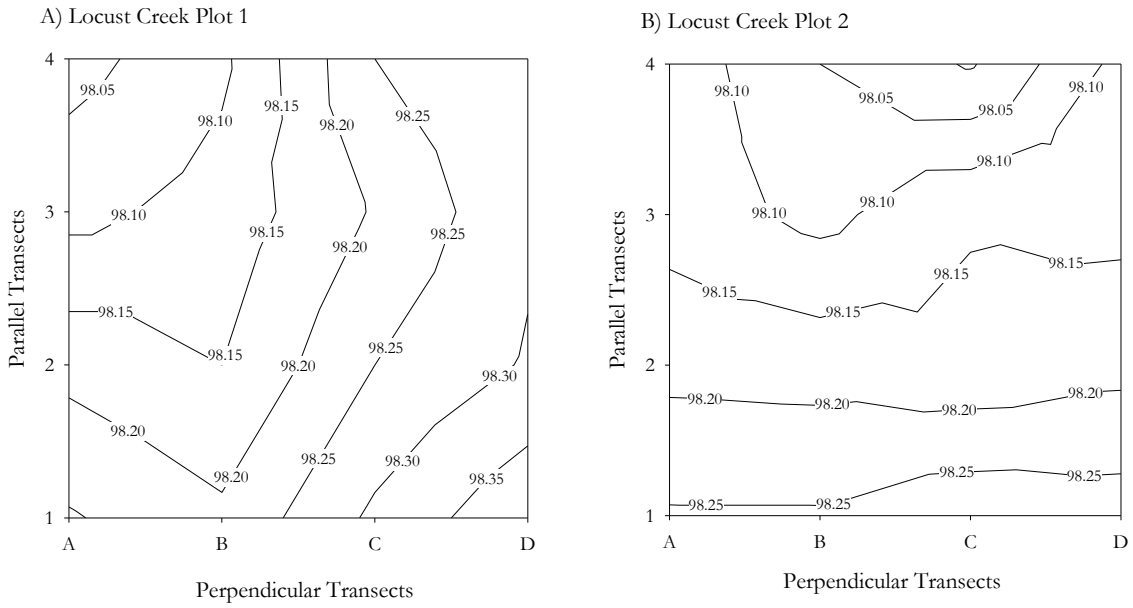

Figure 5.6. Elevation (cm) maps of Locust Creek Plots (30 m x 30 m): A) Locust Creek Plot 1, B) Locust Creek Plot 2. Elevations for each quadrat were taken with standard survey equipment using a reference point of 100 cm. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank).

Planform analysis for Locust Creek plot 1 revealed a gently rolling topography. Concave features were found at or near quadrats B1, C3 and A4, while convex features were found at or near quadrats D1, C2 and D4 (Figure 5.7A). Locust Creek plot 2 was notable in its lack of variation (Figure 5.7B); one convex feature of note was located near quadrat A4. Two potential concave features can be seen near quadrats C2 and C4 (Figure 5.7B).

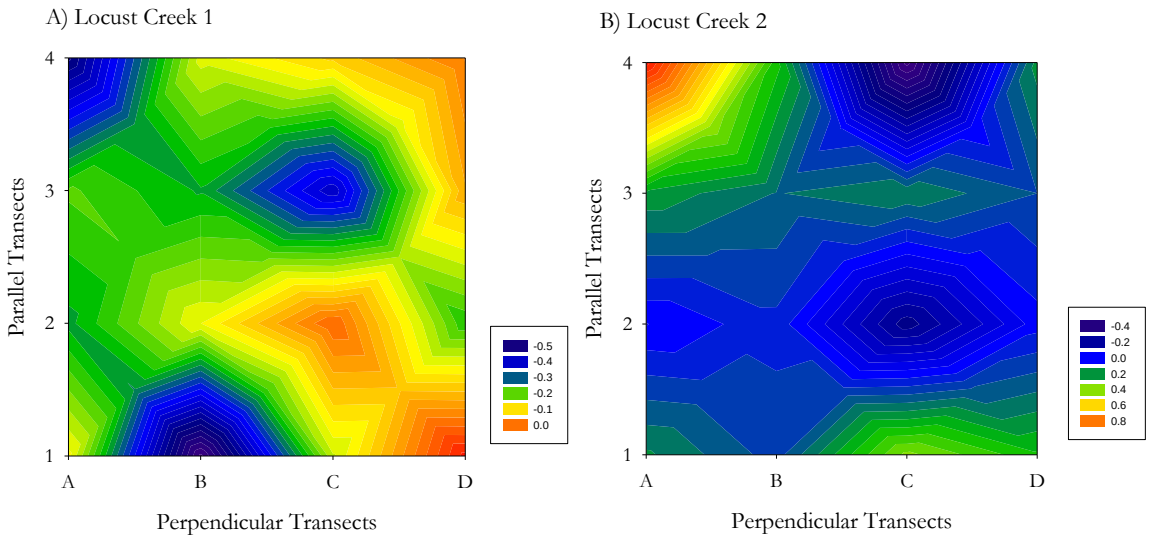

Figure 5.7. Planform maps of Locust Creek Plots (30 m x 30 m): A) Locust Creek Plot 1, B) Locust Creek Plot 2. Elevations from each quadrat, from mid-points between quadrats and from points 5 m outside the plot were used for planform calculations. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank). Note different scales for each plot; colors in violet and blue end of spectrum represent depressions or concave parts of the landscape, while colors in the yellow-red range indicate convex parts of the landscape.

Flow accumulation in plot 1 was greatest in quadrat B2 (FA = 79.7). Other quadrats with high FA included B3 (FA = 43.6) and B4 (FA = 30.1). In plot 1, slope was greatest in quadrat A3 (slope = 0.95°) and C2 (slope = 0.92°). Quadrats D3, C1 and A2 also had high slopes (slope = 0.86°, 0.74°, and 0.73° respectively). Flow accumulation was generally lower in plot 2 than in plot 1, with the highest FA observed in quadrat C4 (FA = 43.7) and the second highest FA observed in quadrat C2 (FA = 22.05). The highest slope in plot 2 was observed in quadrat C4 (slope = 1.3°). Slope was also high in quadrats A3 (slope = 0.9°), C3 (slope = 0.8°) and B3 (slope = 0.7°).

Thompson River

At first inspection, Thompson River seemed more similar to Locust Creek than to Yellow Creek. However, the soils at Thompson River were not as varied in texture or as stratified as those at Locust Creek (i.e. the matrix at Thompson River was siltier than the matrix at Locust Creek). Soil textures at the Thompson River plots ranged from silt loam to silty clay loam with a very general fining as distance from stream increased. Soil colors were darker, which could be indicative of organic matter or could have been due to reduced conditions; colors ranged from very dark grayish brown (10YR 3/2) to dark grayish brown (10 YR 4/2) to brown (10YR 5/3). Mottles, which are indicative of changes in redox potentials associated with inundation, were generally absent.

Elevation patterns at Thompson River plot 1 resembled those at Yellow Creek plot 1 and Locust Creek plot 1, in that elevation gradually decreased as distance from river increased (Figure 5.8A). Again the gradient was minimal, with a 0.3 cm change in elevation across the floodplain. Thompson River plot 2 showed little variation in elevation, having only a 0.15 cm change in elevation across the floodplain and relatively large areas with no changes in elevation (Figure 5.8B).

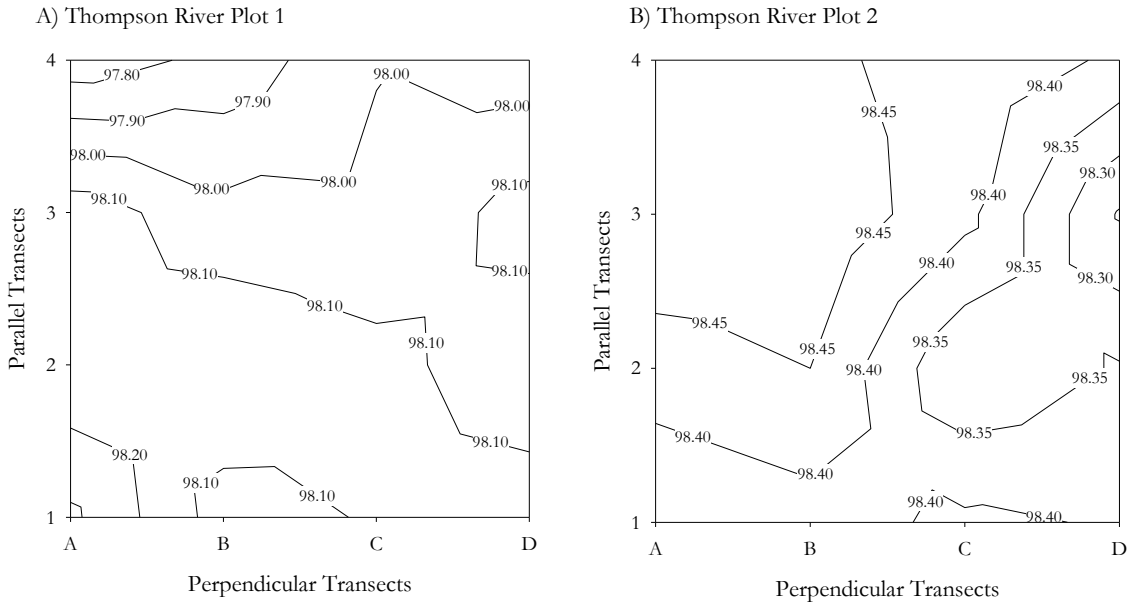

Figure 5.8. Elevation maps of Thompson River Plots (30 m x 30 m): A) Thompson River Plot 1, B) Thompson River Plot 2. Elevations for each quadrat were taken with standard survey equipment using a reference point of 100 cm. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank).

Planform analysis for Thompson River plot 1 indicated that this plot was centered on a relatively featureless portion of the floodplain. Two small convex areas were apparent at quadrats A3 and D3 of this plot, while concave areas were observed at quadrats B1 and C1 (Figure 5.9A); the landscape changed little over the rest of the plot. Plot 2 seemed to have more variation in landforms (Figure 5.9b). Two small concave areas were observed at quadrats C2 and D3. Convex features were observed at quadrats C1, D2, and D4, with an almost ridge like feature extending from quadrat B2 to B3.

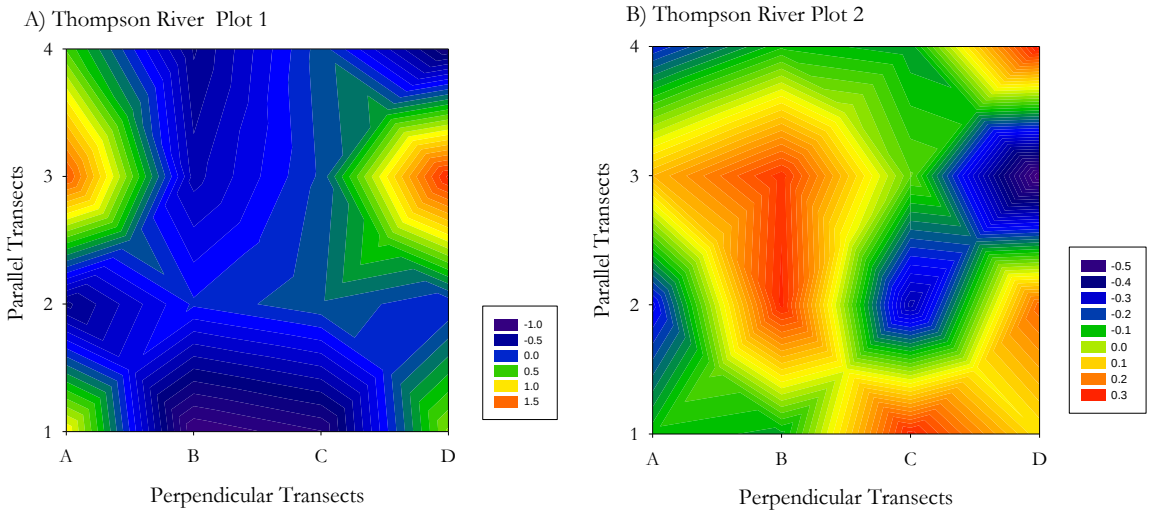

Figure 5.9. Planform maps of Thompson River Plots (30 m x 30 m): A) Thompson River Plot 1, B) Thompson River Plot 2. Elevations from each quadrat, from mid-points between quadrats and from points 5 m outside the plot were used for planform calculations. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank). Note different scales for each plot; colors in violet and blue end of spectrum represent depressions or concave parts of the landscape, while colors in the yellow-red range indicate convex parts of the landscape.

The Thompson River plots showed little variation in FA. In plot 1, the greatest FA was recorded in quadrats B4 (FA = 33.5), B3 (FA = 13.5) and A4 (FA = 8.1). And in plot 2, quadrats C3 (FA = 12.3) and B4 (FA = 10) had the greatest FA. All other quadrats in both plots had FA = 5.0. The highest slopes (overall) were recorded in Thompson River plot 1. Quadrats A3 (slope = 2.4°), C2 (slope = 2.2°) and A4 (slope = 2.1°) were the most notable. Other quadrats in plot 1 with high slopes were B3 (slope = 1.8°) and C3 (slope = 1.7°). In plot 2, quadrat D4 had the greatest slope (slope = 1.1°), followed by quadrat C3 (slope = 1.0°). Other quadrats with high slopes included C1, A3 and B1 (slopes = 0.8°, 0.8°, and 0.7° respectively).

Chemical Characterization of Soils

Chemical properties were evaluated at three levels: i) creek (3 sites), ii) plot within creek (2 plots/site), and iii) distance from river within plot (4 parallel transect lines beginning at 20 m from the river). While these evaluations revealed no significant differences for NH_4-N (Table 5.1), differences in NO_3-N (Table 5.2) were detected at all levels. On the creek level, Locust Creek had significantly higher levels of NO_3-N ($F = 5.21$, $P < 0.01$); conversely, NO_3-N levels at Yellow Creek and Thompson River were not different from each other. Plot comparisons for each river revealed significant difference in NO_3-N levels on the Thompson River plots with Thompson River plot 1 having nearly twice the NO_3-N as plot 2 ($P < 0.0001$). Locust Creek plots were not different from each other, nor were Yellow Creek plots. Significant differences in NO_3-N levels with distance from river were observed at Yellow Creek plot 2 ($F = 8.98$, $P < 0.01$) and at Locust Creek plot 2 ($F = 9.92$, $P < 0.01$). At Yellow Creek plot 2, the 20 m transect line had significantly higher levels of NO_3-N than the other transects. At Locust Creek plot 2, the 40 m transect line had significantly higher levels of NO_3-N than the other transects. (See Appendix E for contour maps depicting the levels of NO_3-N and NH_4-N across each plot.)

Table 5.1. Soil NH_4-N at the three study sites. Values represent least square means of NH_4-N (mg kg^{-1}) levels for creek, plot within creek and distance from river within plot. No significant differences were observed.

	Yellow		Locust		Thompson	
Creek	6.13		5.27		5.47	
Plot(Creek)						
1	6.07		5.03		4.07	
2	6.20		5.50		6.87	
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	5.61	6.90	5.13	4.53	5.07	3.33
30 m	5.82	7.25	5.51	4.24	3.49	9.85
40 m	5.21	5.61	4.86	4.32	3.76	8.94
50 m	7.57	5.16	4.50	8.32	4.21	4.48

Table 5.2. Soil NO_3-N at the three study sites. Values represent least square means of NO_3-N (mg kg^{-1}) levels for creek, plot within creek and distance from river within plot. Means with at least one letter in common are not significantly different ($\alpha = 0.05$) in soil NO_3-N content at that level of comparison (i.e. plots at the same creek were compared only to each other and transect lines at the same plot were compared only to each other).

	Yellow		Locust		Thompson	
Creek	0.99 **b**		1.43 **a**		1.24 **b**	
Plot(Creek)						
1	1.09		1.63		1.88 **a**	
2	0.89		1.23		0.60 **b**	
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	1.65	1.31 **a**	1.59	1.07 **b**	1.32	0.61
30 m	0.93	0.78 **b**	2.28	0.94 **b**	2.39	0.68
40 m	0.91	0.54 **b**	1.22	2.87 **a**	1.76	0.60
50 m	0.68	0.81 **b**	1.37	0.86 **b**	1.91	0.52

Comparisons at the creek and plot level showed similar patterns for TOC (Table 5.3) and TN (Table 5.4). Significantly different levels of both TOC ($F = 58.25$, $P < 0.0001$) and TN ($F = 89.38$, $P < 0.0001$) were observed at the creek level with Yellow Creek having the

greatest levels of TOC and TN, followed by Thompson River and finally by Locust Creek. Plot comparisons revealed differences for these parameters at Locust Creek only; in each case, Locust Creek plot 1 had higher levels of TOC and TN. Thompson River plots and Yellow Creek plots did not differ from each other in terms of TOC or TN. No differences in TOC with distance from river were observed at any of the plots, however differences in TN with distance from river were observed at both Yellow Creek plots. At Yellow Creek plot 1, transect lines at 40 m and 50 m from the river had significantly higher TN levels than transect lines at 20 m and 30 m from the river ($F = 4.62$, $P = 0.03$). Likewise, TN levels increased with distance from river at Yellow Creek plot 2, however the only significant difference observed was between the 20 m and the 50 m transect lines ($F = 4.23$, $P = 0.04$). No differences were detected for C:N ratio at the creek, plot or distance from river levels of comparison (Table 5.5).

Table 5.3. Soil TOC for the three study sites. Values represent least square means of TOC (%) levels for creek, plot within creek and distance from river within plot. Means with at least one letter in common are not significantly different ($\alpha = 0.05$) in soil TC content at that level of comparison (i.e. plots at the same creek were compared only to each other and transect lines at the same plot were compared only to each other).

	Yellow		**Locust**		**Thompson**	
Creek	2.78 **a**		1.51 **c**		1.86 **b**	
Plot(Creek)						
1	2.86		1.75 **a**		1.96	
2	2.71		1.27 **b**		1.77	
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	2.37	2.45	1.73	1.13	1.36	1.66
30 m	2.64	2.65	1.92	1.23	2.14	1.95
40 m	3.45	2.74	1.60	1.48	1.94	1.82
50 m	3.39	2.98	1.75	1.33	2.25	1.61

Table 5.4. Soil TN for the three study sites. Values represent least square means of TN (%) levels for creek, plot within creek and distance from river within plot. Means with at least one letter in common are not significantly different ($\alpha = 0.05$) in soil TN content at that level of comparison (i.e. plots at the same creek were compared only to each other and transect lines at the same plot were compared only to each other).

	Yellow		**Locust**		**Thompson**	
Creek	0.24 **a**		0.13 **c**		0.17 **b**	
Plot(Creek)						
1	0.24		0.15 **a**		0.17	
2	0.24		0.11 **b**		0.16	
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	0.22 **a**	0.22 **a**	0.15	0.10	0.12	0.18
30 m	0.22 **a**	0.23 **ab**	0.16	0.11	0.18	0.17
40 m	0.28 **b**	0.25 **ab**	0.14	0.13	0.17	0.17
50 m	0.28 **b**	0.27 **b**	0.16	0.11	0.20	0.14

Table 5.5. Soil C:N ratio for the three study sites. Values represent least square means of C:N ratios for creek, plot within creek and distance from river within plot. No significant differences were observed.

	Yellow		**Locust**		**Thompson**	
Creek	11.36		11.62		11.14	
Plot(Creek)						
1	11.60		11.51		11.38	
2	11.13		11.74		10.90	
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	10.85	11.19	11.74	11.49	11.42	9.60
30 m	11.83	11.25	11.58	11.56	11.41	11.59
40 m	12.25	10.95	11.42	11.44	11.42	10.94
50 m	11.87	11.09	11.22	12.31	11.29	11.15

Differences in TSP were detected at the creek and at the distance from river levels of comparison (Table 5.6). At the creek level, Locust Creek had significantly less TSP than the other two creeks ($F = 4.37$, $P = 0.02$); TSP levels at Thompson River and Yellow Creek were not different from each other. Comparisons at the plot level were not significantly different. Thompson River plot 2 was the only plot that showed differences in TSP with

distance from river (F = 5.01, P = 0.02). At this plot, TSP at transect lines can be ranked as follows: 40 m > 30 m >20 m > 50 m. The 50 m transect line had significantly lower TSP levels than the 30 and 40 m transect lines (P = 0.02 and P = 0.01 respectively), and the 20 m transect line had significantly lower TSP levels than the 40 m line (P = 0.02). (See Appendix E for contour maps depicting the levels of TSP across each plot.)

Table 5.6. Soil TSP for the three study sites. Values represent least square means of TSP (μg TA g^{-1} soil) levels for creek, plot within creek and distance from river within plot. Means with at least one letter in common are not significantly different (α = 0.05) in soil TSP content at that level of comparison (i.e. plots at the same creek were compared only to each other and transect lines at the same plot were compared only to each other).

	Yellow		**Locust**		**Thompson**	
Creek	10.29 **a**		8.67 **b**		10.53 **a**	
Plot(Creek)						
1	10.75		9.76		10.85	
2	9.83		7.57		10.20	
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	9.64	8.12	9.95	6.77	9.89	8.65 **ab**
30 m	9.73	8.97	9.49	7.82	10.55	11.41 **bc**
40 m	12.99	12.95	9.57	10.07	11.66	12.30 **c**
50 m	12.09	9.84	10.14	6.87	11.07	8.07 **a**

Vegetation Characteristics

Herbaceous vegetation

Percent cover of herbaceous vegetation was evaluated at four nested locations: i) creek, ii) plot within creek, iii) distance from river within plot, and iv) species. Significant differences were observed between Locust Creek and Thompson River (F = 6.46, P = 0.0018) (Table 5.7). Overall, differences between plots were not detected. Locust Creek plot 1 and the two Yellow Creek plots had the highest cover values, while the two

Thompson River plots had the lowest cover. No trends between cover and distance from river were observed; e.g. herbaceous coverage did not increase as distance from river increased but varied from line to line (Table 5.7).

Table 5.7. Average % cover of herbaceous species at creek, plot within creek and distance from river within plot. Means with at least one letter in common are not significantly different ($\alpha = 0.05$) in total cover at that level of comparison (i.e. plots at the same creek were compared only to each other and transect lines at the same plot were compared only to each other).

