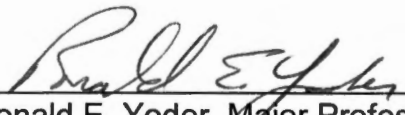
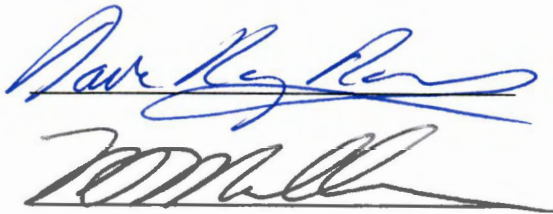


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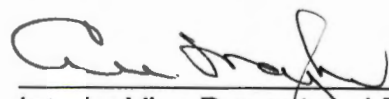
I am submitting herewith a thesis written by Carmen T. Anderson entitled "Examination of Hydrological, Chemical, and Biological Gradients along the Hyporheic Zone of an Upland Agricultural Stream in East Tennessee." I have examined the final copy of this thesis for form and content and recommend that it be accepted in partial fulfillment of the requirements for the degree of Master of Science, with a major in Biosystems Engineering.


Ronald E. Yoder, Major Professor

We have read this thesis
and recommend its acceptance:



Accepted for the Council:


Interim Vice Provost and Dean of
The Graduate School

**EXAMINATION OF HYDROLOGICAL,
CHEMICAL, AND BIOLOGICAL GRADIENTS
ALONG THE HYPORHEIC ZONE OF AN UPLAND
AGRICULTURAL STREAM IN EAST TENNESSEE**

A Thesis

Presented for the

Master of Science Degree

The University of Tennessee, Knoxville

Carmen T. Anderson

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Thesis
2000
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***To my parents Richard and Sandy Truitt
whose love and support have allowed me
to accomplish so much.***

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ABSTRACT

The hyporheic zone, an area of subsurface mixing of stream and groundwater experiences bi-directional movement of water and solutes that reduces pollutant loads in the water. Research of the hyporheic zone in an upland pasture along Hickory Creek in East Tennessee identified patterns of nutrient transformation in both the horizontal and vertical planes. Two transects of shallow groundwater wells were installed to examine the horizontal gradient, and vertical gradients were studied using suction lysimeters installed at three depths near each shallow groundwater well and peepers installed in the stream. Analyses for oxidation-reduction potential, pH, nitrate-N, ammonia-N, sulfate-S, sulfide, chloride, ferric iron, ferrous iron, total organic carbon, biochemical oxygen demand, chemical oxygen demand, fecal coliforms, fecal streptococci, and total solids were conducted on the shallow groundwater, while the suction lysimeter samples and peeper samples were analyzed for nitrate-N, sulfate-S, chloride, and total organic carbon. Additionally, soil samples were collected from around each well and were analyzed for microbial biomass, dehydrogenase activity, L-asparaginases, acid and alkaline phosphomonoesterases, arylsulfatases, soil moisture content, and soil pH. Composite samples from Hickory Creek were analyzed for nitrate-N, sulfate-S, chloride, and total organic carbon concentrations. Piezometer nests located at each shallow groundwater well and at two intermediate locations indicated the occurrence of a wet and a dry season.

Statistical analyses indicated that nitrification potential decreased with increasing distance from the stream while denitrification potential increased with increasing distance from the stream; organic concentrations (biochemical oxygen demand, chemical oxygen demand, and total organic carbon) increased with distance from the stream edge; both the oxidation-reduction potential and the pH decreased as distance from the stream edge increased; and ferrous iron concentrations increased with increasing distance from the stream. Significant trends were noted for microbial biomass and soil moisture content, with both increasing with distance from the stream. Vertically, there was an increase in denitrification potential with depth, a decrease in sulfate-S concentration with depth, and an increase in total organic carbon with depth. Two main points were confirmed by the research, 1.) oxidation and reduction processes were the primary means of constituent transformations and not microbial mineralization, and 2.) the lengths of the water flow paths and the retention times of the water in the hyporheic zone were important factors in determining the ability of the hyporheic zone to provide treatment.

TABLE OF CONTENTS

CHAPTER	PAGE
1. INTRODUCTION	1
BACKGROUND.....	1
OBJECTIVES	2
2. LITERATURE REVIEW	4
HYPORHEIC ZONE	4
NITROGEN	8
Nitrification	11
Denitrification	12
Nitrogen Mineralization/Immobilization	14
Previous Research on Nitrogen in the Hyporheic Zone	15
PHOSPHORUS	21
Biological Subcycle	24
Geochemical Subcycle	26
Previous Research on Phosphorus in the Hyporheic Zone	27
SULFUR	31
Previous Research on Sulfur in the Hyporheic Zone	34
IRON	36

Previous Research on Iron in the Hyporheic Zone	38
FECAL COLIFORMS AND FECAL STREPTOCOCCI	40
Previous Research on Fecal Coliforms and Fecal Streptococci in the Hyporheic Zone	42
ORGANIC CARBON	44
Previous Research on Carbon in the Hyporheic Zone	45
DISSOLVED OXYGEN	49
Previous Research on Dissolved Oxygen in the Hyporheic Zone	50
BIOCHEMICAL OXYGEN DEMAND	53
Previous Research on Biochemical Oxygen Demand in the Hyporheic Zone	54
CHEMICAL OXYGEN DEMAND	55
Previous Research on Chemical Oxygen Demand in the Hyporheic Zone	56
TOTAL SOLIDS	57
Previous Research on Total Solids in the Hyporheic Zone	58
pH	59
Previous Research on pH in the Hyporheic Zone	60
OXIDATION-REDUCTION POTENTIAL	62
Previous Research on Redox Potential in the Hyporheic Zone	64

TEMPERATURE	65
Previous Research on Temperature in the Hyporheic Zone	67
STREAM DISCHARGE	68
Previous Research on Streamflow Effects on the Hyporheic Zone	70
MICROBIAL ASSAYS	72
Microbial Biomass	73
Dehydrogenase	74
L-asparaginase	75
Phosphomonoesterases	75
Arylsulfatase	77
Previous Research on Microbial Assays in the Hyporheic Zone	78
3. MATERIALS AND METHODS	79
SITE DESCRIPTION	79
CLIMATE	85
VEGETATION	88
SOIL TYPE	88
WELLS	94
Construction	94

Installation	98
Well Preparation	108
Sampling	110
SUCTION LYSIMETERS	110
Installation	114
Sampling	125
PIEZOMETERS	125
DATALOGGER	127
Pt ELECTRODES	135
Construction	135
Testing	135
Installation	136
STREAM SAMPLING	139
PEEPERS	139
Construction	139
Installation	143
Sampling	148
STREAM GAUGING	148
SURVEYING/MAPPING	150

SOIL SAMPLES	156
WELL TEST	157
LABORATORY ANALYSES	158
STATISTICAL ANALYSES	160
4. RESULTS AND DISCUSSION	162
FLOW PATTERNS	162
HORIZONTAL CONCENTRATION GRADIENTS	179
Nitrogen	180
Organics	182
Physical Parameters	184
Iron	186
Sulfur	188
Other Constituents	189
Enzymes	189
VERTICAL CONCENTRATION GRADIENTS	193
Nitrate-N	194
Sulfate-S	195
Chloride	195
Dissolved Organic Carbon	196

WELL COMPARISONS	196
5. SUMMARY AND CONCLUSIONS	208
SUMMARY	208
CONCLUSIONS.....	209
REFERENCES	211
APPENDICES	224
VITA	228

LIST OF TABLES

TABLE	PAGE
3.1. Target well depths were selected, but the actual well depths were often less because of the location of the bedrock	104
3.2. Constituent concentrations typically decreased with rinsing	113
3.3. Suction lysimeter depths with respect to an arbitrary benchmark of 30.48 m (100 ft)	123
3.4. Locations and depths of the installed Pt electrodes	137
3.5. Microbial assay methods used	157
3.6. Water chemistry laboratory methods. SM denotes <i>Standard Methods</i>	159
4.1. Mean separation results for Transect 1 Well 2 and Transect 2 Well 2	169
4.2. Mean separation results for all the wells	198

LIST OF FIGURES

FIGURES	PAGE
2.1. Vertical and lateral exchanges of stream and groundwater within the hyporheic zone (shaded area)	6
2.2. The main components of the nitrogen cycle	9
2.3. The phosphorus cycle is divided into two subcycles	23
2.4. Phosphorus cycling in the bottom sediments and overlying waters	29
2.5. The sulfur cycle is similar to the nitrogen cycle	33
2.6. Environmental transformations of iron	37
2.7. The hydrological cycle beginning with precipitation	69
3.1. Morrison and Szecsody (1985) designed a monitoring well and lysimeter combination	95
3.2. Two sets of slots were cut into the PVC pipe to allow for water entry	97
3.3. Site map for the shallow groundwater monitoring wells	101
3.4. A vacuum was applied to the above reservoir to prepare the wells for sampling	109
3.5. Extension tubes allowed for the installation of the lysimeter at three distinct depths	112
3.6. Site map for the suction lysimeters at T1W1	115
3.7. Site map for the suction lysimeters at T1W2	116
3.8. Site map for the suction lysimeters at T1W3	117
3.9. Site map for the suction lysimeters at T1W4	118
3.10. Site map for the suction lysimeters at T1W5	119

3.11.	Site map for the suction lysimeters at T2W1	120
3.12.	Site map for the suction lysimeters at T2W2	121
3.13.	Site map for the suction lysimeters at T2W3	122
3.14.	The peeper was designed to allow for vertical constituent measurements	142
3.15.	Site map of the hyporheic wells generated from survey data	154
3.16.	Site map for the wetland wells generated from survey data	155
4.1.	Groundwater flow patterns during the wet season using piezometer A at T1W2	164
4.2.	Groundwater flow patterns during the dry season using piezometer A at T1W2	165
4.3.	Groundwater flow patterns during the wet season using piezometer B at T1W2	166
4.4.	Groundwater flow patterns during the dry season using piezometer B at T1W2	167
4.5.	DOC concentrations increased after the initial sample was collected	173
4.6.	This well exhibited a similar trend to Transect 1 Well 2, but at a slower rate	174
4.7.	The largest changes in DOC concentrations occurred at Transect 1 Well 3	175
4.8.	Equilibrium was quickly reached at this well	176

LIST OF PLATES

PLATES	PAGE
3.1. Cattle feeding on hay near the study site	80
3.2. Hay is cultivated on the farm	81
3.3. The cattle have free access to Hickory Creek	82
3.4. This spring is located near a feeding area for the cattle and flows into Hickory Creek	83
3.5. Damaged tile has allowed this wetland to reemerge	84
3.6. Water exits the tile system at one of two points along Hickory Creek. This exit is immediately downstream of the study site	86
3.7. The marsh, fed by a spring, is located upstream of the study site and a small stream carries water from the marsh to Hickory Creek	87
3.8. Over 50% of the vegetation in the wetland is bulrush, and as the wetland spreads, so do the bulrushes	89
3.9. A Giddings sampler truck was used to take core samples of the soil	90
3.10. Hollow steel tubes were used to take the core samples	91
3.11. The samples were removed from the hollow steel tubes and laid on divided PVC pipe	92
3.12. The preliminary analysis of the core samples was conducted in the field	93
3.13. Sampling tubing was dedicated to each well and remained in the well throughout the study	99

3.14.	Corrugated pipe was placed over each monitoring well for protection	100
3.15.	The Gopher™ one-man auger was used to dig the well holes, but actually required two personnel to operate	102
3.16.	A 15.24-cm (6-in.) drill bit with extensions was used to auger the hole	103
3.17.	A hand-held bucket auger was used to remove excess soil prior to the installation of the wells	106
3.18.	Bentonite and grout layers were used to prevent water flow downward along the well casing	107
3.19.	The installation of the lysimeters was done using a preconstructed model	124
3.20.	A hand held pump was used to create a vacuum while a second person placed a clamp ring over the tubing to maintain the vacuum	126
3.21.	Piezometers were located around each monitoring well	128
3.22.	A CR10 datalogger by Campbell Scientific was used to collect data at 15-min intervals	129
3.23.	All wiring was run through PVC pipe to prevent damage	130
3.24.	The rain gauge was located in an open are between the wetland and the stream	132
3.25.	This TFLI was located along Transect 1	133
3.26.	TFLI 2 was located along Transect 2	134
3.27.	While the measurement of this Pt electrode is not at the study site, the general principle is demonstrated	138
3.28.	A 40-ml sample was taken from the creek hourly, and each bottle contained a 6-hr composite	140

3.29.	A steel cage constructed by Wesley Wright protected the ISCO sampler from damage by cattle or storms	141
3.30.	The peeper consisted of 10 chambers for vertical water sampling	144
3.31.	Assembly of the peepers was completed while submerged in deionized water	145
3.32.	Using a hammer, the peepers were installed into the hyporheic zone	146
3.33.	A nylon rope tied to the peeper was secured to a nearby tree to prevent loss in case of flooding	147
3.34.	The discharge of the stream was measured using a current meter and measurements of the cross sectional area of ten segments	149
A-1	Appendices CD-ROM	pocket

CHAPTER 1

INTRODUCTION

BACKGROUND

Fresh water is critical for human existence with each person requiring two liters of water per day for drinking. Without water, a human being could survive only a few days. Of all the water present on earth, less than three percent is available for human consumption; however, "less than one hundredth of 1% of the water on earth exists in the atmosphere, rivers, or lakes" where it is accessible (Arms, 1990). Arms (1990) further states that 20% of the fresh water in the United States was deemed unsafe for human consumption. The Tennessee Department of Environment and Conservation notes that approximately 26% of the state's streams are below standards established by the State of Tennessee (TDEC, 1998). Human life, as we know it, exists in part due to the quality of water around us. Our daily lives revolve around school, drinking, bathing, and recreation; activities that are directly or indirectly connected to water. Our health and enjoyment in association with these activities depends on the quality of water from the tap and from natural reservoirs such as streams, rivers, and lakes.

Non-point sources of pollution such as urban, industrial, and agricultural run-off affect waterways in East Tennessee, thus affecting the quality of water for use by local citizens. Streams naturally contain organisms that decompose many pollutants that enter the system, but the recent increase in human, industrial, and agricultural activity has produced a quantity of contaminants in excess of the stream's self cleansing abilities (Arms, 1990). Researchers have examined methods such as maintenance of riparian or vegetative buffer strips for controlling non-point sources of pollution before they enter the stream (Jordan et al., 1993; Smith, 1992; Cooper et al., 1995; Lawrance et al., 1984; Gilliam, 1994; Lawrance et al., 1997), but questions remain concerning processes that reduce pollutant loads as contaminants move toward and reach streams. One area that demonstrates promise for removing pollutants is the hyporheic zone, an area of subsurface mixing of stream and groundwater where bi-directional movement of water and solutes occurs (Triska et al., 1993; Hill, 1996).

OBJECTIVES

The objective of this study was to identify the hydrological, biological, and chemical processes occurring in the hyporheic zone and to characterize the effect of these processes on the water quality of an upland stream located in a grazing area with cattle access to the stream.

Specific objectives were as follow:

1. Determine the existence of any horizontal trends in the hyporheic zone related to a.) oxidation reduction potential (redox), b.) pH, c.) nitrate-N ($\text{NO}_3\text{-N}$), d.) ammonium-N ($\text{NH}_3\text{-N}$), e.) sulfate-S ($\text{SO}_4\text{-S}$), f.) sulfide (S^{2-}), g.) chloride (Cl), h.) ferric iron (Fe^{3+}), i.) ferrous iron (Fe^{2+}), j.) total organic carbon (TOC), k.) biochemical oxygen demand (BOD_5), l.) chemical oxygen demand (COD), m.) fecal coliforms (FC), n.) fecal streptococci (FS), o.) total solids (TS), p.) microbial biomass, q.) dehydrogenase, r.) arylsulfatase, s.) L-asparaginase, t.) soil pH, and u.) soil moisture content.
2. Determine the existence of any vertical trends in the hyporheic zone related to a.) nitrate-N ($\text{NO}_3\text{-N}$), b.) sulfate-S ($\text{SO}_4\text{-S}$), c.) chloride (Cl), and d.) total organic carbon (TOC).
3. Determine if these trends were correlated to groundwater flow paths, seasonality, and/or microbial activity.

Knowledge gained from this study will assist in developing a more clear understanding of the role of the hyporheic zone in water quality. Information gained from the study may be used in a variety of applications including the reproduction or enhancement of hyporheic processes by controlling horizontal and vertical trends, possible usage in stream restoration projects, and better stream management practices.

CHAPTER 2

LITERATURE REVIEW

HYPORHEIC ZONE

Much research acknowledges the effectiveness of Best Management Practices (BMPs) for reducing the introduction of pollutant loads into waterways. Research in Kentucky has focused on the function of grass filter strips in preventing fecal coliforms and fecal streptococci from entering streams (Coyne et al., 1998). Jennings et al. (1998) noted that a riparian BMP system in North Carolina had an annual pollutant load reduction for nitrate plus nitrite, total solids, and median fecal coliform and fecal streptococci bacteria concentrations of 40, 80, and 90%. Research by Barone et al. (1998) demonstrated the effectiveness of vegetative filter strips using filters 8.5 m wide with a reduction of 97% for flow weighted sediment concentrations (settling of soil particles in runoff), 92% for total phosphorus, 84% for total Kjeldahl nitrogen, and 94% for *Escherichia coli*. However, little research on the ability of the hyporheic zone to remove nutrients and fecals has been performed.

The setting for a large portion of research on the hyporheic zone was northwestern California, areas in or near Canada, and desert regions (Grimm and

Fisher, 1984; Hill, 1990; Triska et al., 1990; Duff and Triska, 1990; Hendricks and White, 1991; Triska et al., 1993; Hill and Lymburner, 1998). Thus, little research has been done on the hyporheic zone in the southeastern United States, especially in the area of agriculture. This study attempts to address this issue and fill a gap in hyporheic research.

Along the stream side, an area exists that experiences bi-directional movement of surface water and dissolved solutes due to “channel hydrodynamics” (Triska et al., 1993) (Figure 2.1). Within the hyporheic zone, a hydrological link connects sediments to the stream (Findlay, 1995); surface water and groundwater mix in the subsurface creating hyporheic or interstitial waters (Hill, 1996). Hill and Lymburner (1998) defined the outer boundary of the hyporheic zone as the location where greater than 10% of channel water is present. The composition of the stream and groundwater can undergo biological transformations within the hyporheic zone as physical processes force water into contact with sediments and into aerobic and anaerobic microsites (Findlay, 1995; USGS, 1998). Hyporheos, aquatic invertebrates inhabiting the hyporheic zone, obtain nutrients from the groundwater while the surface water provides the hyporheos with organic matter and dissolved oxygen (Boulton et al., 1998). Algae that grew in the top inches of hyporheic sediment recovered quickest in areas of upward water movement, thus assisting in stream recovery following damaging storms (USGS, 1998).

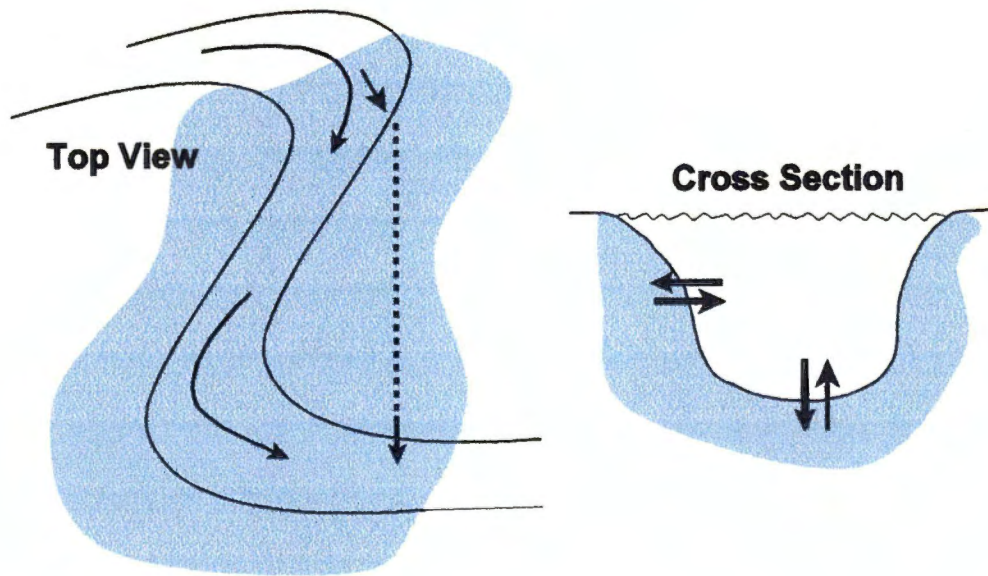


Figure 2.1. Vertical and lateral exchanges of stream and groundwater within the hyporheic zone (shaded area). Adapted from Findlay (1995).

The significance of the hyporheic zone largely depends not only on the volume of flow through it, but also the retention time (the time the water remains in the hyporheic zone). Based upon flow velocities and length of flow paths, Findlay (1995) developed a hypothetical model to identify the relative contribution of hyporheic metabolism to the overall stream ecosystem. He noted that rapid flow following short flow paths through the hyporheic zone would most likely not alter water chemistry for the system, but slow flow coupled with a long flow path possessed the potential to make significant changes in the water chemistry. The longer the water remained in the hyporheic zone, the greater the contact time between the water and the hyporheic sediments; hence, the more time the microbes had to transform contaminants. He also stated that the amount and activity of hyporheic sediments impacted the contribution of the hyporheic zone to the stream system. Active sediments were significant contributors to stream health; the opposite of inactive sediments. From these observations, Boulton et al. (1998) emphasized geological characteristics such as depth to bedrock (and hence the size of the hyporheic zone) as important parameters in determining the contribution of the hyporheic zone to the stream's water chemistry. Microbial and hyporheal metabolism and rates of nutrient and carbon transformation and uptake were considerably higher in streams with large hyporheic zones than in those with small zones (Mulholland et al., 1997; Boulton et al., 1998).

The majority of research conducted on the hyporheic zone has focused upon nitrogen cycling and oxygen levels, although other aspects such as stream metabolism, hyporheic zone size, phosphorus, and organic carbon levels have

also been studied (Grimm and Fisher, 1984; Triska et al., 1990; Hill, 1990; Duff and Triska, 1990; Hendricks and White, 1991; Findlay et al., 1993; Triska et al., 1993; Pusch, 1996; Mulholland et al., 1997; Hill and Lymburner, 1998; Chafiq et al., 1999). Hill (1996) noted that a more interdisciplinary approach that includes a blend of hydrology, chemistry, and biology must be used when evaluating the processes that occur in the hyporheic zone.

This study accomplished the interdisciplinary goal by simultaneously examining the hydrological, chemical, and biological processes of the hyporheic zone. Not only were groundwater flow paths determined, weather monitoring performed, and hyporheic waters analyzed for various chemical constituents, but microbial assays were also conducted. By analyzing the soil within the hyporheic zone for microbial population size and activity, the biological portion of the hyporheic zone was more thoroughly examined. The introduction of microbial enzymatic analyses into hyporheic research was a novel approach; one that may provide insight into hyporheic activities.

NITROGEN

The majority of the research conducted on the hyporheic zone has focused on the role of nitrogen cycling (Figure 2.2). The transformation of nitrogen is an important area of concern in water quality, especially when nitrogen is in the form of nitrates or nitrites. Nitrates and nitrites are often carried to waterways as a

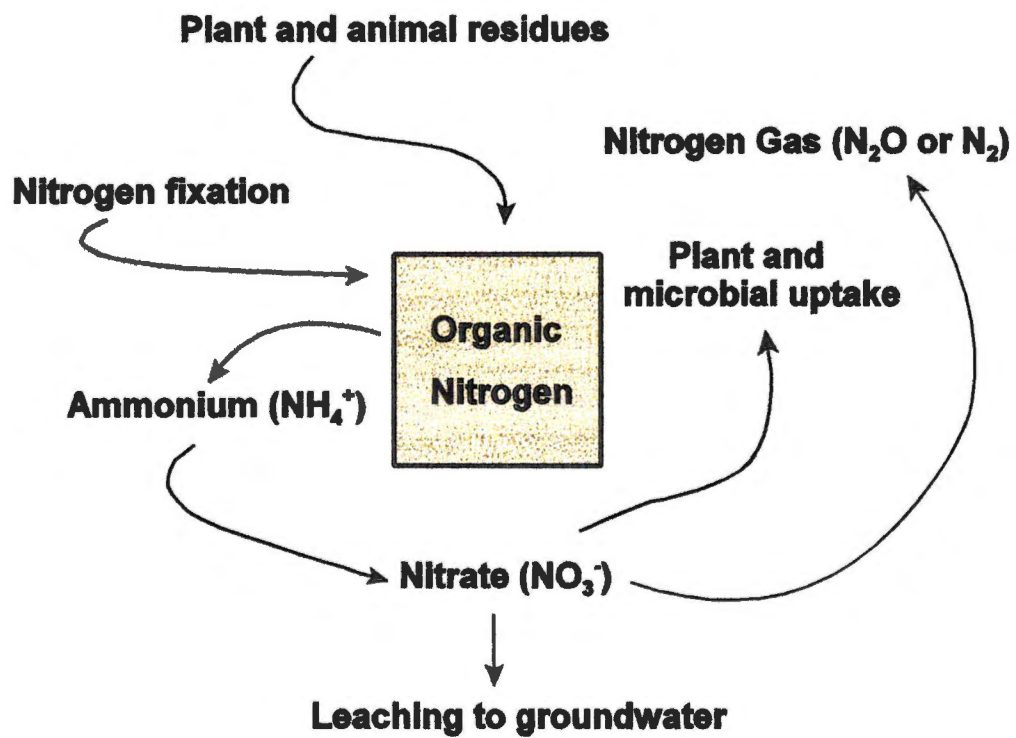


Figure 2.2. The main components of the nitrogen cycle. Adapted from Magdoff (1992).

form of non-point source pollution. Sources of nitrates and nitrites include animal wastes from both livestock and wildlife, fertilizers, natural deposits in the soil or groundwater, septic tank effluent, and raw sewage. National primary drinking water standards established by the Environmental Protection Agency (EPA) dictate that the level of $\text{NO}_3\text{-N}$ in potable water should not exceed 10 mg/L and the level of $\text{NO}_2\text{-N}$ should not exceed 1 mg/L (EPA, 1999). Currently, no maximum contaminant levels have been established for ammonium-nitrogen. Levels of nitrates and nitrites above the regulated level can lead to methemoglobinemia or "blue baby syndrome" in infants (EPA, 1998). Blue baby syndrome occurs when the bacteria in the infant's stomach converts nitrate to nitrite. Nitrite enters the infant's bloodstream, thus affecting the ability of the infant's blood to carry oxygen. The infant is essentially suffocated (Santa Clara Valley Water District, 1997). Excess nitrates and nitrites can also cause problems in ruminant animals such as cattle. A web page from Cornell University (1998) states that high nitrite concentrations in the rumen can lead to the production of methemoglobin, a substance that interferes with the cell's ability to carry oxygen.

Eutrophication is another process enhanced by excessive levels of nitrates in fresh waters. Hanson et al. (1994) noted that nitrates were a contributing factor to the eutrophication of fresh waters. The process of eutrophication occurs when high concentrations of nutrients promote an excessive amount of algal growth that leads to the depletion of oxygen from the water system. Reduction

in the level of oxygen is harmful to aquatic forms of life such as fish (Manahan, 1993; Daniel et al., 1998). For example, trout and salmon require a dissolved oxygen level of greater than 6.0 mg/L for healthy trout and greater than 7.0 mg/L for spawning trout (Roberts and Shepherd, 1997) while small mouth bass can tolerate oxygen levels as low as 4.2 mg/L (Stickney, 1986).

NITRIFICATION

The process of nitrification involves the transformation of ammonium to nitrite by *Nitrosomonas* in the first phase followed closely by the conversion of nitrite to nitrate by *Nitrobacter* in the second step (Myrold, 1998; Brady and Weil, 1999). The bacteria that oxidize ammonium to nitrate are chemoautotrophs meaning they obtain their energy from the oxidation of inorganic compounds and their carbon from carbon dioxide (Alexander, 1998). The two steps of the oxidation reaction are represented by the following equations:

Step 1



Step 2



Nitrite formed in the first step remains in the environment for short periods, since the availability of nitrite is typically the limiting factor in nitrification. This fact is fortunate, for the accumulation of nitrite in the soil or waterways can be toxic to plants and animals (Brady and Weil, 1999).

Several factors affect the process of nitrification including ammonia level, aeration, moisture, and temperature. Nitrification begins with ammonium; hence without an adequate supply of ammonium, nitrification will not occur. However, if the level of ammonium becomes too high, nitrification may be inhibited. Another requirement for nitrification to occur is the presence of oxygen, since the nitrifying organisms are aerobic. In soils, aeration is related to moisture; the optimum moisture conditions for nitrification occur when 60% of the soil pore volume is filled with water. Finally, the temperature of the environment that the nitrifying organisms inhabit must be appropriate. Optimum rates of nitrification occur in the temperature range of 20 to 30°C, but nitrification has been observed at temperatures as low as 5°C and as high as 50°C (Brady and Weil, 1999).

DENITRIFICATION

Denitrification is the process by which nitrate is converted to gaseous forms of nitrogen, mainly dinitrogen and nitrous oxide, and released into the atmosphere. Depending on the environmental conditions and active organisms, the exact process will vary, but the general equation for denitrification is from

nitrate to nitrite to nitric oxide gas (NO) to nitrous oxide gas (N₂O) to dinitrogen gas (N₂):



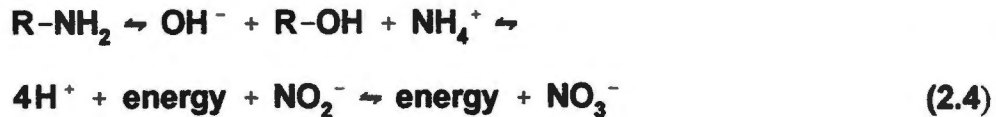
Oxygen is released at each step: two molecules between nitrate to nitrite and nitrite to nitric oxide gas, and one molecule from nitric oxide gas to nitrous oxide gas and nitrous oxide gas to dinitrogen gas (Brady and Weil, 1999).

As with nitrification, several environmental factors can affect the rate of denitrification. The most important factor controlling the process of denitrification is anaerobic conditions (Myrold, 1998); specifically, less than 0.2 mg/L of dissolved oxygen in solution. The entire system need not possess anaerobic conditions, but rather microsites of low oxygen concentration can provide a suitable environment for denitrification (Brady and Weil, 1999). Smith and Tiedje (1978) found that denitrifying enzymes were present under aerobic conditions. Hence, the researchers concluded that either denitrification occurs at anaerobic microsites within an overall aerobic environment, or denitrifying enzymes remain functional in the presence of oxygen until favorable conditions ensue. Following the presence of anaerobic conditions, the availability of nitrate then carbon are next in order of importance in determining denitrification rates (Myrold, 1998). Optimum temperatures for denitrification are between 25 and 35°C, but the process can occur at temperatures as low as 2°C and as high as 50°C.

Additionally, a low pH (less than 5.0) can inhibit denitrification (Brady and Weil, 1999).

NITROGEN MINERALIZATION/IMMOBILIZATION

Nitrogen mineralization refers to the conversion of organic nitrogen, present in organic compounds, to the inorganic forms of ammonium and nitrate (Skukla et al., 1998). When nitrogen mineralization results in only the production of ammonium, it is termed ammonification. Regardless of whether the end product of nitrogen mineralization is ammonium, nitrate, or both, the reaction is driven by a series of steps involving both extracellular and intracellular enzymes (Myrold, 1998). Extracellular enzymes are protein catalysts excreted by microorganisms into the surrounding environment to break down compounds into manageable components that can be absorbed by the microbes (Alexander, 1998). Intracellular enzymes are similar to extracellular enzymes, except they catalyze reactions inside the cell. In nitrogen mineralization, extracellular enzymes degrade nitrogen containing organic compounds such as proteins, microbial cell walls, and nucleic acids. The amino acids released from the process are then absorbed into the active microbial cell where intracellular enzymes complete the transformation to ammonium (Myrold, 1998). The reaction for nitrogen mineralization and subsequent immobilization is as follows, with R-NH₂ representing the amino compound (Brady and Weil, 1999).



Nitrogen immobilization, simultaneous to the occurrence of nitrogen mineralization, involves the conversion of ammonium and nitrate to inorganic forms of nitrogen through microbial assimilation (Myrold, 1998; Brady and Weil, 1999).

PREVIOUS RESEARCH ON NITROGEN IN THE HYPORHEIC ZONE

Grimm and Fisher (1984) researched nitrogen cycling in the hyporheic zone of Sycamore Creek located in the Sonoran Desert of Arizona. Research was conducted during the summer: a time of peak solar radiation and temperature. They discovered that a large portion of nitrate removal from the system occurred through assimilation by sediment microbes rather than by physicochemical reactions such as sorption. Assimilation occurs when microbes incorporate substances such as inorganic nutrients into their biomass, thus rendering the nutrients unavailable (Alexander, 1998). The researchers did not specifically test for nitrate removal in the field, but laboratory analysis suggested that the desert stream sediments were active in nitrate removal. However, the oxygen concentrations (greater than 3.85 mg/L) coupled with the low level of organic matter suggested that denitrification was not the main cause of nitrate removal. Grimm and Fisher (1984) further theorized that denitrification is

potentially a significant process in streams with a high organic matter content and slow moving interstitial water.

The relationship between nitrogen chemistry and groundwater flow paths along a small headwater catchment near Toronto, Canada was examined by Hill (1990). He examined the nitrate and ammonium concentrations of the groundwater transported to the stream along three different pathways: shallow groundwater emerging as springs, deep groundwater becoming rivulets, and deep groundwater entering the stream as bed and bank seepage. Hill (1990) noted higher ammonium levels and lower nitrate and chloride concentrations were present in bank seeps in comparison to rivulets. Nitrate and chloride concentrations in springs were higher than those of rivulets. He concluded that differences in nitrate and chloride concentrations among the springs, rivulets, and bank seepage were due to initial chemical differences in shallow versus deep groundwater with deep groundwater sustaining bank seepage. Variations in the ammonium concentrations between rivulets and bank seeps most likely resulted from aerobic and anaerobic conditions. Likely, little reduction of ammonium occurred in deep groundwater emerging along the streamside; however, greater rates of N mineralization and immobilization occurred in the aerobic rivulets (Hill, 1990).

A study along Little Lost Man Creek in northwestern California by Triska et al. (1990) simultaneously examined nitrate and ammonium gradients in subsurface waters along the hyporheic zone. Additionally, Triska et al. (1990) researched temporal variations in the contaminant nitrogen concentrations,

determined nitrification potential in hyporheic sediments through laboratory slurry studies, and conducted a field experiment with an ammonium injection to confirm nitrification potential (Triska et al., 1990). Nitrate concentrations in the channel water exhibited seasonal and diurnal variations with nitrate concentrations increasing from May to late August and decreasing by 25-40% during the daytime suggesting uptake by hyporheos. High ammonium concentrations correlated with low nitrate concentrations and dissolved oxygen levels, hence channel waters exhibited the lowest ammonium concentrations with amounts doubling at inland sites. Neither seasonal nor diurnal patterns were present. Laboratory tests revealed nitrification potential was highest in sediments closely connected to the main channel by subsurface flow. As distance from the wetted channel increased, the potential for nitrification decreased. Ammonium amendments to the slurries revealed small effects on nitrate concentrations. Field experiments with ammonium injections resulted in only a 12% increase in nitrate production indicating that sorption largely contributed to ammonium retention (Triska et al., 1990).

Along the same creek as described above, Duff and Triska (1990) examined denitrification potentials in hyporheic sediments. Again, the researchers noted that nitrate concentrations increased during the summer. Nitrate concentrations at the most inland well were significantly lower than in the stream water. Laboratory analysis of sediment slurries revealed that the potential for denitrification significantly increased with distance from the wetted channel. Carbon additions to the sediment slurries had little effect on denitrification alone,

but the rates of denitrification increased with the addition of nitrate. As with research performed by Triska et al. (1990), dissolved oxygen concentrations decreased laterally from the stream channel, and thus were correlated with increased denitrification rates.

Hendricks and White (1991) theorized that the biological and chemical processes that occurred in the stream bed would be reflected in the exiting hyporheic water. They studied the East Branch of Maple River, a third order river in the northern part of Michigan. Nitrate concentrations in the hyporheic zone were three times the level found in surface water and five to six times that of groundwater. Decreases in hyporheic nitrate concentrations were observed with depth, and an overall increase in nitrate concentrations occurred with increasing distance downstream beneath the riffle. Ammonium concentrations were slightly irregular, but mimicked nitrate concentrations with depth and distance downstream. Groundwater ammonium concentrations were higher than surface water levels by approximately 2.5 times. The researchers noted that as the water moved further downstream, it began to predominately resemble the groundwater plus the reactions that had taken place in the hyporheic zone (Hendricks and White, 1991).

In 1993, Triska et al. again studied nitrogen cycling in the hyporheic zone, but with a stronger focus on water exchange between the channel and the hyporheic zone. The researchers discovered that groundwater supplied the hyporheos and the surface biota with dissolved solutes while surface water introduced dissolved oxygen to the hyporheic zone. The mixing of surface and

groundwater occurred vertically and laterally in the subchannel. Using injection studies, the researchers identified no direct correlation between distance from the channel and exchange of surface and groundwater in the hyporheic zone. The travel time of water through the hyporheic zone, a result of the permeability of hyporheic sediments, resulted in chemical gradients. Sites with high nitrate and dissolved oxygen levels and low ammonium and dissolved organic carbon concentrations exhibited short travel times through the hyporheic zone; those with long travel times had reversed chemical gradients. Ammonium was sorbed to the clay sediments of the hyporheic zone, thus building a large pool of substrate for nitrification under aerobic conditions (Triska et al., 1993). This study also confirmed previous research by Triska et al. (1990) and Duff and Triska (1990) by finding that denitrification potential increased and nitrification potential decreased with distance from the wetted channel.

Hill and Lymburner (1998) researched concentration patterns of ammonium and nitrate along Glen Major stream and Duffin Creek located northeast of Toronto, Canada. The researchers analyzed water chemistry in piezometer transects located perpendicular to each stream. Solute injection experiments were conducted to determine element transformations at stream-subsurface exchange sites. Based on the definition that the outer boundary of the hyporheic zone contained greater than 10% surface water, background Cl concentrations were used to determine the interface with the assumptions that water in the hyporheic zone only comes from stream water and groundwater plus the source of water is temporally and spatially uniform with respect to Cl.

Considerable variations in depth of the outer boundary of the hyporheic zone were found ranging from less than 4 to 30 cm. Along Duffin Creek, nitrate concentration declined in the upper 5 to 10 cm, with levels at lower depths similar to those of groundwater. Ammonium concentrations exhibited no vertical or longitudinal trends. Similarly at Glen Major, nitrate concentrations decreased with depth, and ammonium concentrations at depths of 2 to 15 cm were higher than those of the stream water. The authors noted zones of high nitrate or ammonium levels were not evident in the sediments and concluded that nitrification and N mineralization in the hyporheic zone were limited, or a balance between production and uptake existed (Hill and Lymburner, 1998).

As seen in the above sections, research into nitrogen cycling in the hyporheic zone has focused primarily on nitrate and ammonium concentrations at various locations within the hyporheic zone. A large portion of the research into nitrification and denitrification potentials was conducted in the laboratory on sediment slurries. In this study, both the concentration of nitrate and ammonium were examined at varying distances from the stream to determine the in-field nitrification and denitrification potentials. However, unlike previous research, examination of soil enzymes was also performed to determine the potential for nitrogen mineralization in the hyporheic zone; thus the combination of soil assays with water chemistry analyses and a hydrological examination of the groundwater flow paths provided for a more complete understanding of nitrogen transformations in the hyporheic zone.

PHOSPHORUS

Phosphorus, like nitrogen, is an important parameter in determining water quality in fresh waters. As with excessive amounts of nitrates, high levels of phosphates can lead to eutrophication. Currently, the appropriate land application of animal waste results in a 4 to 8 fold greater land requirement to satisfy the phosphorus loading rates as compared to the nitrogen loading rates (Beal et al., 1999). So, to satisfy the nitrogen requirements of the land, an excessive amount of phosphorus is subsequently applied. Microorganisms assimilate phosphorus that is in the form of orthophosphate (Correll, 1998). Sources of phosphorus pollution to the environment occur from phosphates in household detergents (Manahan, 1993; Gale et al., 1994; USGS, 1998), industrial water softening processes (Snoeyink and Jenkins, 1980), concentrated animal feeding operations (Parry, 1998), and runoff from agricultural lands treated with fertilizers (Lowrance et al., 1984; Jordan et al., 1993). Daniel et al. (1998) noted that long term application of phosphorus as fertilizer resulted in soils with elevated phosphorus levels, thus drastically increasing the potential of phosphorus runoff. With the continual exchange of nitrogen and carbon between the atmosphere and the water, the control of phosphorus becomes quite important because phosphorus is seen as the limiting element in eutrophication in freshwater environments (Daniel et al., 1998). Phosphates are transported to surface waters via surface flows, because phosphorus binds to sediments

(Correll, 1998). Tan (1982) referred to research by Bolt (1976) who noted that orthophosphate ions are strongly adsorbed to clay soils. When phosphorus concentrations in waters reach 20 µg/L, the potential for occurrence of eutrophication drastically increases (Cornell, 1998).

The phosphorus cycle is divided into a biological subcycle and a geochemical subcycle. The P cycle contains inorganic forms of phosphorus and organic forms of phosphorus with the solution phosphorus pool serving as the centroid of the cycle (Figure 2.3). The inorganic forms of phosphorus are subdivided into calcium-bound inorganic phosphorus that predominate under alkaline conditions, and iron- or aluminum-bound phosphorus that are prevalent under acidic conditions (Brady and Weil, 1999). Plants and microorganisms obtain orthophosphate from the solution phosphorus pool (Mullen, 1998). Particulate phosphorus is the fraction eroded with the soil while dissolved phosphorus is present in runoff (Brady and Weil, 1999). Phosphorus can travel along five basic pathways: 1) uptake of soil phosphorus by plants; 2) recycling of phosphorus through the return of plant and animal residues; 3) mineralization-immobilization (simultaneous processes); 4) fixation reactions at clay and oxide surfaces; and 5) solubilization of inorganic phosphorus. Phosphorus is rendered relatively unavailable to plants and microorganisms through precipitation reactions under aerobic conditions that occur between insoluble phosphates and ferric iron, calcium, or aluminum; phosphate adsorption onto clay particles or aluminum and ferric hydroxides and oxides; and the incorporation of phosphorus into biomass (Stevenson, 1986). Brady and Weil (1999) define adsorption as the

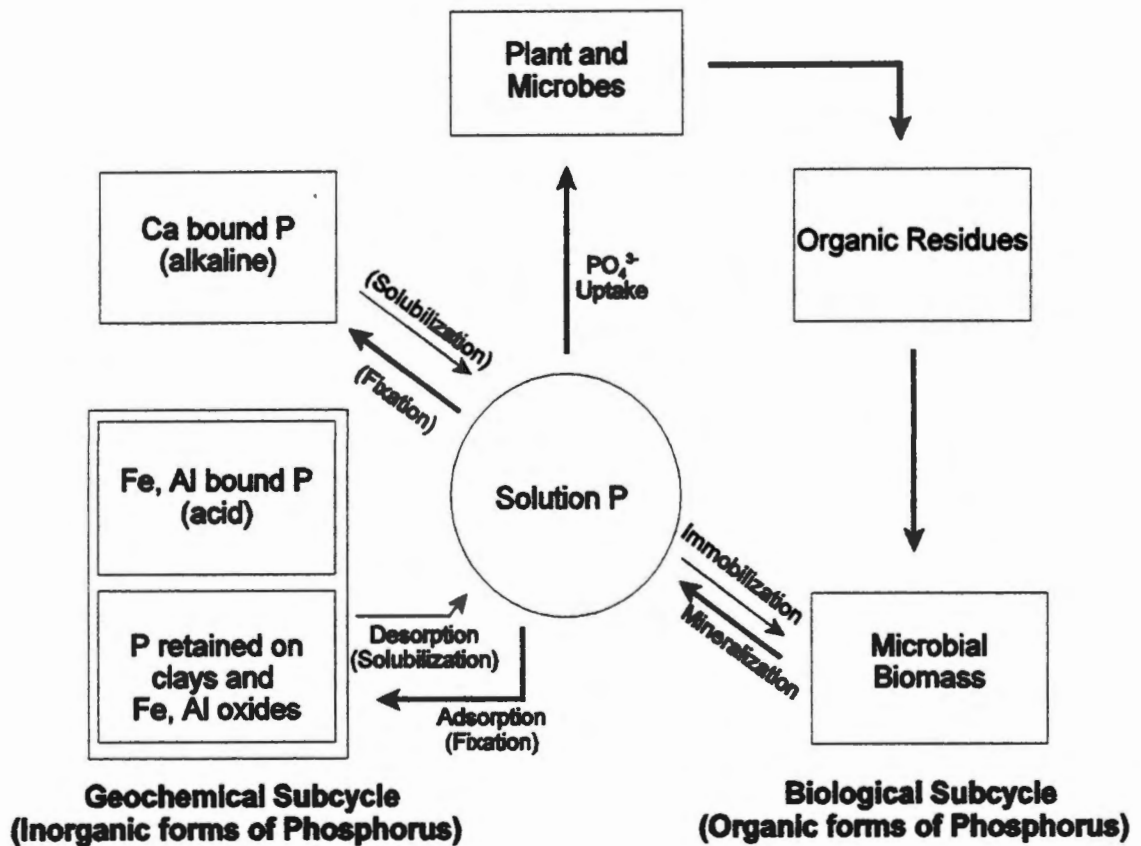


Figure 2.3. The phosphorus cycle is divided into two subcycles.
Adapted from Mullen (1998).

attraction of ions to a solid or soil surface while absorption is the movement of ions into plants or microbes. According to Mitsch and Gosselink (1993), the cycling of phosphorus in wetlands with mineral soils tends to follow the pathways of “sedimentation and resuspension.”

BIOLOGICAL SUBCYCLE

The first realm of the phosphorus cycle involves the biological subcycle: uptake of soil phosphorus by plants and microorganisms, recycling of phosphorus through plant and animal residues, mineralization and immobilization, and solubilization of inorganic phosphorus. Plants take up phosphorus in the inorganic form orthophosphate (H_2PO_4^- and HPO_4^{2-}). H_2PO_4^- is the dominant form of orthophosphate at a pH below 7.2 and HPO_4^{2-} is the primary species at a pH above 7.2 (Mullen, 1998). Maximum available phosphorus tends to occur at the intermediate pH values of 6 to 7, for calcium phosphates tend to accumulate in neutral to alkaline soils and iron and aluminum phosphates accumulate in acidic soils. Above a pH of 7, iron and aluminum phosphates are insoluble; below a pH of 6, calcium phosphates are insoluble. The decay of plants and animals provides the soil with a means of recovering lost phosphorus, for once phosphorus is removed from the soil through harvesting or erosion, replenishment occurs only from external sources (Stevenson, 1986).

Mineralization is the process of converting an element from an organic form to an inorganic form through microbial decomposition. Immobilization is the

conversion of an element from an inorganic form to an organic form in microbial or plant biomass. In immobilization, the microorganisms and plants accumulate and then transform the inorganic phosphorus (Mullen, 1998). Extracellular enzymes called phosphatases catalyze reactions that release phosphorus from its inorganic forms into the soil solution. The alternative wetting and drying of soil enhances the mineralization of organic phosphorus (Stevenson, 1986). Once phosphorus is mineralized, plants and microbes are free to take up the inorganic compound (typically as orthophosphate), or the formation of insoluble inorganic complexes can occur. The extent to which mineralization or immobilization is carried out is regulated by the carbon to phosphorus (C:P) ratio. A certain amount of phosphorus must be present in the soil for carbon assimilation to occur ($C:P < 200/1$). If insufficient phosphorus is present to assimilate the carbon ($C:P > 300/1$), immobilization occurs. However, if more phosphorus is present in the soil solution than needed for carbon assimilation, mineralization of orthophosphate occurs (Mullen, 1998).

The solubilization of inorganic phosphorus occurs when the insoluble forms such as calcium, iron, and aluminum phosphates are released as soluble forms through the action of organic acids and chelates (Stevenson, 1986). A chelate is formed when an electron donor such as an organic compound joins with a metal ion. Soil scientists generally believe that chelates are more mobile in soil and more easily available for plant and microbial uptake than the metal ion (Wagner and Wolf, 1998; Mullen, 1998). Nitrifying bacteria and sulfur-oxidizing bacteria can promote the solubilization of insoluble phosphate salts. Sulfate-

producing bacteria produce hydrogen sulfide under waterlogged or anaerobic conditions. Hydrogen sulfide can displace metal cations from phosphates, thus releasing the phosphate (Mullen, 1998).

GEOCHEMICAL SUBCYCLE

The geochemical subcycle of the phosphorus cycle involves fixation reactions at clay and oxide surfaces. Fixation reactions can limit the availability of phosphates in the soil through the conversion of H_2PO_4^- to insoluble forms. According to Tan (1982), phosphorus that is not extractable from soil by dilute acids is denoted as fixed. A large amount of the phosphorus in water soluble forms that is applied to soils remains as such for a short period of time. A large amount of the phosphorus applied to soils in a water soluble form (fertilizers) is quickly converted to insoluble or complex forms. Research has shown clay mineralogy to have an influence on phosphate fixation. Acidic to slightly acidic conditions result in the highest rate of phosphorus sorption onto clay (Mitsch and Gosselink, 1993). Phosphate fixation is also affected by pH. The precipitation of highly insoluble iron, or aluminum phosphates or the adsorption of phosphates to oxide surfaces, occurs in highly acidic soils. In neutral to alkaline soils (calcareous soils), the formation of less soluble di- and tri- calcium phosphates occurs. Additionally, the adsorption of phosphate to oxide surfaces (replacement of H_2O and/or OH) greatly affects the availability of phosphate.

PREVIOUS RESEARCH ON PHOSPHORUS IN THE HYPORHEIC ZONE

Little research focusing on phosphorus in the hyporheic zone has been performed. Hendricks and White (1991) studied soluble reactive phosphorus ($\text{PO}_4\text{-P}$) in the hyporheic zone of a northern Michigan river. The researchers noted that the soluble reactive phosphorus was approximately three to four times greater in samples from the hyporheic zone at a 10-cm depth than those of surface water and groundwater. Below the 10-cm depth, the soluble reactive phosphorus decreased with depth. The researchers showed that the high level of soluble reactive phosphorus in the 10- to 20-cm range was related to oxygen concentrations. Groundwater soluble reactive phosphorus was approximately twice the level of that found in the surface water. Hendricks and White (1991) hypothesized that soluble reactive phosphorus was immediately consumed by hyporheos as it was desorbed from sediments, thus remaining near or below the detection limit (Hendricks and White, 1991).

Mullholland et al. (1997) examined phosphorus uptake in an eastern Tennessee stream and a North Carolina stream located in the Appalachian Mountains using radiotracer injections of $^{33}\text{PO}_4$ and $^3\text{H}_2\text{O}$. The researchers theorized that uptake of ^{33}P would be seen in a decreased ratio of $^{33}\text{P}:^3\text{H}$ at the downstream stations. This declining ratio would reflect the residence time of water in the hyporheic zone, thus indicating the importance of the hyporheic zone in phosphorus uptake. If the $^{33}\text{P}:^3\text{H}$ ratio remained constant at the downstream station, little phosphorus uptake occurred. Results indicated that a larger

hyporheic zone resulted in a greater uptake of phosphorus. The availability of a labile organic carbon source coupled with a strong microbial need for phosphorus led to a greater rate of phosphorus uptake (Mulholland et al., 1997). Labile indicates a nutrient's or compound's open accessibility for plant uptake, or ease in transformation by microorganisms (Brady and Weil, 1999).

Boulton et al. (1998) summarized research performed in stream ecology studies, especially those with relevance to the hyporheic zone. They cited work performed by Dahm et al. (1987) who noted the effects of environmental variables such as oxygen depletion, redox changes, and desorption of phosphorus on the concentration of phosphorus in hyporheic waters; these ideas were also presented in research by Hendricks and White (1991). A larger volume of research dealing with phosphorus focuses on other freshwater bodies. Correll (1998) observed that in bottom sediments, particulate phosphorus was gradually released into the pore water by microbial activity. From this point, the dissolved phosphorus diffused into the overlying waters or became bound to particulate surfaces (Figure 2.4). Aluminum or ferric hydroxides can form strong bonds with dissolved phosphorus. Oxidized bottom sediment layers that contain ferric hydroxides form a barrier that traps phosphates before they enter the overlying water column. Under anoxic conditions, the reduction of ferric ions can weaken this bond, thus freeing the phosphate. However, anoxic conditions are rare in streams without high nutrient concentrations. Boers and De Blas (1991) conducted research on phosphorus release in interstitial water in the Loosdrecht Lake in the Netherlands. They noted that the retention in, and release of,

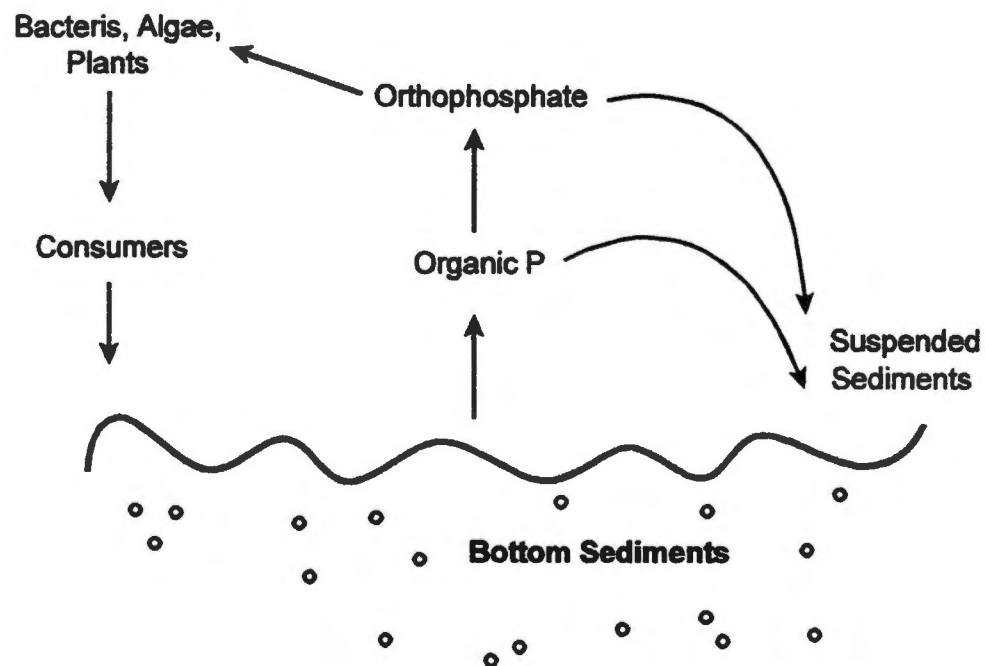


Figure 2.4. Phosphorus cycling in the bottom sediments and overlying waters. Adapted from Correll (1998).

phosphorus in lake sediments is important in phosphorus cycling. Bray et al. (1973) noted that phosphorus can precipitate with iron. Oxidation reactions have a role in altering the phosphate concentrations of interstitial waters during sample handling. The small amount of water that filters into the sample oxidizes ferrous iron, and the ferric iron produced removes inorganic phosphate from solution either by precipitating it directly as an iron phosphate, or by first precipitating as iron hydroxide and subsequently scavenging phosphate from solution by sorption. Currently, researchers believe that iron essentially binds phosphorus instead of forming a precipitate. Under the appropriate conditions, phosphorus may be released (Correll, 1998; Gale, 1998). According to Gburek and Sharpley (1998), a better understanding of the relationship between hydrology and variable phosphorus sources, sinks, temporary storage, and transport processes is needed for better phosphorus management.