	Yellow		**Locust**		**Thompson**	
Creek	14.47 **ab**		27.60 **a**		20.59 **b**	
Plot(Creek)	**Total SP**	**Cover**	**Total SP**	**Cover**	**Total SP**	**Cover**
1	14	14.29	8	30.41	9	20.09
2	11	14.71	8	24.70	11	21.09
Distance(Plot)	**1**	**2**	**1**	**2**	**1**	**2**
20 m	20.00	15.71	32.20	24.18	21.67	19.15
30 m	15.00	15.42	24.50	31.94	17.11	19.94
40 m	15.75	10.63	27.50	21.27	25.86	24.92
50 m	10.00	17.14	39.38	20.59	17.11	20.89

Different herbaceous species and low-lying vines dominated each site; dominant species also varied for plots within site (Table 5.8). ANOVA revealed significant differences in species at the creek level and for all plots except the Yellow Creek plots. These differences can be attributed to primarily to *Laportea canadensis* (Canadian woodnettle) and *Parthenocissus quinquefolia* (Virginia creeper), which had the highest overall cover values and were significantly different than most other species. At Locust Creek, 8 species were recorded in each plot; and of these, Canadian woodnettle and Virginia creeper had cover levels that were significantly higher than the other species found in these plots (plot 1: F =

15.11, $P < 0.0001$; plot 2: $F = 21.14$, $P < 0.0001$) . At Thompson River plot 1, 9 species were identified; Canadian woodnettle again had significantly greater cover than other species ($F = 19.91$, $P < 0.0001$). Eleven species were recorded at Thompson River plot 2. Of these species, Virginia creeper dominated and was significantly more abundant than all others except Canadian woodnettle ($F = 6.13$, $P = 0.0016$). Canadian woodnettle had the second greatest cover level; its cover was significantly greater than most other species found at the plot. Yellow creek plots 1 and 2 had 14 and 11 species respectively. No significant differences in species cover were observed for these plots. See Appendix F for a composite species list and species wetland indicator status for the three study sites.

Table 5.8. Average cover values (%) of the five most common herbaceous species and low-lying vines at each plot and locale.

Plot	Scientific Name	Common Name	Average % Cover
Yellow Creek Plot 1	*Aster* sp.	aster	6.19
	Boehmeria cylindrica	false nettle	6.19
	Carex squarrosa	squarrose sedge	5.00
	Elymus virginicus	Virginia wildrye	2.81
	Pilea pumila	Canadian clearweed	2.31
Yellow Creek Plot 2	*Viola* sp.	violet	8.75
	Aster sp.	aster	5.31
	Boehmeria cylindrica	false nettle	4.69
	Chasmanthium latifolium	Indian woodoats	2.19
	Menispermum canadense	common moonseed	1.56
Locust Creek Plot 1	*Laportea canadensis*	Canadian woodnettle	59.38
	Parthenocissus quinquefolia	Virginia creeper	15.63
	Zizia aurea	golden zizia	6.06
	Elymus virginicus	Virginia wildrye	5.31
	Rudbeckia laciniata	cutleaf coneflower	3.44
Locust Creek Plot 2	*Laportea canadensis*	Canadian woodnettle	47.50
	Parthenocissus quinquefolia	Virginia creeper	4.63
	Viola sp.	violet	9.31
	Rudbeckia lacinata	cutleaf coneflower	3.44
	Zizia aurea	golden zizia	1.63
Thompson River Plot 1	*Laportea canadensis*	Canadian woodnettle	47.50
	Viola sp.	violet	9.31
	Gratiola neglecta	clammy hedgehyssop	7.19
	Elymus virginicus	Virginia wildrye	6.38
	Parthenocissus quinquefolia	Virginia creeper	4.63
Thompson River Plot 2	*Parthenocissus quinquefolia*	Virginia creeper	32.19
	Laportea canadensis	Canadian woodnettle	20.06
	Amphicarpaea bracteata	American hogpeanut	11.38
	Zizia aurea	golden zizia	10.38
	Elymus virginicus	Virginia wildrye	6.38

Herbaceous species richness, diversity and evenness were compared at the creek and plot within creek levels. Species richness was not significant at either of these levels of comparison. Species richness per quadrat varied from a low of 2 species/m^2 to a high of 7 species/ m^2. When averaged for the plot, Yellow Creek plots had the greatest species richness with 4.88 and 4.81 species m^{-2} respectively; Thompson River plots had intermediate

species richness with 4.75 and 4.5 species m^{-2} respectively and Locust Creek plots had the lowest species richness with 4.56 and 4.32 species m^{-2} respectively (Table 5.9). When averaged for the creek, again Yellow Creek had the greatest species richness followed by Thompson River and Locust Creek (Table 5.9).

Table 5.9. Herbaceous species richness, diversity and evenness for study sites. Values represent least square means of species richness, diversity and evenness for creek and plot within creek. Species richness = # spp m^{-2}; diversity and evenness were calculated using herbaceous cover measurements. Means with at least one letter in common are not significantly different ($\alpha = 0.05$) in total cover at that level of comparison (i.e. plots at the same creek were compared only to each other)

	Yellow		**Locust**		**Thompson**	
Richness: Creek	4.84		4.44		4.63	
Plot(Creek)	4.88	4.81	4.56	4.31	4.75	4.50
Diversity: Creek	1.56		1.37		1.34	
Plot(Creek)	1.73	1.39	1.45	1.29	1.26	1.42
Evenness: Creek	0.86 **a**		0.74 **b**		0.71 **b**	
Plot(Creek)	0.90	0.82	0.81 **a**	0.68 **b**	0.68	0.75

Diversity values did not differ at either level of comparison. Species diversity of plots ranged from 1.73-1.26 (plot diversity rankings from highest to lowest: Yellow 1, Locust 1, Thompson 2, Yellow 2, Locust 2, Thompson 1). On the creek level, Yellow Creek was more diverse than Locust Creek and Thompson River which had quite similar diversity measures (Table 5.9).

Evenness comparisons were significant at both creek and plot within creek levels. Similar to the pattern observed with diversity measures, Yellow Creek had the most even distribution of herbaceous species, while the distribution of herbaceous species at Locust Creek and Thompson River were less even than Yellow Creek but quite similar to each other

(F = 76.5, P < 0.01) (Table 5.9). Plot comparisons at each site reveal significant differences between the Locust Creek plots (P = 0.03); Yellow Creek plots did not differ from each other, nor did Thompson River plots.

Shrub layer

The shrub layer at Yellow Creek was significantly different than the shrub layers of the other two creeks (F = 4.51, P =0.02), however no differences between plots within creeks were observed, nor were any differences in number of woody stems with distance from river detected. The shrub layer at Yellow Creek was the densest of the three creeks with 3-8 species per line and 1-50 stems per species. On the other hand, the shrub layer at Locust Creek was nearly absent; half of the belt transects had no shrubs and the remainder had only 1-2 species per line and 1-3 stems per species. The shrub layer at Thompson River was intermediate with 0-4 species per line and 1-16 stems per species. Species differences were observed (F = 2.53, P = 0.01). *Toxicodendron radicans* (poison ivy) had the highest number of stems and was significantly different from most other species. Poison ivy was particularly dense at Yellow Creek and contributed to the high number of woody stems observed at those plots.

Overstory

Crown class designations (i.e. dominant, codominant, intermediate and suppressed) were used to determine dominant tree species for each site and plot. Overstory dominants differed among the sites and between plots within a site. *Acer saccharinum* (silver maple) and *Platanus occidentalis* (American sycamore) were common components of plot overstories, occurring on half of the plots. Five other species: *Celtis occidentalis* (hackberry), *Fraxinus*

pennsylvanica (green ash), *Juglans nigra* (black walnut), *Populus deltoides* (eastern cottonwood), and *Quercus palustris* (pin oak) were found on 2 of the 6 total plots and another 5 species were among the dominant species at one of the plots (Table 5.10).

Table 5.10. Species dominance at the three study sites as determined by crown class determinations.

Plot	Dominant Species	Common Name
Yellow Creek Plot 1	*Acer saccharinum*	silver maple
	Quercus palustris	pin oak
Yellow Creek Plot 2	*Quercus macrocarpa*	bur oak
	Quercus palustris	pin oak
	Celtis occidentalis	hackberry
	Fraxinus pennsylvanica	green ash
	Carya laciniosa	shellback hickory
Locust Creek Plot 1	*Platanus occidentalis*	American sycamore
	Populus deltoides	eastern cottonwood
	Acer saccharinum	silver maple
Locust Creek Plot 2	*Populus deltoides*	eastern cottonwood
	Platanus occidentalis	American sycamore
	Acer saccharinum	silver maple
	Acer negundo	box elder
Thompson River Plot 1	*Celtis occidentalis*	hackberry
	Juglans nigra	black walnut
	Fraxinus pennsylvanica	green ash
Thompson River Plot 2	*Platanus occidentalis*	American sycamore
	Juglans nigra	black walnut
	Morus rubra	red mulberry
	Ulmus americana	American elm

Overstory vegetation was compared at the: i) creek, ii) plot within creek and iii) species levels using the following parameters: i) basal area (BA), ii) relative dominance, iii) relative density, and iv) importance values. No significant differences in these parameters were detected at the creek or plot within creek levels of comparison. However, a

disproportionate dominance by a single species was observed in most plots (Table 5.11). Yellow Creek plot 1 was dominated by silver maple which had over twice as much BA as the next ranking species; while Yellow Creek plot 2 was dominated by *Quercus macrocarpa* (bur oak) which had 3-5 times as much BA as most other overstory species at this plot. Silver maple dominated both plots at Locust Creek. Dominance by silver maple was obvious in Locust Creek plot 1; in this plot, silver maple had 4-30 times the BA of other overstory species. However dominance at Locust Creek plot 2 was shared by silver maple, *Acer negundo* (box elder), and American sycamore. The dominate species at Thompson River plot 1 was hackberry with 4 times the BA of the next ranking species; while at Thompson River plot 2, dominance was shared between American sycamore and *Ulmus americana* (American elm). The canopy at Thompson River was more open with more frequent canopy gaps than the canopy at Locust Creek. Also, Thompson River plot 2 had a high number of small diameter American elm. As is generally the case, the species that was dominant in terms of BA was not necessarily the most frequently encountered species in the plot. For example, at Yellow Creek plot 2, bur oak had the greatest BA but *Carya laciniosa* (shellbark hickory) had the greatest relative dominance.

Table 5.11. Summary of overstory characteristics by plot. Data represents average basal area, relative dominance, relative density and importance value for each species at each plot. Importance values were calculated by summation of relative dominance and relative density measures; the maximum importance value is therefore 200.

Creek	Species	BA (m^2 ha^{-1})	Relative Dominance	Relative Density	Importance Value
Yellow Plot 1	*Acer saccharinum*	15.96	54.16	39.53	93.69
	Quercus palustris	7.13	24.21	6.98	31.19
	Fraxinus pennsylvanica	3.19	10.84	37.21	48.05
	Ulmus rubra	1.50	5.10	6.98	12.08
	Carya laciniosa	1.15	3.90	4.65	8.55
	Morus rubra	0.42	1.42	2.33	3.75
	Carya sp	0.11	0.38	2.33	2.71
	Total BA	**18.37**			
Yellow Plot 2	*Quercus macrocarpa*	15.55	46.55	11.54	58.09
	Carya laciniosa	5.73	17.16	34.61	51.77
	Fraxinus pennsylvanica	3.68	11.01	19.23	30.24
	Celtis occidentalis	3.62	10.85	23.08	33.93
	Quercus palustris	3.35	10.04	3.85	13.89
	Ulmus rubra	1.03	3.07	3.85	6.92
	Morus rubra	0.43	1.30	3.85	5.15
	Total BA	**22.96**			
Locust Plot 1	*Acer saccharinum*	33.20	62.10	29.79	91.89
	Platanus occidentalis	7.40	13.84	2.13	15.97
	Populus deltoides	5.71	10.68	2.13	12.81
	Ulmus americana	3.04	5.69	25.53	31.22
	Celtis occidentalis	1.93	3.61	21.28	24.89
	Acer negundo	1.74	3.26	8.51	11.77
	Ulmus rubra	0.28	0.53	2.13	2.66
	Fraxinus pennsylvanica	0.09	0.17	4.26	4.43
	Carya laciniosa	0.06	0.11	4.26	4.37
	Total BA	**34.44**			
Locust Plot 2	*Acer saccharinum*	14.51	32.82	20.00	52.82
	Acer negundo	11.91	26.92	26.67	53.59
	Platanus occidentalis	8.77	19.82	6.67	26.49
	Populus deltoides	5.54	12.53	3.33	15.86
	Celtis occidentalis	2.35	5.31	23.33	28.64
	Ulmus americana	0.40	0.89	10.00	10.89
	Morus rubra	0.39	0.89	3.33	4.22
	Carya laciniosa	0.36	0.81	6.67	7.48
	Total BA	**22.96**			

Table 5.11 Continued Creek	Species	BA (m^2 ha^{-1})	Relative Dominance	Relative Density	Importance Value
Thompson Plot 1	*Celtis occidentalis*	18.66	64.81	51.61	116.42
	Fraxinus pennsylvanica	4.66	16.17	22.58	38.75
	Juglans nigra	2.28	7.91	3.23	11.14
	Quercus macrocarpa	1.59	5.54	9.68	15.22
	Carya laciniosa	1.28	4.43	9.68	14.11
	Ulmus americana	0.33	1.14	3.23	4.37
	Total BA	**18.37**			
Thompson Plot 2	*Platanus occidentalis*	12.00	42.87	19.95	62.82
	Ulmus americana	10.06	35.97	62.71	98.68
	Juglans nigra	4.06	14.50	11.02	25.52
	Celtis occidentalis	0.76	2.70	5.08	7.78
	Morus rubra	0.47	1.66	0.85	2.51
	Carya laciniosa	0.35	1.25	1.69	2.94
	Acer negundo	0.24	0.87	0.85	1.72
	Ulmus rubra	0.05	0.18	0.85	1.03
	Total BA	**22.96**			

There were no differences among sites in species richness, diversity and evenness. However, Locust Creek plots were the richest, with 8-9 species per plot; Yellow Creek had 7 species on each plot and Thompson River had 6-8 species per plot (Table 5.12). Regardless of whether diversity and evenness were calculated using relative dominance or relative density, the results were similar (Table 5.12). When relative dominance was used to calculated diversity and evenness, Locust 2 had the highest diversity and Yellow 2 was the most even. When relative density was used to calculate these measures, Locust 2 had the highest diversity and evenness. Thompson River plots generally had the lowest diversity and evenness.

Table 5.12. Overstory richness, diversity and evenness by plot. Richness represents total number of species observed in each 30 m^2 plot. Diversity and evenness were calculated in two ways: 1) using relative dominance of each species and ii) using relative density of each species. No significant differences were observed for these parameters regardless of method of calculation.

		Richness	Relative Dominance		Relative Density	
Creek	**Plot**	**(spp plot^{-1})**	**H'**	**J'**	**H'**	**J'**
Yellow	1	7	1.28	0.66	1.42	0.73
Yellow	2	7	1.54	0.79	1.65	0.85
Locust	1	9	1.25	0.57	1.76	0.80
Locust	2	8	1.58	0.76	1.83	0.88
Thompson	1	6	1.13	0.63	1.35	0.75
Thompson	2	8	1.28	0.62	1.20	0.58

Correlation and Regression Analysis

Correlation analysis was used to determine: i) the association among soil chemical variables, ii) the degree of correlation between herbaceous and shrub layer characteristics, iii) if correlations existed between site microtopographical characteristics and soil chemical variables or understory vegetation characteristics, iv) if correlations existed between and soil chemical variables and understory vegetation characteristics. Regression analysis was used to determine: i) if any of the site microtopographical characteristics could be used to explain either variation in soil chemical variables or variation in understory vegetation characteristics and ii) if any of the soil chemical variables could be used to explain the variation observed in understory vegetation characteristics.

Total organic C and TN were positively correlated ($r = 0.96$, $P < 0.0001$) as were TOC and C:N ratio ($r = 0.27$, $P = 0.01$). Soil TSP content was positively correlated with TOC ($r = 0.46$, $P < 0.0001$) and TN ($r = 0.42$, $P < 0.0001$). No other correlations were detected between soil chemical parameters.

Herbaceous cover was negatively correlated with number of woody stems ($r = -0.74$, $P < 0.001$), shrub species richness ($r = -0.76$, $P < 0.0001$) and shrub diversity ($r = -0.61$, $P = 0.0014$). However, herbaceous evenness was positively correlated with number of woody stems ($r = 0.47$, $P = 0.02$) and shrub species richness ($r = 0.44$, $P = 0.03$). As anticipated, richness and diversity of the herbaceous layer were correlated ($r = 0.60$; $P = 0.0018$) and diversity and evenness of the herbaceous layer were correlated ($r = 0.78$; $P = <0.0001$). Similar relationships were found for the shrub layer. The number of woody stems was positively correlated with shrub species richness ($r = 0.83$; $P < 0.0001$), and shrub diversity ($r = 0.62$; $P < 0.002$). Shrub species richness was correlated with shrub diversity ($r = 0.91$; $P < 0.0001$) and shrub evenness ($r = 0.67$; $P < 0.001$). Shrub diversity and evenness were also correlated ($r = 0.86$; $P < 0.0001$).

Microtopographical characteristics were not correlated with each other; i.e., there were no significant correlations among planform, FA, slope or elevation. Slope was positively correlated with NO_3-N ($r = 0.28$, $P < 0.01$) and negatively correlated with NH_4-N ($r = -0.24$, $P = 0.03$), TN ($r = -0.61$, $P < 0.0001$) and TOC ($r = -0.60$, $P < 0.0001$). Elevation was negatively correlated with NO_3-N ($r = -0.24$, $P = 0.02$), TN ($r = -0.33$, $P < 0.002$), and TOC ($r = -0.38$, $P < 0.001$). Herbaceous percent cover was positively correlated with FA ($r = 0.35$, $P < 0.001$), slope ($r = 0.57$, $P < 0.0001$) and elevation ($r = 0.24$, $P = 0.03$). Meanwhile, number of woody stems was negatively correlated with slope ($r = -0.61$, $P < 0.0001$) and elevation ($r = -0.26$, $P < 0.02$). Herbaceous species richness was not correlated with any of the microtopographical parameters. Calculations of herbaceous diversity and evenness and shrub richness, diversity and evenness were made on a plot (i.e. not quadrat) basis, and therefore comparisons with microtopographical parameters were not possible.

Herbaceous cover was negatively correlated with both TOC and TN (Table 5.13). Conversely, the number of woody stems was positively correlated with these same variables (Table 5.13). No correlations were observed between the soil chemical variables of NO_3-N, NH_4-N and TSP and i) herbaceous cover, ii) number of woody stems, iii) herbaceous species richness, diversity and evenness or iv) shrub species richness, diversity or evenness. However, both TOC and TN were positively correlated with herbaceous diversity and evenness as well as shrub richness and diversity (Table 5.13); no correlations between TOC or TN with herbaceous species richness or shrub evenness were observed.

Table 5.13. Pearson correlation coefficients for observed significant correlations between soil chemical variables and understory vegetation characteristics. The first number for each comparison represents the Pearson correlation coefficients (r) and the second number represents the P value for the comparison.

	Herb Cover	**Herb Diversity**	**Herb Evenness**	**Woody stems**	**Shrub Richness**	**Shrub Diversity**
TN	-0.57 < 0.0001	0.45 0.03	0.56 < 0.01	0.64 < 0.0001	0.81 < 0.0001	0.68 < 0.001
TC	-0.51 < 0.0001	0.44 0.03	0.55 < 0.01	0.60 < 0.0001	0.78 < 0.0001	0.68 < 0.001

Regression analyses showed that slope and elevation could explain some of the variation observed in soil chemical parameters. Specifically, these two microtopographical parameters explained 15% of the variation observed in NO_3-N ($P < 0.001$) (Figure 5.10), and 41% of the variation observed in both TOC ($P < 0.0001$) (Figure 5.11) and TN ($P < 0.0001$) (Figure 5.12). Soil NO_3-N levels increased with increases in slope and with decreases in elevation. On the other hand, both TOC and TN exhibited inverse

relationships with slope and elevation. Models that would adequately explain variation in NH_4N, C:N ration or TSP were not observed.

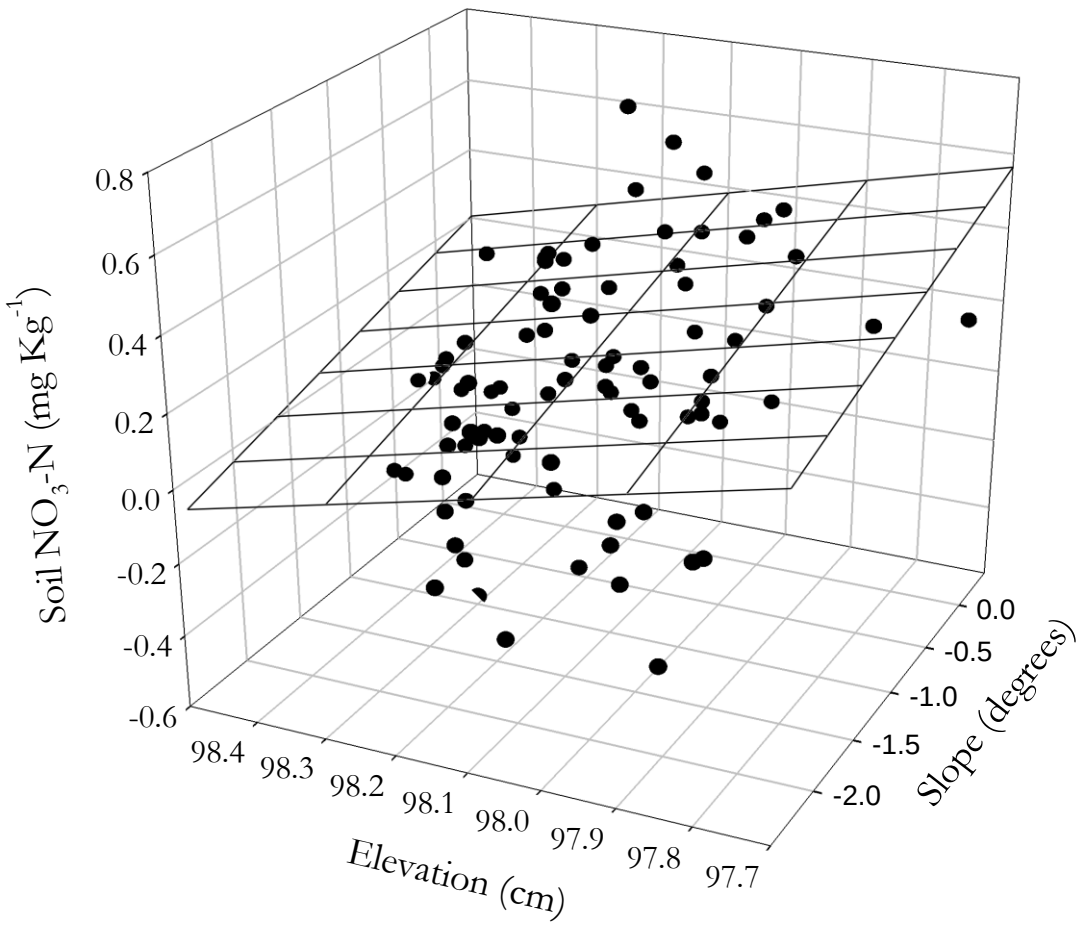

Figure 5.10. Relationship between NO_3-N and slope and elevation. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: NO_3-N = 44.49 + 0.10 (slope) - 0.45 (elevation) which shows that nitrate increases with increases in slope and with decreases in elevation. Elevation is measured in cm and is calculated from a reference height of 100 cm. Slope is measured in degrees; values are log transformation of actual slopes.