Concentrations of orthophosphate were examined in this study along with ferric and ferrous iron levels. Previous research has examined the biological aspects of the phosphorus cycle, but has somewhat neglected the geochemical portion. By examining ferric and ferrous iron concentrations in addition to orthophosphate levels, this study attempted to equally address both the biological and geochemical subcycles of the phosphorus cycle.

SULFUR

Sulfur, an important element in the survival of plants and microorganisms, is required in the synthesis of certain critical amino acids (Germida, 1998; Brady and Weil, 1999). At times, too much sulfur is present in the environment bringing about acid rain and acid mine drainage which damage waterways (Germida, 1998). Sources of sulfur include organic matter, soil minerals, and atmospheric sulfur gases (Brady and Weil, 1999). The amount of sulfur in the atmosphere is relatively small in comparison to the entire pool of sulfur, but the amount has more than doubled since the Industrial Revolution (Germida, 1998). Organic matter contains sulfur in organic forms while soil minerals are comprised in part by inorganic sulfur. Organic sulfur is further divided into carbon-bonded sulfur, present in amino acids and proteins, and ester or organic sulfur that has oxygen bonded to sulfur instead of carbon. Organic forms of sulfur enter the environment through the decomposition of organic matter. The other primary source of sulfur is soil minerals that supply inorganic forms of sulfur, the most common being sulfate and sulfide. Inorganic sulfur enters the environment through the weathering of rocks and minerals or the addition of fertilizers. Similar to nitrogen, sulfur is removed from the system by leaching, volatilization, and runoff (Germida, 1998; Brady and Weil, 1999).

The sulfur cycle is quite similar to the nitrogen cycle, for sulfur undergoes mineralization/immobilization reactions, oxidation-reduction reactions, and

volatilization (Figure 2.5). Mineralization involves the conversion of organic sulfur containing molecules to inorganic forms while immobilization occurs when microorganisms assimilate the inorganic sulfur compounds. Both plants and microorganisms use inorganic forms of sulfur. Sulfur mineralization produces hydrogen sulfide under anaerobic conditions and sulfate in the presence of oxygen (Bear, 1964). Mineralization of organic sulfur must occur before plants and microorganisms can use the sulfur in compounds. Mineralization of ester or organic sulfur results in the direct release of sulfates while mineralization of carbon-bonded sulfur produces hydrogen sulfide compounds which undergo oxidation to sulfates (Brady and Weil, 1999). The counterpart of mineralization reactions is immobilization reactions. Similar to nitrogen, sulfur immobilization is related to the quantity of carbon present. Brady and Weil (1999) report that sulfur immobilization occurs at a C/S ratio of 400:1.

The oxidation of sulfur is performed by numerous types of microorganisms such as chemoautotrophs, photoautotrophs, and chemoheterotrophs. Chemoautotrophic bacteria, photoautotrophic, and chemoheterotrophic bacteria obtain their energy and carbon from inorganic compounds and carbon dioxide, light and carbon dioxide, and organic compounds for both, respectively (Alexander, 1998). The basic reaction is represented by the following equation that involves the transformation of numerous sulfur compounds in the order of elemental sulfur, thiosulfate, tetrathionate, and finally sulfate (Germida, 1998):

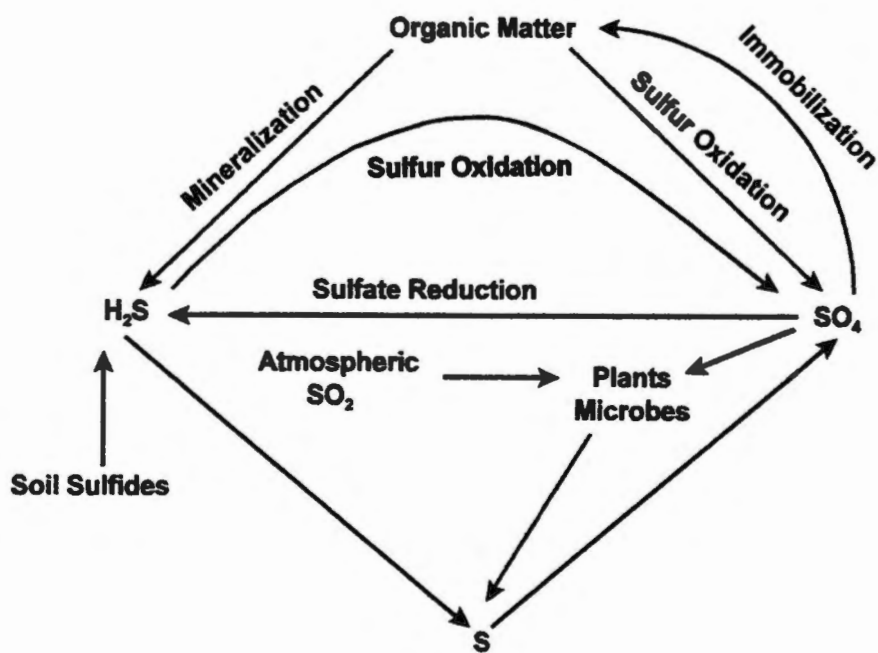


Figure 2.5. The sulfur cycle is similar to the nitrogen cycle. Adapted from Bear (1964).



Researchers believe the chemoautotrophic, photoautotrophic, and chemoheterotrophic organisms work harmoniously to oxidize sulfur compounds in agricultural soils. Although sulfur oxidation can occur chemically such as the conversion of sulfite (SO_3^{2-}) to sulfate (SO_4^{2-}), scientists believe the majority of sulfur oxidation is mediated by microbial activity (Brady and Weil, 1999). Coupled with oxidation reactions are reduction reactions. The reduction of sulfur compounds is significant in anaerobic waterlogged soils, but not in aerobic soils. The process of sulfur reduction involves the transformation of sulfate to hydrogen sulfide. Other inorganic sulfur compounds that are not completely oxidized to sulfate may also undergo reduction to hydrogen sulfide (Bear, 1964; Germida, 1998; Brady and Weil, 1999).

Sulfur volatilization occurs with the production of sulfur gases such as hydrogen sulfide. This reaction typically occurs under waterlogged conditions. A portion of the gas produced escapes to the atmosphere while the rest remains in the soil or water to undergo further reactions (Germida, 1998; Brady and Weil, 1999).

PREVIOUS RESEARCH ON SULFUR IN THE HYPORHEIC ZONE

Sulfur transformations in the hyporheic zone have only received cursory examination. Williams (1989) attempted to relate water chemistry with the

organisms inhabiting the hyporheic zone of two Canadian channels. He found that sulfide concentrations were an average of four to seven times greater in the hyporheic zone than in the stream water. The pattern of sulfide concentration at Rouge River resembled that of Duffin Creek with sulfide concentrations two to five times greater at hyporheic sites than in the surface water, resulting in a 40% difference in hyporheic sulfide concentrations between the two channels (Williams, 1989).

Since very little research into the role of sulfur transformation in the hyporheic zone has been examined, other research into the characteristics of sulfur may prove helpful. Barth (1986) stated that hydrogen sulfide acts as an indicative parameter and confirmation for redox (Eh) measurements in identifying reduced conditions. The cycling of sulfur can affect iron concentrations in the system, thus affecting the cycling of phosphorus. Feng and Hsieh (1998) examined sulfate reduction in freshwater wetland soils. They and other researchers have noted that sulfate reduction and metal sulfide formation were significant mechanisms of iron removal, acidity, and heavy metals in wetlands (Teal and Peterson, 1991; Marnette et al., 1993). In anaerobic sediments, sulfates are reduced to sulfides that may in turn bind to ferrous ions preventing the ferrous ions from diffusing into the water column (Marnette et al., 1993; Correll, 1998). Although sulfate concentrations in freshwaters are usually low (50 to 450 μM) (Spratt and Morgan, 1990; D'Angelo and Reddy, 1994), contaminants such as animal feces can substantially increase sulfate concentrations in fresh waters (Feng and Hsieh, 1998). Elsgaard et al. (1994) noted that for a

freshwater environment, the rate of sulfate reduction is often limited by a low sulfate concentration. However, they also reported that the sulfate reducing microbes were well adapted for low sulfate concentrations. The reduction of sulfate is regulated by the amount of sulfate or electron acceptors present, the electron donors present in the anaerobic environment, pH, and temperature (Skyring, 1987; Okabe and Characklis, 1991; Marnette et al., 1993; Elsgaard et al., 1994). Sulfate reduction has been observed in the pH range of 3.8 to 9.9 (Elsgaard et al., 1994).

Sulfur concentrations in the hyporheic zone have received cursory examination primarily because sulfate is not considered an important water quality parameter while sulfide is viewed as moderately important. Of the various forms of sulfur, sulfide has been given the most attention. This study examines both the sulfate-S and sulfide concentrations at various locations in the hyporheic zone. Additionally, soil assays were performed to determine the potential of sulfur mineralization; a novel approach in hyporheic research.

IRON

Iron is an essential mineral for life (Arms, 1990). Transformations of iron in the environment affect the availability of the micronutrient to plants and microbes (Figure 2.6). Although iron is one of the most abundant elements in the soil environment, soluble iron is scarce under aerobic condition (Mullen, 1998).

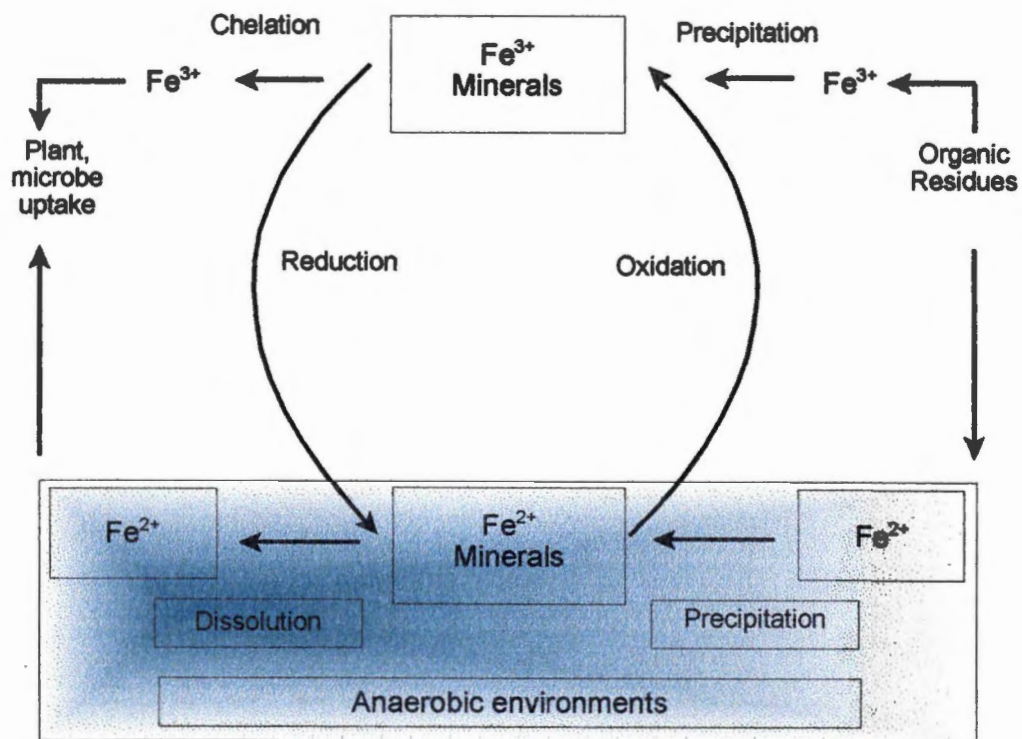


Figure 2.6. Environmental transformations of iron. Adapted from Mullen (1998).

Iron exists either in the ferrous or the ferric forms. Ferrous forms are divalent while ferric compounds are trivalent (Hem and Cropper, 1959). The oxidation and reduction of iron is the primary means of iron transformation. Because the process can occur either biotically or abiotically, researchers often have difficulty identifying the origin. Under aerobic condition with a pH greater than 3, ferrous iron (Fe^{2+}) is rapidly oxidized to ferric iron (Fe^{3+}), abiotically. Certain microorganisms possess the capability to oxidize Fe^{2+} under acidic conditions. As with nitrogen, phosphorus, and sulfur, mineralization reactions occur as organic forms of iron are converted to inorganic states. Microorganisms also solubilize inorganic forms of iron (Mullen, 1998). When chelation of iron occurs as represented in the following equation, iron is available for uptake.



The compound FeOOH is unavailable for uptake while the Fe chelate is available for assimilation (Brady and Weil, 1999).

PREVIOUS RESEARCH ON IRON IN THE HYPORHEIC ZONE

Currently, no studies are available on iron cycling in the hyporheic zone. Observations concerning iron transformations in terrestrial and aquatic environments may prove beneficial when examining the hyporheic zone for

trends. Under the natural conditions of waterways, oxidizing conditions result in iron precipitation as ferric hydroxides, with $\text{Fe}(\text{OH})_3$ as the most frequently observed form (Hem and Cropper, 1959). Ferric iron is a crucial electron acceptor in streams, although the reduction of ferric iron can lead to corrosion of steel structures present in the water (Mullen, 1998). The oxidation of Fe^{2+} can cause the removal of inorganic phosphates from solution through binding (Correll, 1998). The amount of iron present in hyporheic soils may affect the removal of phosphorus compounds from surface waters and groundwaters. Iron phosphates tend to accumulate in acidic soils, remaining insoluble at a pH above 7 (Stevenson, 1986). Soils with a high iron concentration, especially clays, provide a medium for phosphorus fixation. Also, the reduction of ferric iron in phosphate minerals can lead to the release of phosphate (Mullen, 1998). Sulfur compounds are affected by the presence of ferrous ions (Correll, 1998).

The uniqueness of this hyporheic study in comparison to others was the fact that no other such research has monitored iron cycling. The primary purpose of examining the ferric and ferrous iron concentrations was to provide assistance for interpreting the phosphorus data. However, useful knowledge about hyporheic functions may be garnered by examining iron concentrations in relation to hydrological, chemical, and biological processes.

FECAL COLIFORMS AND FECAL STREPTOCOCCI

The transmission of human and animal pathogens poses a serious health threat. Contamination of fresh water with fecal coliforms and/or fecal streptococci hinders the usefulness of waterways for both humans and livestock. Fecal coliforms and fecal streptococci are found in the intestines of warm blooded animals, and fecal coliform concentrations in fresh waters indicate the amount of potential pathogenic microorganisms present in the water reservoir. The primary concern of water quality in connection with indicator organisms is the transmission of disease, especially between humans. Since the turn of the century, the United States Public Health Department has used fecal contamination of drinking water as a basis for determining the quality of water.

To assess the contamination level of water by pathogenic organisms, scientists have monitored indicator organisms. Indicator organisms such as fecal coliforms and fecal streptococci are selected because they meet a number of criteria: 1) presence in the intestines of warm blooded animals, 2) presence and absence correlated with pathogens, 3) presence in numbers greater than those of pathogens, 4) resistance to environmental factors in the same manner as pathogens, 5) easily and rapidly detectable, and 6) nonpathogenic (Bitton, 1994). The maximum contaminant level for fecal coliforms set by the Tennessee Department of Environment and Conservation (1999) modeled after guidelines

established by the Environmental Protection Agency (1999) is the repetition of a positive sample.

Fecal coliforms such as *Escherichia coli* are typically found in humans, while fecal streptococci are normally found in animals. The ratio of fecal coliforms to fecal streptococci (FC/FS ratio) indicates the origin of fecal contamination in water; whether human or animal. A FC/FS ratio of four or greater indicates that the source of contamination is human, while a FC/FS ratio of 0.7 or less indicates animal origins (Doran and Linn, 1979; Doran et al., 1981; Tiedemann et al., 1988; Bitton, 1994; APHA et al., 1992; Cook et al., 1998).

Questions have arisen related to the accuracy and dependency of using the FC/FS ratio to quantify the source of microbial contamination. Prior to the 17th edition of the Standard Methods for the Examination of Water and Wastewater, the American Public Health Association recommended using the FC/FS ratio. However, the APHA et al. (1992) currently does not recommend the use of the FC/FS ratio because of the variable survival rate of fecal streptococci. The streptococci species of predominant animal origin, *S. bovis* and *S. equinus*, quickly die-off whereas the predominately human originated species, *S. faecalis* and *S. faecium*, tend to survive for longer periods of time (APHA et al., 1992). Some researchers have noted erroneous results in which the FC/FS ratio indicated that the contamination was human in origin when they knew this was false (Howell et al., 1996; Wiggins, 1996; Edwards et al., 1997). However, previous research by Doran and Linn (1979), Doran et al. (1981), and Tiedemann

et al. (1988) and recent research by Edwards et al. (1997) and Cook et al. (1998) all use FC/FS ratios. Care must be taken when interpreting the FC/FS ratio. Observations of the watershed and upstream uses assist in determining the validity of the FC/FS ratio obtained.

PREVIOUS RESEARCH ON FECAL COLIFORMS AND FECAL STREPTOCOCCI IN THE HYPORHEIC ZONE

Currently, no studies have examined fecal coliforms or fecal streptococci in the hyporheic zone. Previous studies have attempted to define primary production and respiration rates (Grimm and Fisher, 1984, Pusch, 1996; Mulholland et al., 1997) in the hyporheic zone while Chafiq et al. (1999) examined the relationship between microbial and bacterial biomass with depth. Williams (1989) researched correlations between the taxa or hyporheos in the hyporheic zone with the water chemistry. Most research that may prove applicable to the hyporheic zone has focused on fecal coliforms and fecal streptococci within stream sediments.

Research into the role of stream sedimentation and fecal coliforms has resulted in interesting discoveries. Bottom sediments appear to act as reservoirs for fecal coliforms. Research by Stephenson and Rychert (1982) indicated a definite relationship between the concentrations of *E. coli* in bottom sediments as compared to the overlying waters. The researchers found that fecal coliforms in

the sediment were 2 to 270 times greater than that in the overlying water. Van Donsel and Gelreich (1971) noted similar results with the concentration of sediment fecal coliforms 100 to 1000 times greater than that of the overlying water. Stephenson and Rychert (1982) noted that disturbance of the bottom sediment resulted in higher *E. coli* concentrations in the overlying water downstream from the resuspension of fecal coliforms. Along with sedimentation, the particle size of the sediment appears to have a crucial affect on the survival rate of fecal coliforms. Howell et al. (1996) found that the mortality rates of fecal coliforms were much lower in clay-sized sediment as opposed to coarser sediments. Similarly, fecal streptococci mortality rates declined in clay sized sediments. However, the mortality rates of fecal streptococci did not dramatically increase in coarse sediments. Sherer et al. (1992) also noted a relationship between sediment type and fecal survival rates, with higher survival rates occurring in fine sediments. Burton et al. (1986) observed that *E. coli* survived longer in sediments containing at least 25% clay. The carbon content of the sediments also aids in the longevity of fecal bacteria. Stephenson and Rychert (1982) discovered a relationship between the *E. coli* concentration in the bottom sediment and the percent of organic carbon in the sediment. Higher numbers of *E. coli* in the bottom sediments corresponded to a greater percentage of organic carbon in the bottom sediments.

Other factors such as temperature and ultraviolet light play important roles in the mortality rates of fecal organisms. Howell et al. (1996) observed that

temperature had a significant effect on both fecal coliforms and fecal streptococci. Edwards et al. (1997) noted that the concentrations of fecal coliforms and fecal streptococci were highest during the summer months, whether nutrient or temperature related. Fecal coliforms appeared to decrease in numbers in the daytime (Gannon et al., 1983). The researchers allowed the fecal coliforms to experience both sedimentation and exposure to direct sunlight to reduced light, thus determining that ultraviolet light increased the mortality rates.

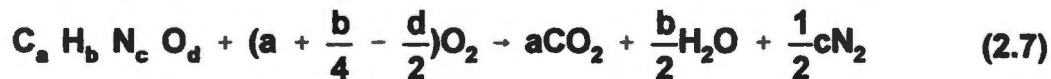
Water quality research often examines the concentrations of fecals because they are among the primary indicators of a stream's health. However, research into the hyporheic zone has not closely examined any forms of fecal contamination. This point is interesting in light of the fact that much research has been conducted upon fecal survival rates among the bottom sediments of streams (which are essentially hyporheic sediments). However, no research has focused upon the ability of the hyporheic zone to remove these contaminants from that water. Measurements of both fecal coliforms and fecal streptococci were both performed to gain an understanding of the ability of the hyporheic zone in controlling these constituents.

ORGANIC CARBON

The organically bound carbon in a sample is defined as the total organic carbon (TOC). Total organic carbon is comprised of dissolved organic carbon

(DOC), particulate organic carbon (POC), and total inorganic carbon (TIC).

Dissolved organic carbon is the fraction of carbon that remains in a sample after it is filtered through a 0.45 µm filter (Robinson, 1998). The carbon that remains on the 0.45 µm filter is the POC. Carbonate, bicarbonate, and dissolved carbon dioxide comprise the fraction of TOC known as TIC (APHA, 1992). The oxidation of organic matter ($C_aH_bN_cO_d$) results in the production of carbon dioxide (CO_2), water (H_2O), and nitrogen gas (N_2) as represented in the following equation.



When measuring TOC, the concentration of carbon dioxide is actually measured. Hence, knowledge of the amount of carbon that is converted to carbon dioxide on a 1:1 ratio (C_a : aCO_2) is desired (Robinson, 1998).

PREVIOUS RESEARCH ON CARBON IN THE HYPORHEIC ZONE

The majority of research into carbon cycling in the hyporheic zone has focused upon dissolved organic carbon. Hendricks and White (1991) in their study of the hyporheic zone of a northern Michigan stream examined dissolved organic carbon. The mean DOC concentrations in surface water were 12.4 mg/L, and the mean concentration in groundwater was 2.5 mg/L. The DOC levels in the hyporheic zone were higher than those of both surface water and groundwater. The researchers cited sources that indicated concentrated DOC

levels often occurred in sediments at depths of 10 to 20 cm (Crocker and Meyer, 1987; Rutherford and Hynes, 1987).

Another reference indicated the minimal contribution of groundwater to DOC levels (Wallis et al., 1981). Hendricks and White (1981) discovered that DOC levels decreased with depth in the hyporheic zone. Similar results were obtained by Schindler and Krabbenhoft (1998) who noted an order of magnitude decrease in DOC concentration from a 15-cm to a 61-cm depth. They also noted that the DOC concentrations did not decrease with increasing distance downstream. Meyer and Tate (1983) discovered that DOC concentrations in groundwater were lower than in the surface water of a North Carolina stream.

Dissolved organic carbon levels in the hyporheic zone of Little Lost Man Creek in northwestern California were high when the water traveled long distances through the hyporheic zone, but they were low at locations with short travel times according to Triska et al. (1993). The researchers found DOC levels between 1.2 and 2.0 mg/L in stream water and the portion of the hyporheic zone that consisted mainly of stream water. The section of the hyporheic zone that received a greater than 75% contribution from groundwater had DOC levels between 2.7 and 5.3 mg/L. Triska et al. (1993) felt that the low DOC levels measured in the hyporheic zone did not significantly limit the rate of denitrification. The researchers discovered that DOC concentrations were significantly higher for areas with the greatest potential for denitrification.

Findlay et al. (1993) examined the relationship between microbial metabolism and dissolved organic carbon concentrations in the hyporheic zone of

Wappinger Creek in New York state. Additionally, the researchers measured dissolved inorganic carbon (DIC). Laboratory experiments with hyporheic sediments revealed that water with a higher DOC content supported a larger microbial population. Oxygen and DOC concentrations displayed similar trends with both decreasing along the flow path of the water. However, DIC levels increased with distance along the flow path indicating that DOC and oxygen depletion coupled with DIC generation were the result of biotic activities. Findlay et al. (1993) performed a stoichiometric analysis of the DOC, O₂, and DIC levels to determine if the concentration changes were truly the result of biotic activity and not DOC adsorption to sediments. Again, they concluded that microbial activity mainly accounted for the concentration changes and that adsorption of DOC to sediments was not a significant factor.

In a 1995 article, Findlay attempted to explain the important components of carbon cycling in the hyporheic zone. Findlay noted that carbon enters the hyporheic zone in two main forms: particulate organic carbon (POC) and dissolved organic carbon (DOC). A combination of stream disturbances coupled with stream water and groundwater flows transport POC and DOC into the hyporheic zone. Hyporheos also assist in transporting POC into the hyporheic zone. Unlike DOC which is frequently found in the hyporheic zone, POC inputs are more sporadic. Findlay noted that few microbes are capable of using DOC as their carbon source; hence, he noted the importance of POC inputs into the hyporheic zone.

In their examination of the hyporheic zone of the Rhone River in France, Chafiq et al. (1999) noted that dissolved organic carbon concentrations decreased in samples downstream similar to results obtained by Findlay et al. (1993). They noted that DOC concentrations for the system were more than adequate (greater than 1.5 mg/L), but POC levels were thought to limit biological activity. POC concentrations were highly correlated with sediment depth and microbial populations and activity. Such a relationship did not exist between DOC and the biological factors. The researchers theorized that the more readily available forms of DOC were more important than the quantity, since an adequate supply was available but not well correlated to biological activities.

The amount of organic carbon present in hyporheic sediment may affect the rate of denitrification (Myrold, 1997; Ingersoll and Baker, 1998), *E. coli* concentrations, and microbial metabolism (Pusch, 1996). The rate of microbial metabolism can have an effect on nitrogen, phosphorus, iron, and sulfur cycling; higher levels of carbon require higher rates of respiration. As oxygen is depleted from the environment, the microbes require an alternate terminal electron acceptor in the form of nitrogen, iron or sulfur. Additionally, a growing microbial population will utilize greater amounts of phosphorus.

Almost all of the previous research performed on the hyporheic zone focused upon DOC measurements as an indicator of carbon transformations in the hyporheic zone with a portion of the authors also examining POC and DIC. Dissolved organic carbon measurements were also performed with this research

to assist in quantifying the influence of either the stream water or groundwater at various locations within the hyporheic zone.

DISSOLVED OXYGEN

The health of a stream can be characterized by its dissolved oxygen (DO) level. Numerous chemical, physical, and biological processes affect the dissolved oxygen level of water systems (APHA, 1992). Aquatic life survives only if the DO level of the stream is adequate, such as with fish. As previously mentioned, a minimum dissolved oxygen level of 5.5 mg/L is required for trout and salmon to survive. Small mouth bass can tolerate levels as low as 4.2 mg/L (Stickney, 1986). Fish deposit their oxygen dependent eggs on top of the stream sediments (Findlay, 1995). The maximum concentration of DO in water depends a number of factors including temperature, pressure, and ionic strength (APHA, 1992; Robinson, 1998). DO levels also affect hyporheic reactions such as denitrification which proceeds in the absence, or near absence, of oxygen (Myrold, 1998).

PREVIOUS RESEARCH ON DISSOLVED OXYGEN IN THE HYPORHEIC ZONE

Virtually all studies of the hyporheic zone include measurements of dissolved oxygen. Grimm and Fisher (1984) used the changes in oxygen concentrations between upstream and downstream samples to calculate the metabolism of a desert stream. The researchers noted that oxygen concentrations of the hyporheic water were slightly lower than those of surface waters with the DO level decreasing with depth. Oxygenated interstitial waters revealed the movement of surface water into the hyporheic zone. Oxygen consumption rates of sediment cores ranged between 1.64 and 2.41 mg O₂ L⁻¹ h⁻¹. Grimm and Fisher (1984) concluded that surface waters continually replenished hyporheic DO levels, for hyporheic waters would be depleted of DO in a few hours without an input. Thus, they noted that respiration in the hyporheic zone was significant. Boulton et al. (1998) also reported the importance of surface waters carrying dissolved oxygen into the hyporheic zone. Hill (1990) measured dissolved oxygen concentrations of shallow groundwater emerging as springs, deep groundwater becoming rivulets, and deep groundwater entering the stream as bed and bank seepage in a Canadian headwater catchment. He noted differences between DO levels of shallow and deep groundwater with DO levels less for deep groundwater.