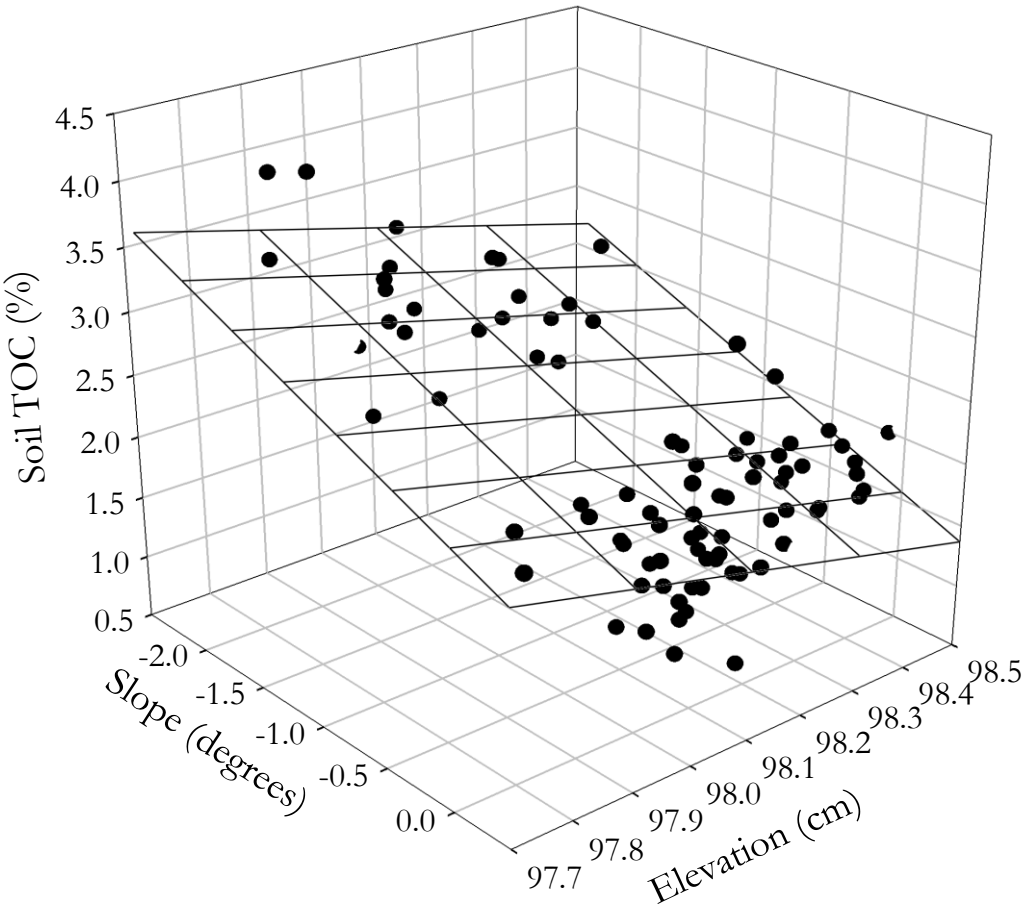

Figure 5.11. Relationship between TOC and slope and elevation. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: TOC = 117.79 - 0.48 (slope) - 1.18 (elevation) which shows that TOC exhibits inverse relationships with slope and elevation. Elevation is measured in cm and is calculated from a reference height of 100 cm. Slope is measured in degrees; values are log transformation of actual slopes.

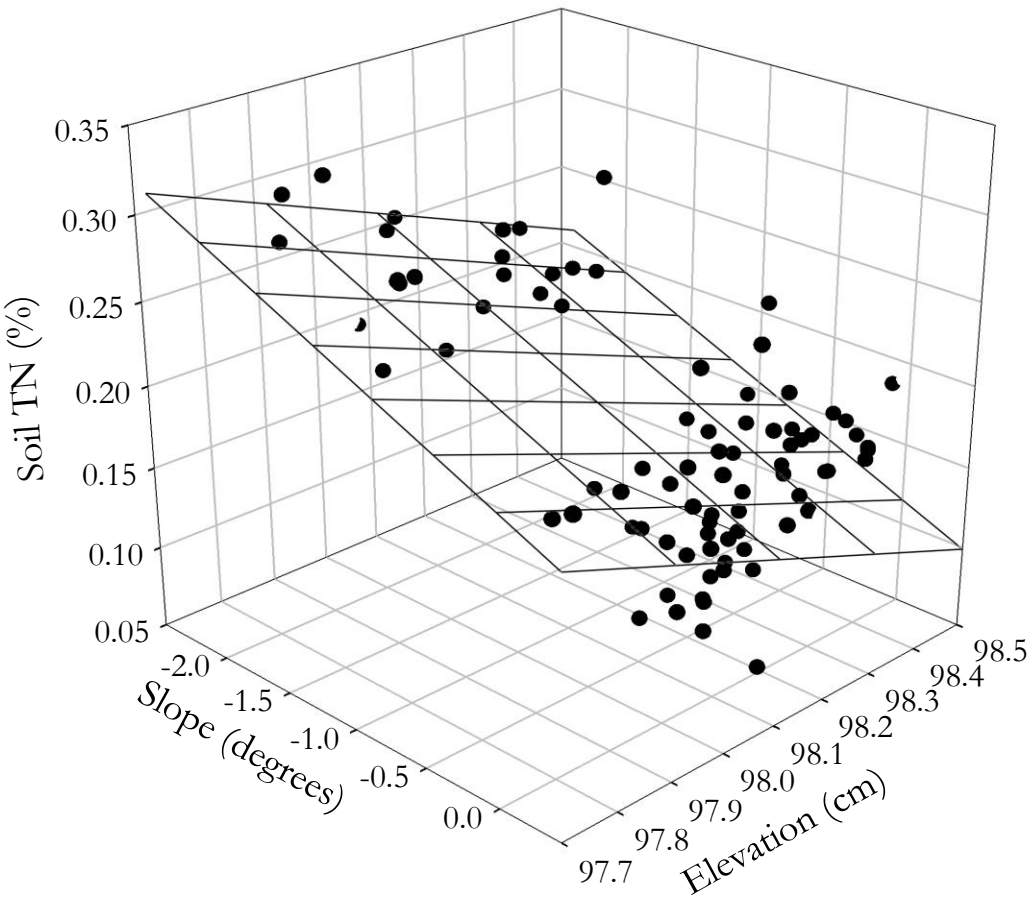

Figure 5.12. Relationship between TN and slope and elevation. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: TN = 12.92 - 0.04 (slope) -0.13 (elevation) which shows that TN exhibits inverse relationships with slope and elevation. Elevation is measured in cm and is calculated from a reference height of 100 cm. Slope is measured in degrees; values are log transformation of actual slopes.

Variation in herbaceous cover and number of woody stems could also be explained by microtopographical variables. In the case of the understory vegetation, however, FA and slope were the most important microtopographical factors. Flow accumulation and slope explained approximately 48% of the variation observed in herbaceous cover ($P < 0.0001$)

(Figure 5.13) and approximately 40% of the variation observed in number of woody stems ($P < 0.0001$) (Figure 5.14). Herbaceous cover exhibited a direct relationship with FA and slope, while number of wood stems exhibited an inverse relationship with these parameters.

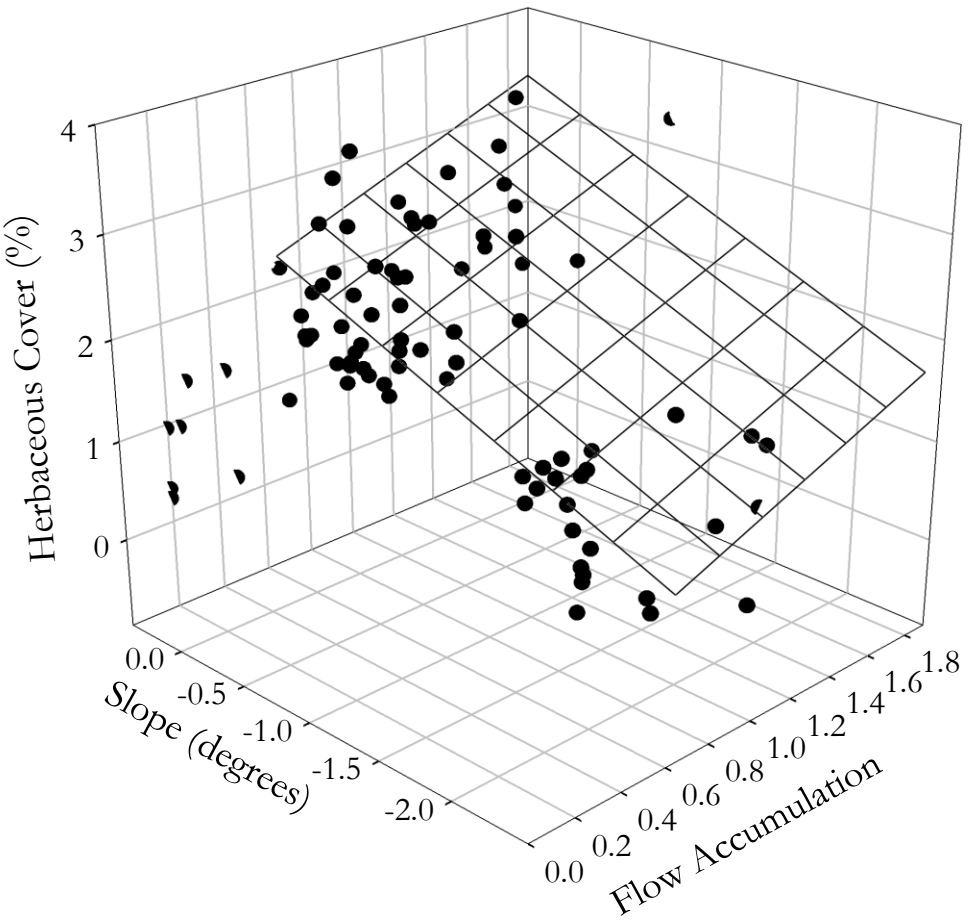

Figure 5.13. Relationship between herbaceous cover, flow accumulation and slope. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: herbaceous cover = 1.44 + 0.86 (FA) + 0.60 (slope) which shows that herbaceous cover exhibits a direct relationship with FA and slope. Note: herbaceous cover values were transformed (arcsine square root); transformed values are presented. Flow accumulation represents the number of quadrats contributing water to a particular quadrat. Slope is measured in degrees; values are log transformation of actual slopes.

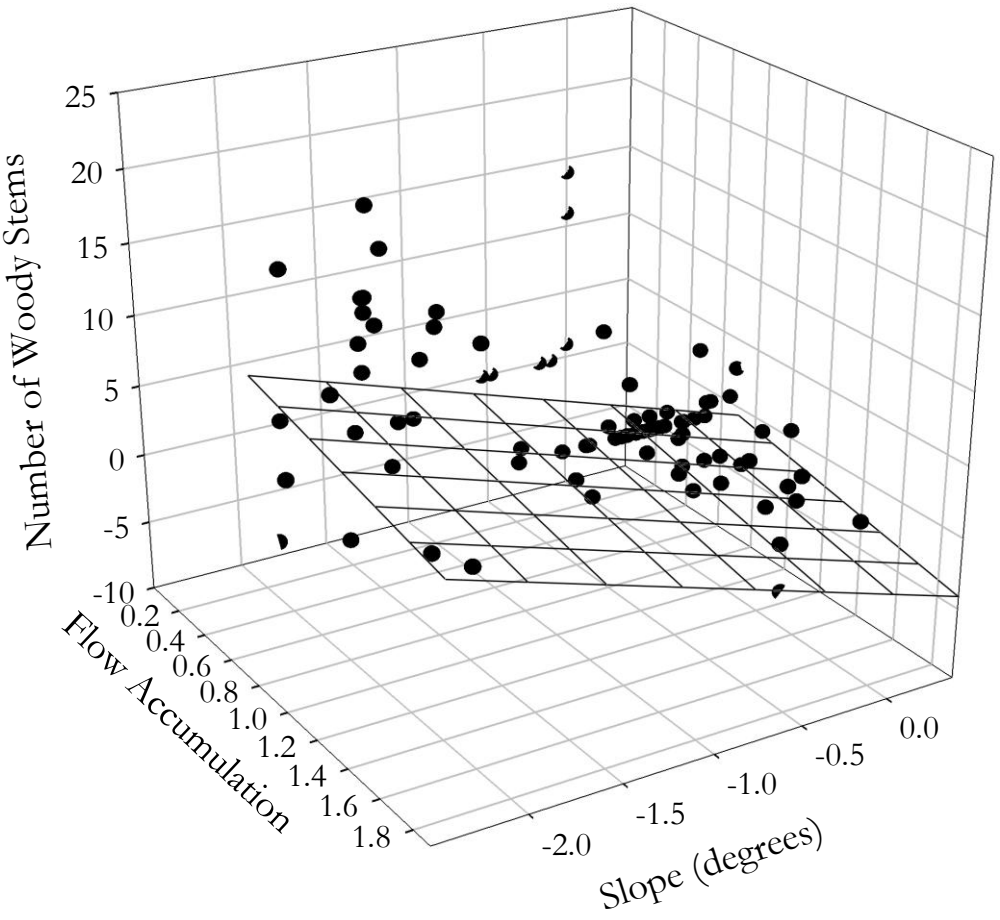

Figure 5.14. Relationship between number of woody stems, flow accumulation and slope. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: number of woody stems = 3.07 - 2.95 (FA) -3.97 (slope) which shows that number of woody stems exhibits an inverse relationship with FA and slope. Flow accumulation represents the number of quadrats contributing water to a particular quadrat. Slope is measured in degrees; values are log transformation of actual slopes.

Variation in understory vegetation could also be explained by soil chemistry. Observed variation in herbaceous cover and herbaceous evenness as well as in number of woody stems, shrub richness and shrub diversity was best explained by TN content of the soil. TN explained approximately 33% of the variation observed in herbaceous cover ($P <$

0.0001) (Figure 5.15) and 31% of the variation observed in herbaceous evenness ($P < 0.01$) (Figure 5.16). Approximately 41% of the variation observed in number of woody stems ($P < 0.0001$) (Figure 5.17), 66% of the variation observed in shrub richness ($P < 0.0001$) (Figure 5.18) and 46% of the variation observed in shrub diversity ($P < 0.001$) (Figure 5.19) can be explained by TN content of the soil.

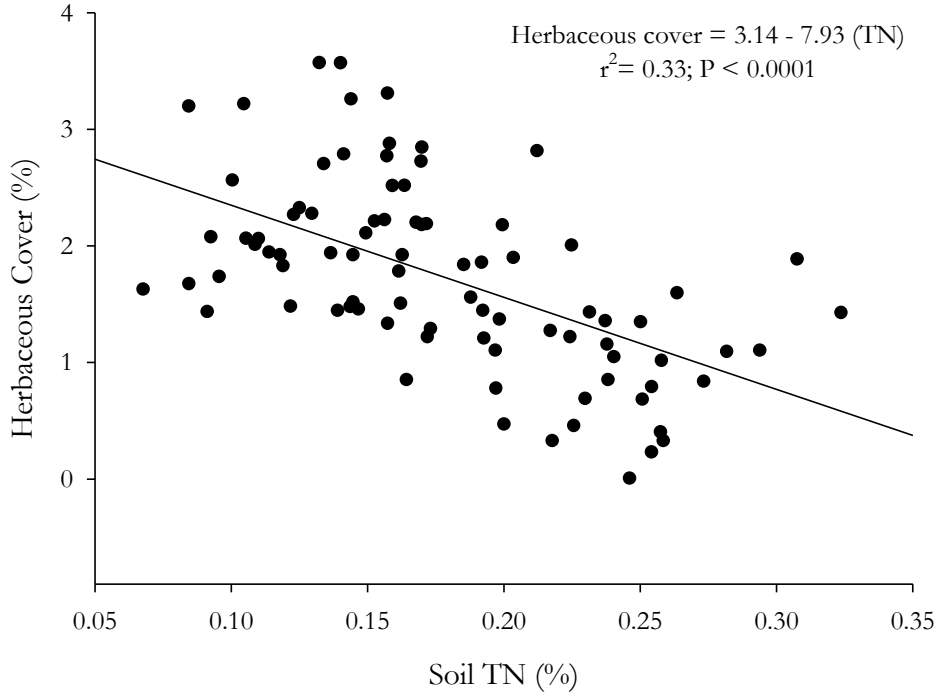

Figure 5.15. Relationship between herbaceous cover (arcsine square root) and soil TN.

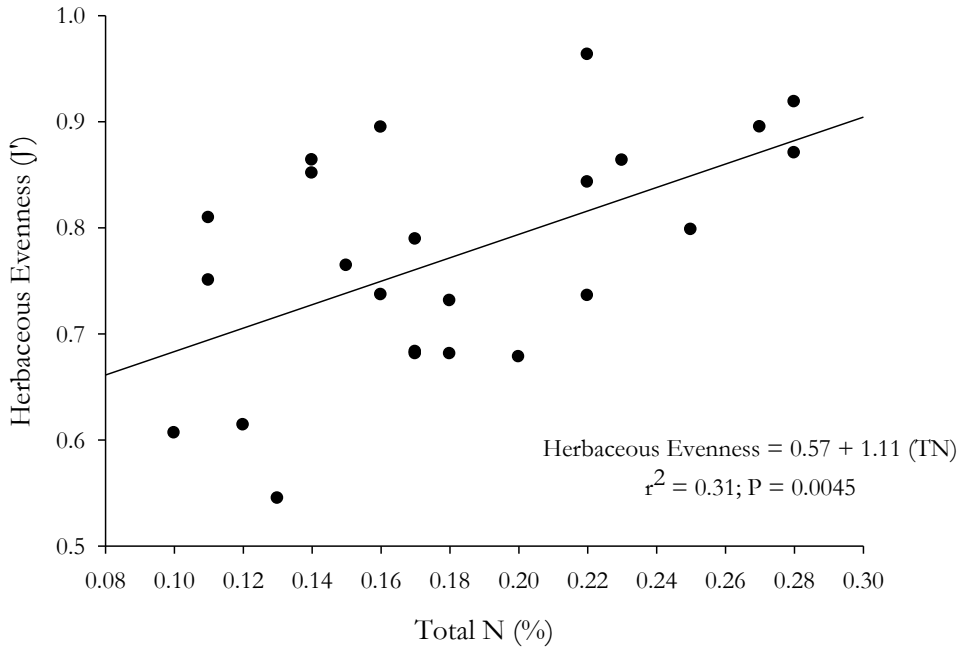

Figure 5.16. Relationship between herbaceous evenness and soil TN.

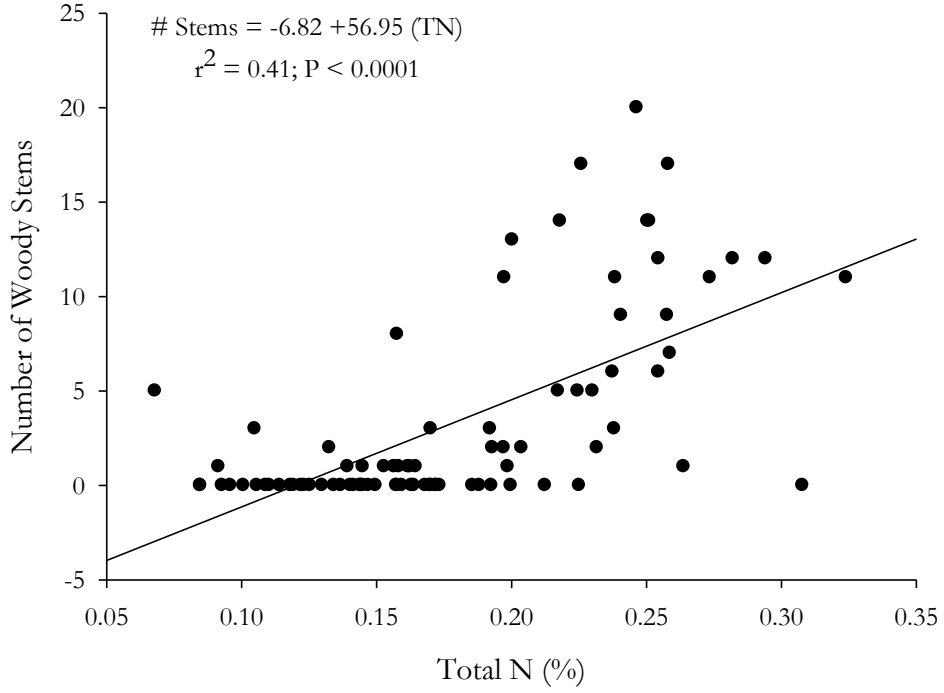

Figure 5.17. Relationship between number of woody stems and soil TN.

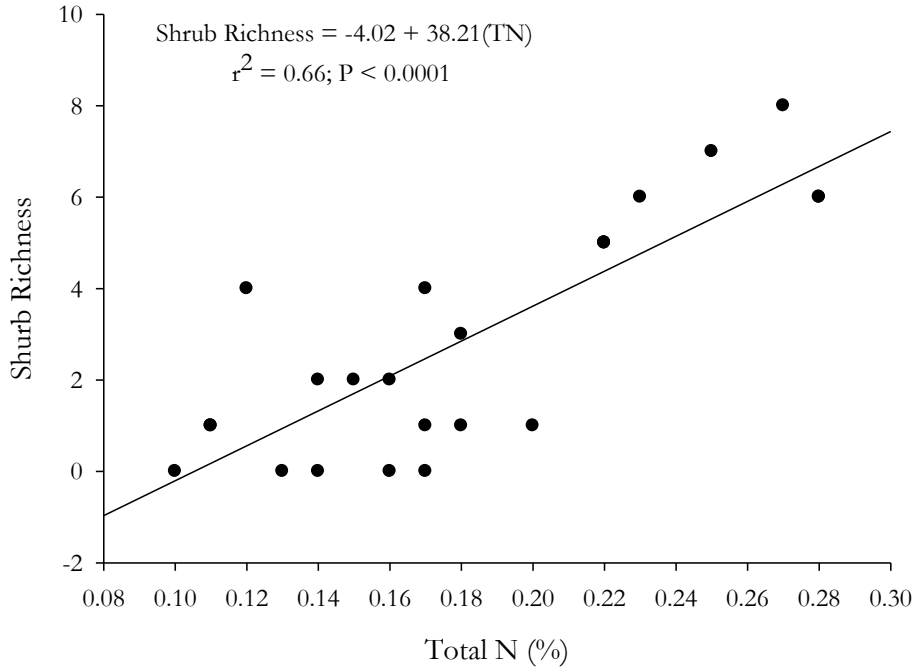

Figure 5.18. Relationship between shrub richness and soil TN.