Duff and Triska (1990) discovered that dissolved oxygen levels declined with lateral distance from Little Lost Man Creek in northwestern California. The researchers believed that the hyporheic zone received DO from the inward (stream to bank) lateral movement of stream water. As expected, DO concentrations dropped with the rising temperature, since water holds less DO at higher temperatures. However, the DO level at more inland sites fell below levels expected for the recorded temperatures indicating high demands by the microbial community. Similar results were reported by Triska et al. (1990).

Examination of the hyporheic zone of Maple River in northern Michigan by Hendricks and White (1991) included measurements of dissolved oxygen. Surface water continually had higher DO levels than groundwater which remained fairly constant at 2 mg/L. The researchers stated that summer DO values varied considerably, but those taken in February displayed more regular patterns. Concentrations of hyporheic DO remained similar to those of surface waters until a depth of 50 cm. Additionally, DO concentrations decreased with distance downstream.

Triska et al. (1993) noted that at Lost Little Man Creek, dissolved oxygen concentrations were high in areas of the hyporheic zone that experienced a short travel time, but were low in areas with a long travel time. They found trends similar to that reported by Duff and Triska (1990) in that DO levels decreased with distance from the channel. Surface waters were typically saturated with DO, but wells located farthest inland had anoxic conditions. The researchers discovered that the stream water traveled up to 18 m inland.

In the process of researching the hyporheic zone of Wappinger Creek in New York, Findlay et al. (1993) measured dissolved oxygen levels. Surface waters were often saturated with DO. Dissolved oxygen levels decreased along the well transect that was somewhat parallel to the stream from the upstream to the downstream point. After stoichiometric analysis, Findlay et al. (1993) believed the decrease in DO was a result of biological activity.

Mulholland et al. (1997) used dissolved oxygen measurements of upstream and downstream sites along two southeastern streams in the United States for the purpose of calculating gross primary production and community respiration. Results of the analysis indicated that respiration was the main activity in both streams. However, the stream with the larger hyporheic zone exhibited higher respiration rates. The researchers concluded that the size of the hyporheic zone has a notable impact on stream respiration.

Hill and Lyburner (1998) measured DO levels in a study of the hyporheic zones of Duffin Creek and Glen Major in Canada. Groundwater DO levels were significantly lower than surface water for both streams. Dissolved oxygen concentrations at Glen Major declined with depth. A similar trend was reported for Duffin Creek. However, the researchers did not find longitudinal trends with DO levels.

Dissolved oxygen measurements of samples from the Rhone River in France were taken during research of the hyporheic zone by Chafiq et al. (1999). The researchers discovered that DO levels decreased with distance downstream. They also noted a decrease of DO concentrations with depth. Chafiq et al.

(1999) theorized that variations in dissolved oxygen levels over the course of the study were related to changing discharge. One sampling point had the highest DO levels during high flow while peak DO levels occurred at a low discharge for two others.

Similar to previous studies of the hyporheic zone, this research used dissolved oxygen measurements to assist in identifying the processes that were likely occurring in the hyporheic zone. Earlier research provided a clear link between DO concentration gradients and particular chemical and biological processes. Since all of the fore mentioned research occurred in other geographical areas, measurements of DO may provide a connection between the multiple systems.

BIOCHEMICAL OXYGEN DEMAND

Water quality researchers often use the measurement of the five-day biochemical oxygen demand (BOD_5) to determine the amount of degradable organic matter in water (APHA, 1992; Robinson, 1998). The term BOD_5 indicates the amount of oxygen needed by bacteria and other microorganisms to decompose the organic matter present in the water sample and is represented by the following equation (Jenkins et al., 1980):

Microorganisms + Organic Matter + O₂ →

More Microorganisms + CO₂ + H₂O + Residual Organic Matter (2.8)

A high BOD₅ value equates to a high-strength waste or a high level of degradable organic matter; a low BOD₅ level represents a low-strength waste or a low degradable organic matter content. The subscript "5" on the term BOD₅ indicates the typical five-day period required to administer the test (APHA, 1992; Hammer and Hammer, 1996; RRDEC, 1998; Robinson, 1998). Measurements of BOD₅ can include those not only of carbonaceous organic matter, but also nitrogenous organic compounds unless a nitrification inhibitor is added to the samples (Jenkins et al., 1980; APHA, 1992).

PREVIOUS RESEARCH ON BIOCHEMICAL OXYGEN DEMAND IN THE HYPORHEIC ZONE

Biochemical oxygen demand is frequently used at wastewater treatment facilities, but researchers have also used the test to gauge the health of streams (Benefield and Randall, 1985). According to Arms (1990), if the BOD of a water system results in an oxygen level below 4 mg/L, the water is extremely polluted. Researchers of the hyporheic zone have not frequently measured the biochemical oxygen demand. Williams (1989) measured the one-day BOD concentration of water sampled from the hyporheic zone. He found a higher

biochemical oxygen demand in samples immediately below the stream bed at Duffin Creek. However, Rouge River samples taken under the bank had a greater one-day biochemical oxygen demand. The BOD at Rouge River displayed a high positive correlation with the carbon dioxide concentration, but no significant correlation was established between BOD and other measurements at Duffin Creek.

Measurements of BOD₅ in the hyporheic zone are lacking at best. This study attempts to use BOD₅ concentrations as an indicator of hyporheic water quality; an approach not yet utilized.

CHEMICAL OXYGEN DEMAND

Chemical oxygen demand (COD) is a measure of the amount of oxygen required to chemically oxidize all organic matter in a sample. COD measures approximately 95 to 100% of the theoretical oxidation of the organic matter in the sample (APHA, 1992; Robinson, 1998). Typically, potassium dichromate is used as the oxidant with an acid solution and elevated temperatures (i.e., digestion).

The equation for the reaction is as follows:



where

$$c = \frac{2}{3}n + \frac{a}{6} - \frac{b}{3}$$

Any remaining potassium dichromate not reduced in the reaction is measured to determine the oxygen demand of the sample. The presence of chloride can cause interferences with COD results (Jenkins et al., 1980). Since COD measurements do not distinguish between organic and inorganic matter, substances such as nitrites, ferrous iron, and sulfides may be oxidized (Benefield and Randall, 1985).

PREVIOUS RESEARCH ON CHEMICAL OXYGEN DEMAND IN THE HYPORHEIC ZONE

The measurement of chemical oxygen demand is typically used in wastewater treatment facilities because the test is run in a considerably shorter time than BOD₅ (Robinson, 1998). Currently, there are no reports of COD measurements in hyporheic zone research. As with BOD₅, COD may prove to be a useful parameter in gauging hyporheic water quality, but the avenue has yet to be explored.

TOTAL SOLIDS

Agricultural lands generate over half of the estimated three billion tons of sediment deposited annually in waterways (Knight et al., 1998). Knight et al. (1998) referred to research by Wedemeyer et al. (1976) that indicates gill damage in fish begins to occur at suspended solid concentrations of 80 to 100 mg/L. Knight et al. (1998) also noted that besides damaging fish and other aquatic invertebrates, suspended solids contribute to pesticide and metal transport.

The solids in a water system can be divided into numerous categories such as total solids, total dissolved solids, total suspended solids, and volatile solids. Other divisions exist, but they are seldom used in natural water systems. APHA (1992) defines total solids as the residue remaining after evaporating the water in the sample at 103 to 105°C; hence, gases and water are not part of the measurement. Total dissolved solids and total suspended solids are determined after the sample is filtered. The portion of the sample that passes through the filter is evaporated at 180°C to determine total dissolved solids while the fraction remaining on the filter is dried at 103 to 105°C to give total suspended solids (APHA, 1992). Volatile solids, mostly materials of organic form with some inorganics, are generally "lost" or converted to carbon dioxide and water when the sample is heated at 550°C (Robinson, 1992).

PREVIOUS RESEARCH ON TOTAL SOLIDS IN THE HYPORHEIC ZONE

The solids present in hyporheic water affect the lives of aquatic organisms. Boulton et al. (1998) reported that the influx of fine sediments into the hyporheic zone can clog pathways, thus reducing the flow of water. The reduction in the flow of water into the hyporheic zone also resulted in the reduction of oxygen and nutrient inputs. Most research into the hyporheic zone does not examine forms of solids in the water. Williams (1989) examined suspended solids while attempting to correlate water chemistry with the type of organisms inhabiting the hyporheic zone. He noted that suspended solids were usually higher in the hyporheic zone of Duffin Creek than in surface waters. Suspended solids were highly correlated with organic matter content ($r^2=0.76$), alkalinity ($r^2=0.87$), and conductivity ($r^2=0.87$); moderately correlated to sulphide ($r^2=0.63$) and carbon dioxide ($r^2=0.59$) concentrations. Along the Rouge River, suspended solids concentrations were only slightly higher than surface waters. Established correlations were moderate between suspended solids and oxygen concentration ($r^2=-0.63$), conductivity ($r^2=0.64$), alkalinity ($r^2=0.59$), and pH ($r^2=0.61$). A strong correlation was discovered between suspended solids and one day BOD ($r^2=0.71$) using an $r^2=0.70$ as the categorical separation point. Others have researched activities within sediment samples, but have not studied the role of sediments in the hyporheic waters (Duff and Triska, 1990; Muholland et al., 1997; Chafiq et al., 1999).

One unique point of this study is that, similar to Boulton et al. (1998), measurements of TS were taken for the purpose of determining their relation to other constituents. This is an area that has proven to be beneficial as seen by the results of Boulton et al. (1998), but one that lacks a thorough examination.

pH

The acidity or alkalinity of a solution is described by pH. The measurement of pH indicates the negative logarithm of the hydrogen ion concentration where p is the symbol used to indicate a negative logarithm and H represents the hydrogen ion. Commonly, the equation for pH is written as follows:

$$\text{pH} = -\log[\text{H}^+]$$
(2.10)

However, $[\text{H}^+]$ in the equation actually represents the hydronium ion, H_3O^+ (Whitten et al., 1988). Regions of pH have been established based on $[\text{H}^+]$ and $[\text{OH}^-]$ concentrations with the scale ranging from 0 to 14. At neutrality, the concentration of hydrogen ions and hydroxyl ions are equal, thus indicating a pH of seven. If the concentration of hydrogen ions is greater than the concentration of hydroxyl ions, the solution is acidic. Conversely, an alkaline solution contains a greater concentration of hydroxyl ions than hydrogen ions. For an acidic

solution, the pH is less than seven; for an alkaline solution, the pH is greater than seven (Omega Engineering, Inc., 1995; Whitten et al., 1988).

The pH of a stream can provide insight to its health. The change in pH of stream water, groundwater, or water of the hyporheic zone can indicate the production of certain products. The process of nitrification results in the release of hydrogen ions, thus making the surrounding environment more acidic. The transformation of nitrate to ammonium and the process of denitrification both use hydrogen ions (Myrold, 1998; Mullen, 1998). The reduction of sulfur produces hydrogen sulfide using hydrogen ions in the process (Manahan, 1993; Germida, 1998). Oxidation-reduction potential is pH dependent, and must be evaluated with pH in mind (Gambrell, 1975; APHA, 1992; Gale, 1998).

PREVIOUS RESEARCH ON pH IN THE HYPORHEIC ZONE

Williams (1989) measured the pH of samples taken at Duffin Creek and Rouge River in Canada with the goal of discovering a correlation between the organisms inhabiting the various sections of the hyporheic zone and the chemistry of the water. The pH at Duffin Creek remained in the range of 7.4 to 8.0 with the pH slightly lower in samples of hyporheic waters. He found that the level of carbon dioxide was highly negatively correlated with pH ($r^2=-0.80$) and moderately correlated with oxygen concentrations ($r^2=0.67$). At Rouge River, the measured pH was in the same range of 7.4 to 8.0. Similarly, the pH of the hyporheic waters was a little lower than that of the other samples from the river.

Once again, pH was highly negatively correlated with carbon dioxide levels ($r^2=-0.88$) and moderately correlated with oxygen concentrations ($r^2=0.62$). However, additional correlations were established at Rouge River. The pH of the samples had a negative, moderate correlation with biochemical oxygen demand, conductivity, and alkalinity.

The pH was measured in the field during a study performed on the hyporheic zone of a north Michigan stream by Hendricks and White (1991). The average pH of surface waters ranged between 7.5 and 8.5 while that of groundwater was between 6.5 and 7.0. The researchers noted that surface water was between 0.5 to 1 pH unit higher than hyporheic waters; a significant increase. As indicated by the above values, groundwater was 1 to 2 pH units lower than surface water. The measured pH values coincided with other research noted by Hendricks and White (1984) that indicated a decrease in pH of approximately 1 unit for hyporheic water compared to surface waters (Williams and Hynes, 1974; Williams, 1984, 1989).

Mulholland et al. (1997) noted a pH difference between the Walker Branch stream (pH 8.1) in East Tennessee and that of Hugh White Creek in North Carolina (pH 6.7). The authors did not theorize on the reasons for the differences in pH, but they did state that the hyporheic zone of Hugh White Creek was twice that of the Walker Branch stream, and that the size of the hyporheic zone was related to nutrient transformations.

Chafiq et al. (1999) measured pH of all samples taken during research of the hyporheic zone of the Rhone River in France. Little was done with the pH

data except to acknowledge that decreases in pH and oxygen content during the summer months were the result of high levels of very fine sediments. Very fine sediments were defined as those less than 50 μm in diameter. The researchers felt that the decreased microbial activity observed in hyporheic sediments was from these changes.

The pH of the hyporheic waters were measured in this study to aide in identifying chemical and biological processes that occurred in the hyporheic zone. As seen by the previous research, the use of pH for this purpose has been under utilized although some correlations have been established between pH and other contaminants. Similar to the earlier studies, any changes in the pH between the stream water and the hyporheic waters were noted.

OXIDATION-REDUCTION POTENTIAL

Electrochemistry deals with the electrical current that is driven by and produces chemical reactions (Whitten et al., 1992). Oxidation-reduction reactions are the focus of electrochemistry, for they involve the transfer of electrons. Oxidation denotes the process by which a compound donates electrons, thus becoming oxidized; reduction indicates the process in which a compound gains electrons (Hartel, 1997; Sawyer and McCarty, 1978). Oxidation-reduction reactions occur together; for every oxidation reaction, a reduction reaction takes place. The oxidation-reduction or redox potential of electrochemical reactions is

based upon the Nernst equation (Plambeck, 1996). The pH of the solution affects the potential of the system. Accounting for the pH of the system, the Nernst equation is written as follows:

$$E = E^0 - \frac{59}{n} \log \frac{(\text{Red})}{(\text{Ox})} + 59 \frac{m}{n} \text{ pH} \quad (2.11)$$

where

E is the potential for nonstandard conditions, V

E^0 is the potential for standard conditions, V

Red is the reduced species, M

Ox is the oxidized species, M

m is the number of protons, H^+

n is the number of electrons, e^-

Standard conditions are those at 25°C, 1 atmosphere, and 1 molar ionic strength (Whitten et al., 1992). For practical purposes, a unity between protons and electrons is assumed, thus resulting in a 59 mV change in the redox potential per unit of pH (Gambrell, 1975; Patrick et al., 1996). In natural or field conditions, factors such as the presence of silicates, carbonates, and oxyhydroxides that are found naturally in soils affect the redox potential (Patrick et al., 1996).

The measure of the redox potential is used as an indicator of the type of chemical reactions in the soil environment. Analysis of hyporheic water should be

conducted as the primary source of information with the redox value acting as a secondary indicator, since several factors influence the redox potential. Such information can assist in determining which reactions are occurring and whether the reactions are nitrification, denitrification, ammonification, sulfur reduction, or iron reduction (McBride, 1994; Patrick et al., 1996). According to Bower (1984) soil types are characterized by their redox potential range indicated in parentheses: oxidized soils (greater than 400 mV), moderately reduced soils (100 to 400 mV), reduced soils (-100 to 100 mV), and highly reduced soils (below -300 to -100 mV). Once oxygen is depleted from the system, microorganisms utilize other compounds as terminal electron acceptors in the order of nitrate, ferrous iron, and sulfate. Denitrification occurs at a redox potential between 280 to 220 mV, reduction of ferric iron occurs in the 110 to 80 mV range, and sulfate reduction happens within the redox range of -140 to -170 mV (Brady and Weil, 1999).

PREVIOUS RESEARCH ON REDOX POTENTIAL IN THE HYPORHEIC ZONE

Research of the hyporheic zone that uses oxidation-reduction potential as a secondary indicator is limited to one study. Hendricks and White (1991) measured the oxidation-reduction potential of surface water, groundwater, and hyporheic waters with field meters during their study in northern Michigan. Data for only one year were collected pertaining to the redox potential. The researchers found that the redox potential of the surface water (229 mV) was

considerably higher than that of the groundwater (45 mV). Redox potential in the hyporheic zone was considerably varied with the lowest value occurring at depths of 10 to 20 cm in the sediments. Upstream and downstream hyporheic redox values exhibited differences, with the upstream section displaying redox values similar to those of the surface water.

Research into riparian buffers by Jacobs and Gilliam (1985) exemplifies the promise of using redox potential to aid in defining chemical transformations. They used the redox potential coupled with a water analysis for nitrate-N and TKN to determine whether or not the reduction of nitrogen was due to denitrification.

This study further explored the usefulness of the oxidation-reduction potential by obtaining measurements for a portion of the hyporheic water samples. As with pH and DO, the oxidation-reduction potential is a valuable secondary indicator in understanding hyporheic processes; like pH, it is under utilized.

TEMPERATURE

Temperature is an important parameter in microbial activity as evidenced by the various habitats of microorganisms. Some dwell in cold environments, some in warm environments, and others in hot environments. Microorganisms are divided into three classes according to the temperature ranges at which they

survive. Microorganisms that survive in the temperature range of -10 to 10°C are termed psychrophilic. Microbes that prefer temperatures between 40 to 80°C are thermophilic. The majority of microorganisms are mesophilic meaning they prefer temperatures in the range of 10 to 40°C (Alexander, 1997). Microbially mediated reactions are dependent on temperature, thus water chemistry can vary with temperature. Optimum levels of nitrification occur in the temperature range of 20 to 30°C; optimum denitrification rates occur between 25 and 35°C (Brady and Weil, 1999). According to Howell et al. (1996), a significant correlation exists between the mortality rates of fecal coliforms and temperature; a similar correlation exists between fecal streptococci mortality rates and temperature. Temperature increases from 4 to 35°C resulted in higher mortality rates for both species, so the death rate increases with temperature. Sulfate reduction is also influenced by temperature. Elsgaard et al. (1994) noted that microbial sulfate reduction in hydrothermal sediments occurred at optimum rates for mesophilic species from 34 to 45 °C and from 56 to 65°C for thermophilic species. The level of dissolved oxygen in water is dependent on temperature with the solubility decreasing with increasing temperatures (APHA, 1992). Seasonality has an effect on the type of vegetative growth around the hyporheic zone and in the stream. Processes such as denitrification are dependent upon carbon availability with leaves being a large source (Myrold, 1998; Ingersoll and Baker, 1998).

PREVIOUS RESEARCH ON TEMPERATURE IN THE HYPORHEIC ZONE

A large portion of the research on the hyporheic zone occurred during summer months (Duff and Triska, 1990; Triska et al., 1993), especially with studies that examined microbial community respiration and productivity. Respiration experiments were conducted during summer months for the purpose of obtaining maximum values (Grimm and Fisher, 1984). Pucsh (1996) examined hyporheic respiration during the spring and summer with average water temperatures of 5°C and 16°C, respectively. He noted that the increase in temperature over the summer months resulted in higher respiration rates. Duff and Triska (1990) used temperature measurements of water samples to calculate the saturation percentage of dissolved oxygen. Williams (1989) used temperature measurements in a similar fashion; he found a moderate correlation between dissolved oxygen concentrations and temperature in water samples from Duffin Creek in Canada. The other waterway he examined in the study, Rouge River, exhibited no significant correlations between temperature and other parameters. Hendricks and White (1991) used temperature measurements to distinguish between surface water and groundwater. Surface waters of Maple River in northern Michigan peaked at 26 to 27°C during the summer, and groundwater samples remained constant at 8 to 9°C. Summer conditions resulted in warm surface waters moving to a depth of 50 cm in the hyporheic zone. Winter conditions reversed temperature trends between surface water and the warmer groundwater. The researchers discovered that temperature patterns

were similar to chemical gradients for soluble reactive phosphorus, winter dissolved oxygen, and chloride.

As with many of the earlier studies, contaminant trends underwent analysis for seasonality, especially those thought to be more biologically driven (i.e., BOD₅). However, unlike previous research, this study attempted to establish connections between temperature and a wide range of constituents, not merely one or two.

STREAM DISCHARGE

The beginning of the hydrologic cycle is typically designated as precipitation (Figure 2.7). Precipitation falls on both the stream surface and the land surface as rain or snow. Rain and melted snow can undergo the process of evapotranspiration, become runoff, or can infiltrate into the soil. The soil, until it reaches saturation, is capable of holding the moisture. Under saturated conditions, precipitation may flow overland becoming runoff. The water that infiltrates into the soil may become interflow and return to the surface or it may recharge groundwater storage zones. Precipitation that becomes runoff will typically enter some type of small channel and will continue to travel to a larger, more defined channel (Freeze and Cherry, 1979). A significant portion of the water that enters a channel comes from baseflow (Yoder and Yoder, 1999).

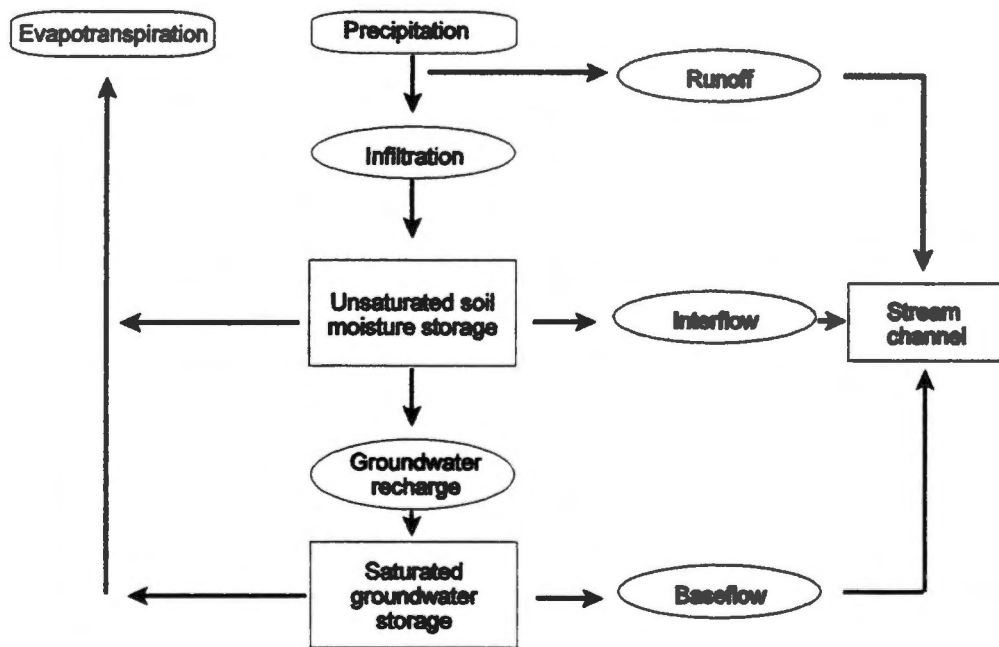


Figure 2.7. The hydrological cycle beginning with precipitation. Adapted from Freeze and Cherry (1979).

Each of the above components adds to the total volume of stream flow. Rates of stream discharge may affect the volume of water moving from the stream into the hyporheic zones. Different stages of the stream may produce flow paths of varying lengths, thus affecting the residence time of the water in the hyporheic zone. As previously mentioned, the residence time and length of flow path are two factors important in the contribution of the hyporheic zone to the water chemistry of the stream. Sherwani and Moreau (1975) stress the importance of collecting mass flow data of pollutants for a complete water quality study of a stream. By monitoring the discharge of the Haw River in North Carolina, Sherwani and Moreau (1975) discovered moderate correlations between all of the following: temperature and flow, BOD₅, and Kjeldahl nitrogen.

PREVIOUS RESEARCH ON STREAMFLOW EFFECTS ON THE HYPORHEIC ZONE

An understanding of the typical flow of a stream system is necessary in water quality studies because cases of seasonal variation or instances of drought or flooding may require monitoring of several years to accurately predict trends (Sherwani and Moreau, 1975). A large portion of studies on the hyporheic zone mention the average discharge and slope for the stream of interest (Duff and Triska, 1990; Triska et al., 1990; Hill, 1990; Findlay, 1995; Triska et al., 1993; Pusch, 1996; Mulholland et al., 1997; Hill and Lymburner, 1998; Chafiq et al.,

1999). However, few hyporheic studies attempt to correlate stream discharge with water chemistry. Findlay (1995) mentions research by Verier and Naiman (1992) that demonstrates the effects on river discharge variations on hyporheic DOC levels. Valett et al. (1994) in a study of Sycamore Creek in Arizona observed that floods and the length of time between floods influenced the direction and magnitude of hyporheic water exchange with the stream. Generally, vertical upwelling of hyporheic waters increased following floods. Pre-flood values for vertical upwelling waters were exceeded for 10 to 12 days after flooding. Mulholland et al. (1997) demonstrated that two southeastern United States streams with similar flows had hyporheic zones with different characteristics. Boulton et al. (1998) identified the hyporheic zone as a safe haven for stream and hyporheic organisms from strong currents generated in the stream during periods of high discharge. Chafiq et al. (1999) researched fractions of sediments in the hyporheic zone and noted that the level of fine sediments and particulate organic matter associated with the fine sediments decreased with high discharges at depths of 20 and 40 cm. The researchers also noted that hyporheic DOC concentrations increased and microbial biomass and activity decreased with increasing discharge. They theorized that high levels of discharge improve the influx of organic matter into the stream bed coupled with the removal of fine sediments, thus assisting in the restoration of the stream.

Another unique aspect of this study was that it attempted to establish relationships between the various contaminant concentrations and the discharge rate of the stream. Often, stream discharge merely receives a cursory glance,

usually one sentence stating the average flow rate. Others have made theoretical observations without scientific backing. This study attempted to provide a solid data set to enforce many of the discharge related observations made in earlier research of the hyporheic zone.

MICROBIAL ASSAYS

All chemical reactions related to microorganisms are catalyzed by enzymes. Benefield and Randall (1985) refer to microorganisms as “bags of enzymes.” Enzymes are catalytic proteins specific to the type of reactions and substrates with which they work; they are not altered by the reaction (Prescott et al., 1996; Fuhrmann, 1998). Enzymes work by lowering the energy of activation for the reaction. The energy of activation is the energy required to break existing chemical bonds within the specific substances, thus better controlling the rate and extent of the reactions (Fuhrmann, 1998). Enzyme assays are a means of understanding enzymatic reactions by controlling the substrate concentration, temperature, pH, and ionic strength. Conditions are such that the concentration of substrate is great enough to “saturate” the enzymes. The rate of product formation is then monitored (Tabatabai, 1994). To utilize compounds containing nitrogen, phosphorus, sulfur, or iron, microorganisms must use enzymes to assist in the degradation of these compounds (Fuhrmann, 1998). Microbially mediated reactions are important components of hyporheic water quality (Grimm

and Fisher, 1984; Williams, 1989; Hill, 1990; Triska et al., 1990; Duff and Triska, 1990; Henricks and White, 1991; Triska et al., 1993; Findlay, 1995; Mulholland et al., 1997; Hill and Lymburner, 1998; Chafiq et al., 1999).