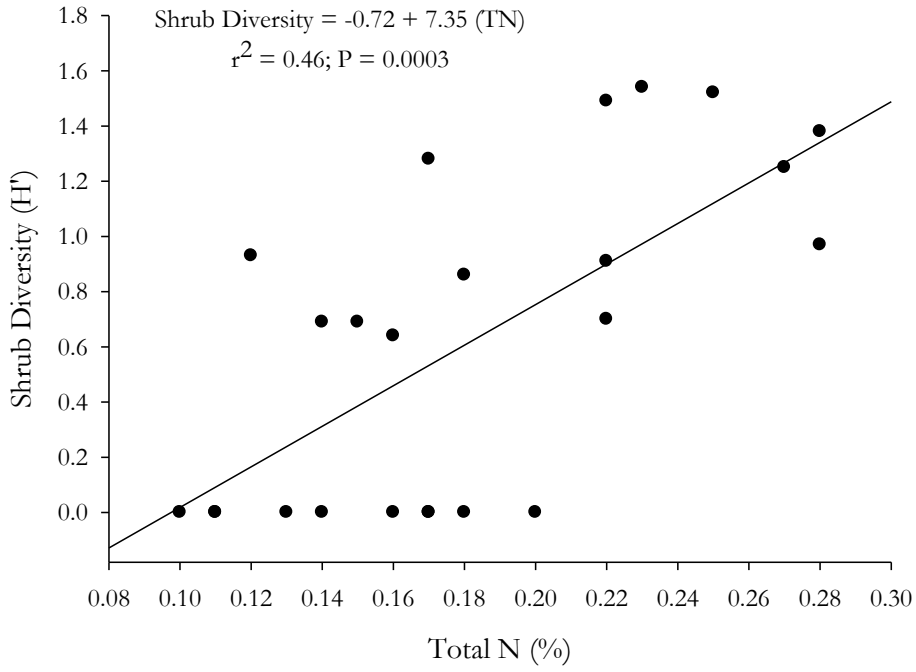

Figure 5.19. Relationship between shrub diversity and soil TN.

Herbaceous species diversity was best explained by a multiple linear regression model that combined TN and the C:N ratio. These two soil parameters, when combined, explained 27% of the variation observed in herbaceous diversity (P = 0.01) (Figure 5.20). Herbaceous species diversity exhibits a direct relationship with TN and C:N ratio. No regression models using soil chemical parameters adequately explained the variation observed in herbaceous species richness or shrub evenness.

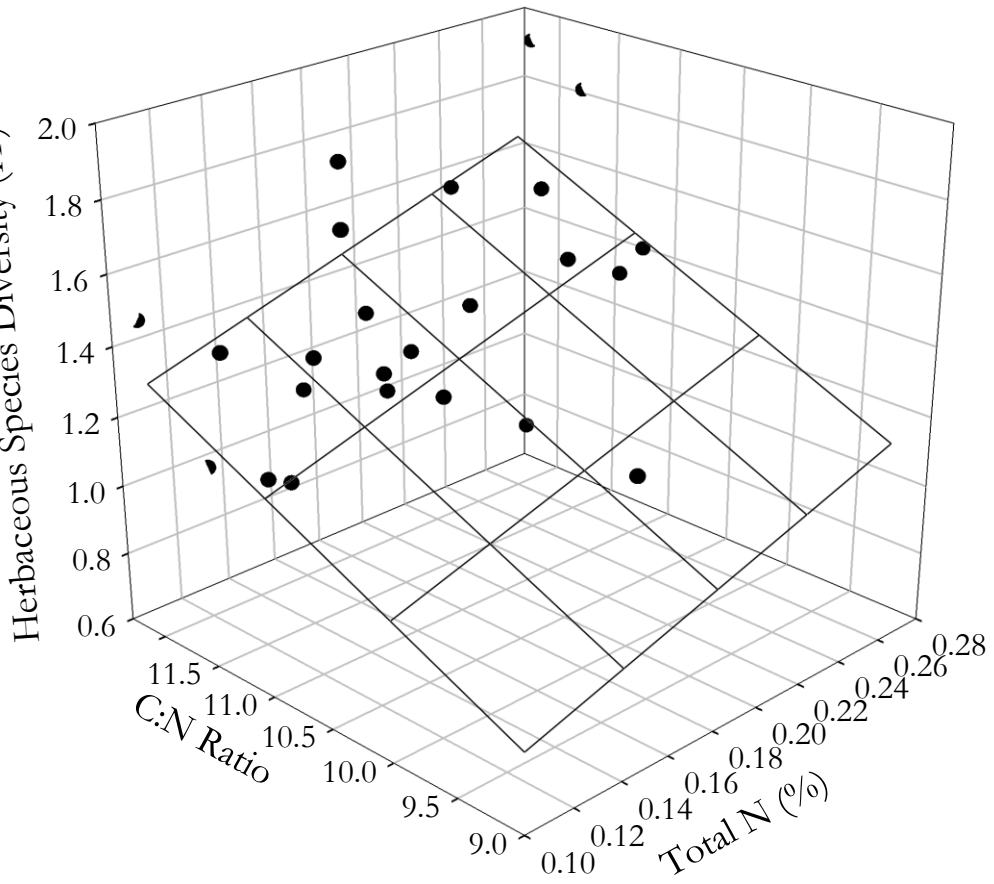

Figure 5.20. Relationship between herbaceous species diversity and soil TN and C:N ratio. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: herbaceous species diversity = -0.92 + 2.19 (TN)+ 0.17 (C:N ratio) which shows herbaceous species diversity exhibits a direct relationship with TN and C:N ratio.

Regression analyses revealed that shrub richness and shrub evenness together (i.e. multiple linear regression) could explain approximately 64% of the variation observed in herbaceous cover ($P < 0.0001$) (Figure 5.21). Herbaceous cover was inversely related with shrub richness and directly related with shrub evenness. Meanwhile, number of woody stems explained approximately 22% of the variation observed in herbaceous evenness ($P = 0.02$) (Figure 5.22). There was a direct relationship between herbaceous evenness and number of woody stems.

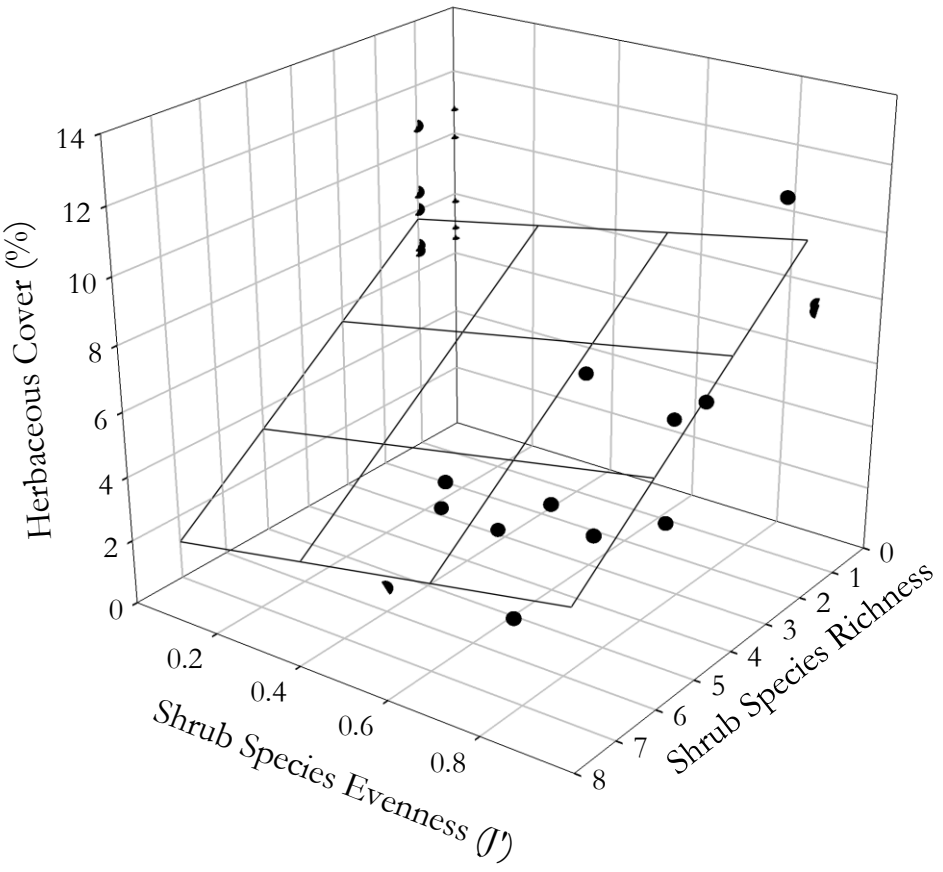

Figure 5.21: Relationship between herbaceous cover and shrub richness and shrub evenness. Scatter plot represents actual data points. Planar surface represents predicted values from the regression equation: herbaceous cover = 8.80 – 1.09 (shrub richness) + 2.59 (shrub evenness) which shows that herbaceous cover exhibits an indirect relationship with shrub richness and a direct relationship with shrub evenness. Note: herbaceous cover values were transformed (arcsine square root); transformed values are presented.

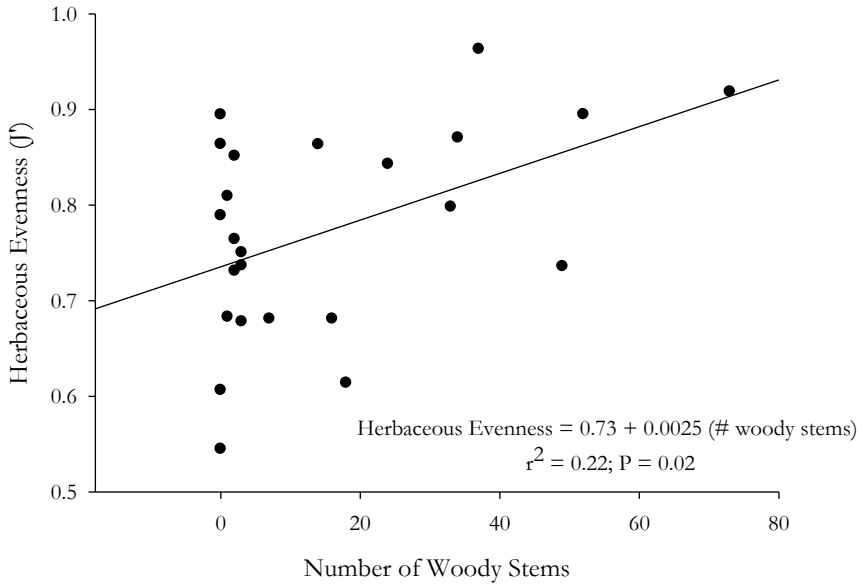

Figure 5.22: Relationship between herbaceous evenness and number of woody stems.

Multivariate Analysis

Detrended Correspondence Analysis (DCA) was used to examine the patterns in understory species (i.e. herbaceous species and woody stems analyzed separately), soil chemicals and microtopography across the sites/ plots. Results from these analyses were uninformative, failing to clarify patterns with one exception: herbaceous species cover. Therefore, only the results for the herbaceous species cover analysis will be addressed. Graphical representation of the herbaceous species cover DCA revealed a separation of Yellow Creek plots from a tight grouping of Locust Creek and Thompson River Plots (Figure 5.23). Three axes were included in the analysis; much of the variation (63%) was described by the first axis; the 2nd and 3rd axes contributed little additional information (Table 5.14). *Boehmeria cylindrica* and *Carex squarrosa* showed high positive correlation with the first axis (r = 0.60 and r = 0.58 respectively), while *Parthenocissus quinquefolia*, *Laportea canadensis*,

and *Zizia aurea* showed high negative correlation with the first axis (r = -0.65, r = -0.47 and r = -0.47 respectively) (Table 5.15). Total variance ("inertia") in the species data was 3.14.

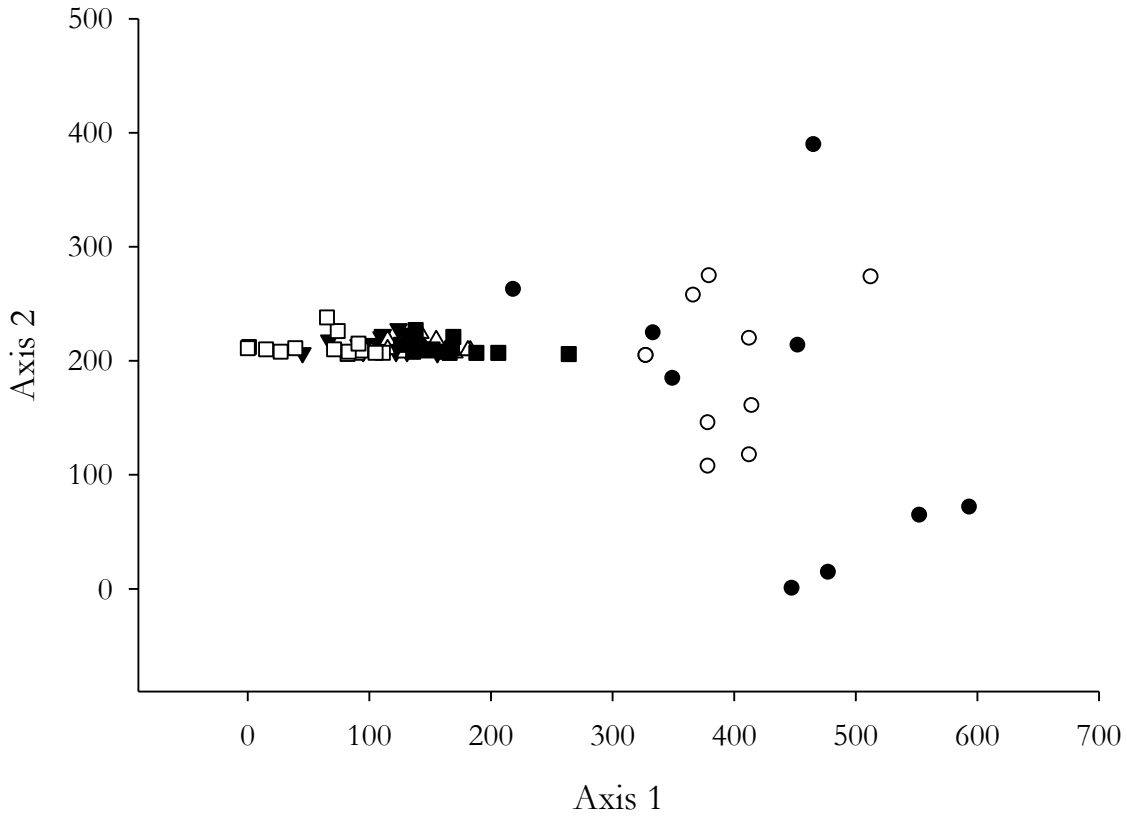

Figure 5.23. DCA analysis of herbaceous species cover. Circles designate Yellow Creek quadrats, triangles designate Locust Creek quadrats and squares designate Thompson River quadrats; open symbols designate quadrats at plot 1 for the respective streams and closed symbols designate quadrats at plot 2 for the respective streams.

Table 5.14. Eigenvalues and proportion of variance represented for each axes for the DCA analysis of herbaceous species cover.

	Axis 1	**Axis 2**	**Axis 3**
Eigenvalue	0.84	0.31	0.19
Proportion of variance represented by axis	0.63	0.01	0.02

Table 5.15. Correlations of herbaceous species with DCA axes. For this analysis, rare species were down weighted relative to their frequency; weights applied are listed.

	Weights Applied	**Axis 1 r**	**Axis 2 r**	**Axis 3 r**
Aster spp.	0.609	0.50	0.29	0.28
Amphicarpaea bracteata	0.590	-0.31	0.07	-0.03
Boehmeria cylindrica	0.582	0.60	-0.42	0.07
Carex squarrosa	0.857	0.58	-0.65	0.33
Elymus virginicus	1.000	-0.26	0.20	-0.08
Gratiola neglecta	0.901	-0.10	0.06	0.53
Impatiens capensis	0.578	-0.06	0.03	-0.26
Laportea canadensis	1.000	-0.47	0.20	0.12
Parthenocissus quinquefolia	1.000	-0.65	0.14	-0.25
Rudbeckia laciniata	0.611	-0.17	0.04	-0.32
Viola spp.	1.000	0.30	-0.05	-0.40
Zizia aurea	1.000	-0.47	0.13	-0.25

Canonical Correspondence Analysis (CCA) was used to examine the relationships between understory species and soil chemicals and between understory species and site microtopography. Herbaceous species and woody stems were analyzed separately. CCA was also used to explore the relationship between soil chemical variables and site microtopography. As with the DCA analyses, graphs resultant from the CCA analyses generally failed to clarify patterns in the data. The exception was again with the herbaceous species cover data, where patterns were observed for both soil chemistry and site microtopography. As before, only these analyses will be discussed.

CCA analysis of herbaceous species cover with soil chemical variables resulted in the separation of the Yellow Creek quadrats/plots from the other two sites (Figure 5.24). The Yellow Creek quadrats/plots were concentrated along the negative portion of CCA Axis 1, while the Locust Creek and Thompson River quadrats/plots were concentrated towards the positive end of this Axis. Both groupings of quadrats/plots extended over the range of Axis

2. The first Axis explained approximately 11% of the quadrat/plot variation; the second and third axes contribute inconsequentially for a 15.9% cumulative % of variation explained (Table 5.16). Total variance ("inertia") in the species data for this data was 3.37.

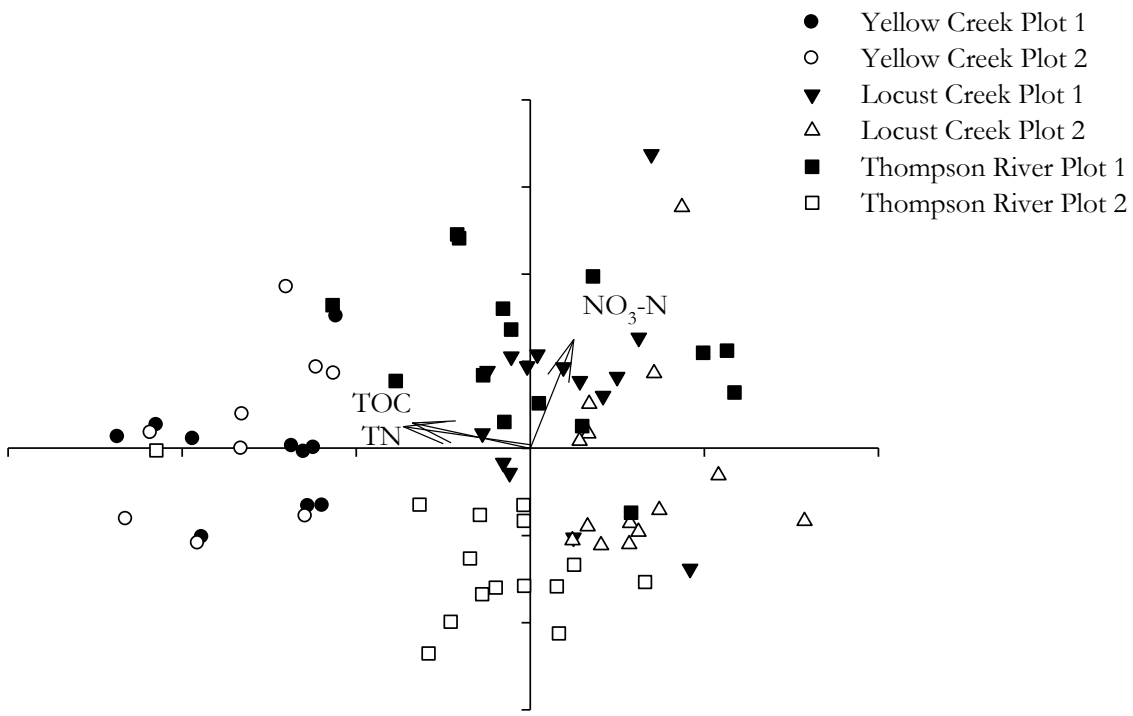

Figure 5.24. CCA analysis of herbaceous species cover with soil chemical variables. Plot axes are centered at the origin. Vectors for TN, TOC and NO_3-N represent the contribution of these soil chemicals to plot separation; the angle and length of the line indicate the direction and strength of the relationship.

Table 5.16. CCA analysis of herbaceous species cover with soil chemical variables; * = Correlation between sample scores for an axis derived from the species data and the sample scores that are linear combinations of the environmental variables.

	Axis 1	**Axis 2**	**Axis 3**
Eigenvalue	0.36	0.13	0.05
Pearson Correlation Spp.—Envt.*	0.68	0.58	0.42
Variance in Species Data			
% of Variance Explained	10.7	3.7	1.5
Cumulative % Explained	10.7	14.4	15.9

Inter-set correlations of the soil chemicals with the CCA axes indicated that TOC and TN were highly negatively correlated with Axis 1, while NO_3-N was highly positively correlated with Axis 2 (Table 5.17). High concentrations of TOC and TN and low concentrations of NO_3-N are associated with the Yellow Creek quadrats/plots. Lower concentrations of TOC and TN are associated with Locust Creek and Thompson River quadrats/plots.

Table 5.17. Inter-set correlations of soil chemistry variables with CCA axes.

	Correlations		
Variable	**Axis 1**	**Axis 2**	**Axis 3**
TSP	< -0.01	0.03	-0.33
NO_3-N (log)	0.20	0.55	-0.02
NH_4-N (log)	-0.14	-0.03	0.03
TN	-0.61	0.12	-0.16
TOC	-0.55	0.12	-0.10
C:N	0.06	0.05	< 0.01

The graphical presentation of the CCA analysis for herbaceous species cover vs. site microtopography showed nearly the same pattern as the CCA analysis for herbaceous species cover vs. soil chemical variables. Again, the Yellow Creek quadrats/plots were grouped along the negative portion of Axis 1, away from Locust Creek and Thompson River quadrats/plots (Figure 5.25). In this analysis, however, the Yellow Creek quadrats/plots were also concentrated along the positive portion of Axis 2. The Locust Creek and Thompson River plots were grouped along the positive portion of Axis 1 and extended over the range of Axis 2. Axis 1 explained about 14% of the variation, while axis 2 and 3 explained additional 2.8% and 3.2% variation respectively for a cumulative % of 18.9% of the variation explained (Table 5.18). Total variance ("inertia") in the species data = 3.37.