MICROBIAL BIOMASS

For biological reactions to occur, the presence of a sufficient microbial population is required. The rate of nutrient cycling and pollutant degradation is controlled by the size of the microbial population (Jones, 1997). Parkinson and Paul (1982) explained the theory behind microbial biomass measurements as developed by Jenkinson (1966). A fumigated soil has a lower respiration rate than a nonfumigated soil immediately after removal of the fumigant. Once the fumigant is removed, the fumigated soil experiences a significant increase in the rate of respiration for a short time, exceeding that of the nonfumigated soil. Jenkinson (1966), as relayed by Parkinson and Paul (1982), theorized that the carbon in the dead microorganisms was much more easily mineralized than that of living microorganisms. Hence, he concluded that the release of carbon dioxide in the fumigated soil was the result of decomposition of the dead microbial cells in the fumigated soil. The difference between the extractable total organic carbon in the fumigated and nonfumigated soil samples is the measure of the microbial biomass; corrections are made for dilution and extraction efficiency (Parkinson and Paul, 1982; Vance et al., 1987; Kirchner, 1999).

DEHYDROGENASE

The enzyme dehydrogenase that is involved in microbial respiration is a parameter often used to determine the average activity of the microbial population in the soil. The oxidation of soil organic matter by microbes is largely controlled by the enzyme dehydrogenase. A general equation for dehydrogenation follows (Tabatatai, 1994):



where

XH_2 is an organic compound

A is the hydrogen acceptor

Dehydrogenase enzymes assist in transferring hydrogen ions from substrates to the final hydrogen acceptor. Under aerobic conditions, oxygen is the terminal electron acceptor; anaerobic conditions result in the use of oxidized compounds (Benefield and Randall, 1985, Tabatabai, 1994). Dehydrogenase activity is determined by the rate of transformation of 2,3,5 - triphenyltetrazolium chloride (TTC) to triphenylformazan (TPF) and is a colormetric measurement (Bollag and Stotzky, 1990; Tabatabai, 1994).

L-ASPARAGINASE

For nitrogen cycling, the enzyme L-asparaginase plays an important role in the production of ammonium and nitrate through nitrogen mineralization. The L-asparaginase enzyme produces L-aspartic acid and ammonia (NH_3) through catalyzation of the hydrolysis of L-asparagine. Nitrogen mineralization can further proceed to produce nitrate. According to Wriston (1971) as reported by Tabatabai (1994), L-asparaginase which occurs in both plants and microorganisms is abundantly present in the environment. Soil samples are incubated at 37°C with 0.1-M THAM at pH 10 (a buffered L-asparagine solution) and toluene, a microbial inhibitor. The subsequent production of ammonium is related to L-asparaginase activity.

PHOSPHOMONOESTERASES

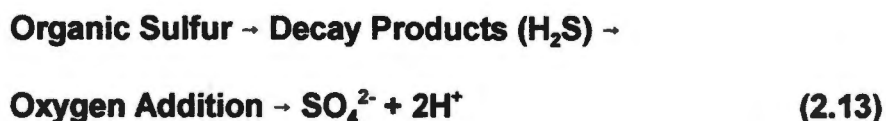
Phosphomonoesterases (phosphoric monoester hydrolases) assist in the mineralization of organic phosphorus to orthophosphate after which the orthophosphate is available for plant uptake, immobilization by microbial cells, or bound in insoluble inorganic complexes with Fe and Al (Burns, 1978; Tabatabai, 1994; Mullen, 1998). The phosphomonoesterase enzymes are divided into acid phosphatase and alkaline phosphatase because each performs optimally in under either acidic or alkaline conditions: acid phosphatase in acidic soils and alkaline

phosphatase in alkaline soils. Alkaline phosphatase activity is derived solely from microorganisms, but acid phosphatase activity is produced in both microorganisms and plants (Dick et al., 1983; Kirchner, 1997). Estimates of acid and alkaline phosphatase activity in soils is through colormetric determinations based on the amount of *p*-nitrophenol released from phosphatase activity. A buffered sodium *p*-nitrophenol solution with a pH of 6.5 for acidic phosphatase activity and a pH of 11 for alkaline phosphatase activity is added to soil samples, in addition to toluene. Soils are incubated at 37°C (Tabatabai, 1994).

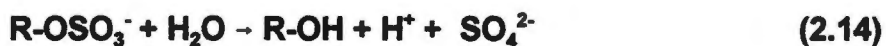
Juma and Tabatabai (1978) discovered that the activity of phosphomonoesterases was highest in surface soils and decreased with depth. They also noted that the presence of orthophosphate in soils inhibited the enzymes. Burns (1978) reported that little or no seasonal variation occurs with the concentration of phosphatase enzymes; however, the enzyme concentration is influenced by fertilization and the type of plant cover. The addition of labile carbon increases the rate of phosphorus mineralization, and thus the phosphatase enzymatic activity. In a separate study, Juma and Tabatabai (1997) concluded that the presence of ferric and ferrous irons among other trace elements in soils were inhibitory to acid phosphatase activity, but nitrate, nitrite, chloride, and sulfate showed no effects on the acid or alkaline phosphatase activity.

ARYLSULFATASE

The enzyme arylsulfatase is involved in the mineralization of sulfur (Tabatabai, 1994) which is denoted by the following equation (Brady and Weil, 1999):



The arylsulfatase anion is hydrolyzed by the arylsulfatase enzyme through severing of the O-S bond (Tabatabai, 1994; Kirchner, 1997):



The mineralization of organic sulfur caused by the enzyme arylsulfatase is a significant factor in the cycling of sulfur. The release of *p*-nitrophenol from soil incubated at 37°C with a *p*-nitrophenol sulfate solution and toluene indicates the activity of the enzyme arylsulfatase (Tabatabai, 1994). Research performed by Tabatabai and Bremmer (1970) indicates that arylsulfatase activity decreases with soil depth. Significant correlations exist between arylsulfatase activity and organic carbon content of the soil (Burns, 1978). Cooper (1972) as reported by Burns (1978) discovered increased arylsulfatase activity in Nigerian soils after

the rainy season, potentially indicating that maximum arylsulfatase activity occurs in wetter soils.

PREVIOUS RESEARCH ON MICROBIAL ASSAYS IN THE HYPORHEIC ZONE

Microbial numbers and enzymatic activity are often an overlooked component in hyporheic research. Chafiq et al. (1999) examined total microbial biomass and hydrolytic activity in sediment samples taken from the hyporheic zone of the Rhone River in France. They noted that the highest concentration of biomass and metabolic activity occurred at the surface of the sediment layer and declined with depth. A positive correlation was discovered between fine sediments and microbial biomass and activity. Chafiq et al. (1999) theorized that the combination of organic and inorganic fine particles controlled these biological factors in the hyporheic zone. Currently, no other studies have examined microbial concentrations and enzymatic activity in the hyporheic zone. Hence, the most unique aspect of this study revolves around the incorporation of biological data into the hydrological and chemical analysis of the hyporheic zone. A few of the previous researchers of the hyporheic zone have examined the various species present in the hyporheic zone, but none have examined the size, activity, or enzymatic potential of the microbes that inhabit the hyporheic zone.

CHAPTER 3

MATERIALS AND METHODS

SITE DESCRIPTION

The study site is situated on a small 40-hectare (100-acre) beef cattle and hay farm in west Knox County, TN near Beaver Ridge, approximately 40 km (25 miles) from downtown Knoxville (Plates 3.1 to 3.2). Research was conducted on Hickory Creek (Latitude 35°53' N, Longitude 84°13' W), an East Tennessee upland stream that runs through a grazing pasture where cattle have complete access to the stream (Plate 3.3). Hickory Creek is located along the southeastern portion of the farm. The local geology consists primarily of limestone bedrock with overlying soils classified as Typic Endoaquept, Glossic Fragiudult, and Oxyaquic Hapludalf (refer to Appendix A). Topographical information is presented in Appendix M. Two springs are located approximately 230 m (250 yards) upstream from the study site. One spring (SF), near the feeding area for the cattle, produces a small surface stream that flows into Hickory Creek (Plate 3.4). The flow from the other spring (SR) is transported to the creek by an underground tile system installed in the early 1960s. A wetland is reemerging approximately 115 m (125 yards) southeast of the study site (Plate 3.5). According to Richard Truitt (1999), the section of the grazing pasture



Plate 3.1. Cattle feeding on hay near the study site.



Plate 3.2. Hay is cultivated on the farm.



Plate 3.3. The cattle have free access to Hickory Creek.



Plate 3.4. This spring is located near a feeding area for the cattle and flows into Hickory Creek.



Plate 3.5. Damaged tile has allowed this wetland to reemerge.

containing the wetland was swampy and unsuitable for productive use until the installation of the tile system. Over the past ten years, the tile has begun to disintegrate, allowing portions of the soil to once again become saturated (Plate 3.6). A marsh that is fed by a third spring is located further upstream (Plate 3.7). Flow exits the marsh in the form of a small stream and enters the creek approximately 320 m (350 yards) upstream from the study site. Hayfields within the watershed were fertilized with Triple 19™ phosphate fertilizer during the months of March and April of 1999. Tractor ruts throughout the farm created during the wet winter months were disced and leveled during June of 1999. The field containing the study site and surrounding fields were mowed in July 1999.

CLIMATE

East Tennessee is located in a mesothermal climate characterized as a humid and temperate region with temperatures in the warmest month exceeding 22°C (Espenshade et al., 1990). Knox County experiences four distinct seasons: a mild winter, spring, warm summer, and fall. Typical winter lows are about -6°C (21°F) and are normally experienced during the months of January and February. The spring and fall seasons are mild with temperatures ranging from 15 to 25°C (59 to 77°F). The summer months are humid with highs reaching 35°C (95°F) (Lee, 1999). Annual precipitation averages 119.6 cm (47.1 in.) with an average of 30 cm (11.8 in.) of precipitation as snowfall (NOAA, 1999; Lee, 1999).



Plate 3.6. Water exits the tile system at one of two points along Hickory Creek. This exit is immediately downstream of the study site.



Plate 3.7. The marsh, fed by a spring, is located upstream of the study site and a small stream carries water from the marsh to Hickory Creek.

VEGETATION

The pasture land is mainly covered in Johnson grass, fescue, timothy, and clover. At the study site along Hickory Creek, the primary form of vegetation is deciduous hardwood trees with a few evergreens: Boxelder (*Acer Negundo*), Sycamore (*Platanus occidentalis*), Sugar Maple (*Acer saccharum*), Sweet Gum (*Liquidambar Styraciflua*), Black Walnut (*Juglans nigra*), Pignut Hickory (*Carya glabra*), Blue Beech (*Carpinus caroliniana*), Red Cedar (*Juniperus virginiana*), and Yellow Buckeye (*Aesculus octadra*). Over 50% of the wetland is covered in bulrush (*Juncus*) with cocklebur plants scattered about (Plate 3.8).

SOIL TYPE

On December 3, 1998, Dr. J. T. Ammons and his graduate students from the Plant and Soil Sciences Department at the University of Tennessee plus a crew from the United States Department of Agriculture, Natural Resources Soil Conservation Service (USDA, NRCS) Knox County division visited the study site to take soil core samples along the two proposed transects of the study site (Plates 3.9-3.12) in order to classify the soils. The field notes from the coring crew are in Appendix A. Five core sites were sampled: two in the wetland, two on the south side of the creek approximately 10 m (32.8 ft) from the creek, and one on the north side of the creek approximately 5 m (16.4 ft) from the creek.



Plate 3.8. Over 50% of the vegetation in the wetland is bulrush, and as the wetland spreads, so do the bulrushes.



Plate 3.9. A Giddings sampler truck was used to take core samples of the soil.



Plate 3.10. Hollow steel tubes were used to take the core samples.



Plate 3.11. The samples were removed from the hollow steel tubes and laid on divided PVC pipe.



Plate 3.12. The preliminary analysis of the core samples was conducted in the field.

Attempts were made to sample closer to the stream (within a few meters from the edge of the bank), but the crew could not position the sampling truck closer to the stream. All samples were analyzed in the Soil Pedology Laboratory of the Plant and Soil Sciences Department at the University of Tennessee.

WELLS

CONSTRUCTION

The initial design of the shallow groundwater sampling wells included the installation of multiple suction lysimeters at various depths. Morrison and Szecody (1985) developed a sleeve lysimeter and monitoring well combination that provided the researchers with the ability to sample from both the saturated and unsaturated zones of the soil within the same area (Figure 3.1). A series of lysimeters (as many or few as desired) were attached to the outside of the monitoring well. Caution is required when determining the minimum distance between the porous sections of the lysimeters. When two porous sections of the lysimeters are too close, flow interference between the two units can occur. The researchers, based on theoretical calculations, indicated that with a 5.1-cm (2-in.) diameter lysimeter, the radius of influence was approximately 10 cm (3.9 in.) in coarse sands and 65 cm (25.6 in.) in fine soils. The smaller the diameter of the lysimeter, the smaller the radius of influence. A bentonite seal was placed above

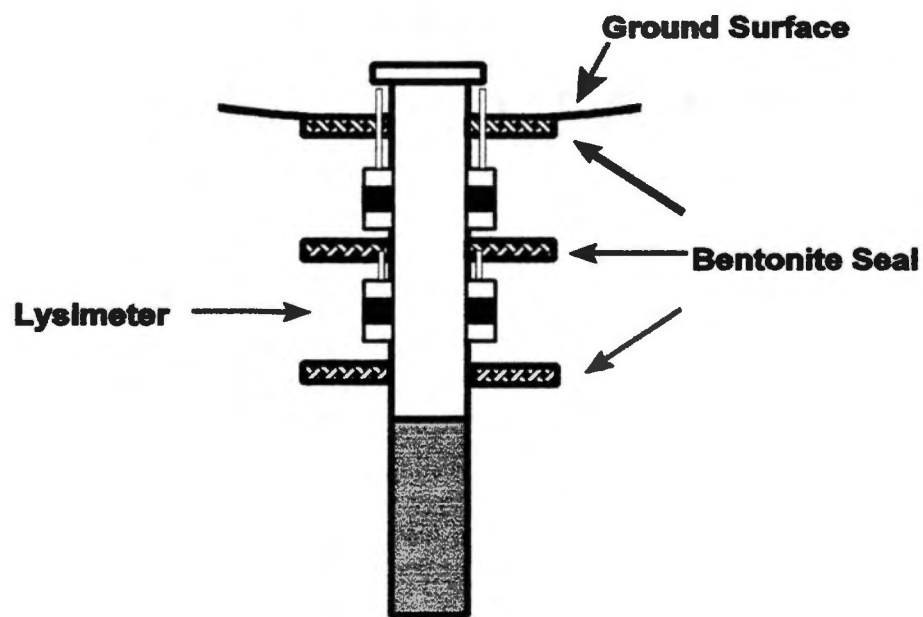


Figure 3.1. Morrison and Szecsody (1985) designed a monitoring well and lysimeter combination.

and below each lysimeter to assist in preventing vertical solute flow between sampling depths.

Construction of the shallow groundwater monitoring wells was modeled after those used by Rainwater (1996). Each well consisted of a 3-m (9.8-ft) section of 3.8-cm (1.5-in.) diameter schedule 40 PVC pipe. An end cap was placed on the bottom end of the PVC pipe. Twelve, 0.79-mm (1/32-in.) slots 12.7 cm (5 in.) in length were cut into the PVC pipe at even spacing around the circumference of the pipe. Two sets of the slots were cut, one beginning 15.2 cm (6 in.) from the end of the pipe with a 5.1-cm (2-in.) spacing between the sets (Figure 3.2). A 174-ml reservoir below the well slits allowed for the collection of any sediments to aide in the prevention of clogging of the slots. The top of the PVC pipe was left unaltered to allow for any necessary trimming during installation. The cap for the well was constructed, but not attached to the monitoring well. A 7.6-cm (3-in.) to 3.8-cm (1.5-in.) reducing coupler was attached to a section of 7.6-cm (3-in.) diameter PVC schedule 40 pipe of a nonspecific length (ranging from approximately 15.2 cm (6 in.) to 30.5 cm (12 in.)). A straight coupler was then placed on the section of 7.6-cm (3-in.) diameter PVC pipe to allow for the placement of an endcap. No PVC glue was used in the construction of the wells; this was to prevent any possible contamination from the glue. The end cap used in construction of the well cap had a 9.53-mm (3/8-in.) diameter hole drilled in the center to allow for entry of the sampling tubing. The sampling tubing consisted of Masterflex™ L/S 17 Tygon tubing with a 9.53-mm (3/8-in.) outside diameter. Excess tubing was wrapped

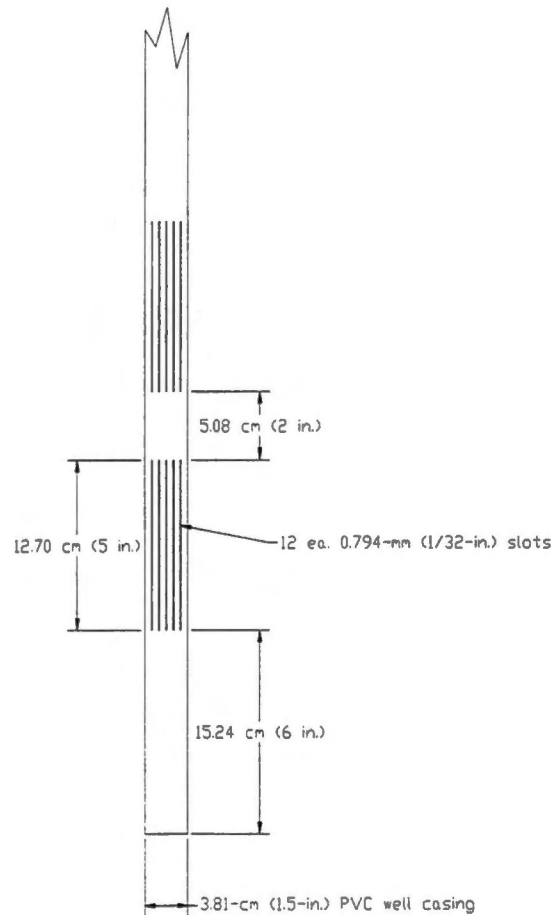


Figure 3.2. Two sets of slots were cut into the PVC pipe to allow for water entry.

around the well casing and remained dedicated to its respective well throughout the study period (Plate 3.13). Dedicated tubing decreased the chance of cross contamination between well samples. The portion of the well extending above the ground was covered with black corrugated PVC pipe of either 30.5-cm (12-in.) or 38.1-cm (15-in.) diameter and the appropriately sized cap (Plate 3.14). A description of the suction lysimeter portion of the well/lysimeter unit modeled after that described by Morrison and Szecody (1985) appears in a later section.

INSTALLATION

Prior to the installation of the monitoring wells and subsequently the suction lysimeters, flags were placed along two prospective transects. A Pentax™ PTS-III total station surveying unit was used to ensure that both transects were parallel and that the proposed well locations fell along a straight line. The transects were designated as Transect 1 and Transect 2. Transect 1 contained five wells while Transect 2 had three. The wells within each transect were assigned numbers beginning with one (Figure 3.3). Only the installation procedure for the monitoring wells is described in this section; a description of the lysimeter installation appears in a later section.

On December 7, 1998 through December 9, 1998, the well holes were dug using a Gopher™ one-man auger with a 15.2-cm (6-in.) diameter drill bit (Plates 3.15 to 3.16). Target depths were selected for the wells based upon the height of the bank in relation to a low flow level of Hickory Creek (Table 3.1). After drilling



Plate 3.13. Sampling tubing was dedicated to each well and remained in the well throughout the study.



Plate 3.14. Corrugated pipe was placed over each monitoring well for protection.

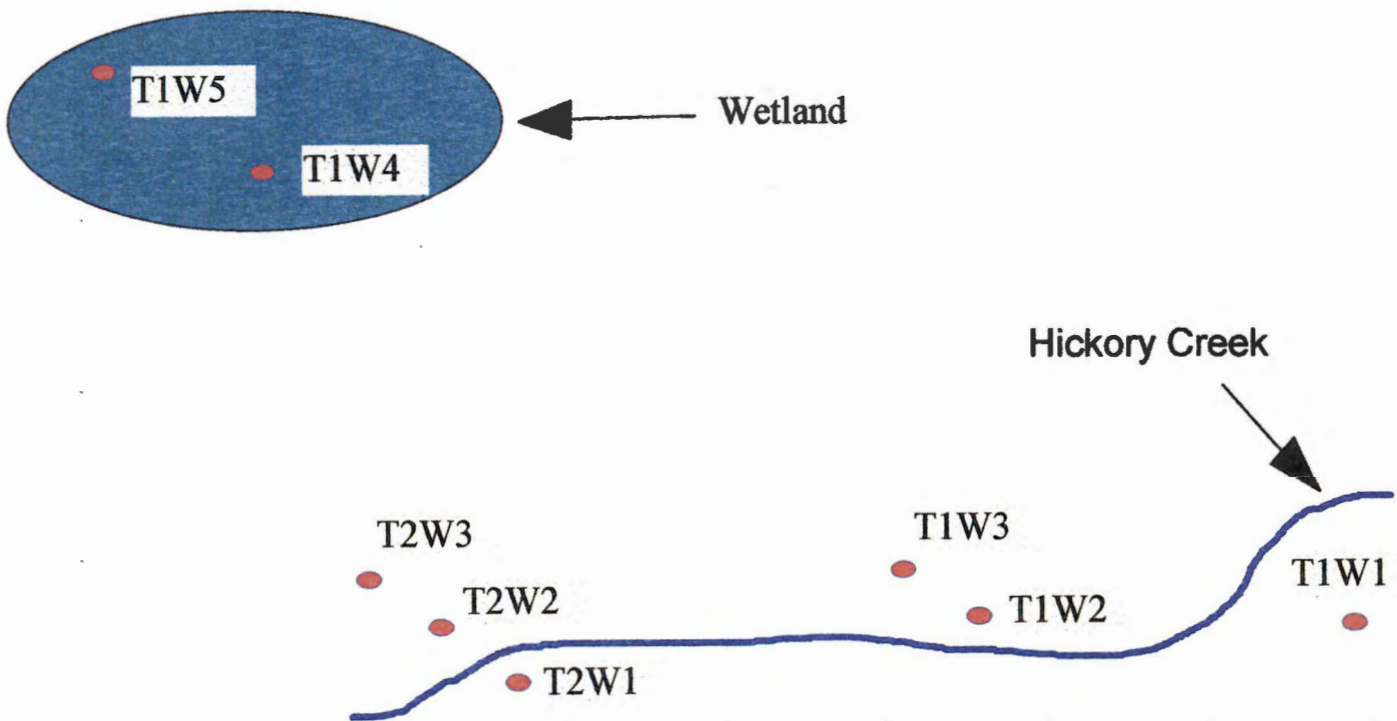


Figure 3.3. Site map for the shallow groundwater monitoring wells.



Plate 3.15. The Gopher™ one-man auger was used to dig the well holes, but actually required two personnel to operate.



Plate 3.16. A 15.24-cm (6-in.) drill bit with extensions was used to auger the hole.

Table 3.1. Target well depths were selected, but the actual well depths were often less because of the location of bedrock.

Well	Target Depth (m, (ft))	Actual Depth (m, (ft))
Transect 1 Well 1	1.524 (5.0)	1.295 (4.25)
Transect 1 Well 2	1.372 (4.5)	1.499 (4.917)
Transect 1 Well 3	1.372 (4.5)	1.511 (4.958)
Transect 1 Well 4	1.372 (4.5)	1.397 (4.583)
Transect 1 Well 5	1.372 (4.5)	1.181 (3.875)
Transect 2 Well 1	1.981 (6.5)	1.829 (6.0)
Transect 2 Well 2	1.372 (4.5)	1.27 (4.167)
Transect 2 Well 3	1.372 (4.5)	1.651 (5.417)

the holes, a bucket auger was used to remove excess soil and debris (Plate 3.17). Bedrock was struck at some of the well locations before reaching the desired depth. Plastic was used to cover the freshly drilled well holes until installation of the wells and lysimeters occurred a few days later. The well holes were cleaned with the bucket auger a second time immediately prior to installation of the wells.

The monitoring wells and lysimeters were installed simultaneously on January 11, 1999. A 2.54-cm (1-in.) thick layer of Driller's Service, Inc. (DSI) number 2 filter sand was placed at the bottom of the well hole. The end of the well casing with the precut slits was then placed into the well hole on top of the sand. Approximately 48.3 cm (19 in.) of the DSI number 2 filter sand was poured around the well casing to cover the slits and prevent the influx of fines into the well casing. The sand layer was tamped with a blunt stick to remove air pockets. A layer of Enviroplug™ bentonite between 3.81 cm (1.5 in.) and 5.1 cm (2 in.) was then placed over the sand. Water was poured onto the bentonite and allowed to set. The bentonite layer was used to prevent the sampling of water above the well slots, so that only the water at the appropriate depth was sampled. Installation of the lysimeters occurred above the first bentonite layer and is discussed in a later section. Above the lysimeters, a top layer of bentonite was placed followed by a layer of Quikrete™ non-shrink precision grout to prevent the flow of surface waters (precipitation) into the annulus around the well (Plate 3.18). Field notes for each individual well are included in Appendix B.



Plate 3.17. A hand-held bucket auger was used to remove excess soil prior to the installation of the wells.



Plate 3.18. Bentonite and grout layers were used to prevent water flow downward along the well casing.

WELL PREPARATION

Prior to sampling from the monitoring wells, cleaning from the installation procedure was required. To remove a large amount of the sediment that was introduced into the wells during installation, a large reservoir was created for use with a vacuum (Figure 3.4). The reservoir consisted of a 15.2-cm (6-in.) diameter 30.5-cm (12-in.) section of schedule 40 PVC pipe. A 15.2-cm (6-in.) end cap was glued onto one end of a PVC section and a 15.2-cm (6-in.) male adapter was glued to the other end. Approximately 5.1 cm (2 in.) below the connection point of the PVC pipe and the male adapter, a 1.27-cm (1/2-in.) fitting was installed. The fitting provided a connection point for the tubing used to extract debris from the well. A 15.2-cm (6-in.) PVC screw cap was attached to the male adapter. A 9.53-mm (3/8-in.) fitting was inserted into the screw cap to provide a vacuum connection. Teflon™ tape was used with the screw cap and both fittings to provide an airtight seal. Tubing was connected to the 9.53-mm (3/8-in.) fitting. A 9.53-mm (3/8-in.) shut off valve was connected to the other end of the 9.53-mm (3/8 in.) tubing. A second piece of 9.53-mm (3/8-in.) diameter tubing was connected to the other end of the shut-off valve to allow for a vacuum gauge connection. Additional tubing was connected to the vacuum gauge to provide for a vacuum tank shut off valve. The reservoir apparatus and the vacuum tank were used at each well to assist in preparation of the wells by removing sediments and debris deposited in the wells during installation.

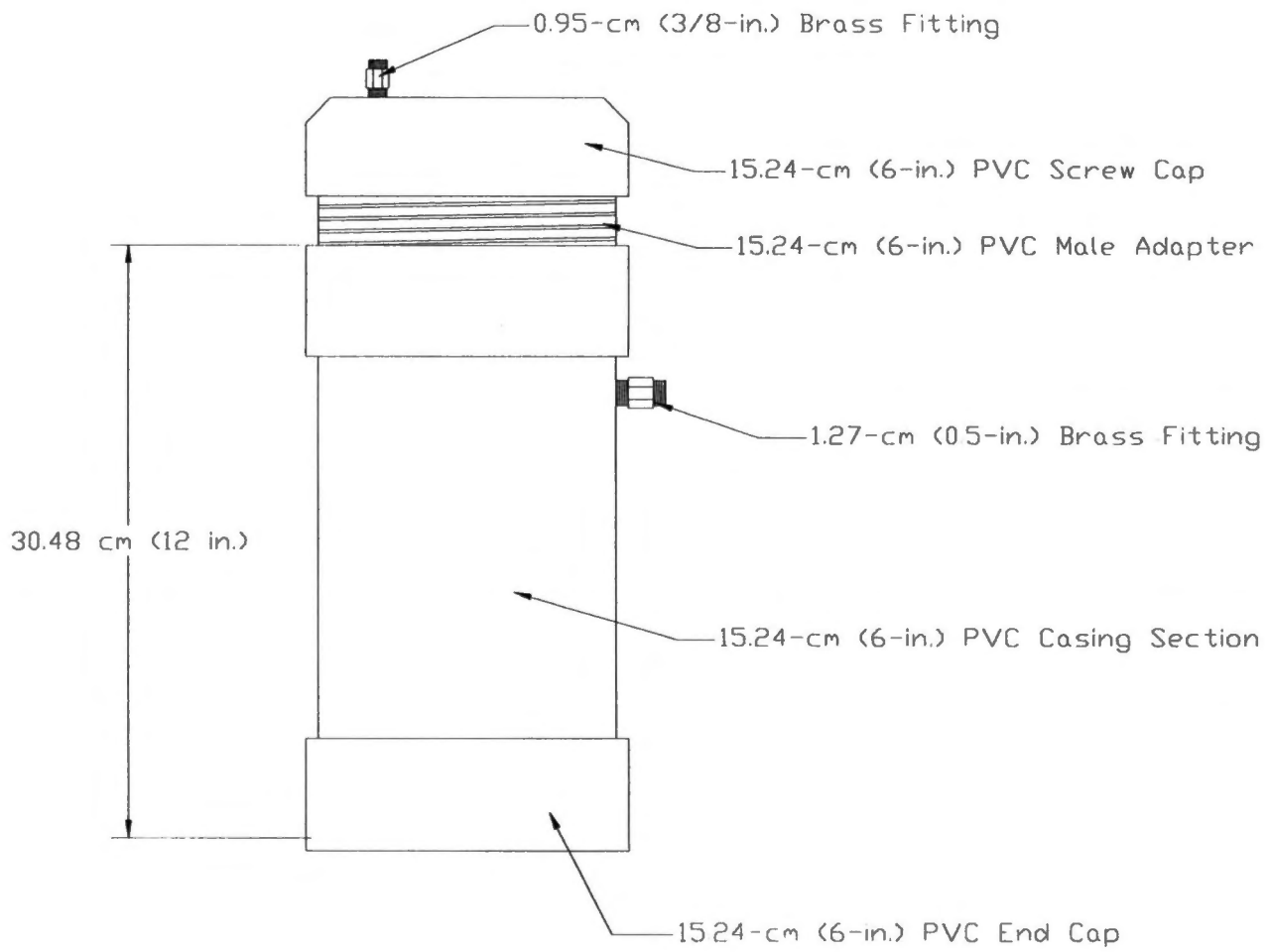


Figure 3.4. A vacuum was applied to the above reservoir to prepare the wells for sampling.

SAMPLING

Similar to the cleaning reservoir described above, the samples from the monitoring wells were collected with a reservoir system. Nalgene™ bottles with a 500-ml capacity were used as the reservoir. Tops, dedicated to each monitoring well, were modified to provide a connection for the well sampling tubing and a hand held pump. Two 6.35-mm (1/4-in.) diameter holes were drilled into each cap, and two sections of 6.35-mm (1/4-in.) Nalgene™ rigid tubing approximately 5.1 cm (2 in.) in length were inserted into the holes. To one section of the Nalgene™ rigid tubing, a tubing barb was added to connect a 7.62-cm (3-in.) piece of 9.53-mm (3/8-in.) Tygon™ tubing. A hand held pump was connected to the Tygon™ tubing from the modified bottle top while the Nalgene™ rigid tubing line from the bottle top was connected to the monitoring well sampling tubing. By creating a vacuum with the hand held pump, the sample was drawn into the sampling bottle that acted as a reservoir.

SUCTION LYSIMETERS

Two different types of suction lysimeters were used during the study. The lysimeters initially installed with the wells failed to perform, and after a series of tests and attempted solutions, they were abandoned for a preconstructed type. A description of the initial construction is included in Appendix C.

Preconstructed lysimeters were ordered from Soil Moisture Equipment Corporation. After examination of the company's website, the model 1905 Slim Tube Soil Water Sampler™ was selected because the lysimeter was equipped with many desirable features: a 2.22-cm (7/8-in.) outside diameter and a 1-bar high flow porous ceramic cup (Figure 3.5). Extension tubes 15.24 cm (6 in.) in length provided greater control over the length of the lysimeter. Based upon the average well depths, lysimeters 30.48 cm (12 in.), 60.96 cm (24 in.), and 91.44 cm (36 in.) in length were selected. The 30.48-cm (12-in.) and the 60.96-cm (24-in.) samplers were the basic models and consisted of a screw top extraction cap, a 2.22-cm (7/8-in.) diameter 1-bar, high flow screw in porous ceramic cup, two O-rings, and the appropriate extension tubes. The screw top extension cap was equipped with precut butyl tubing that allowed for connection of the hand held vacuum pump and clamping rings to maintain the vacuum once drawn. According to the company, butyl is considered a "cleaner" product since it is manufactured with fewer components. The porous ceramic cups remained in deionized water until required during assembly and were added to the lysimeter apparatus in the field. One lysimeter was completely constructed and tested to determine if it held a vacuum for 24 hours before the installation procedure.

Prior to assembly, the porous ceramic cups were soaked in deionized water for a 24-hr period. The porous ceramic cups were divided into two groups to provide a replicate sample. The water in which the cups were soaked was tested on both an ion chromatograph and a total organic carbon analyzer to determine if the cups introduced contaminants into the system and if introduced,

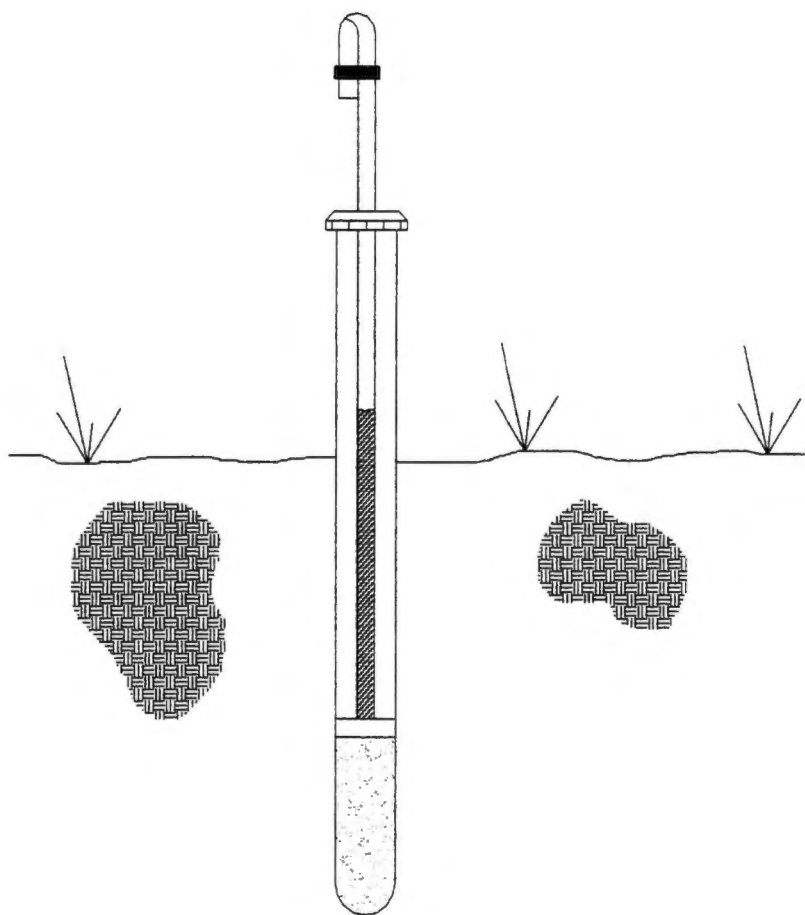


Figure 3.5. Extension tubes allowed for the installation of the lysimeter at a three distinct depths.

would the concentrations of the contaminants decrease over time, thus becoming nonsignificant. The process of soaking the cups was repeated in triplicate. Results from the soaking indicated that anion ($\text{SO}_4\text{-S}$ and Cl) concentrations decreased with each additional soaking (Table 3.2). Fluoride concentrations did not decrease rapidly. Nitrate-nitrogen was not detected (ND) in any sample. However, TOC concentrations did not decline in the same manner, for the third treatment exhibited higher TOC concentrations than the second. Deionized water tested at the same time did not reveal any TOC contamination. A definitive answer is not available for this unexpected trend. In their catalog, Soil Moisture Equipment Corporation (1999) indicated that an equilibrium with any ion exchange sites on the porous ceramic cups would be reached after approximately five to eight pore-volume had traveled through the cups.

Table 3.2. Constituent concentrations typically decreased with rinsing.

Test & Replicate	$\text{NO}_3\text{-N}$	$\text{SO}_4\text{-S}$	Cl	F	TOC
1 A	ND	1.649	0.206	0.083	85.26
1 B	ND	5.001	0.373	0.175	90.22
2 A	ND	ND	0.156	0.186	9.802
2 B	ND	ND	0.172	0.19	12.87
3 A	ND	ND	0.134	0.158	17.83
3 B	ND	ND	ND	0.153	34.92

INSTALLATION

A decision was made to install the newly constructed lysimeters within a 1-m radius of the monitoring wells whenever possible. The same apparatus used to auger the well holes, the Gopher™ was used to dig the lysimeter holes. A total of six holes were required at each monitoring well site; required depths of the holes, two each, were 30.48 cm (12 in.), 60.96 cm (24 in.), and 91.44 cm (36 in.) (Figures 3.6 to 3.13). Table 3.3 contains a listing of the lysimeter depths with respect to an arbitrary benchmark at an elevation of 30.48 m (100 ft). A hand-operated bucket auger was used to remove any excess soil from the holes to provide a clean installation. A soil slurry was created using soil from the appropriate depth. A 5.08-cm (2-in.) thick layer of soil slurry was placed into the lysimeter hole prior to the insertion of the lysimeter. Additional soil slurry was added until the porous ceramic cup was completely covered. Based on advice by Brad Salsbury, P.E., a project engineer for Soils and Materials Engineering, a 30.48-cm (12-in.) layer of bentonite was laid over the soil slurry. For the 30.48-cm (12-in.) deep holes, a 20.32-cm (8-in.) layer of bentonite was added. Water was added to the bentonite, and the bentonite was allowed to harden. After all of the lysimeters were installed and the bentonite layer had hardened, soil was added to fill the hole (Plate 3.19).

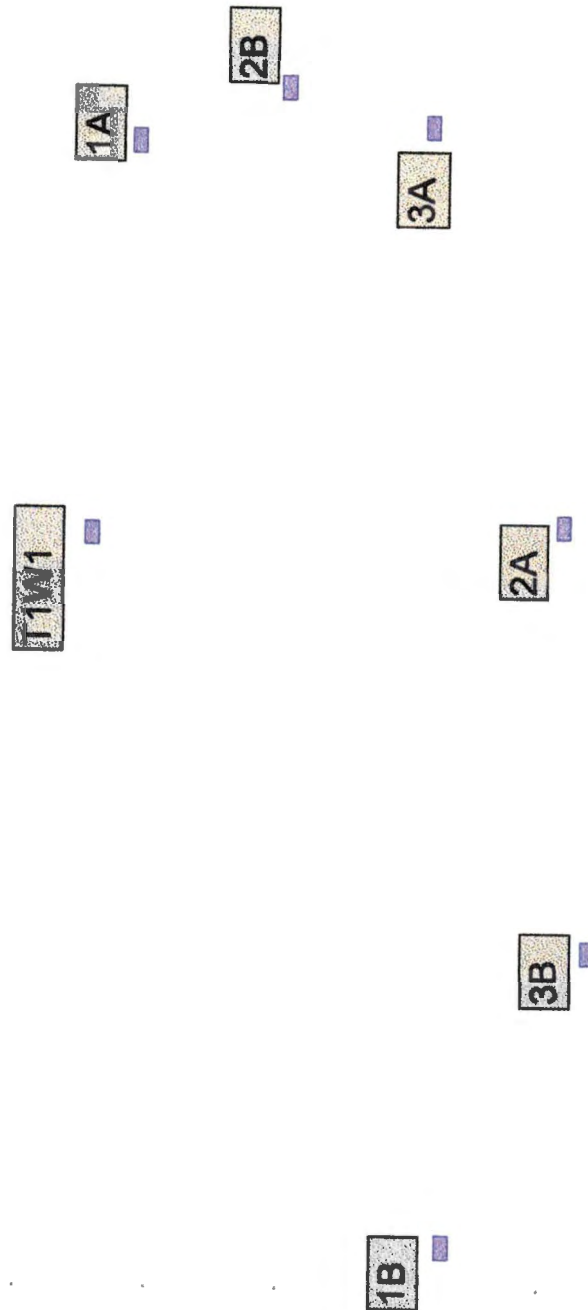


Figure 3.6. Site map for the suction lysimeters at T1W1.

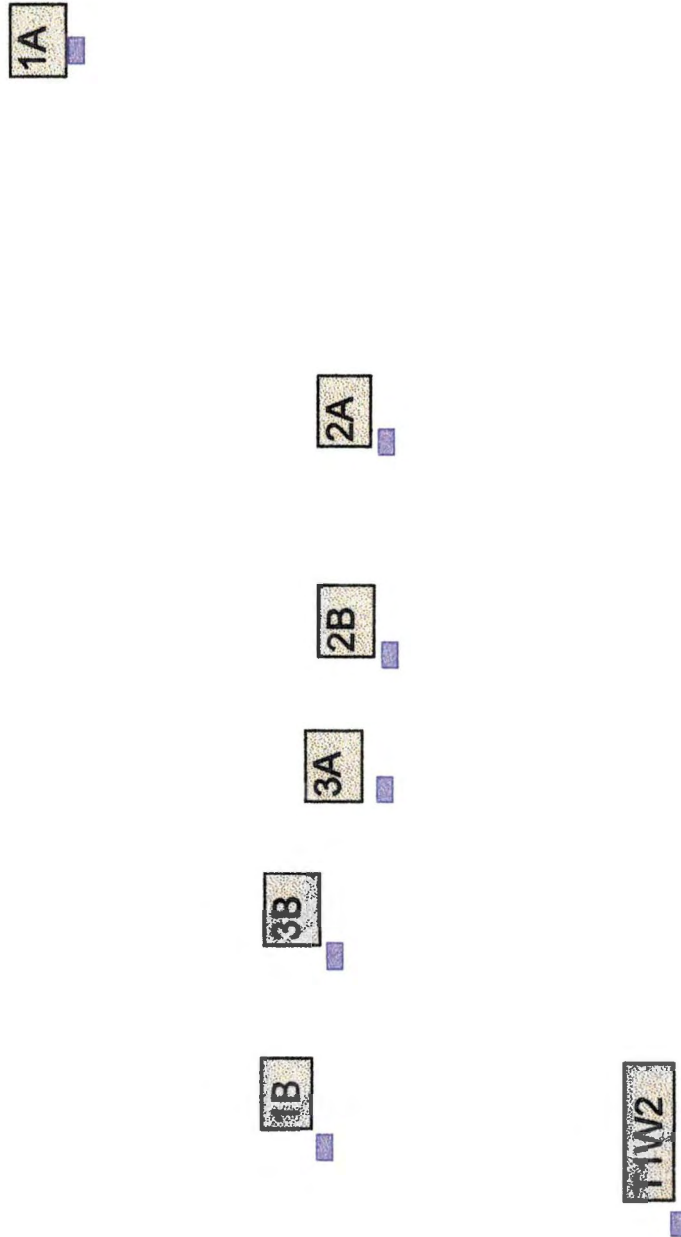


Figure 3.7. Site map for the suction lysimeters at T1W2.

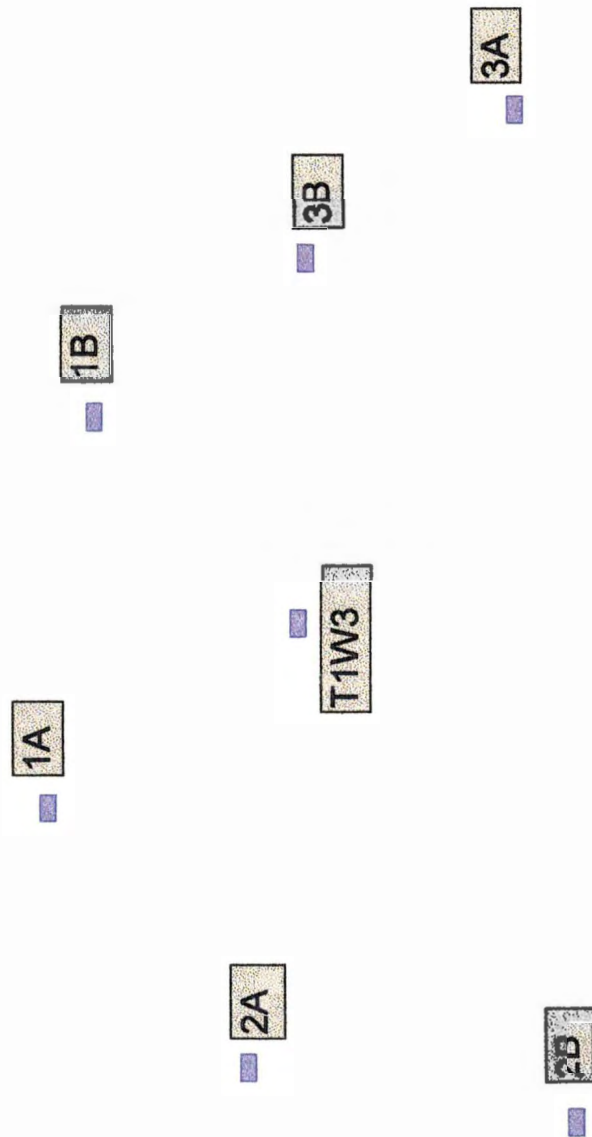


Figure 3.8. Site map for the suction lysimeters at T1W3.

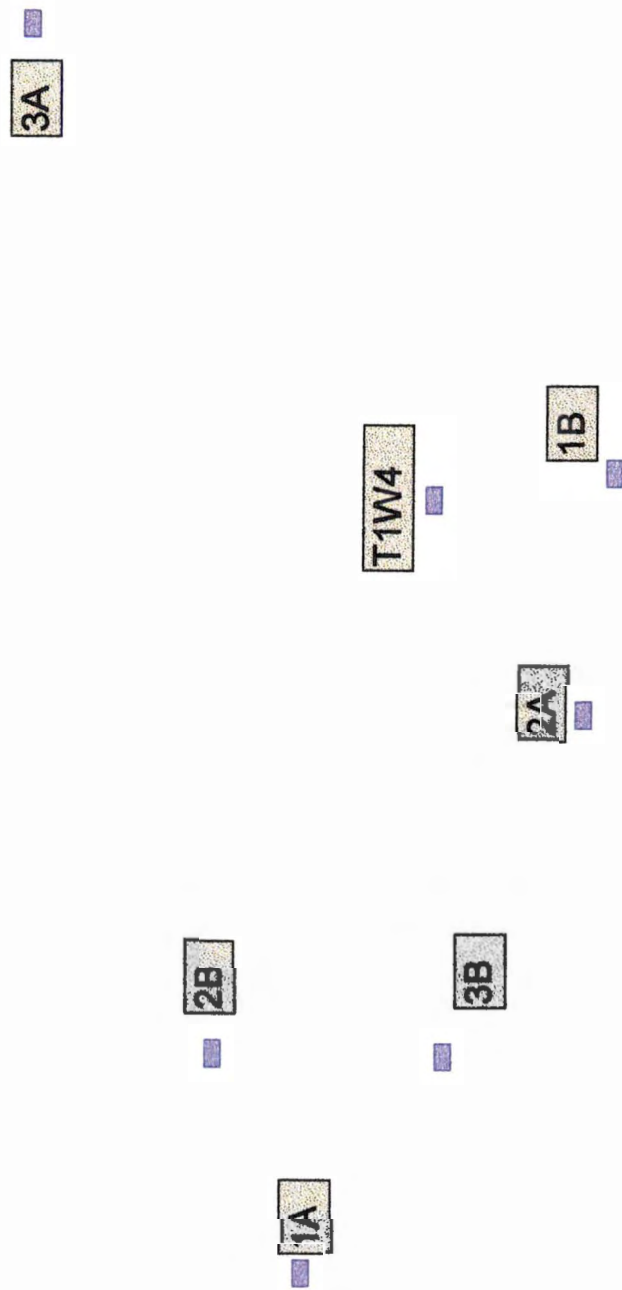


Figure 3.9. Site map for the suction lysimeters at T1W4.

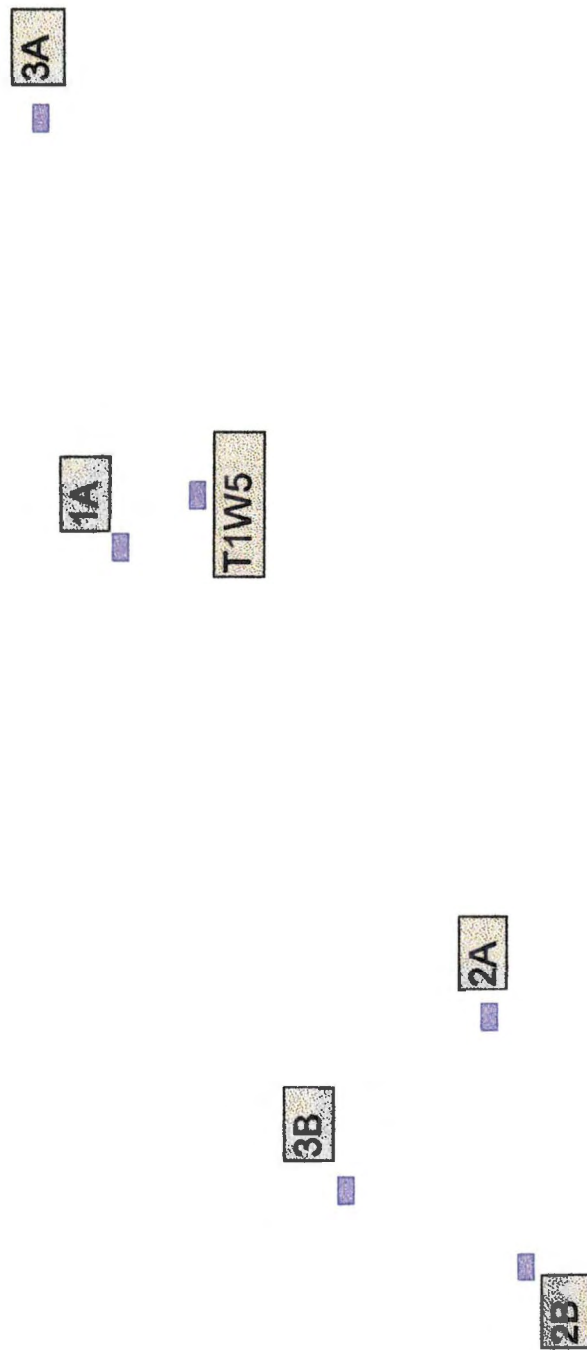


Figure 3.10. Site map for the suction lysimeters at T1W5.

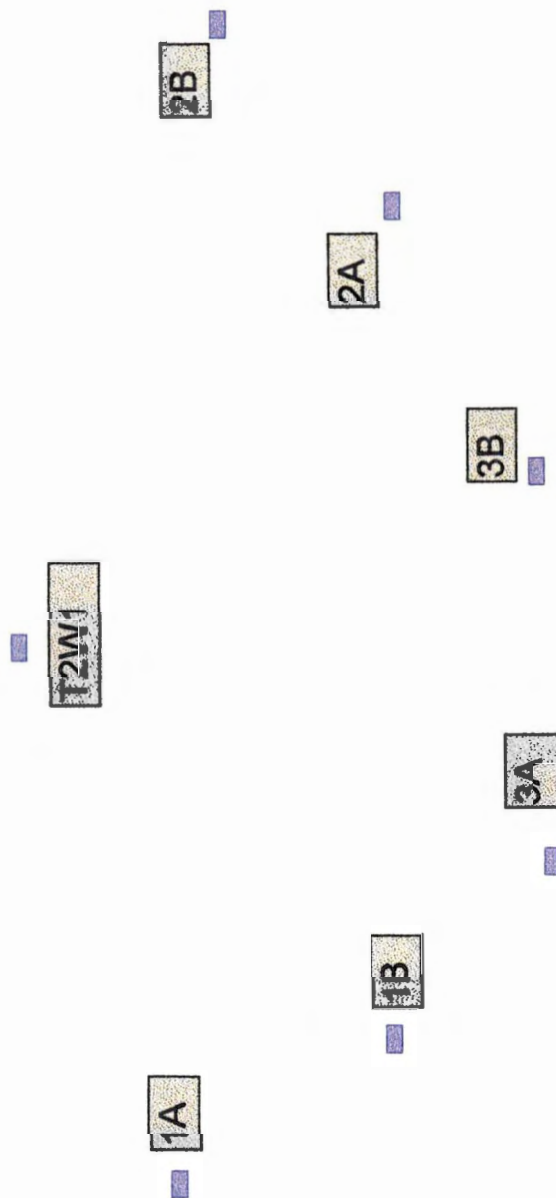


Figure 3.11. Site map for the suction lysimeters at T2W1.

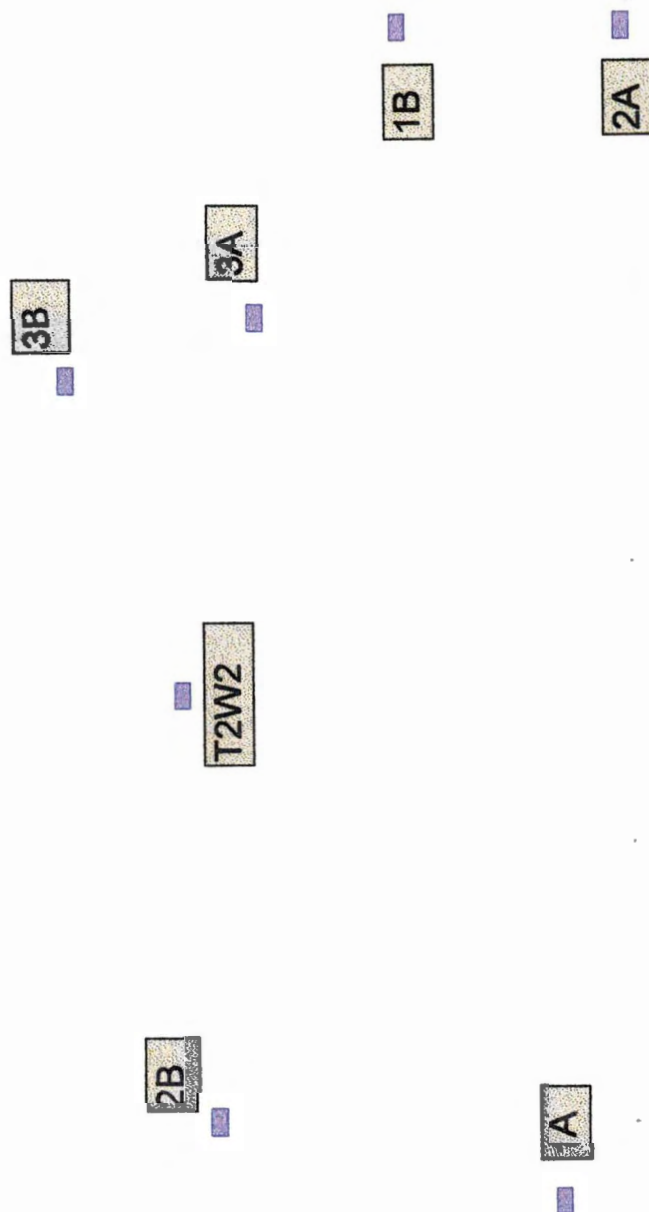


Figure 3.12. Site map for the suction lysimeters at T2W2.