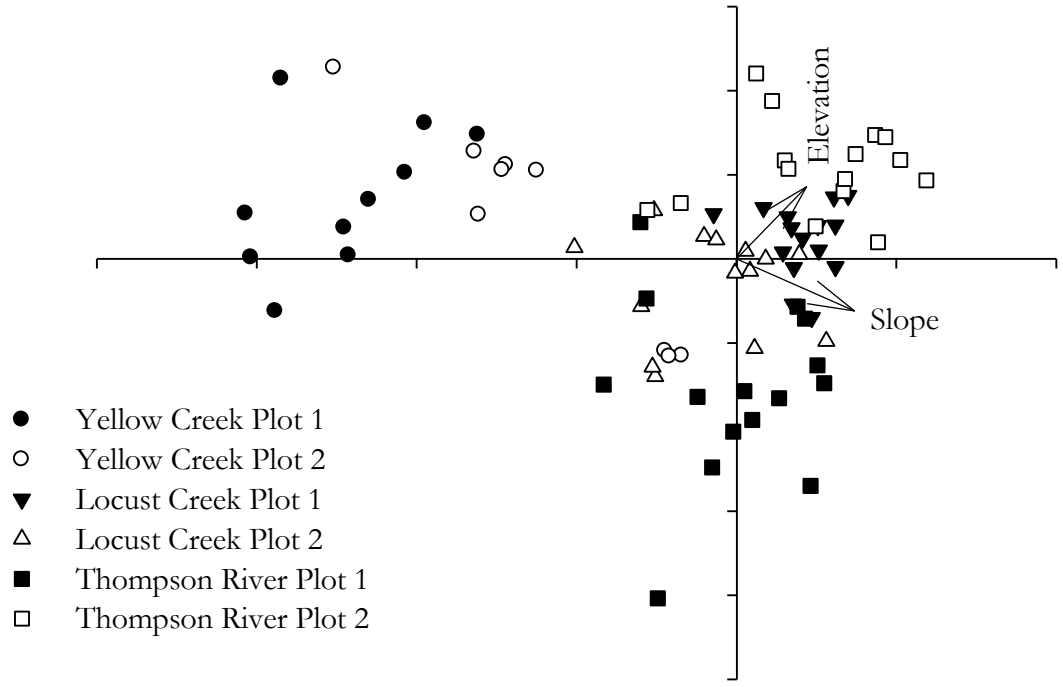

Figure 5.25. CCA analysis of herbaceous species cover with site microtopography variables. Plot axes are centered at the origin. Vectors for slope and elevation represent the contribution of these variables to plot separation; the angle and length of the line indicate the direction and strength of the relationship.

Table 5.18. CCA analysis of herbaceous species cover with site microtopography variables; * = Correlation between sample scores for an axis derived from the species data and the sample scores that are linear combinations of the environmental variables.

	Axis 1	**Axis 2**	**Axis 3**
Eigenvalue	0.47	0.09	0.08
Pearson Correlation Spp.—Envt.*	0.76	0.50	0.52
Variance in Species Data			
% of Variance Explained	13.8	2.8	2.3
Cumulative % Explained	13.8	16.6	18.9

Inter-set correlations of the site microtopography variables with the CCA axes are given in Table 5.19. Of these microtopographical variables, slope has the highest correlation with axis 1 while elevation has the highest correlation with axis 2. This would imply that the Yellow Creek quadrats/plots were associated with low slopes and high elevations. It would also mean that the Locust Creek and Thompson River quadrats/plots are associated with higher slopes. However since the Locust Creek/ Thompson River grouping spans the gradient of axis 2, little can be said as to how these quadrats/plots respond to elevation. In other words, these sample units are found across the range of measured elevations.

Table 5.19. Inter-set correlations of site microtopography variables with the CCA axes.

	Correlations		
Variable	**Axis 1**	**Axis 2**	**Axis 3**
Planform	-0.06	-0.12	0.01
Flow Accumulation (log)	0.14	0.02	0.50
Slope (log)	0.63	-0.26	-0.09
Elevation	0.40	0.40	-0.15

Discussion

Site Soil Characteristics

Past soil inundation can be recorded in soil properties such as soil color. There are three aspects to color: hue, the quality of the pigmentation; value, the lightness or darkness (i.e. the amount of light reflected); and chroma, the richness or brightness of the pigment (Brady and Weil, 2002). Most soil hues are combinations of red and yellow; these hues are related to the Fe^{+3} content of the soil. The reduced form of Fe^{+} (i.e. Fe^{+2}) is colorless,

soluble and can move through the soil (Vepraskas and Faulkner, 2001). Therefore, with inundation and subsequent reduced soil conditions, red, yellow and brown colors are effectively "washed" out of the soil, leaving behind a grey color. This chemical reduction of Fe^{+} (and/or Mn^{+}) and subsequent loss of soil color is referred to as "gleying" and resultant soils have what is referred to as a "gleyed condition". Subsoil horizons that have undergone strong gleying are given the Bg designation which indicates a low chroma and a high value (chroma < 2 and value >4) (Brady and Weil, 2002; Vepraskas, 2001). These low chroma matrices may have reddish mottles which denote redox concentrations or places where Fe^{+2} accumulates upon oxidation.

Soil samples collected along the floodplains of Yellow Creek, Locust Creek and Thompson River show varying degrees of reduced conditions. Soil samples from Yellow Creek displayed the most reduced conditions of the three sites. These soils had a reduced matrix (chroma = 1) with occasional mottles of a more oxidized character (chroma > 2). The gleyed condition of these soils could be due to influence of the creek, i.e. a high water table and/or frequent inundation of the floodplain. The fine texture of the soils (silty clay with ~ 40% clay) also contributes to the gleyed condition. The high clay content of the soil may either limit the drainage of water from the floodplain, causing ponding after heavy rain events or may contribute to soil moisture through capillary action and the pulling of water up into the upper soil horizons from the water table below.

Soil samples from Locust Creek were of more intermediate character, i.e. not as reduced as those observed at Yellow Creek but more reduced than those observed at Thompson River. Locust Creek soil samples had an oxidized matrix (i.e., values ≤ 4 and chroma > 2) with occasional reduced mottles (e.g. grayish brown, 10 YR 5/2). Therefore, while soil inundation is evident from the presence of reduced mottles, flooding is likely

short-lived. Either the flood waters recede quickly or the soils drain quickly due to their more coarse texture. Soils at Locust Creek had less clay than those at Yellow Creek and were characterized as silt loam or silty clay loam. In addition to the higher silt content of these soils, they were also well stratified with fine and very fine sands. This combination of characteristics would allow the soils at Locust Creek to drain faster than the soils at Yellow Creek. In addition, Locust Creek appeared to be a faster moving, higher energy stream. These types of systems tend to be more "flashy" than slack water systems like Yellow Creek. Where water levels in slack water systems tend to rise and fall more slowly, water levels in flashy systems tend to rise and fall quickly. The available discharge data for Locust Creek shows a relatively low base-flow for the system, punctuated with periods of very high flow (Figure 5.2a). These high discharge periods appear as sharp peaks, with discharge rising and falling quickly, not gradually. A visit to both Yellow Creek and Locust Creek in early 2007 following a period of heavy rain revealed the differences in these systems. The floodplain of Yellow Creek had several areas of ponding while the floodplain of Locust Creek showed evidence of high water (i.e. displaced plant debris) but there were no obvious areas of ponding.

The soils observed at Thompson River showed the least reduced characteristics of the three systems. Soil colors were darker (values ≥ 3 and chroma ≤ 3) which could be an indication of accumulated soil organic matter or of soil inundation. The lack of other redoximorphic features (i.e. mottles) lends support to the soil organic matter hypothesis. Like the soils found at Locust Creek, the soils observed at Thompson River were coarser and were characterized as silt loams or silty clay loams. As discussed previously, the higher silt and sand content of these soils would contribute to better drainage following flood events. Discharge data reveals that Thompson River is also a flashy system, however its

drainage area and base-flow discharge are 3-4 times greater than Locust Creek. Despite this larger size, it is possible that the Thompson River floodplains examined in this study actually flood less frequently than the selected Locust Creek floodplains due to a much higher bank height. Despite the close proximity to the other sites (and thus the assumption that heavy rains fell there also), a visit to the Thompson River site in early 2007, showed little evidence of flooding. For example, there was no evidence of standing water on the site (as was observed at Yellow Creek) and the litter layer was not disturbed (as was observed at Locust Creek).

Microtopography and Soil Chemistry

Unlike those in the Ozarks (Sagers and Lyon, 1997; Lyon and Sagers, 1998), riparian forests in the Grand River Hills and the Chariton River Hills showed very little topographical relief. Such distinctions are expected with landscapes that differ in glacial history and geological processes. The current study areas were located in a section of the Central Dissected Till Plains characterized by broad valley bottoms and smooth uplands with less than 30 m of relief (Nigh and Schroeder, 2002). Thus when distance from river was used as a proxy for flood frequency (and it was assumed that locations closest to the river would flood more frequently), relationships between flood frequency and levels of inorganic-N or TSP in the soil were not detected. Microtopographical variations can be expected to exist, however, even on apparently featureless landscapes. Measures such as elevation, planform, flow accumulation and slope should provide an indication of where water might be expected to accumulate and thus have an impact on soil nutrient cycling and vegetative growth. For example, areas of higher elevation would see less accumulation of water and sediments. Likewise areas with higher degree slope would see greater movement of water away from the

area. On the other hand, areas with lower elevation, less slope or concave features would see greater accumulations of water and dissolved substances. And in these areas one might expect to see greater concentrations of soil chemicals including inorganic-N, TOC or TSP.

The microtopographical analyses confirmed the observation that the floodplains were gently sloping away from the creeks. In other words, the highest elevations were typically encountered along the transect lines closest to the creek while lower elevations were observed along the 50 m transect line. This finding could help explain why distance from river was not a good proxy for flood frequency and why the expected trends (increased NO_3-N, decreased NH_4-N, decreased TSP and increased herbaceous and shrub vegetation richness and diversity with distance from river) were not observed. Other microtopographical variables that describe how water flows and where water might be expected to accumulate in the landscape may further explain the observed soil chemical patterns. Spatial heterogeneity of soil characteristics could also be a factor. Soils, in general and riparian soils in particular, are known to be highly heterogeneous (Lyon and Sagers, 1998), and the sampling methodology applied here (i.e. sampling at 10 m intervals) may have been too coarse to capture the true variation of soil chemicals across the sampling plots. Caution must also be applied when scaling up to the landscape level. The relationships between microtopography, soil chemistry and understory vegetation observed within the plots may not be representative of the relationships between these parameters along the entire floodplain of these streams.

Among the microtopographical parameters, slope and elevation were good predictors for soil chemical patterns. A positive correlation was observed between slope and NO_3-N and negative correlations were observed between slope and NH_4-N, TN or TOC. Therefore, in areas with steeper slopes, higher concentrations of NO_3-N and lower

concentrations of NH_4-N, TN or TOC were encountered. A negative relationship between elevation and NO_3-N, TN or TOC was also observed. This would indicate that higher positions in the landscape had lower values of these soil chemical parameters. While the effect of slope and elevation are opposite in terms of NO_3-N, they are in agreement for TOC and TN. Total organic C and TN concentrations could be said to increase along a gradient from higher elevation to lower elevation with the greatest changes in TOC and TN corresponding to the areas with the greatest changes in elevation (i.e. highest slope). Places in the landscape with higher elevation and slope would be expected to be better drained; i.e. a greater percentage of pore space would be allocated to air rather than water. In these "aerobic" portions of the landscape, decomposition processes may cycle C and N more rapidly resulting in less C and N in the soil and more C and N incorporated into living biomass. Places in the landscape with lower elevation and/or with gradual slopes may be expected to accumulate water. In these places, a greater percentage of pore space may be allocated to water over air resulting in pockets of anaerobic conditions. In these anaerobic pockets, decomposition would be slowed resulting in accumulation of C and N in the soil. Slope and elevation appear to have opposite effects on soil NO_3-N. Following the logic above that places in the landscape with greater elevation are more aerated these landscape positions would be excepted to have a higher nitrate:ammonium ratio. While ammonification can occur in either aerobic or anaerobic environments, nitrification is the dominate process in aerobic environments, thus NO_3-N would accumulate at the expense of NH_4-N. However, NO_3-N is the more mobile form of inorganic-N, therefore in areas with steeper slopes, one might expect NO_3-N to be leached from the soils.

Despite observing only slight changes in elevation across our sites, correlations between various site parameters and elevation were found. In addition to the correlations

with soil chemical parameters discussed above, elevation was also negatively correlated with number of woody stems. These results are similar to other studies where elevation was found to be the primary determinant of site characteristics (Lyon and Sagers, 1998; Turner et al., 2004; Grell et al., 2005). For example, in a study of old-growth bottomland hardwood-loblolly pine forests in southern Arkansas, Grell et al. (2005) found elevation to be correlated with 75% of environmental variables measured. Likewise, importance values for 35% of seedling species, 30% of overstory species, 22% of herbaceous species and 8% of sapling species differed significantly by elevation class in this study. In a comparison between upland and floodplain sites, Holmes et al. (2005) found that TN as well as concentrations of NO_3-N, P, K, Ca, Mg, Mn, and Zn were all significantly higher on floodplain landforms than upland landforms. In addition, these landforms differed in groundflora composition as well as in soil organic matter, pH and texture (Holmes et al., 2005). While Grell et al. (2005) and Holmes et al. (2005) made comparisons on a more coarse scale; Beatty (1984) considered microtopographical effects of forest floor treefall mounds and pits on soil nutrient levels within a maple-beech forest. In this study, the mounds were found to be of poorer nutrient content and to have less organic matter than the pits. This is in agreement with the observation that areas higher in elevation in the studied riparian forest floodplains (similar to the mounds in Beatty (1984)) had lower concentrations of TN and TOC.

Putative Response of Vegetation to Soil Chemistry and Microtopography

Sorting out the vegetation responses to soil chemistry and floodplain microtopography is problematic. Since the effects of soil chemistry and micropography on understory vegetation were analyzed separately, it cannot be said for certain which, or if, a parameter (chemistry or microtopography) is acting as the dominant structuring agent.

Further, the herbaceous and shrub layers not only respond differently to these parameters but they are also negatively correlated with each other. Therefore, it is not known if they are responding to each other or to gradients in soil chemistry or microtopography. For example, herbaceous cover and number of woody stems are both correlated with TN; however, herbaceous cover is negatively correlated with TN while number of woody stems is positively correlated with TN. It is possible that the shrubs are out-competing the herbaceous plants on the microsites with greater TN, relegating the herbaceous plants to microsites with lower TN. On the other hand, herbaceous plants might be better able to tolerate lower TN and are therefore found on these microsites preferentially.

The herbaceous and shrub layers also respond differently to microtopographical variation. Herbaceous cover was positively correlated with flow accumulation and slope, while number of woody stems was negatively correlated with slope and elevation. Likewise regression analysis showed a positive relationship between the herbaceous layer and flow accumulation and slope and a negative relationship between the shrub layer and these variables. If these microtopographical observations are considered in light of the expected effect of water movement and water accumulation on the landscape, then herbaceous cover is greater in wetter areas and number of woody stems is greater in drier areas. Here again similar questions apply: i) are the herbaceous species better competitors for the more moist positions in the landscape or ii) are woody species somehow more tolerant of drier conditions?

Differential response of forest layers to environmental gradients has been observed in other studies (Sagers and Lyon, 1997; Lyon and Sagers, 1998; Lyon and Sagers, 2002; Grell et al., 2005; Lyon and Gross, 2005) and in these studies site heterogeneity was hypothesized as the cause for this uncoupling. For example, Lyon and Gross (2005)

observed 4 distinct assemblages of overstory species that responded to soil and topography characteristics; however, no distinct shrub assemblages were identified and shrubs were not correlated with environmental parameters. Lyon and Sagers (1998) failed to find any significant coupling between tree and herbaceous layer assemblages. Vegetation layers certainly respond to environmental gradients, but different layers may track different gradients and the same gradient may not be perceived equally by all layers (Sagers and Lyon, 1997). In the current study, it is possible that the shrub layer is responding to variations in microtopography and/or soil nutrients and thereby exerting pressure on the herbaceous community or the converse may be true. In the case of the Locust Creek sites, the herbaceous community may be the more dominant component of the understory. At these sites, a very dense layer of Canadian woodnettle carpeted the forest floor. These plants were 0.5-1.5 m in height and were spaced in such close proximity that little, if any, light penetrated to the true forest floor. In these sites, the shrub layer was nearly absent and a tree regeneration layer was not observed.

The results of the current study suggest the importance of microtopography for floodplain restoration efforts. The floodplains in this study appeared uniform at first glance; however, differences in soil chemistry across the floodplains became apparent when microtopographical measures were taken into account. While not measured directly, differences in soil moisture may also be assumed; water would be expected to accumulate at lower elevation points or in convex landscape features. Other studies have documented the need for considering microtopographical influences along with flood tolerance ratings during floodplain restoration efforts. Grell et al. (2005) recommend flood tolerant species on areas that experience annual flooding and moderately tolerant to intolerant species on higher sites that rarely flood. Likewise, McLeod et al. (2000) noted differential survival due to planting

elevation; flood tolerant species had greatest survival in lowest elevation sites and flood intolerant species had greatest survival in relatively drier sites. Restoration plans should, therefore, include a fine-scale evaluation of elevation contours and species selection based on relative flood tolerance.

Floodplain Heterogeneity

In a study of the Vindel River and its tributaries (northern Sweden) Nilsson et al. (1994) observed large floristic differences between the main channel and the tributaries. They also observed that the tributaries showed certain floristic characteristics of their own, such as proportions of woody plants or altitudinal patterns of species richness. A study of three tributaries in the southern region of the Merrimack River watershed in Massachusetts and New Hampshire also demonstrated the variability in species composition of river systems in close proximity of each other (Lyon and Gross, 2005). In their study, the three riparian systems shared common overstory dominants, but exhibited greater variability in the non-dominant tree species.

Yellow Creek, Locust Creek and Thompson River are all in relative close proximity and all are tributaries of the Grand River. Results similar to Nilsson et al. (1994) and Lyon and Gross (2005) might be expected and were observed. Like Nilsson et al. (1994), each tributary had its own pattern of vegetation as well as its own pattern of soil nutrients. Some species were found on all three sites, but each site had species that were unique to the site. For example, silver maple and American sycamore were among the dominant tree species on all three sites but black walnut was only found at the Thompson River sites and box elder was only found at Locust Creek Plot 2. Likewise in the understory, Canadian woodnettle and Virginia creeper were dominant species at the Locust Creek and Thompson River sites, while

species such as black snakeroot (*Sanicula odorata*) and Muskingum sedge (*Carex muskingumensis*) were found at one site and at only a few quadrats at that site. This would be very similar to Lyon and Gross' (2005) observation the two most dominant species were found at all three rivers but 55.8% of the species recorded were found only at 1 or 2 plots. Also, like Nilson et al. (1994), proportions of species varied. For example, Yellow Creek had a very dense shrub layer dominated by poison ivy. On the other hand, the shrub layer was virtually absent at Locust Creek and the herbaceous layer at this site was dominated by Canadian woodnettle and Virginia creeper. The Yellow Creek, Locust Creek and Thompson River sites also varied from each other with regard to soil nutrients. In this case, Yellow Creek had higher amounts of TOC and TN than the other two sites; Locust Creek had the lowest levels of TOC and TN, but the highest levels of NO_3-N.

These creek level differences were highlighted in the multivariate analyses and could explain the correlation and regression patterns already discussed. Both DCA and CCA analyses of the herbaceous species component resulted in the separation of the Yellow Creek quadrats/plots from the other two sites. Yellow Creek had the densest shrub layer and the highest TOC and TN levels. On the other hand, Locust Creek had the densest herbaceous layer and the lowest TOC and TN levels. In general the herbaceous and shrub layers were negatively correlated with each other. These layers were also shown to respond differently to soil nutrients; TOC and TN in particular. The differential response of the herbaceous and shrub layers to TOC and TN may have more to do with the site (Yellow Creek vs Locust Creek) than to do with actual concentrations of C or N. For example, disturbance regimes and past management may vary between the three sites. These factors are known to influence species composition and diversity. Locust Creek and Thompson River appear to be "flashy" systems; hydrographs from these rivers reveal low base-flows punctuated by

short periods of high flow. In these systems, floods may be more dramatic/forceful and woody species may succumb to physical damage during flood periods, resulting in low numbers of woody stems on these floodplains. Lumping of the study sites for correlation and regression analysis allowed for determination of overall trends; however this lumping may have created patterns that don't actually exist.

Conclusions

The overall objective of this research was to determine if the relationship between riparian plant community composition and soil chemistry is mediated via flooding. Distance from river was used as a proxy for flood frequency. Landscape positions closer to the river were expected to flood more frequently than landscape positions further from the river. However, due to the flat terrain of selected floodplains, no differences in soil chemical variables, or understory vegetation characteristics were observed with increased distance from river. Microtopographical variations were thus considered to determine if landscape positions that accumulate water (i.e., concave features) would have different soil chemistry and understory vegetation than other landscape positions. Microtopographical analysis showed that concave landscape features were associated with higher levels of TN and TOC. No other clear relationships between soil chemistry and microtopography were observed. Landscape positions that accumulate water were associated with higher herbaceous cover, while those that were better drained were associated with higher numbers of woody stems.

Understory vegetation was also affected by soil chemistry variables, but not as anticipated. Based on greenhouse experiments (Chapter 3), understory richness and diversity were expected to correlate with landscape positions with higher total N but with lower TSP levels than neighboring landscape positions. While soil TN was correlated with understory

vegetation, soil TSP was not. In addition, the different understory components (i.e., herbaceous cover vs. number of woody species) responded differently to TN levels. Herbaceous cover was negatively correlated with TN, but woody stems were positively correlated with TN. In addition, the understory components were negatively correlated with each other. The relationship of understory vegetation with microtopography and soil chemistry is thus complicated by the potential interaction between the herbaceous and woody species that make up this layer. Multivariate statistics showed differences between sites, highlighting the need for additional study sites and/or additional plots within sites.

The final objective of this study was to determine overstory differences between watersheds in the Central Dissected Till Plains of northwest Missouri. Each of the rivers included in this study had its own pattern of overstory vegetation. Some species, such as *Acer saccharium*, *Fraxinus pennsylvania* and *Platanus occidentalis*, were common components of the overstories; while other species, such as *Juglans nigra* and *Acer negundo,* were only found at a single site. A disproportionate dominance by a single species was observed in most plots.

This study demonstrates the importance of studying multiple structural layers of a forested ecosystem when attempting to quantify the effects of a disturbance, such as flooding. Although the method used to sample the overstory did not allow for comparisons of the overstory response to flooding with the shrub layer or herbaceous layer responses, the response of these latter two layers could be compared. The shrub and herbaceous layers were found to respond differently to gradients in soil chemical and site topography variables. However, these relationships may not have been evident if the microtopography of the sites had not been quantified. In riparian systems with clear elevation gradients such as those found in the Missouri Ozarks, distance from the river may indeed be a good proxy for flood frequency. However, in systems such as those found in the Central Dissected Till Plains of

northwest MO, where topography is relatively flat and elevation changes only gradually over great distances, microtopographical quantification of the landscape is necessary to determine where water might accumulate and thus were soils might remain inundated for longer periods of time. Therefore, in systems similar to those of the current study, care should be taken to sample not only all vegetational layers but also as much site heterogeneity as possible.