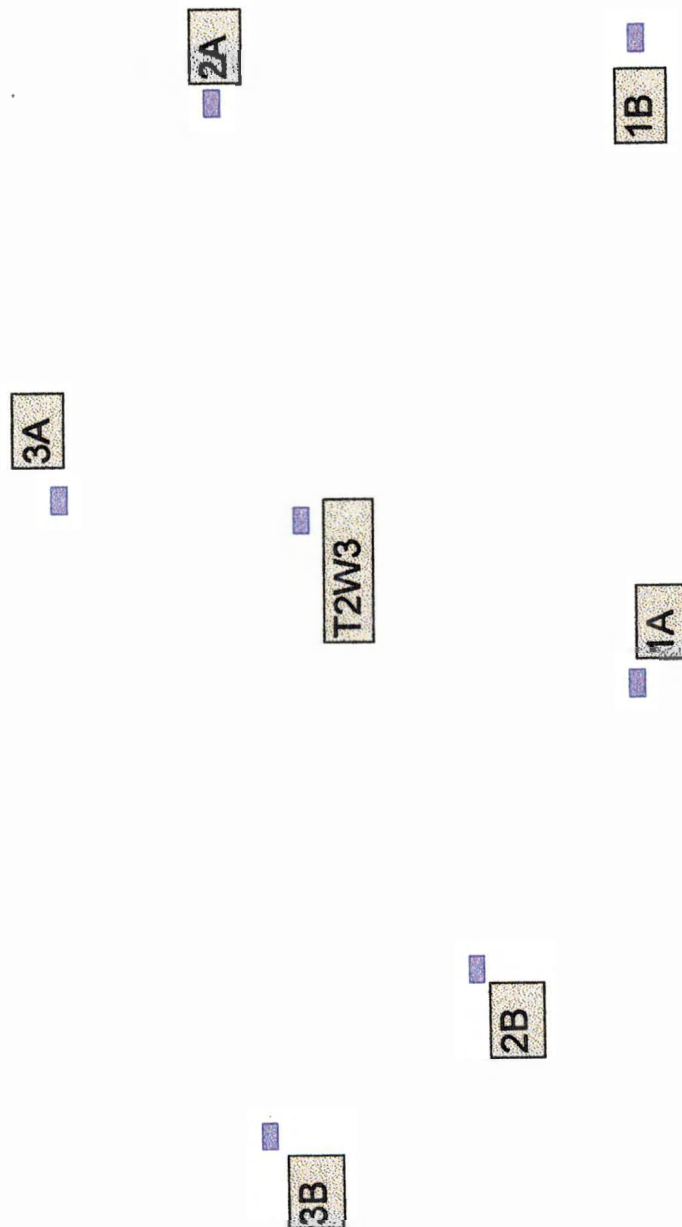


Figure 3.13. Site map for the suction lysimeters at T2W3.

Table 3.3. Suction lysimeter depths with respect to an arbitrary benchmark of 30.48 m (100 ft).

Location	Depth, m (ft)					
	1A	1B	2A	2B	3A	3B
T1W1	29.89	29.84	29.59	29.64	29.33	29.31
	98.06	97.90	97.09	97.25	96.23	96.15
T1W2	29.71	29.71	29.59	29.52	29.22	29.20
	97.46	97.46	97.08	96.85	95.85	95.80
T1W3	29.99	30.00	29.70	29.68	29.42	29.36
	98.39	98.44	97.43	97.38	96.51	96.33
T1W4	30.15	30.12	29.89	29.85	29.55	29.55
	98.93	98.81	98.05	97.94	96.94	96.96
T1W5	30.18	—	29.90	29.89	29.52	29.70
	99.03	—	98.10	98.05	96.85	97.45
T2W1	30.73	30.75	30.45	30.46	30.19	30.16
	100.82	100.90	99.91	99.92	99.06	98.96
T2W2	29.84	29.89	29.56	29.71	29.41	29.43
	97.90	98.05	96.98	97.46	96.29	96.55
T2W3	30.09	30.06	29.81	29.77	29.53	29.48
	98.73	98.61	97.80	97.67	96.87	96.73



Plate 3.19. The installation of the lysimeters was done using a preconstructed model.

SAMPLING

A hand held pump was used to create a vacuum on the lysimeters, and a clamp ring was used to maintain the vacuum (Plate 3.20). Syringes were modified based upon a design by Soil Moisture Corporation and used as the sample collection apparatus. A sixteen-gauge needle was blunted and cleaned in acid, then attached to a 60-cm³ syringe. A 2.54-cm (1-in.) section of Super Blue Silicone Fuel Tubing™ from DU-BRO was connected to the blunted needle to provide a connection for the sampling tubing. Nylon tubing (p/n MYT001) from Soil Moisture Equipment Corporation with a 2.38-mm inside diameter was cut to the appropriate length and connected to the Super Blue Silicone Fuel Tubing™. A sampling syringe was dedicated for each lysimeter. To obtain the sample, the butyl tubing was removed from the lysimeter and the nylon tubing from the sampling apparatus was inserted. The syringe was used to withdraw the sample.

PIEZOMETERS

The direction of groundwater flow was monitored with piezometers constructed of 2.54-cm (1-in.) schedule 40 steel pipe. The pipe was cut with a bandsaw to the appropriate lengths, and one end of the pipe was threaded to allow for a 2.54-cm (1-in.) PVC screw cap to prevent the entry of rainfall into the piezometer. Appendix D contains information pertaining to the lengths of the



Plate 3.20. A hand held pump was used to create a vacuum while a second person placed a clamp ring over the tubing to maintain the vacuum.

piezometers. A hand-operated auger with a 2.54-cm (1-in.) diameter was used to auger each hole. The holes were augered a few centimeters deeper than the required depth to prevent soil from clogging the end of the piezometer. Initially, an attempt was made to place a metal bolt in the driven end of the piezometer during installation. Nests of piezometers were installed around each monitoring well and at two additional locations between the creek and the wetland (Plate 3.21). Since bedrock was struck at each site during the installation of the monitoring wells, a decision was made to place the deepest piezometer directly above the bedrock. Attempts were made to install subsequent piezometers with a minimum depth separation of 25.4 cm (10 in.).

DATALOGGER

The data for battery voltage, panel temperature, rain fall, stream stage, creek temperature, and soil and well water temperature for four monitoring wells were collected on a CR10™ Campbell Scientific datalogger at 15-min intervals for the duration of the study (Plate 3.22). The datalogger was located in a fenced portion of the study site with wiring enclosed in 1.27-cm (½-in.) PVC pipe to prevent damage from cattle and rodents (Plate 3.23). Battery voltage was monitored to ensure that the solar panel used to recharge the battery was functioning properly, hence delivering sufficient power to the datalogger. Rainfall was monitored with a Rain Collector II™ tipping bucket rain gauge from Davis

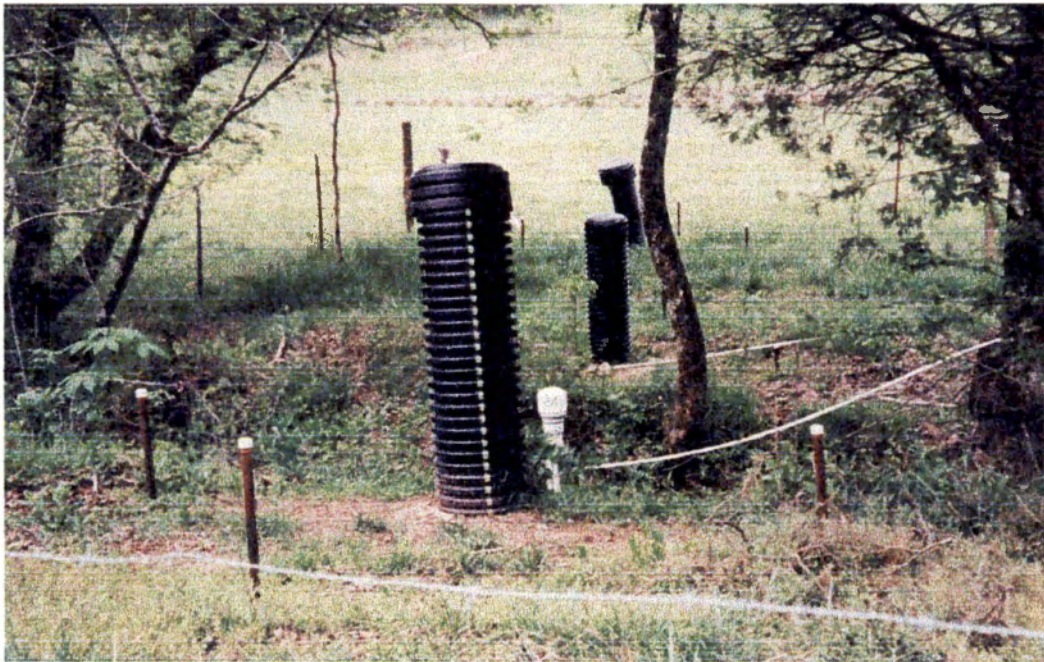


Plate 3.21. Piezometers were located around each monitoring well.

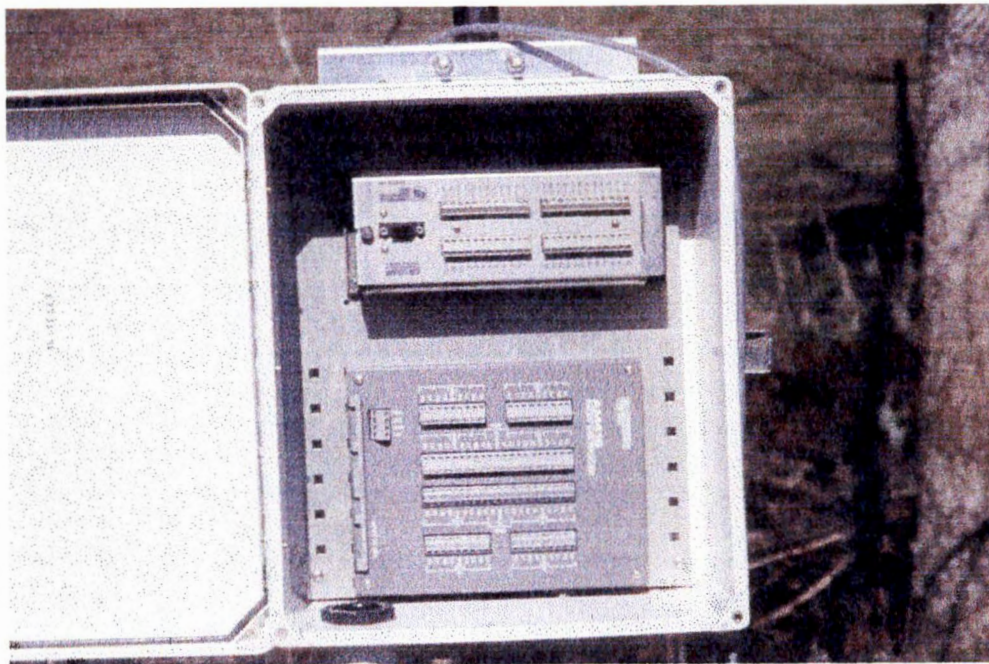


Plate 3.22. A CR10 datalogger by Campbell Scientific was used to collect data at 15-min intervals.

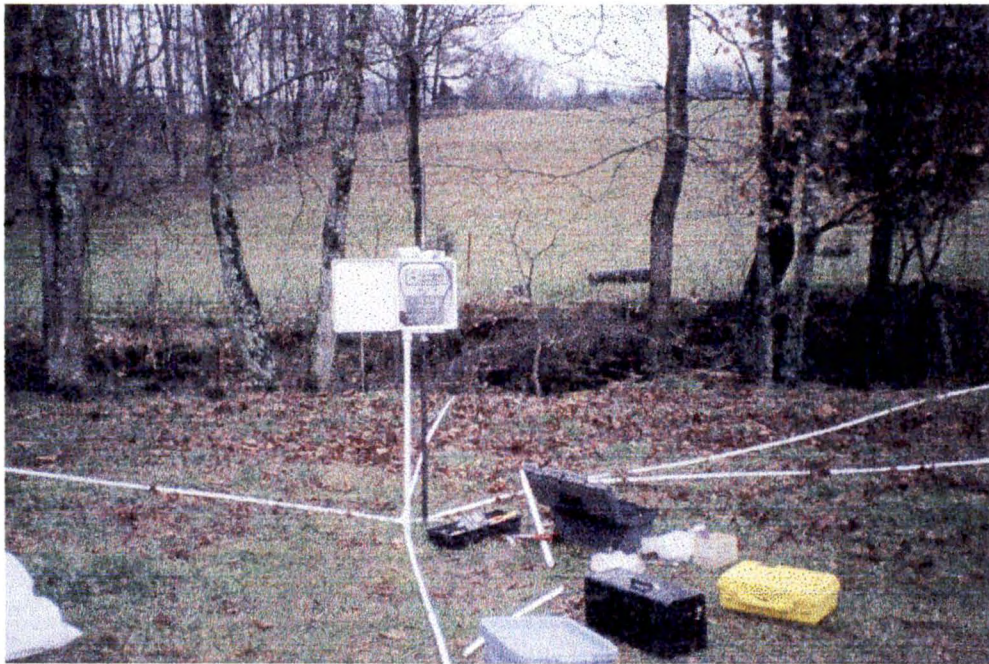


Plate 3.23. All wiring was run through PVC pipe to prevent damage.

Weather Systems (Plate 3.24). A bird perch constructed of 1.27-cm ($\frac{1}{2}$ -in.) PVC pipe was placed next to the rain gauge to offer the birds an alternate resting area. The stage of Hickory Creek was monitored using two Tennessee Fluid Level Indicators (TFLIs) (Yoder et al., 1999). Two TFLIs were used to allow for replication (Plates 3.25 to 3.26). Strong storms during the summer months of 1999 required that the TFLIs be moved closer to the stream bank; at their initial location, the high flows generated by the storms toppled the TFLIs. Plates of the storm damage along with the calibration results for the TFLIs are included in Appendix E. Temperatures from the creek and the soil and well water were measured with type T (copper-constantan) thermocouples. A thermocouple was wrapped around a steel post driven into the stream bed for monitoring the temperature of Hickory Creek. The thermocouples used to measure the soil temperature were inserted approximately 5.08 cm (2 in.) into the soil next to monitoring wells Transect 1 Well 2, Transect 1 Well 3, Transect 2 Well 2, and Transect 2 Well 3. Well water temperature was taken by attaching a thermocouple to a plastic rod and inserting the rod into the monitoring well. The same wells monitored for soil temperature were also monitored for well water temperature. A copy of the CR10 program is included in Appendix E.

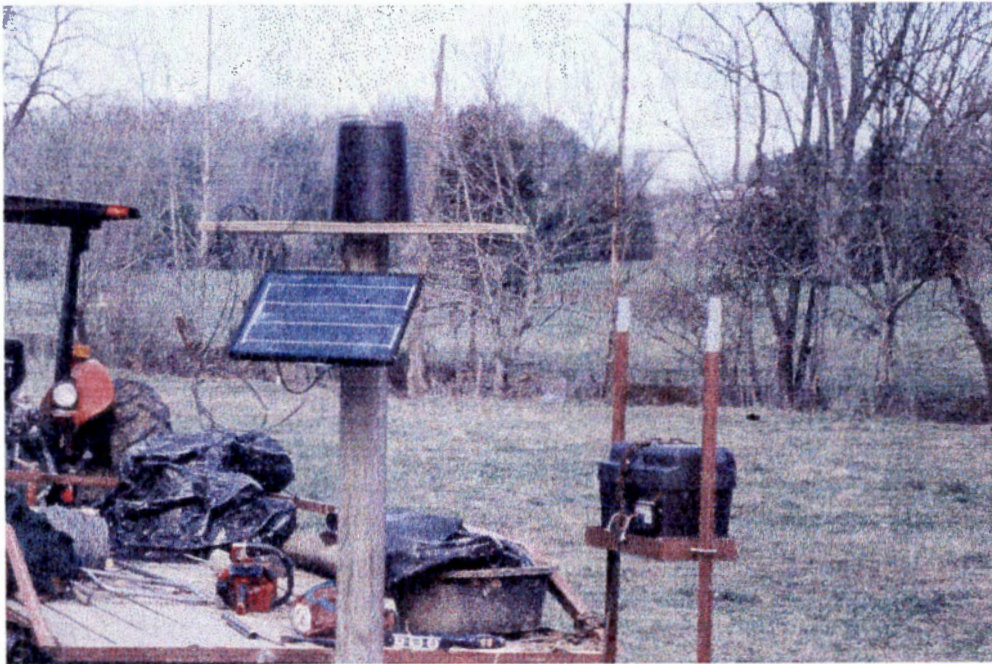


Plate 3.24. The rain gauge was located in an open area between the wetland and the stream.



Plate 3.25. This TFLI was located along Transect 1.

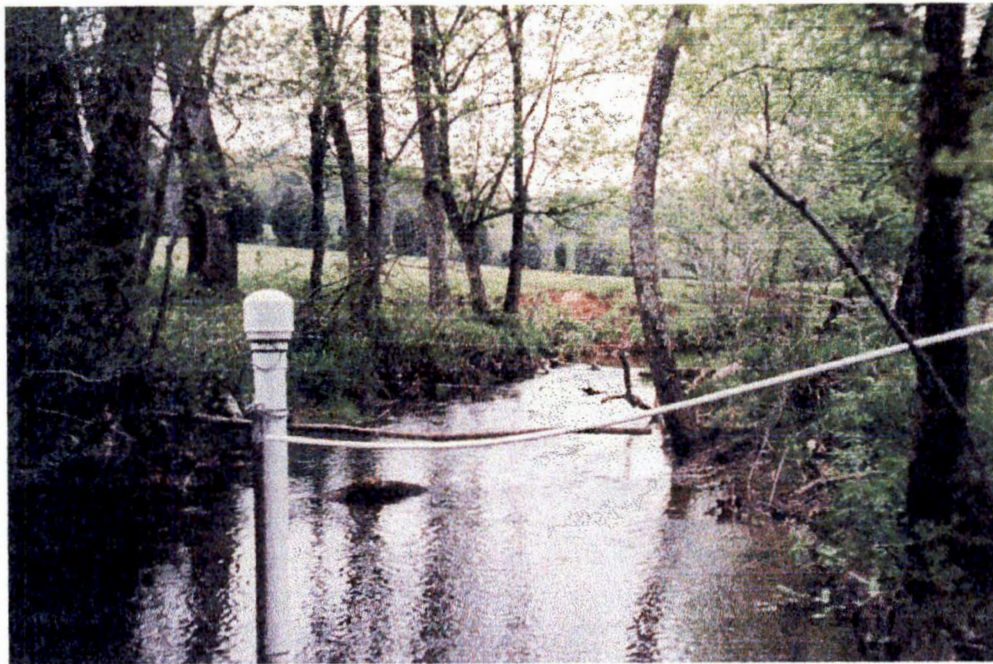


Plate 3.26. TFLI 2 was located along Transect 2.

Pt ELECTRODES

CONSTRUCTION

The Pt electrodes were constructed using 24-gauge Pt wire (99.95% Pt wire) and 12-gauge copper wire with a waterproof insulation. The Pt wire was cut into 7.62-cm (3-in.) segments, and impurities were removed by soaking the Pt segments in a 1:1 mixture of nitric and hydrochloric acids for approximately four hours. Following the acid bath, the Pt segments were stored in deionized water until used. Because of the difficulty in drilling a hole to insert the Pt wire, the copper and Pt wires were welded together. Approximately 7.62 cm (3 in.) of insulation was stripped from the unwelded end of the copper wire. A waterproof epoxy was applied to both the weld and the point of contact between the copper wire and the insulation to prevent the intrusion of water into the electrode. Heat shrink tubing was applied over the welded end of the electrode to provide additional protection and another coat of epoxy was applied.

TESTING

Prior to installation, the Pt electrodes were tested in accordance with method 2580.B of the *Standard Methods for the Examination of Water and Wastewater* 18th edition (1992). The Pt electrodes were tested using ZoBell's

solution with a calomel reference electrode at 25°C. A constant temperature was maintained by using a water bath (Fisher Scientific Isotemp 10135). To read the redox potential with the Pt electrodes, a pH meter with a mV mode was required. An adapter by Fisher (p/n 13-620-498) was used to connect the calomel reference electrode to another copper wire with an alligator clip that was attached to the end of the Pt electrode. A liquid connection was maintained throughout the experiment. Results from the experiment are included in Appendix F. Eighteen Pt electrodes that were constructed and tested, were installed at the study site.

INSTALLATION

Pt electrodes were installed around each monitoring well and along the stream side in between monitoring wells. Locations of each electrode are given in Table 3.4. A slim metal pilot rod was first driven approximately 30.48 cm (12 in.) into the soil and removed; the Pt electrode was then inserted into the pre-made hole. A pH meter by Hach with the apparatus described in the above section was used to measure the redox potential. Using an old screwdriver and water, a mud paste was made within 1-m (3.28-ft) horizontal distance of the Pt electrode. To measure the redox potential, the calomel reference electrode was then inserted into the mud paste and the alligator clip was attached to the end of the Pt electrode (Plate 3.27).

Table 3.4. Locations and depths of the installed Pt electrodes.

Electrode	Location	Depth (m, (ft))
1	Transect 2 Well 2	0.508 (1.667)
2	Transect 2 Well 2	0.508 (1.667)
3	Transect 1 Well 4	0.610 (2.0)
4	Transect 1 Well 5	0.610 (2.0)
5	Stream side between Transect 1 Well 2 and Transect 2 Well 2	0.610 (2.0)
6	Stream side between Transect 1 Well 1 and Transect 2 Well 1	0.610 (2.0)
7	Stream side between Transect 1 Well 2 and Transect 2 Well 2	0.305 (1.0)
8	Along stream bank in front of Transect 2 Well 1	0.610 (2.0)
9	Not installed	—
10	Transect 1 Well 1	0.305 (1.0)
11	Not installed	—
12	Transect 1 Well 3	1.219 (4.0)
13	Transect 1 Well 2	0.305 (1.0)
14	Transect 1 Well 1	0.762 (3.5)
15	Not installed	—
16	Transect 2 Well 3	0.610 (2.0)
17	Transect 1 Well 1	0.305 (1.0)
18	Transect 1 Well 3	1.067 (3.5)
19	Transect 1 Well 1	0.914 (3.0)
20	Along stream bank in front of Transect 2 Well 1	0.610 (2.0)
21	Transect 1 Well 4	1.524 (5.0)



Plate 3.27. While the measurement of this Pt electrode is not at the study site, the general principle is demonstrated.

STREAM SAMPLING

Samples of stream water were collected on an hourly basis with an ISCO 3700™ sampler (Plate 3.28). Each of the twenty-four bottles contained a composite of six samples, thus representing a six-hour period. The sampling bottles held a maximum of 250 ml, so the selected sample size was 40 ml. Samples were collected at least once every six days. The sampling inlet was connected to a steel post driven into the stream bed and was located between Transect 1 Well 2 and Transect 2 Well 2. To ensure the collection of samples during times of low flow, the sampling inlet was placed near the bottom of the stream bed. A steel cage protected the ISCO 3700™ sampler from flooding and vandalism (Plate 3.29).

PEEPERS

CONSTRUCTION

A design similar to the one developed by Steinmann and Shotyk (1996) was used in this study with minor modifications (Figure 3.14). The main body of each peeper was constructed of 1.91-cm (3/4-in.) thick PVC sheet cut to the desired specifications. Both outer sides of the peeper were made of 0.48-cm (3/16-in.) thick PVC sheet and were cut to match the main body. Holes were

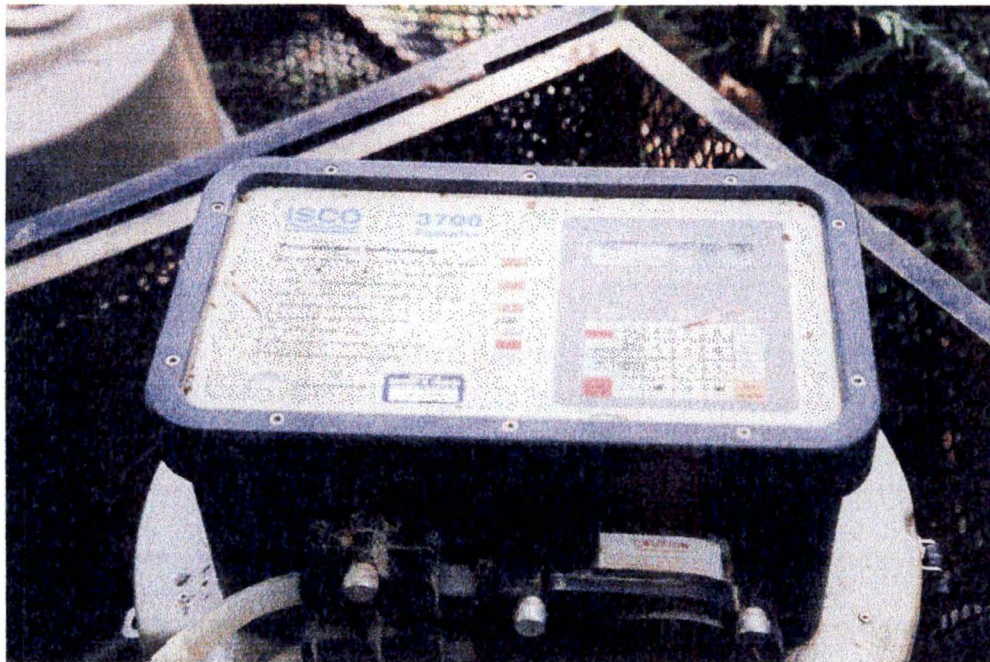


Plate 3.28. A 40-ml sample was taken from the creek hourly, and each bottle contained a 6-hr composite.



Plate 3.29. A steel cage constructed by Wesley Wright protected the ISCO sampler from damage by cattle or storms.

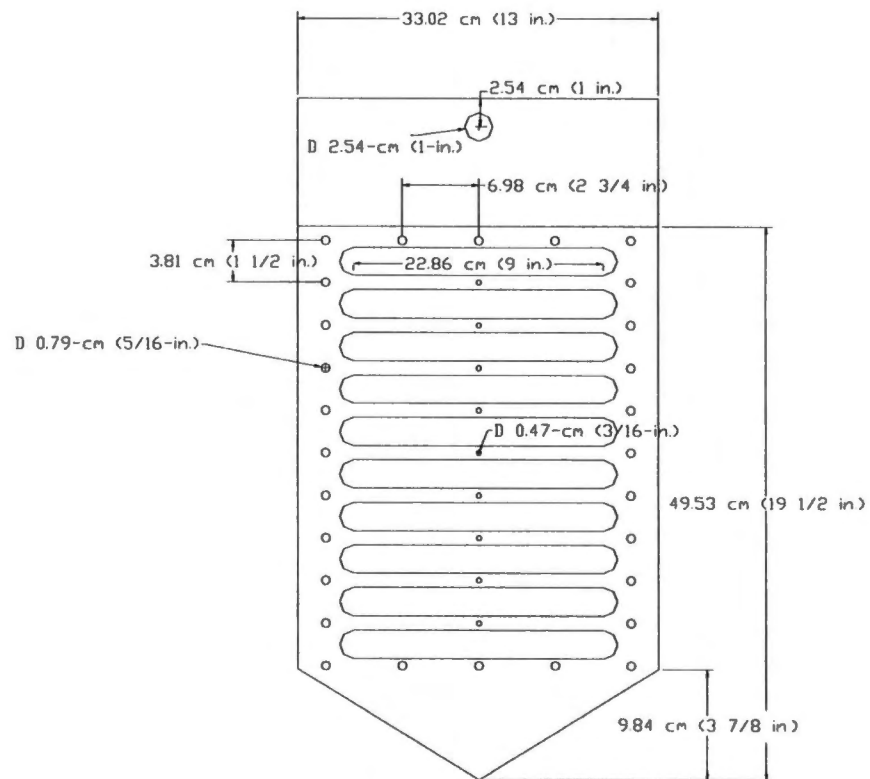


Figure 3.14. The peeper was designed to allow for vertical constituent measurements.

drilled through the main body and both outer sides for 0.95-cm (3/8-in.) stainless steel screws along the perimeter and 0.64-cm (1/2-in.) screws between the chambers. HT-200™, 0.2 micron filter paper by Pall Gelman Laboratories was placed between each outer side and the main body. A layer of nylon Weedblock™ was placed over the filter paper to prevent damage to it during the installation process. The screws were inserted and tightened slightly. A total of eight peepers were constructed (Plate 3.30). In between sampling periods, the peepers were disassembled, cleaned with phosphate-free soap, rinsed with deionized water, and reassembled.