The current study also highlights the issue of scale in studying ecosystem processes. While sites (i.e., Yellow Creek vs Locust Creek vs Thompson River) varied in terms of soil nutrient status (for example: TOC, TN, NO_3-N, TSP) and understory vegetation, plots within sites and quadrats within plots generally did not vary. The sampling distance of 10 m may very well have been too coarse to capture the variation occurring at the plot level. As with any landscape scale study, a compromise between sampling at a fine enough scale to quantify processes and yet at a large enough scale as to be able to draw conclusions about the system as a whole had to be made. While tentative conclusions about the relationships between flooding and soil chemical/nutrient status and understory vegetation may be made, additional studies of these riparian forests at a finer scale and of additional riparian forests in the Central Dissected Till Plains are necessary before generalizations about these relationships can be made.

CHAPTER 6

SUMMARY, CONCLUSIONS AND IMPLICATIONS

Prior to this study, research into the effects of flooding on soil properties was limited primarily to the characterization of soil oxygen status and subsequent changes in soil chemistry. For example, it is well known that inundation results in anaerobic soil conditions which, in turn, result in the reduction of compounds, such as NO_3^-, MnO_2, and $Fe(OH)_3$, as soil microorganisms carry out anaerobic respiration. Changes in soil N status with flooding as well as the effects of flooding on litter decomposition have likewise been investigated. Investigations in multi-cropped rice systems have shown that the accumulation of phenolic compounds in the soil with flooding may lead to decreased available-N for plants. However, few studies have examined the effect of flooding on soil microbial communities. Furthermore, few studies have examined alterations in soil properties due to flooding collectively or at multiple spatial scales. This study helps clarify the relationships between soil microbial community characteristics and soil chemical alterations due to periodic flooding. Likewise, this study is unique in its analysis of ecosystem processes related to floods on multiple levels; patterns in soil chemistry changes with flooding observed at the greenhouse level were compared with those in a field-laboratory and with those in natural settings.

Objective 1: Effects of Flooding and Residues on Soil Chemistry

Flooding Effects on Soil Oxygen Status

Redox potential was used to monitor changes in soil oxygen status with flooding. Redox potential decreased with the onset of flood waters (i.e. saturation) and increased with

the removal of flood waters. Decreases in redox potential with flooding may occur rapidly. Under simulated flood conditions in the greenhouse, all flood treatments were anoxic (i.e., anaerobic) within 7-10 days of inundation. On the other hand, under field conditions (i.e. in the FTL), anoxic conditions were not detected until 12-18 days after onset of flood conditions. Whether the water was flowing or stagnant did not affect redox potentials. In the greenhouse, water in the flowing flood treatment had significantly higher dissolved oxygen levels than water in the stagnant flood treatment. However, redox potentials for the flowing flood treatment remained only slightly greater (and at times not significantly different) than that of the stagnant treatment. Contrary to expectations, the oxygen present in the flowing flood waters was not able to effectively diffuse through the soil. In the FTL, automated dissolved oxygen sensors at the soil surface indicated that the 5WF flood treatment had lower soil dissolved oxygen than the 5WS flood treatments. Manual monitoring showed no difference in dissolved oxygen of these flood waters and automated redox sensors (5 cm depth) indicated no difference in soil redox potential between these two treatments. In both cases (i.e. flowing and stagnant flood waters), soils developed anoxic conditions.

Flooding and Residue Effects on Soil Inorganic-N and Polyphenolics

Simulated floods in the greenhouse and FTL had no effect on soil TN but did affect the form of inorganic-N present in the soil. Stagnant and flowing flood treatments in the greenhouse resulted in a dominance of NH_4-N, while the control treatment had a dominance of NO_3-N; the intermittent treatment had a mixture of NH_4-N and NO_3-N. Incorporation of grass residues (rather than legume or tree residues) resulted in higher inorganic-N concentrations. Flood treatments in the FTL did not differ in their effect on soil NO_3-N or

soil NH_4-N. Soil NO_3-N levels decreased with flooding while NH_4-N increased with flooding; both of these trends were regardless of flood treatment differences (i.e., duration and flow rate).

Total soluble polyphenolics accumulated in the soil due to simulated floods in the greenhouse. However, toward the termination of the experiment, TSP levels were declining. Grass residue incorporation resulted in greater accumulation of TSP than tree or legume residues. In the FTL, the control and 3WF flood treatments had lower levels of TSP than both of the 5-week flood treatments. Therefore, the expectation that TSP would accumulate in the soil under flood conditions was upheld.

Objective 2: Effect of Soil Chemistry Changes on Germination and Seedling Growth

Simulated floods in the greenhouse were followed up with a germination experiment to determine if changes in soil chemistry due to flood and residue treatments had an effect on seed germination and seedling growth. Germination was lowest with soils previously under stagnant flood or grass residue treatments. Germination and shoot length were negatively correlated with soil NH_4-N and TSP concentrations. Soil TSP levels explained about 17% of the variation in total germination and about 18% of the variation in shoot length.

Phenolic compounds in the soil due to flooding may have allelopathic effects on riparian vegetation. Low molecular weight phenolic compounds have been shown to affect germination (Blum et al., 1991; Reigosa et al., 1999; Muscolo et al., 2001), while polyphenolic compounds may affect soil nutrient status (Browaldh, 1997; Mafongoya et al., 1998; Suominen et al., 2003 Mungai and Motavalli, 2006). This study focused on soluble polyphenolic compounds which are capable of binding proteins and thus rendering them

unavailable to plants. Reduced shoot growth in seedlings germinating in soil from stagnant floods may be due to N limitations from polyphenolic-N binding.

Effects of soil TSP on N availability depends on the residence time of phenolic compounds in soil. In the greenhouse experiment, TSP concentrations increased up to and peaked at day 35. The subsequent decline in TSP may be due to microbial succession. Once simple carbon compounds in the soil are exhausted, microbes will utilize the more recalcitrant compounds such as lignins and tannins for their metabolism. Phenolic compounds that are altered or degraded by microorganisms may lose their N-binding capacity and thus no longer influence N availability.

In natural systems, the accumulation of TSP and its influence on soil N may be inconsequential. Floodplains are open systems; water and nutrients converge and then flow through these wetlands. Thus, soluble phenolic compounds may flow through the floodplain rather than accumulate in the soil. Floods in riparian forests typically occur during the dormant season and any phenolic compounds that do accumulate will likely be metabolized by microorganisms prior to the growing season. Long-term accumulation of TSP in the FTL is also unlikely, since flood treatments occur only once during the growing season and flood durations are generally short. Any accumulation of TSP during flood treatments will be mitigated during the long periods between floods; therefore, the effect of TSP accumulation in natural systems or in the FTL may be transient.

Objective 3: Effects of Flooding on Soil Microbial Communities

Flood-related changes in the soil microbial community were observed in the greenhouse but not in the FTL. Under field settings, soil microbial community structure was influenced by sampling depth and sampling season; soil heterogeneity may also have played a

role as soils were not uniform across the FTL facility. Under stagnant flood conditions in the greenhouse, microbial biomass and monounsaturated fatty acid markers were reduced as well as the response of aerobic bacteria, Gram-negative bacteria and mycorrhizae fungi. Stress indicators, which often increase under low oxygen conditions, increased under stagnant as well as flowing flood conditions in the greenhouse. Changes in microbial community function accompanied these changes in microbial community structure. Both C- and N-utilization decreased under stagnant and flowing flood conditions in the greenhouse. Inundation of FTL soils resulted in decreased C-utilization and overall, changes in N-utilization correlated with changes in soil microbial community structure.

Soil microorganisms are sensitive to disturbance and vary in their ability to tolerate anaerobic conditions. Anaerobic soil conditions were observed in the flowing and stagnant flood treatments in the greenhouse flood simulations, explaining the increased stress indicators (under both treatments) as well as the decreased microbial biomass (under the stagnant treatment).

Implications of Soil Microbial Community Changes

A decline in mycorrhizae fungi as observed under stagnant flood conditions in the greenhouse has implications for the decomposition of recalcitrant compounds. Actinomycetes and fungi are the primary decomposers of complex nutrients such as lignin and humus. Decreases in the fungal component due to flooding, therefore, could cause the accumulation of phenolic based compounds, and may impact N availability. Fungi are the dominant saprophytic heterotrophs in many forest soils, especially acidic soils as fungi are more tolerant of acid conditions than other soil microbes. The implications of inundation of forest soils are thus greater than those for grassland or agricultural soils. Changes in soil

microbial community structure may subsequently affect above-ground components of the ecosystem due to the critical roles that bacteria and fungi play in decomposition and nutrient cycling. However, flood timing and duration will influence this effect. If flooding occurs during the dormant season, or if floods are of short duration, the overall effect on the microbial community will be minimal. Microbial populations, with their short generation times will be able to quickly rebound from the effects of flooding when favorable conditions for their reproduction and growth are returned to the soil.

Objective 4: Influence of Microtopography on Soil Chemistry and Vegetation

The floodplains examined in this study were broad and generally flat; where an elevation gradient existed, the highest landscape positions were the closest to the creek. Therefore, distance from river was not a good proxy for flood frequency in this study. The floodplains had subtle relief with convex as well as concave features scattered across the floodplains. Water accumulating in landscape depressions may alter nutrient cycling and thus vegetation on a very fine scale. Higher slopes may result in the leaching of NO_3-N as well as changes in the relative amounts of TOC or TN. In this study, both TOC and TN increased with increased elevation gradient; i.e. the greatest changes in these measures were correlated with the greatest changes in elevation and slope.

The understory vegetation may respond to any number of factors including the microtopography of the site, the gradients of soil nutrients present or to neighboring vegetation. Each layer of understory vegetation may respond differently to these factors such that the layers are uncoupled. For example, the shrub layer may respond to soil TOC and TN, with increased shrub diversity and richness under high nutrient conditions, while the herbaceous layer may respond more to the resultant shade from the shrub layer than to

soil nutrient gradients. In this study, the shrub layer was correlated with landscape positions that had higher TN, while herbaceous plants were correlated with low TN sites. Greater amounts (i.e. % cover) of herbaceous plants were found in areas of greater water accumulation.

The riparian communities in this study had species in common, but each also had unique species. In addition, the relative importance of species varied. For example, Yellow Creek had the most dense shrub layer, while Locust Creek had the most dense herbaceous layer. These two creeks also had contrasting soil nutrient status; Yellow Creek had the highest TOC and TN levels, while Locust Creek soils had the lowest levels of these variables. Site differences in soil chemistry and vegetational composition may be due to disturbance regimes as well as past management practices; in addition, watershed characteristics (especially hydrology) may play a role in system characteristics. The site differences observed in this study seem to explain other results. The probable relationship between the shrub layer and high soil TOC and TN, and likewise the inverse relationship between the herbaceous layer and these variables, may be entirely linked to the sites selected. Additional studies at these sites and along other floodplains in the Central Dissected Till Plains of Missouri are necessary to better understand the findings of this study.

The Importance of Studying a System on Multiple Levels

Research into ecological processes often poses a dilemma: is it better to sacrifice natural settings and conduct studies in highly controlled artificial settings or to sacrifice control and conduct research in natural settings? If a highly controlled artificial setting is chosen, then the question of applicability arises; i.e. are the findings from a controlled experiment applicable to the natural environment? Can experiments made on one scale

translate to a different scale? This study addressed this dilemma by approaching the research questions in multiple settings: greenhouse, field-laboratory, and floodplain, with varying degrees of control. Under the highly controlled setting of the greenhouse, flooding affected soil oxygen status and soil inorganic-N. These changes, along with the accumulation of phenolic compounds in the soil, affected post-flood germination and seedling growth. Furthermore, flood-induced soil chemistry changes resulted in changes in microbial community structure and function.

As the study progressed to the FTL, the challenges of field studies become obvious. In the case of the FTL, maintaining controls that represented non-flood conditions were difficult. Within two weeks of the initiation of flood treatments in adjacent FTL channels, soils in the control channels were equally saturated as the soils in the experimental channels. The FTL is a large facility; each flood channel measures 6 x 180 m for a total lab area of 72 x 180 m. Therefore, the FTL crosses gradients in soil texture and soil chemical properties that exist in the Sulfur Creek floodplain. Soils within a channel vary along the length of the channel; likewise adjacent channels have different soil properties (e.g. soil texture, pH and CEC). This high degree of variability confounds findings. Flooding affected soil NO_3-N and TSP but not NH_4-N, TOC, TN, the C:N ratio or soil microbial community structure. T-tests revealed some changes in these soil properties with sampling depth and season; however, the high degree of variability coupled with the low number of soil samples may have contributed to the lack of significant results.

While some trends were similar between the greenhouse and the FTL floods, there were notable differences. In both experiments, stagnant and flowing flood treatments resulted in anoxic soil conditions; however, under greenhouse conditions the stagnant flood had lower redox potential than the flowing flood, while under FTL conditions, the opposite

was true. Soil inorganic-N and TSP results were similar between the two experiments; however, soil microbial community structures differed. Soils from the FTL supported a different microbial community and greater microbial biomass than soils from the greenhouse experiment. In the FTL, changes in the soil microbial community had more to do with sampling depth and season than with flood treatment; while in the greenhouse, flood treatments were shown to affect soil microbial community structure and function. Different results in the greenhouse and FTL under similar flood treatments again can be attributed to the variability of field conditions.

The variable nature of soil properties also contributed to the results from the floodplain studies. At these sites, soil chemistry and understory vegetation were not affected by flood frequency but rather by microtopographical characteristics. Elevation and slope affected soil chemistry (i.e. NO_3-N, TN and TOC), while flow accumulation and slope affected understory vegetation (i.e. herbaceous cover and number of woody stems). Understory vegetation was related to TN; however competitive interactions between herbaceous and woody species may have played a role in the observed patterns.

In a natural setting, scale dictates. Sampling at a fine scale can reduce sample variability and thus produce reliable results; however, interpretation and extrapolation are limited. Similarly, sampling at a coarse scale may allow for broader applications but results are not applicable at smaller scales. The FTL study would benefit from finer scale sampling; this would involve the installation of additional sensors and the collection of additional soil samples. The floodplain study would benefit from additional plots at the current sites as well as additional sites. Additional plots and sites would address the questions that the multivariate statistics raised regarding the influence of site and soil chemistry on understory vegetation.

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APPENDIX A

Lachet QuickChem Method 12-107-04-1-B for NO_3-N Determination

Soil Nitrate-N

Cadmium Reduction Method

Principle of the Method

1.1 The nitrate ion (NO_3^-) is soluble in any water-based solution. However, because nitrate extraction is done in tandem with ammonium, the extracting solution is 2 M potassium chloride (KCl). Ammonium ions (NH_4^+) on colloidal exchange sites are brought into solution by exchange with potassium (K^+) ions.

1.2 Nitrate is quantitatively reduced to nitrite by passage of the sample through a copperized cadmium column. Once reduced the nitrite is then determined by using a modified Griess-Ilosvay method in which nitrite is diazotized with sulfanilamide followed by coupling with N-(1-naphthyl) ethylenediamine dihydrochloride. The resulting water soluble dye has a magenta color which is read at 520 nm.

1.3 The procedure outlined here is for use with a Lachet Flow Injection Autoanalyzer.

Range and Sensitivity

2.1 The procedure has a soil extract detection range of 0.02 to 20.0 ppm NO_3-N.

Interferences

3.1 If unfiltered suspended matter in the reduction column may restrict sample flow.

3.2 High concentrations of iron, copper and other heavy metals can result in low values.

Precision and Accuracy

4.1 Coefficients of variation of 2.1 to 3.4% have been reported.

Equipment

5.1 Balance or 10 g scoop (NCR-13).

5.2 50 mL Erlenmeyer flasks.

5.3 Extracting solution dispenser (25 mL).

5.4 Reciprocating shaker, capable of 180 or more opm (oscillation per minute).

5.5 Nitrate-free filter paper (Schleicher and Schuel # SA720).

5.6 Filter funnels.

5.7 30 mL receiving beakers.

5.8 10 mL test tubes.

5.9 A refrigerator.

5.10 Flow Injection Autoanalyzer.

Reagents

6.1 2 M Potassium Chloride (KCl) Extracting Solution

Dissolve 150 g of KCl in one liter volumetric flask and bring to volume with deionized water. Thoroughly mix then transfer to a clean, labeled plastic bottle.

6.2 15 M Sodium Hydroxide(NaOH) Solution

Dissolve 150 g of NaOH very slowly in 250 mL of distilled water in a 500 mL beaker. CAUTION: solution becomes very hot! Swirl until completely dissolved. Cool and store in a glass flask.

6.3 Ammonium Chloride Buffer (pH 8.5).

In a hood, add 500 mL of deionized water, 105 mL of concentrated HCl, 95 mL NH_4OH, and 1.0 g of disodium EDTA to a one liter volumetric flask. Adjust the pH to 8.5 with 15 M NaOH solution. Dilute to the mark, invert to mix, then store in a glass flask.

6.4 Sulfanilamide Color Reagent

Dissolve 40.0 g of sulfanilamide and 1.0 g of N-1-naphthylethylenediamine dihydrochloride (NED) into 600 mL of deionized water in a

one liter volumetric flask. Add 100 mL of 85% phosphoric acid (H_3PO_4). Stir for 20 minutes. Dilute to the mark and invert to mix. Store in a dark brown bottle. Discard when solution turns pink.

Procedure

7.1 Scoop or weigh 10 g of air-dried soil into a 50 mL Erlenmeyer flask.

7.2 Include at least one blank and one reference sample per run.

7.3 Add 25 mL of 2 M KCl solution using an extracting solution dispenser.

7.4 Shake for 5 minutes at 180-200 oscillations per minute.

7.5 Filter the soil suspension into 30 mL receiving beakers using nitrate-free filter paper that will provide a clean filtrate without contributing measurable amounts of nitrate-N to the filtrate.

7.6 Transfer a portion of the filtrate to 10 mL test tubes for analysis.

7.7 Nitrate-N content of the filtrated soil extracts is determined by using the nitrate reduction method (Quikchem No. 12-107-04-1-B) through the Lachat Flow Injection Analyzer.

Calibration and Standards

8.1 Standard Stock Solution - 1000 ppm as NO_3^--N in 2 M KCl

Weigh 1.444 g of potassium nitrate (KNO_3) into a 200 mL of volumetric flask with 2M KCl extracting solution. Dilute to the mark and invert three times to mix. Store in a refrigerator.

8.2 Working Stock Solution - 100 ppm as NO_3^--N in 2M KCl

Pipet 20 mL of the 1000 ppm standard stock solution into a 200 mL volumetric flask. Dilute to the mark with 2M KCl extracting solution and invert three times to mix.

8.3 Working Standards

Pipette the following volumes of 100 ppm working stock solution into the corresponding volumetric flasks and dilute to volume with extracting solution:

100 ppm Working Solution	Volumetric Flask	Working Standard Conc. NO_3^--N
mL	mL	ppm
5	500	1
25	500	5
25	250	10
50	250	20

Store in glass bottles and keep in the refrigerator until ready to use.

Calculations

$$ppm\ as\ NO_3^- - N\ in\ soil = ppm\ as\ NO_3^- - N\ in\ reading\ x\ \frac{Extracting\ solution\ volume}{soil\ weight}$$

$$ppm\ as\ NO_3^- - N\ in\ soil = ppm\ as\ NO_3^- - N\ in\ reading\ x\ \frac{25}{10}$$

$$ppm\ as\ NO_3^- - N\ in\ soil = ppm\ as\ NO_3^- - N\ in\ reading\ x\ 2.5$$

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APPENDIX B

Lachet QuickChem Method 12-107-06-2-A for NH_4-N Determination

Soil Ammonium-N

Phenolate Method

Principle of the Method

1.4 The extracting solution is 2 M potassium chloride (KCl). Ammonium ions (NH_4^+) on colloidal exchange sites are brought into solution by exchange with potassium (K^+) ions.

1.5 Ammonium is reacted with alkaline phenol. Subsequent reaction with sodium hypochlorite forms indophenol blue. Sodium nitroprusside (nitroferricyanide) is added to enhance sensitivity. With a spectrophotometer absorbance is read at 630 nm.

1.6 The procedure outlined here is for use with a Lachet Flow Injection Autoanalyzer.

Range and Sensitivity

2.1 The procedure has a soil extract detection range of 1.0 to 20.0 ppm NH_4-N.

Interferences

3.3 If unfiltered, suspended matter may interfere with reading absorbance.

3.4 High concentrations of calcium and magnesium ions may precipitate.

Precision and Accuracy

4.1 Coefficients of variation of 2.1 to 3.4 % have been reported.

Equipment

5.1 Balance or 10 g scoop (NCR-13).

5.2 50 mL Erlenmeyer flasks.

5.3 Extracting solution dispenser (25 mL).

5.4 Reciprocating shaker, capable of 180 or more opm (oscillation per minute).

5.5 Filter funnels.

5.6 30 mL receiving beakers.

5.7 10 mL test tubes.

5.8 A refrigerator.

5.9 Flow Injection Autoanalyzer.

Reagents

6.1 2 M Potassium Chloride (KCl) Extracting Solution

Dissolve 150 g of KCl in a one liter volumetric flask and bring to volume with deionized water. Thoroughly mix then transfer to a clean, labeled plastic bottle.

6.2 Sodium Phenolate Solution

In a one liter volumetric flask, dissolve 88 ml of 88% liquefied phonol in about 600 ml of water. While stirring, slowly add 32 g of sodium hydroxide (NaOH). Cool, dilute to volume and invert three times to mix. CAUTION: Wear gloves. Phenol causes severe skin burns and is rapidly absorbed into the skin.

6.3 Sodium hypochlorite

Dilute 250 mL of regular Clorox bleach to 500 mL with water. Degas with helium.

6.4 Buffer

In a one liter volumetric flask, dissolve 50.0 g of disodium ethylenediamine tetraacetate (Na_2EDTA) and 5.5 g of sodium hydroxide (NaOH) in about 900 mL of water. Dilute to volume and invert three times to mix. Degas with helium.

6.5 Sodium Nitroprusside

Dissolve 3.50 g of sodium nitroprusside in one liter of water. Degas with helium.

Procedure

7.1 Scoop or weigh 10 g of air-dried soil into a 50 mL Erlenmeyer flask.

7.2 Include at least one blank and one reference sample per run.

7.3 Add 25 mL of 2 M KCl solution using an extracting solution dispenser.

7.4 Shake for 5 minutes at 180-200 oscillations per minute.

7.5 Filter the soil suspension into 30 mL receiving beakers using nitrate-free filter paper that will provide a clean filtrate without contributing measurable amounts of nitrate-N to the filtrate. This filter paper is available from Schleicher and Schuell Inc., Keene NH.

7.6 Transfer a portion of the filtrate to 10 mL test tubes for analysis.

7.7 Ammonium-N content of the filtrated soil extracts is determined by using the ammonia phenolate method (Quikchem No. 12-107-06-1-B) through the Lachat Flow Injection Analyzer.

Calibration and Standards

8.1 Standard Stock Solution - 1000 ppm as NH_4^+–N in 2 M KCl

Weigh 3.819 g of ammonium chloride (NH_4Cl) into a 200 mL of volumetric flask with 2 M KCl extracting solution. Dilute to the mark and invert three times to mix. Store in a refrigerator.

8.2 Working Stock Solution - 100 ppm as NH_4^+–N in 2 M KCl

Pipet 20 mL of the 1000 ppm standard stock solution into a 200 mL volumetric flask. Dilute to the mark with 2 M KCl extracting solution and invert three times to mix.

8.3 Working Standards

Pipette the following volumes of 100 ppm working stock solution into the corresponding volumetric flasks and dilute to volume with extracting solution:

100 ppm Working Solution	Volumetric Flask	Working Standard Conc. NH_4^+-N
mL	mL	ppm
5	500	1
25	500	5
25	250	10
50	250	20

Store in glass bottles and keep in the refrigerator until ready to use.

Calculations

$$ppm\ as\ NH_4^+ - N\ in\ soil = ppm\ as\ NH_4^+ - N\ in\ reading\ x\ \frac{Extracting\ solution\ volume}{soil\ weight}$$

$$ppm\ as\ NH_4^+ - N\ in\ soil = ppm\ as\ NH_4^+ - N\ in\ reading\ x\ \frac{25}{10}$$

$$ppm\ as\ NH_4^+ - N\ in\ soil = ppm\ as\ NH_4^+ - N\ in\ reading\ x\ 2.5$$

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APPENDIX C

Characterization of Soils within the Flood Tolerance Laboratory

Channel #1
SSURGOII Map Unit: Menfro Silt Loam, 5 to 9 % slopes
Classification: Fine, mixed, active, mesic Aquertic Hapludalfs

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay < .002	Silt .002-.05	Sand .05-.2
Ap	0-7" (0-18 cm)	70% 10YR 3/1, Crushed 30% 10YR 4/1, Crushed	Silty clay loam	35.2	60.6	4.2
Bt_1	7-14" (18-36 cm)	10YR 4/2, Crushed	Silt loam	35.7	61.0	3.4
Bt_2	14-20" (36-51 cm)	80% 10YR 4/2, Crushed 20% 10YR 4/3, Crushed	Silty clay loam	24.3	69.8	5.9

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 10YR 4/6
Bt_1	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 10YR 4/4 Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 10YR 3/4 Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 10YR 2/1 Clay films: 12% Distinct 10YR 4/1
Bt_2	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 5YR 3/4 On surfaces along pores Masses of manganese accumulation: 12% Medium 10YR 2/1 Clay films: 12% Faint 10YR 5/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	20.2	4.0	0.1	0.5	24.7	28.0	26.1	N/A
Bt_1	16.2	3.6	0.1	0.5	20.4	25.9	25.3	N/A
Bt_2	16.5	2.8	0.1	0.4	19.7	23.7	21.6	N/A

Horizon	Base Sat percent	pH		Org C (%)
	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	95	7.3	7.4	1.0
Bt_1	80	6.7	7.0	0.5
Bt_2	91	7.0	7.3	1.2

Channel #2
SSURGOII Map Unit: Nodaway Silt Loam, Occasionally Flooded
Classification: Fine, mixed, superactive, nonacid, mesic Aquertic Hapludalfs

				Total % of < 2mm		
Horizon	**Depth**	**Matrix Color**	**Texture**	**Clay < .002**	**Silt .002-.05**	**Sand .05-.2**
Ap	0-4" (0-10 cm)	10YR 3/2, Broken face	Silt Loam	29.5	65.1	5.4
Btg_1	4-12" (10-30 cm)	10YR 4/1, Broken face	Silty clay loam	30.6	64.9	4.5
Btg_2	12-22" (30-56 cm)	10YR 4/2, Broken face	Silty clay	49.6	47.3	3.1

	Redoximorphic Features
Horizon	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 10YR 4/6 Iron depletions: 1% Fine Faint 10YR 4/2
Ap	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Prominent 10YR 4/6
Btg_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 10YR 4/6 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 10YR 3/3 Iron depletions: 1% Fine Faint 10YR 5/2 Iron-manganese concretions: 1% Fine Distinct 10YR 2/1
Btg_2	

	NH_4OAc Ext. Bases meq per 100 g					**CEC meq per 100 g**		
Horizon	**Ca**	**Mg**	**Na**	**K**	**Sum Base**	**Sum Cats**	**NH_4OAc**	**Bases +Al**
Ap	15.4	3.1	N/A	0.6	19.1	23.0	23.8	N/A
Btg_1	14.7	3.5	0.1	0.4	18.7	23.5	23.7	N/A
Btg_2	19.0	5.0	0.1	0.6	24.8	32.3	33.6	N/A

	Base Sat percent		**pH**		
Horizon	**Sum**	**NH_4OAc**	**$CaCl_2$ (0.01 M)**	**H_2O**	**Org C (%)**
Ap	83	80	7.2	7.5	1.4
Btg_1	80	79	7.0	7.3	1.0
Btg_2	77	74	6.5	6.7	0.68

Channel #3
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Typic Endoaqualfs

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay < .002	Silt .002-.05	Sand .05-.2
Ap	0-7" (0-18 cm)	10YR 4/1, Crushed	Silt loam	29.9	64.0	6.1
Bg_1	7-14" (18-36 cm)	10YR 4/1, Crushed	Silt loam	24.2	69.5	6.3
Bg_s	14-19" (36-48 cm)	70% 10YR 4/1, Crushed 30% 10YR 4/2, Crushed	Silt loam	25.1	68.5	6.4

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 7.5YR 4/6
Bg_1	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 7.5YR 4/6 Iron depletions: 1% Fine Faint 10YR 5/1
Bg_s	Ironstone nodules: 12% Medium Prominent 5YR 4/6 Iron depletions: 1% Fine Distinct 10YR 5/1 Masses of manganese accumulation: 1% Fine Faint 10YR 2/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	17.5	4.0	N/A	0.5	22.0	25.0	24.5	N/A
Bg_1	15.7	2.7	N/A	0.4	18.8	21.3	18.9	N/A
Bg_2	19.1	2.4	N/A	0.5	22.0	N/A	20.1	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	88	90	7.2	7.5	1.0
Bg_1	88	100	7.2	7.5	0.8
Bg_2	N/A	100	7.4	7.7	0.9

Channel #4
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine, mixed, superactive, nonacid, mesic Aeric Endoaqualfs

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay < .002	Silt .002-.05	Sand .05-.2
Ap_1	0-7" (0-18 cm)	10YR 4/2, Crushed	Silt loam	22.4	70.6	7.1
Bt	7-16" (18-41 cm)	50% 10YR 4/2, Crushed 50% 10YR 4/3, Crushed	Silt loam	21.4	71.7	6.9
Abp	16-21" (41-53 cm)	10YR 3/2, Crushed	Silty clay loam	24.2	68.9	6.9

Horizon	Redoximorphic Features
Ap_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 10YR 5/8 Silt coats: 12% Faint 10YR 7/1
Bt	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6 Iron depletions: 12% Fine Faint 10YR 4/1 Ironstone nodules: 1% Fine 5YR 4/6 Clay films: 12% Faint 10YR 3/1
Abp	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/4 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 5YR 5/6 Iron depletions: 1% Medium Distinct 10YR 4/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	13.4	3.6	N/A	0.4	17.4	20.1	17.6	N/A
Bt_1	12.0	2.8	N/A	0.3	15.1	18.6	17.0	N/A
Abp	14.6	3.2	N/A	0.4	18.2	22.4	19.4	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	86	99	7.2	7.6	0.9
Bt_1	81	99	6.8	7.2	0.7
Abp	82	94	6.9	7.2	0.9

Channel #5
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Mollic Fluvaquents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay <-.002	Silt .002-.05	Sand .05-.2
Ap	0-8" (0-20 cm)	10YR 3/2, Broken face	Silt loam	43.1	53.4	3.4
Bg_1	8-16" (20-41 cm)	50% 10YR 4/2, Broken face 50% 10YR 4/2, Broken face	Silt loam	25.6	68.3	6.1
Bg_2	16-23" (41-58 cm)	10YR 4/2, Broken face	Silty clay loam	18.9	76.3	4.7

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 10YR 4/4 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 7.5YR 3/4 Iron depletions: 1% Fine Faint 10YR 4/2 Masses of manganese accumulation: 1% Fine Distinct 10YR 2/1
Bg_1	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 7.5YR 3/4
Bg_2	Masses of oxidized iron (Fe+3) accumulation: 12% Fine Prominent 10YR 4/6 Masses of oxidized iron (Fe+3) accumulation: 12% Fine Distinct 7.5YR 3/4

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	12.5	3.1	N/A	0.4	15.9	18.7	18.0	N/A
Bg_1	10.9	2.9	N/A	0.2	14.0	17.0	16.9	N/A
Bg_2	11.8	3.3	N/A	0.3	15.4	18.7	17.3	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	85	89	7.0	7.3	1.0
Bg_1	83	83	6.8	7.3	0.7
Bg_2	83	89	6.8	7.2	0.6

Channel #6
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Fluvaquentic Endoaquepts

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay < .002	Silt .002-.05	Sand .05-.2
Ap	0-6" (0-15 cm)	10YR 5/1, Broken face	Silt loam	21.1	74.0	4.9
Bw_1	6-11" (15-28 cm)	10YR 4/2, Broken face	Silt loam	21.2	71.4	7.4
Bw_2	11-22" (28-56 cm)	10YR 4/2, Broken face	Silt loam	22.5	69.7	7.9

Horizon	Redoximorphic Features
Ap	N/A
Bw_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 7.5YR 4/6 Iron depletions: 12% Fine Distinct 10YR 5/1 Silt coats: 12% Faint 10YR 5/1 On surfaces along pores
Bw_2	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 7.5YR 3/4 Masses of oxidized iron (Fe+3) accumulation: 12% Fine Prominent 7.5YR 4/6 Iron depletions: 12% Fine Faint 10YR 5/1 Iron-manganese concretions: 1% Fine Distinct 10YR 2/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	11.6	3.9	0.1	0.4	16.0	18.7	18.5	N/A
Bw_1	10.4	3.5	0.1	0.3	14.3	17.1	16.3	N/A
Bw_2	10.9	3.5	0.1	0.4	14.8	17.7	15.8	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	86	87	6.8	7.2	0.8
Bw_1	84	88	6.8	7.2	0.7
Bw_2	84	94	6.6	7.1	0.5

Channel #7
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Mollic Udifluvents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay < .002	Silt .002-.05	Sand .05-.2
Ap	0-7" (0-18 cm)	10YR 3/3, Interior	Silt loam	24.1	70.9	5.0
Bg_1	7-13" (18-33 cm)	90% 10YR 4/2, Interior 10% 10YR 3/3, Interior	Silt loam	21.1	71.4	7.6
Bg_2	13-25" (33-64 cm)	10YR 4/2, Interior	Silt loam	25.9	69.9	4.2

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 5/6 Silt coats: 12% Faint
Bg_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6 Iron depletions: 1% Fine Faint 10YR 4/1
Bg_2	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6 Iron depletions: 1% Fine Faint 10YR 4/1 Masses of manganese accumulation: 1% Fine Faint 10YR 2/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	13.9	3.6	0.1	0.5	18.1	20.5	20.0	N/A
Bg_1	10.7	2.8	0.1	0.3	13.9	16.5	17.2	N/A
Bg_2	11.8	3.3	0.1	0.3	15.6	19.2	18.7	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	88	90	6.6	7.0	1.1
Bg_1	84	81	6.5	6.8	0.7
Bg_2	81	83	6.3	6.7	0.6

Channel #8
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Typic Fluvaquents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay < .002	Silt .002-.05	Sand .05-.2
Ap	0-10" (0-25 cm)	10YR 4/2, Crushed	Silt loam	23.1	70.6	6.3
Bg_1	10-18" (25-46 cm)	10YR 5/2, Crushed	Silt loam	20.6	71.2	8.2
Bg_2	18-24" (46-61 cm)	10YR 5/2, Crushed	Silty clay loam	30.7	62.0	7.4

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 2% Fine Prominent 10YR 5/6
Bg_1	Iron depletions: 5% Fine Faint 10YR 5/1 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 10YR 4/6
Bg_2	Masses of oxidized iron (Fe+3) accumulation: 3% Fine Prominent 10YR 4/6

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	12.7	3.2	0.1	0.6	16.6	21.7	19.9	N/A
Bg_1	11.8	2.8	0.1	0.4	15.1	20.4	17.7	N/A
Bg_2	15.1	4.5	0.1	0.7	20.4	25.5	24.8	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	77	83	6.0	6.3	1.2
Bg_1	74	85	6.5	6.8	0.8
Bg_2	80	82	6.4	6.7	0.9

Channel #9
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Aeric Fluvaquents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay <-.002	Silt .002-.05	Sand .05-.2
Ap	0-6" (0-15 cm)	70% 10YR 4/1, Crushed 30% 10YR 4/2, Crushed	Silt loam	20.2	73.9	5.9
Bg_1	6-17" (15-43 cm)	10YR 4/1, Crushed	Silt loam	22.8	69.4	7.8
Bg_2	17-26" (43-66 cm)	70% 10YR 4/2, Crushed 30% 10YR 4/1, Crushed	Silty clay loam	27.5	68.2	4.4

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6
Bg_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6 Iron depletions: 12% Fine Faint 10YR 5/1 Masses of manganese accumulation: 12% Fine Distinct 10YR 2/1
Bg_2	Iron depletions: 1% Fine Faint 10YR 5/1 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	13.3	2.7	0.1	0.4	16.5	21.8	17.4	N/A
Bg_1	13.1	2.8	0.1	0.4	16.3	21.2	18.0	N/A
Bg_2	14.2	3.1	0.1	0.4	5.8	23.6	20.8	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	76	95	6.0	6.4	1.0
Bg_1	77	91	6.2	6.6	0.8
Bg_2	75	86	6.1	6.4	0.9

Channel #10
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Aeric Fluvaquents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay <-.002	Silt .002-.05	Sand .05-.2
Ap	0-7" (0-18 cm)	10YR 3/2, Crushed	Silt loam	23.2	70.7	6.2
Bg_1	7-15" (18-38 cm)	10YR 4/2, Crushed	Silt loam	22.3	70.7	7.0
Bg_2	15-23" (38-58 cm)	60% 10YR 4/1, Crushed 40% 10YR 4/2, Crushed	Silt loam	18.3	70.2	11.6

Horizon	Redoximorphic Features
Ap	Iron depletions: 12% Medium Faint 10YR 4/1 Masses of oxidized iron (Fe+3) accumulation: 12% Fine and medium Prominent 5YR 3/4
Bg_1	Iron-manganese nodules: 12% Fine Faint 10YR 2/1 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 10YR 4/4 Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 7.5YR 3/4
Bg_2	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 7.5YR 4/6 Masses of oxidized iron (Fe+3) accumulation: 12% Medium Faint 10YR 3/4 Masses of manganese accumulation: 1% Fine Faint 10YR 2/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	11.3	2.7	0.1	0.5	14.6	20.7	18.0	N/A
Bg_1	11.2	2.8	0.1	0.3	14.3	19.9	18.2	N/A
Bg_2	10.2	2.4	0.1	0.2	12.9	16.9	14.9	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	71	81	5.7	6.1	1.3
Bg_1	72	79	5.9	6.3	1.1
Bg_2	76	87	5.9	6.3	0.8

Channel #11
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Aquic Udifluvents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay <-.002	Silt .002-.05	Sand .05-.2
Ap	0-7" (0-18 cm)	50% 10YR 4/2, Crushed 50% 10YR 4/1, Interior	Silt loam	19.9	73.7	6.4
Bg_1	7-15" (18-38 cm)	55% 10YR 4/2, Interior 45% 10YR 4/3, Crushed	Silt loam	19.2	73.8	7.1
Bg_2	15-25" (38-64 cm)	55% 10YR 4/2, Interior 45% 10YR 4/3, Crushed	Silt loam	16.8	71.7	12.1

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6 Masses of oxidized iron (Fe+3) accumulation: 1% Medium Prominent 7.5YR 5/8 Silt coats: 100% Distinct 10YR 6/2 On vertical faces of peds
Bg_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Prominent 7.5YR 4/6 Iron depletions: 1% Fine Faint 10YR 5/1
Bg_2	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Faint 7.5YR 3/4 Iron depletions: 1% Fine Faint 10YR 5/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	11.3	2.8	0.1	0.4	14.6	18.9	18.0	14.6
Bg_1	11.3	2.4	0.1	0.3	14.1	18.2	16.6	14.1
Bg_2	9.1	2.0	0.1	0.3	11.5	14.8	14.3	11.5

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	77	81	5.5	5.9	1.1
Bg_1	77	85	6.1	6.4	0.9
Bg_2	78	80	6.2	6.5	0.6

Channel #12
SSURGOII Map Unit: NODAWAY SILT LOAM, OCCASIONALLY FLOODED
Classification: Fine-silty, mixed, superactive, nonacid, mesic Mollic Udifluvents

Horizon	Depth	Matrix Color	Texture	Total % of < 2mm		
				Clay <-.002	Silt .002-.05	Sand .05-.2
Ap	0-7" (0-18 cm)	10YR 4/2, Interior	Silt loam	21.5	63.0	15.5
Bg_1	7-18" (18-46 cm)	10YR 4/2, Interior	Silt loam	19.6	72.9	7.5
Bg_2	18-25" (46-64 cm)	10YR 4/2, Interior	Silt loam	22.6	70.0	7.4

Horizon	Redoximorphic Features
Ap	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 10YR 5/6 Organic stains: 70% Faint 10YR 2/1
Bg_1	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 10YR 5/6 Iron depletions: 12% Fine Faint 10YR 5/1
Bg_2	Masses of oxidized iron (Fe+3) accumulation: 1% Fine Distinct 10YR 5/6 Iron depletions: 30% Fine Faint 10YR 5/1

Horizon	NH_4OAc Ext. Bases meq per 100 g					CEC meq per 100 g		
	Ca	Mg	Na	K	Sum Base	Sum Cats	NH_4OAc	Bases +Al
Ap	13.0	2.8	N/A	0.5	16.3	20.8	17.9	N/A
Bg_1	10.4	2.4	0.1	0.4	13.2	17.4	14.8	N/A
Bg_2	11.8	2.7	0.1	0.3	14.9	19.5	16.3	N/A

Horizon	Base Sat percent		pH		Org C (%)
	Sum	NH_4OAc	$CaCl_2$ (0.01 M)	H_2O	
Ap	78	91	5.9	6.3	1.2
Bg_1	76	89	5.9	6.3	0.8
Bg_2	76	91	5.7	6.1	0.7

APPENDIX D

Procedure for Acid Detergent Fiber (ADF) Analysis

ANKOM Technology Method 10-21-05

Acid Detergent Fiber in Feeds Filter Bag Technique

(ANKOM^{200})

DEFINITION

This method determines Acid Detergent Fiber, which is the residue remaining after digesting with H_2SO_4 and CTAB. The fiber residues are predominantly cellulose and lignin.

SCOPE

This method is applicable to grains, feeds, forages and all fiber-bearing material.

APPARATUS

1. Analytical Balance—capable of weighing 0.1 mg.
2. Oven—capable of maintaining a temperature of 102±2°C.
3. Digestion instrument—capable of performing the digestion at 100±0.5°C and maintaining a pressure of 10-25 psi. The instrument must also be capable of creating a similar flow around each sample to ensure uniformity of extraction (ANKOM^{200}, 65 rpm agitation, ANKOM Technology).
4. Filter bags—constructed from chemically inert and heat resistant filter media, capable of being heat sealed closed and able to retain 25 micron particles while permitting rapid solution penetration (F57, ANKOM Technology).
5. Heat sealer—sufficient for sealing the filter bags closed to ensure complete closure (1915, ANKOM Technology).
6. Desiccator pouch—collapsible sealable pouch with desiccant inside that enables the removal of air from around the filter bags (MoistureStop Weigh Pouch, ANKOM Technology).
7. Marking pen—solvent and acid resistant (F08, ANKOM Technology).

REAGENTS

1. Acid Detergent Solution—add 20 g cetyl trimethylammonium bromide (CTAB) to 1 L 1.00*N* H_2SO_4 previously standardized (premixed chemical solution available from ANKOM). Agitate and heat to aid solution. (see Notes, *Caution*).

PREPARATION OF SAMPLE

Grind samples in a centrifugal mill with a 2 mm screen or cutter type (Wiley) mill with a 1 mm screen. Samples ground finer may have particle loss from the filter bags and result in low values.

PROCEDURE

1. Use a solvent resistant marker to label the filter bags. Weigh filter bag (W_1) and zero balance.

   Note—Do not pre-dry filter bags; any moisture will be accounted for by the blank bag correction.
2. Weigh 0.45-0.55 g of prepared sample (W_2) directly in filter bag. Avoid placing the sample on the upper 4 mm of the bag.
3. Using a heat sealer, completely seal the upper edge of the filter bag within 4 mm of the top.

   Note—Use sufficient heat to completely seal the filter bag and allow enough cool time (2 sec) before removing the bag from the heat sealer.
4. Weigh one blank bag and include in run to determine blank bag correction (C_1).
5. **Pre-extract only samples containing soybean products or >5% fat:** Extract samples by placing 24 bags with samples into a container with a top. Pour enough acetone into container to cover bags and secure top. Shake the container 10 times and allow bags to soak for 10 minutes. Repeat with fresh acetone. Pour out acetone and place bags on a wire screen to air-dry. **Exception - Roasted soybean:** Due to the processing of roasted soy a modification to the extraction is required. Place roasted soy samples into a container with a top. Pour enough acetone into container to cover bags and secure top. Shake the container 10 times and pour off acetone. Add fresh acetone and allow samples to soak for twelve hours. After soak time, pour out acetone and place bags on a wire screen to air-dry.
6. Place a maximum of 24 bags into the Bag Suspender. All nine trays should be used regardless of the number of bags being processed. Place three bags per tray and then stack trays on center post with each level rotated 120 degrees. Insert the Bag Suspender with bags into the fiber analyzer vessel and place the Bag Suspender weight on top to keep it submerged.

   Note—Prior to inserting the Bag Suspender, if the vessel temperature is warm from a previous run, add cold water and exhaust.

7. When processing 24 sample bags, add 1900-2000mL of ambient temperature AD solution to the fiber analyzer vessel. If processing less than 20 bags, add 100 ml/bag of AD solution (use minimum of 1500 mL to ensure Bag Suspender is covered).
8. Turn Agitate and Heat ON and confirm agitation. Set timer for 60 min and close lid.
9. At end of extraction, turn Heat and Agitate off. Open the drain valve (slowly at first) and exhaust hot solution before opening lid.

   Note—The solution in the vessel is under pressure. The exhaust valve needs to be opened to release the pressure and solution prior to opening the lid.
10. After the solution has been exhausted, close the exhaust valve and open the lid. Add 1900-2000mL of (70-90°C) rinse water. Turn Agitate on and rinse for 5 min. The lid may be sealed with the Heat on or left open with the Heat off. Repeat 5 min. hot water rinses a total of three times or until water is neutral pH.
11. When the rinsing process is complete remove the samples. Gently press out excess water from bags. Place bags in a 250 mL beaker and add enough acetone to cover bags and soak for 3-5 min.
12. Remove bags from acetone and place on a wire screen to air-dry. Completely dry in oven at 102±2°C (most ovens will complete drying within 2-4 hrs).

    Note—Do not place bags in the oven until acetone has completely evaporated.
13. Remove bags from oven, place directly into a collapsible desiccant pouch and flatten to remove air. Cool to ambient temperature and weigh bags.

    Note—Do not use conventional desiccator container.

CALCULATIONS

$$\% \text{ ADF (as-received basis)} = \frac{(W_3 - (W_1 \times C_1))}{W_2} \times 100$$

Where:
- W_1 = Bag tare weight
- W_2 = Sample weight
- W_3 = Dried weight of bag with fiber after extraction process
- C_1 = Blank bag correction (final oven-dried weight divided by original blank bag weight)

NOTES

Caution

Sulfuric acid is a strong acid and will cause severe burns. Protective clothing should be worn when working with this acid. Always add acid to water and not the reverse.

CTAB will irritate the mucous membranes. A dust mask and gloves should be worn when handling this chemical.

Acetone is extremely flammable. Avoid static electricity and use a fume hood when handling.

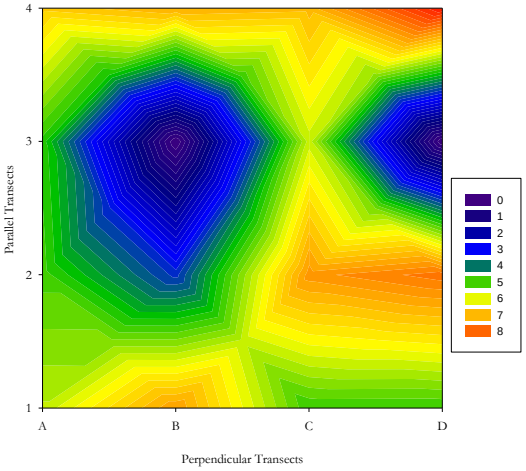
Appendix E:

Floodplain Inorganic-N and Total Soluble Polyphenolics

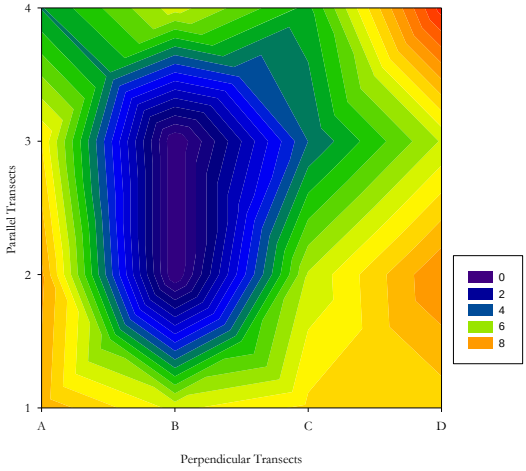
Contour maps of soil chemicals for each plot: 1) Ammonium, 2) Nitrate, and 3) Total Soluble Polyphenolics. Note different scales for each plot. Areas with violet-blue colors had lower concentrations of the chemical, while areas with yellow-red colors had higher concentrations of the chemical and areas with green had intermediate levels of the chemical. Parallel transects numbers correspond to position relative to stream bank; i.e. parallel transect 1 is closest to the stream (20 m distance from stream bank) while parallel transect 4 is farthest from the stream (50 m distance from stream bank).

Soil Ammonium

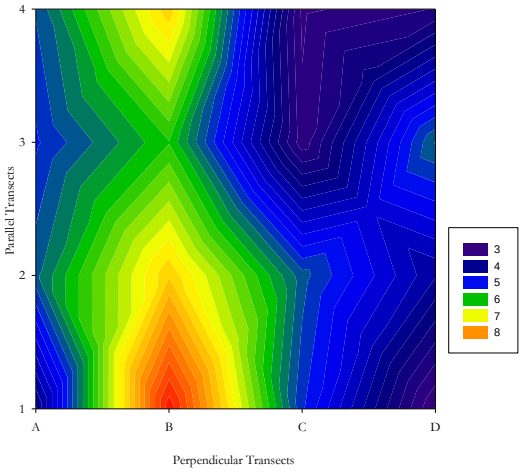
Yellow Creek Plot 1



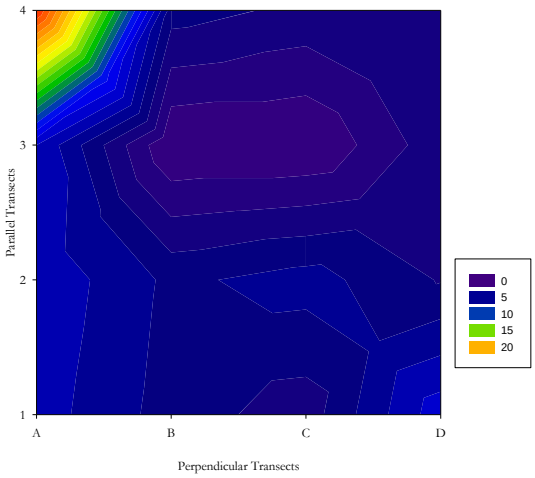
Yellow Creek Plot 2



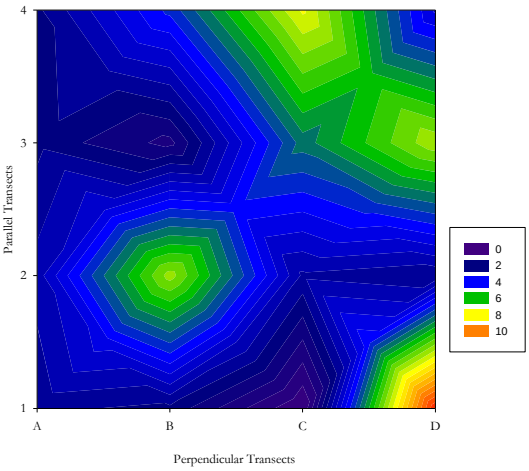
Locust Creek Plot 1



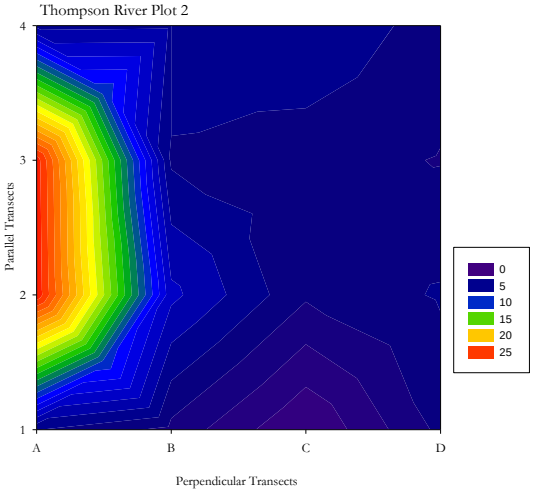
Locust Creek Plot 2



Thompson River Plot 1

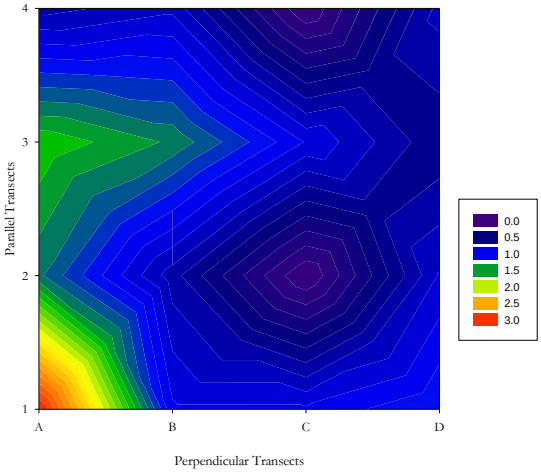


Thompson River Plot 2

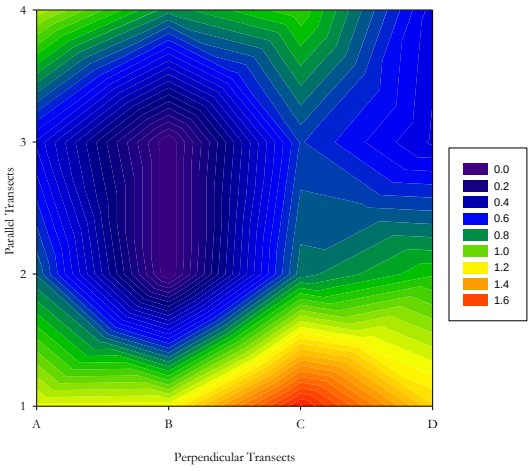


Soil Nitrate

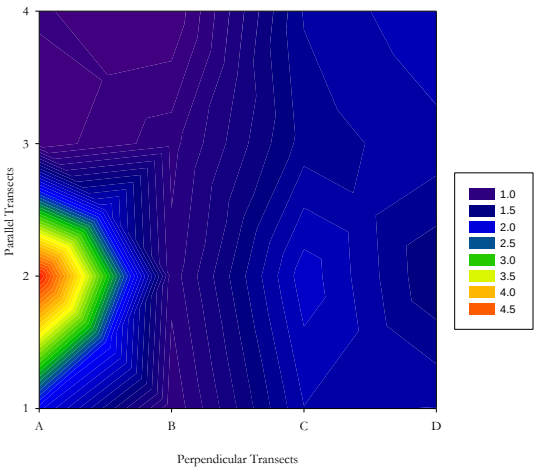
Yellow Creek Plot 1



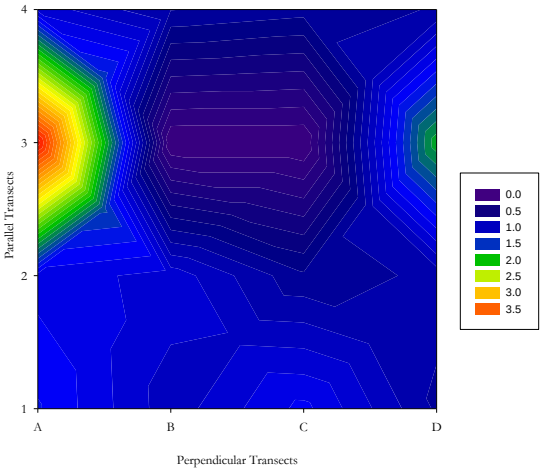
Yellow Creek Plot 2



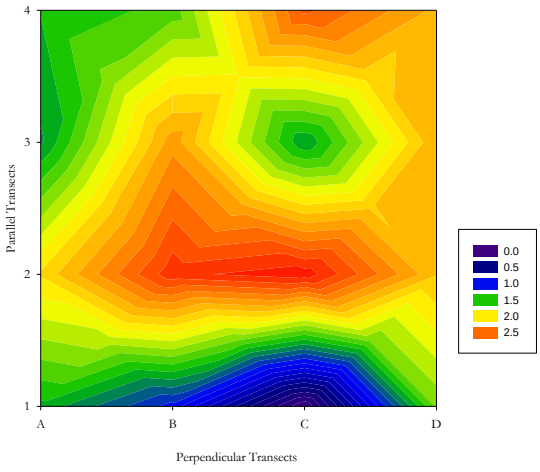
Locust Creek Plot 1



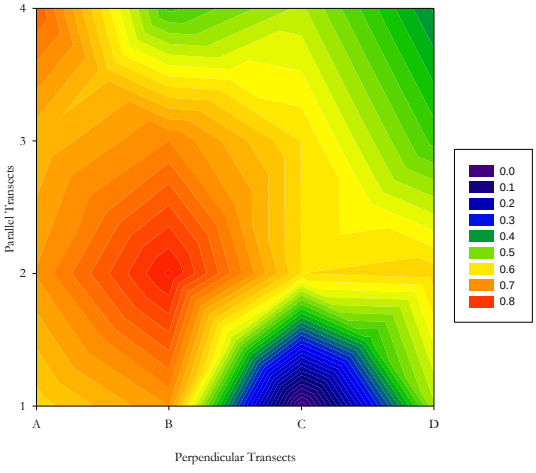
Locust Creek Plot 2



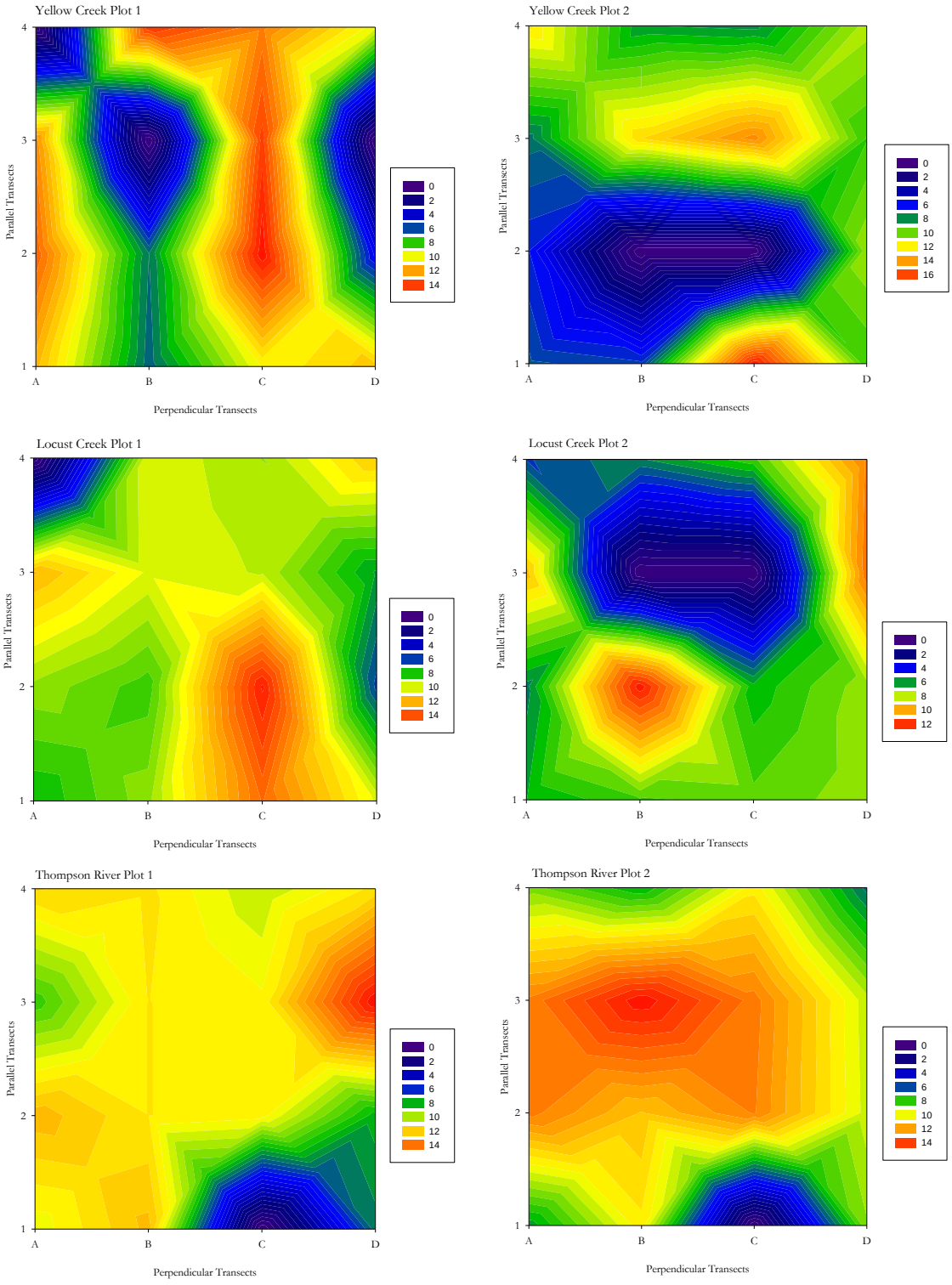
Thompson River Plot 1



Thompson River Plot 2



Soil Total Soluble Polyphenolics



APPENDIX F

Composite Species List for the Three Study Sites

National and regional wetland indicator status for species was taken from the USDA Plants Database (http://plants.usda.gov/wetland.html). National designations represent the range of frequency of occurrence of a species occurring in wetlands vs. non-wetlands across the entire distribution of the species. Regional designations represent the likelihood of species occurring in wetlands versus non-wetlands in the region. Designations are reported for Region 3 which includes: Iowa, Illinois, Indiana, Michigan, Minnesota, Missouri and Wisconsin. Designations are as follows: obligate wetland (OBL) = occurs almost always (~99%) under natural conditions in wetlands; facultative wetland (FACW) = usually occurs in wetlands (~67%-99%) but occasionally found in non-wetlands; facultative (FAC) = equally likely to occur in wetlands or non-wetlands (~34%-66%); facultative upland (FACU) = usually occurs in non-wetlands (~67%-99%), but occasionally found on wetlands (~1%-33%); obligate upland (UPL)= occurs in wetlands in another region, but occurs almost always (~99%) under natural conditions in non-wetlands in the region specified. National status indicators followed by (+) = species is more frequently found in wetlands; if followed by (-) = species is less frequently found in wetlands. Regional status indicators followed by (*) = tentative assignment based on limited information.

A) Yellow Creek Species List

Species	Common Name	National Wetland Indicator Status	R3 Wetland Indicator Status
Acer saccharinum	silver maple	FAC, FACW	FACW
Boehmeria cylindrica	smallspike false nettle	FACW, OBL	OBL
Carex grayii	Gray's sedge	FACW, FACW+	FACW+
Carex muskingumensis	Muskingum sedge	OBL	OBL
Carex squarrosa	squarrose sedge	FACW, OBL	OBL
Carya laciniosa	Shellbark hickory	FAC, FACW	FACW
Celtis occidentalis	Common hackberry	FACU, FAC	FAC-
Chasmanthium latifolium	Indian woodoats	UPL, FACW	FACW
Elymus virginicus	Virginia wildrye	FAC, FACW	FAC-
Fraxinus pennsylvanica	green ash	FAC, FACW	FACW
Glyeria striata	fowl mannagrass	OBL	OBL
Impatiens capensis	Jewelweed	FACW, FACW+	FACW
Menispermum canadense	Common moonseed	FAC	FAC*
Morus rubra	red mulberry	FACU, FAC	FACU
Pilea pumila	Canadian clearweed	FAC, FACW	FACW
Poa compressa	Canada bluegrass	FACU-, FAC	FACU+
Quercus macrocarpa	bur oak	FACU, FAC	FAC-
Quercus palustris	pin oak	FAC, FACW	FACW
Stachys tenuifolia	smooth hedgenettle	FACW-, OBL	OBL
Ulmus rubra	slippery elm	FAC	FAC

B) Locust Creek Species List

Species	Common Name	National Wetland Indicator Status	R3 Wetland Indicator Status
Acer saccharinum	silver maple	FAC, FACW	FACW
Acer negundo	box elder	FAC, FACW	FACW-
Carya laciniosa	Shellbark hickory	FAC, FACW	FACW
Celtis occidentalis	Common hackberry	FACU, FAC	FAC-
Elymus virginicus	Virginia wildrye	FAC, FACW	FAC-
Fraxinus pennsylvanica	green ash	FAC, FACW	FACW
Gratiola neglecta	clammy hedgehyssop	OBL	OBL
Impatiens capensis	Jewelweed	FACW, FACW+	FACW
Laportea canadensis	Canadian woodnettle	FAC, FACW	FACW
Morus rubra	red mulberry	FACU, FAC	FACU
Parthenocissus quinquefolia	Virginia creeper	FACU, FAC	FAC-
Platanus occidentalis	American sycamore	FAC, FACW	FACW
Poa pratensis	Kentucky bluegrass	UPL, OBL	FAC-
Populus deltoides	eastern cottonwood	FAC, FACW	FAC+
Rudbeckia laciniata	cutleaf coneflower	FACU, FACW	FAC
Ulmus americana	American elm	FAC, FACW	FACW-
Ulmus rubra	slippery elm	FAC	FAC
Zizia aurea	golden zizia	FAC-, FAC+	FAC+

C) Thompson River Species List

Species	Common Name	National Wetland Indicator Status	R3 Wetland Indicator Status
Acer negundo	box elder	FAC, FACW	FACW-
Amphicarpaea bracteata	American hogpeanut	FACU, FACW	FAC
Carya laciniosa	Shellbark hickory	FAC, FACW	FACW
Celtis occidentalis	Common hackberry	FACU, FAC	FAC-
Chaerophyllum procumbens	spreading chervil	FAC, FACW	FAC+
Elymus virginicus	Virginia wildrye	FAC, FACW	FAC-
Fraxinus pennsylvanica	green ash	FAC, FACW	FACW
Gratiola neglecta	clammy hedgehyssop	OBL	OBL
Hibiscus laevis	halberdleaf rosemallow	OBL	OBL
Juglans nigra	black walnut	FACU	FACU
Laportea canadensis	Canadian woodnettle	FAC, FACW	FACW
Morus rubra	red mulberry	FACU, FAC	FACU
Parthenocissus quinquefolia	Virginia creeper	FACU, FAC	FAC-
Platanus occidentalis	American sycamore	FAC, FACW	FACW
Quercus macrocarpa	bur oak	FACU, FAC	FAC-
Sambucus canadensis	Common elderberry	UPL, FACW	FACW
Sanicula odorata	Clustered blacksnakeroot	FACU, FAC+	FAC+
Stachys tenuifolia	smooth hedgenettle	FACW-, OBL	OBL
Ulmus americana	American elm	FAC, FACW	FACW-
Ulmus rubra	slippery elm	FAC	FAC
Zizia aurea	golden zizia	FAC-, FAC+	FAC+

VITA

Irene Unger was born September 28, 1968 in St. Louis, Missouri, USA. She received her B.S. in Biology at Truman State University (1990); a M.S. (Research) at St. Louis University (1994) where she focused on the effects of clearcut logging on the herbaceous community of a Missouri deciduous forest. She was an adjunct instructor of Biology at St. Louis Community College for two years before accepting a full-time, tenure tract position in Biology at Missouri State University-West Plains. Irene was at Missouri State University-West Plains for nine years; while there she earned tenure, was promoted to the rank of Assistant Professor and served as Department Chair for the Biology Department. In 2004, she began working towards her doctoral degree in the Forestry Department at the University of Missouri.