INSTALLATION

Prior to installing the peepers in the hyporheic zone of the stream, they were submerged in a container of deionized water (Plate 3.31). Once deionized water filled the chambers, all of the screws were tightened while the peeper remained submerged. Immediately after tightening the screws, the peeper was installed in the hyporheic zone by hammering it to the desired depth (Plate 3.32). The pointed tip of the peeper allowed for smoother installation, but the bedrock in the stream bottom hindered the process of placing the peeper at the desired depths. A section of nylon rope was attached to a pre-drilled hole at the top of the peeper and secured to a nearby tree to prevent loss during storms (Plate 3.33). Four to six peepers were installed at one time.



Plate 3.30. The peeper consisted of 10 chambers for vertical water sampling.

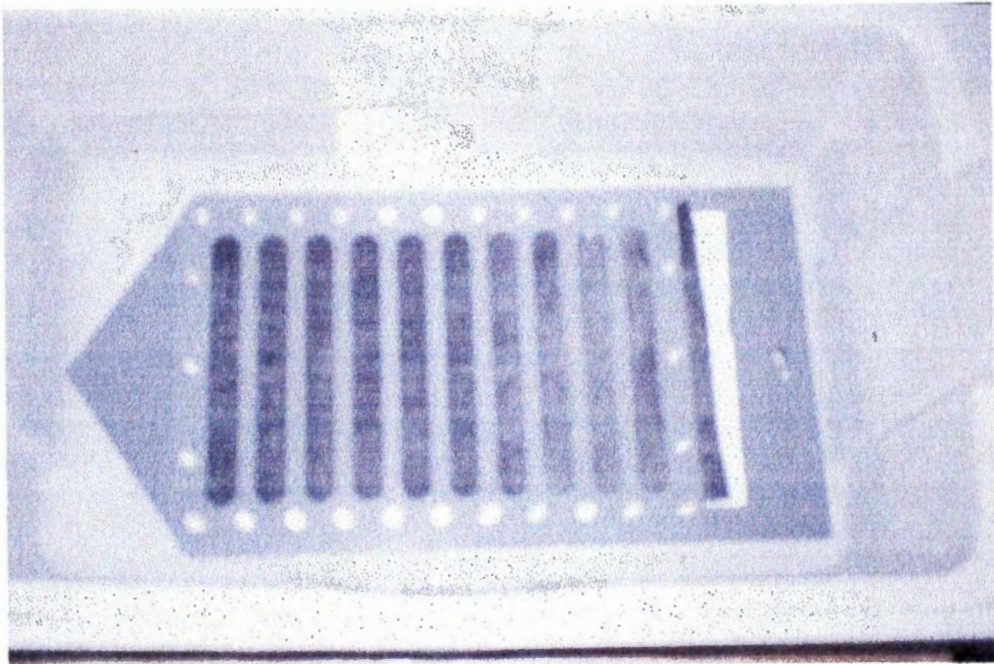


Plate 3.31. Assembly of the peepers was completed while submerged in deionized water.



Plate 3.32. Using a hammer, the peepers were installed into the hyporheic zone.

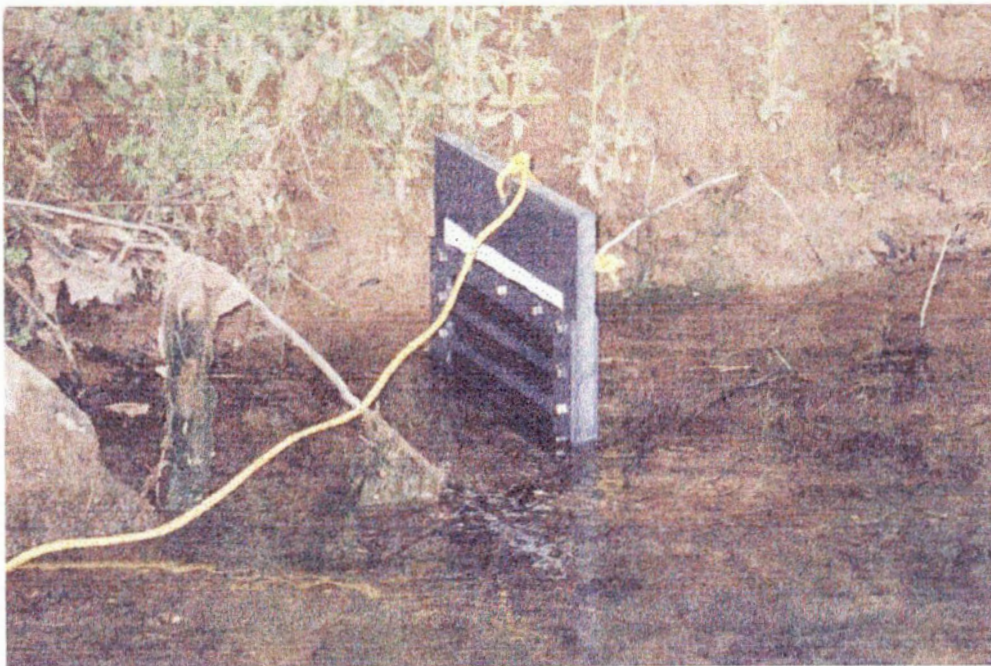


Plate 3.33. A nylon rope tied to the peeper was secured to a nearby tree to prevent loss in case of flooding.

SAMPLING

After at least five weeks in situ, the peepers were removed one at a time for sampling. Samples were collected by cutting a small section of filter paper over the chamber and withdrawing the sample with a 60-cm³ dedicated syringe. At the time of sampling, notes were taken concerning which chambers were or were not covered with soil, water, or neither.

STREAM GAUGING

To compute the discharge of the stream, discharge measurements were required in addition to the recorded stage. A Swoffer™ current meter from Swoffer Instruments, Inc. was used to measure the average velocity at a point over a twenty-second interval (Plate 3.34). The U.S.G.S., U.S. Department of Interior (1997) recommended the technique used to compute the average discharge of the stream. If no water is lost from the open channel and discharge does not change along a reach, the total discharge is the sum of the partial discharges (partial areas multiplied by average point velocity)

$$q = a_1V_1 + a_2V_2 + \dots + a_nV_n \quad (3.1)$$



Plate 3.34. The discharge of the stream was measured using a current meter and measurements of the cross sectional area of ten segments.

where the subscripts designate cross sectional areas and velocities of selected positions along the stream. The total discharge is then the sum of all the partial discharges. The velocity-area concept is the principle behind the computation of discharge using a current meter (U.S. Department of Interior, 1997). To develop a useful discharge curve, data were collected at different times over a wide range of flows.

The stream width of the stream was measured using a 30.48-m (100-ft) tape. The width of the stream was then divided into ten segments and the depth of the water was recorded at each point. If the depth of the water was less than 0.61 m (2 ft), the velocity was recorded at 0.6 of the depth. However, if the depth exceeded 0.61 m (2 ft), the velocity was recorded at points 0.2 and 0.8 of the depth and were averaged. During each stream gauging period, the discharge was computed twice for the stream, once near each TFLI.

SURVEYING/MAPPING

Surveying procedures were done with the assistance of Wesley Wright and were conducted using a Pentax™ PTS-III Electronic total station. An arbitrary North was established for the collection of the points. Recorded data included the horizontal distance, the horizontal angle, and the elevation with reference to a permanent benchmark for the wells, lysimeters, piezometers, peepers, TFLIs, rain gauge, and datalogger. After the data were collected,

simple geometry was utilized to determine the X, Y, and Z coordinates with respect to the benchmark. The permanent benchmark provided the surveyors with the opportunity to survey additional points while using the same initial setup (i.e., the same axes). Topographic data points of the study site were also collected by surveying through use of the total station. The total station allows the collection of data points with an accuracy of approximately 3.05 mm (0.01 ft). A GeoExplorer II hand held Global Positioning System (GPS) unit was then used to place a surveyed "known" point at the proper location on the surface of the Earth. The latitude and longitude of the other surveyed points were determined with reference to the "known" point.

Prior to collecting data with the GPS unit, a number of factors were considered to ensure the collected data were accurate and reliable. Using Pathfinder Office 2.11, PDOPs (Positional Dilution of Precision) and the number of satellites available were determined for the collection of the GPS data on November 16, 1999. The term PDOP refers to the positional dilution of precision and is comprised of both the HDOP (horizontal dilution of precision) and the VDOP (vertical dilution of precision). Depending upon the positions of the satellites used by the GPS hand-held unit, the HDOP and VDOP, and thus subsequently the PDOP, will vary. Ideally, a PDOP of 6 or lower indicates that the data collected are "good." Hence, for this study, GPS data were only collected if the PDOP met the minimum criterion of 6. The resulting information indicated several potential times for collection. Since the collection of GPS data was to occur for two points, a curtain was created based upon the general

limitations of both points. A curtain is simply a mask created in Pathfinder Office 2.11 to better determine the ideal time for data collection. Since GPS requires “line of sight” to satellites to function properly, things such as trees and hills can obstruct the signal; these factors were entered into the program first to determine the ideal time to collect the data with the known obstacles. The two selected points were the permanent benchmark and the well at Transect 2 Well 3. The benchmark was determined to be the most difficult site for collecting data, since it was placed at the edge of a woody area. Transect 2 Well 3 was located in a more open area, but the northern side of the well was blocked by trees along the creek. Therefore, a curtain was developed that encompassed a large portion of the north and the east sides. From the PDOPs generated from this curtain, the time from approximately 8:00 am to 10:00 am was selected.

Data were collected at both points for one hour each to better determine the absolute location of the collection points rather than the relative location. The GeoExplorer II unit was set to collect data every 20 seconds provided the PDOP did not exceed 7. The unit’s PDOP was set at 7 instead of 6 because very few data points were collected at the lower setting. Additionally, the S/N (signal to noise) ratio was set at 5 and the elevation mask at 15°. A signal to noise ratio (S/N) refers to the amount of signal the GPS unit receives versus the amount of noise present. Ideally, the higher the S/N ratio, the better. An elevation mask prevents the GPS unit from using satellites in the data collection process unless they are above the set elevation mask. Typically, the standard elevation mask is 15 degrees. The manual 3-D mode was used to collect latitude, longitude, and

elevation using a minimum of 4 satellites. Data were collected in units of meters. At the benchmark, data for a total of 48 points were collected while at Transect 2 Well 3 153 points of data were collected. The files for each collection were differentially corrected to arrive at an average latitude, longitude, and elevation. Coupled with the lower PDOP value at Transect 2 Well 3 along with the much higher number of collected data points, the values at this location were used as the "truth" from which to reference all of the surveyed points. The "truth" at Transect 2 Well 3 contained the following values for latitude, longitude, and elevation: 35°53'36.80462"N, -84°13'47.17863"W, and 260.315 m above mean sea level. The overall PDOP was 2.8 with the HDOP equal to 1.5 and the VDOP equal to 2.3. Horizontal precision was within 1.192 m.

Once the GPS coordinates were obtained for a known point, the latitude, longitude, and elevation of all the other surveyed points were determined. These calculations were performed in Quattro Pro 8. Since the previously collected survey points were originally referenced to the permanent benchmark, the origin of the data set required shifting to Transect 2 Well 3. ArcView was then used to generate topographical and hydrological maps. Additionally, site maps containing the locations of the wells, piezometers, and suction lysimeters were created with the surveyed data (Figures 3.5 and 3.6). Appendix G contains the surveyed data.

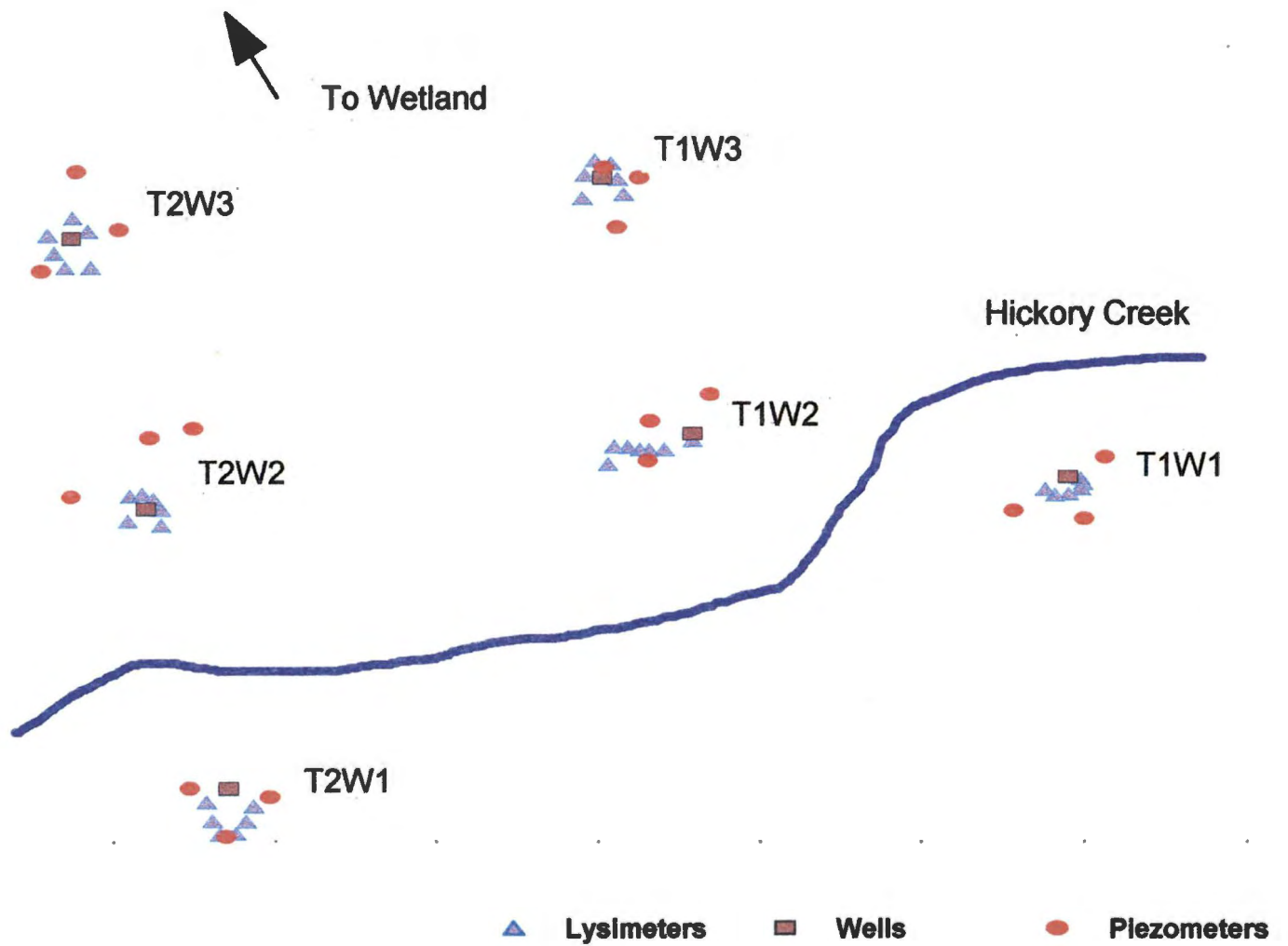
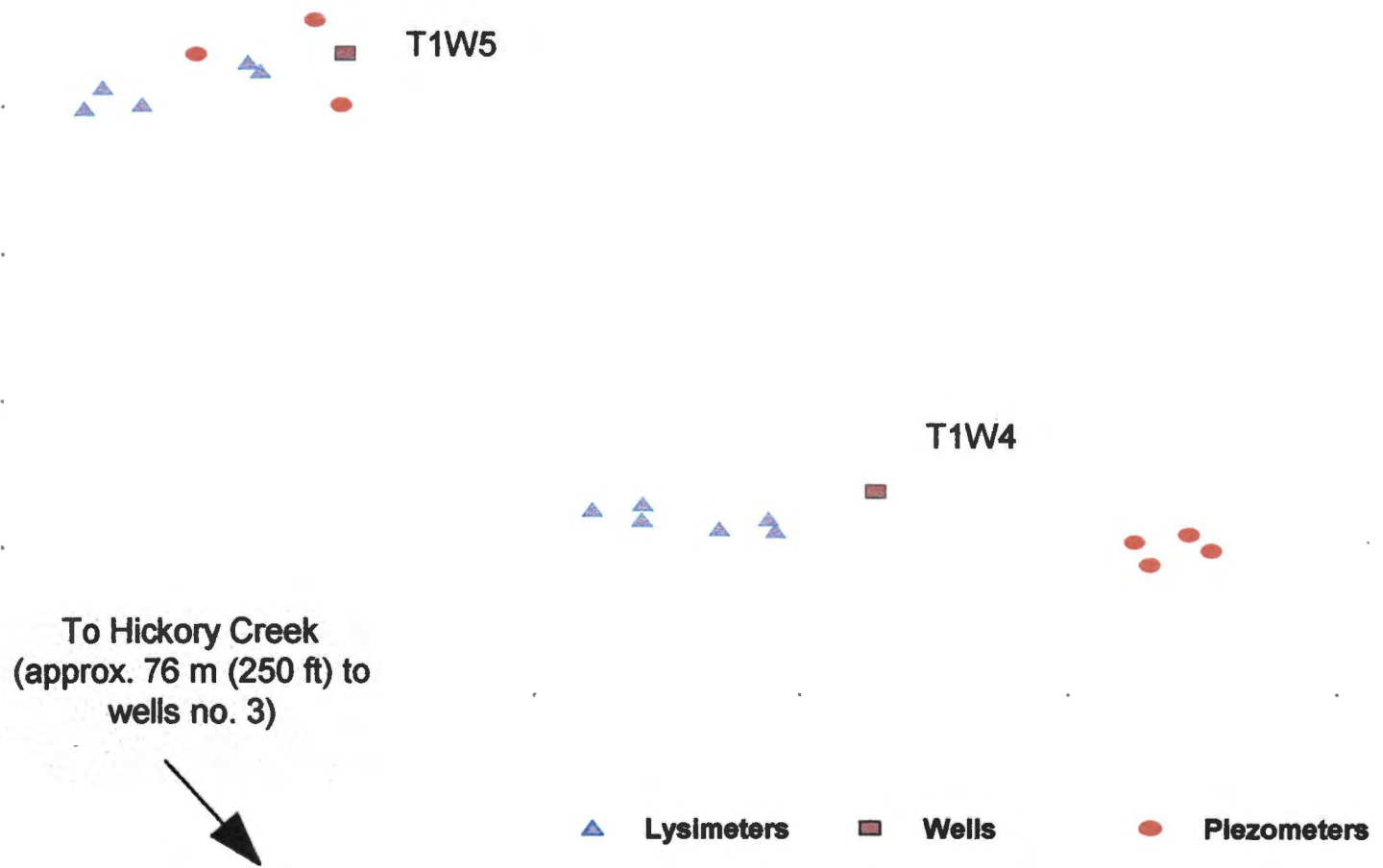


Figure 3.15. Site map for the hyporheic wells generated from survey data.

Figure 3.16. Site map for the wetland wells generated from survey data.



SOIL SAMPLES

Soil core samples, other than those collected for analysis in the Soil Pedology Laboratory at the Department of Plant and Soil Sciences, were taken for analysis of microbial assays. At three depths, soil was collected near each monitoring well: top, middle, and bottom 15.24 cm (6 in.) of soil. The top, middle, and bottom depths were based upon the average depth to bedrock near each monitoring well. Samples were collected with a 2.54-cm (1-in.) hand-operated corer and were collected during the winter, spring, and fall. Summer samples were not collected because of the extreme hardness of the soil; the corer would not penetrate the soil past the top few centimeters. Multiple cores were taken near each monitoring well during each sampling to acquire the necessary volume of soil to perform the analyses. Samples were placed in a cooler for transport and stored at 4°C until analysis. All soil tests were conducted in the Soil Microbiology Laboratory in the Department of Plant and Soil Sciences and included dehydrogenase activity, microbial biomass, alkaline phosphatase, acid phosphatase, arylsulfatase, L-asparaginase, moisture content, and pH. Refer to Table 3.5 for the procedure numbers used for the analyses; all procedures were taken from *Methods of Soil Analysis, Part 2: Chemical and Microbiological Properties* (1994).

Table 3.5. Microbial assay methods used.

Microbial Assay	Procedure
L-asparaginase	37-3.1.3
Acid Phosphatase	37-3.2.3.1
Alkaline Phosphatase	37-3.2.3.1
Arylsulfatase	37-3.3.3.1
Dehydrogenase	37-3.5.3
Microbial Biomass	40-2.1 and Vance et al. (1987)

WELL TEST

Well samples were collected without withdrawing and discarding any well volumes due to small volumes and slow recharge rates. To determine if this decision was a sound one, a well test was conducted at the conclusion of the study. Samples were collected from each monitoring well with the exception of Transect 1 Well 4, Transect 1 Well 5, and Transect 2 Well 1. Transect 2 Well 1 was not sampled because it produced very few samples throughout the course of the study, and the wetland wells (Transect 1 Well 4 and Transect 1 Well 5) were not closely connected to the stream. Four samples were taken from each monitoring well at the following times: 0, 15, 30, and 45 minutes. Samples were collected in the same manner described in the previous section titled **WELLS**. Each sample was placed in a cooler and transported to the Water Quality

Laboratory in the Department of Agricultural and Biosystems Engineering for analysis on the ion chromatograph for $\text{NO}_3\text{-N}$, $\text{SO}_4\text{-S}$, and Cl , and on the total carbon analyzer for TOC.

LABORATORY ANALYSES

All water chemistry analyses were conducted in the Water Quality Laboratory in the Department of Agricultural and Biosystems Engineering. The *Standard Methods of the Examination of Water and Wastewater* 18th edition (1992) were used to determine most of the analytical procedures. Methods developed by the Hach corporation were also used for some of the analyses. All water chemistry analyses were performed by the author with the exception of BOD_5 and the operation of the ion chromatograph and total carbon analyzer. These functions were performed by the Water Quality Laboratory Research Associate, Galina Melnichenko. Information concerning the contaminant and procedure used for the analyses are included in Table 3.6. All bottles used in the collection of samples from the monitoring wells were washed in acid to prevent iron contamination and triple rinsed in deionized water.

Soil samples were analyzed in the Soil Biochemistry Laboratory in the Department of Plant and Soil Sciences. Procedures used for the soil analysis were taken from *Methods of Soil Analysis, Part 2. Chemical and Microbiological Properties* (1994) and are listed in Table 3.5. The method for determining

Table 3.6. Water chemistry laboratory methods. SM denotes *Standard Methods*.

Contaminant	Procedure	Minimum Detection Level
NO ₃ -N	SM 4110 B - ion chromatography	0.001 mg/L
NO ₂ -N	SM 4110 B - ion chromatography	0.001 mg/L
SO ₄ -S	SM 4110 B - ion chromatography	0.001 mg/L
PO ₃ -P	SM 4110 B - ion chromatography	0.001 mg/L
Cl	SM 4110 B - ion chromatography	0.001 mg/L
F	SM 4110 B - ion chromatography	0.001 mg/L
Br	SM 4110 B - ion chromatography	0.001 mg/L
TOC	SM 5310 B - total carbon analyzer	0.01 mg/L
COD	Hach Method 8000	1 mg/L
BOD ₅	SM 5210 B	0.01 mg/L
DO	SM 4500-O G	0.1 mg/L
FC	SM 9230 C	1 CFU
FS	SM 9230 C	1 CFU
NH ₃ -N	Hach Method 8038 (Nessler)	0.01 mg/L
S ²⁻	Hach Method 8131	0.001 mg/L
Fe ²⁺	Hach Method 8146	0.01 mg/L
Fe ³⁺	Hach Method 8146	0.01 mg/L

Table 3.6. continued

Contaminant	Procedure	Minimum Detection Level
Total Iron	Hach Method 8008	0.01 mg/L
TS	SM 2540 B	0.0001 mg/L
pH	SM 4500-H ⁺ B	0.01 pH unit
Redox Potential	SM 2580 B	0.1 mV

microbial biomass was adapted from that presented in the *Methods of Soil Analysis* under the advisement of Dr. Mike Kirchner to model a method presented by Vance et al. (1987). The moisture content of the soil was also determined in a manner similar to that presented in the *Standard Methods* for total solid analysis.

STATISTICAL ANALYSES

Dr. Arnold Saxton assisted with the statistical analysis using SAS (1999). To determine the existence of horizontal trends, a simple linear regression was performed between the contaminants and distance from the edge of the stream. A multiple regression was then performed on the same variables but with ambient temperature and discharge included in the analyses. A second simple linear regression was performed to detect the presence of any horizontal trends between the microbial aspects in relation to distance from the edge of the stream. A comparison between the microbial factors and the examined constituents was

conducted to determine any relationships. Lastly for the horizontal variables, a complete randomized design (crd) was performed to generate an ANOVA table and to perform a mean separation to determine if there were any similarities between the wells. Vertical trends were examined with the peeper data through the use of a simple linear regression. Finally, a simple linear regression was used to determine the effects of purging the well volumes over time with the mini well test. Appendix H contains the SAS program used in the analyses.

CHAPTER 4

RESULTS AND DISCUSSION

FLOW PATTERNS

Previous researchers into the hyporheic zone have defined the area through hydrological connections to the stream. Some researchers have merely defined the hyporheic zone as the area that experiences bi-directional movement of surface water (Triska et al., 1993) while others have attempted to quantify the size of the hyporheic zone by the percentage of stream water present at various spatial measurements (Hill and Lymburner, 1998). To begin understanding the hyporheic zone of Hickory Creek at the study site, the flow patterns between the stream and the groundwater must be known on both a macro and a micro scale. Data measured from the piezometers over the course of the study provided definition of general groundwater flow paths. Appendix I contains the piezometer data taken at nineteen different sampling periods throughout the study. The piezometer data were collected on the same day that the wells were sampled, so contaminant trends associated with the flow paths should be representative of these days; hence horizontal trends within the hyporheic zone could be defined. The presence of a drought beginning in mid August and lasting until mid December caused minor changes to the flow paths. Since two distinct periods

were noted based upon the flow paths, all horizontal trends were analyzed accordantly with a wet and a dry season.

Data from the most deeply installed piezometers (piezometer A) along with the surface water elevation measured with a TFLI were used to determine the direction of water flow along both Transect one and two, in between the wells of the same number but in different transects, and the creek (refer to Figures 3.15 and 3.16). At Transect 1 Well 2, piezometer A and B presented conflicting hydraulic head readings, and thus two distinct seasonal flow patterns (Figures 4.1 to 4.4). Piezometer A had rather high hydraulic head values suggesting that it was located in a different layer (confined water source) than the other piezometers. Readings for piezometer A paralleled those of the creek until a few months into the study. One possibility was that the soil at the piezometer end collapsed, thus placing the piezometer in a “confined” aquifer or water source. Piezometer A extended to a depth in the soil similar to that of Transect 1 Well 2 (within 0.6-m (0.2-ft)) while piezometer B was within 0.2-m (0.7-ft) of the end of the well. Since the well screen was 1-ft in length, and because there was no way to determine the major water flow path without conducting a tracer study (whether the flow was largely controlled by the hydraulic head at piezometer A or piezometer B), a mean separation using SAS™ (1999) was performed upon wells Transect 1 Well 2 and Transect 2 Well 2. The main assumptions for the statistical analysis were:

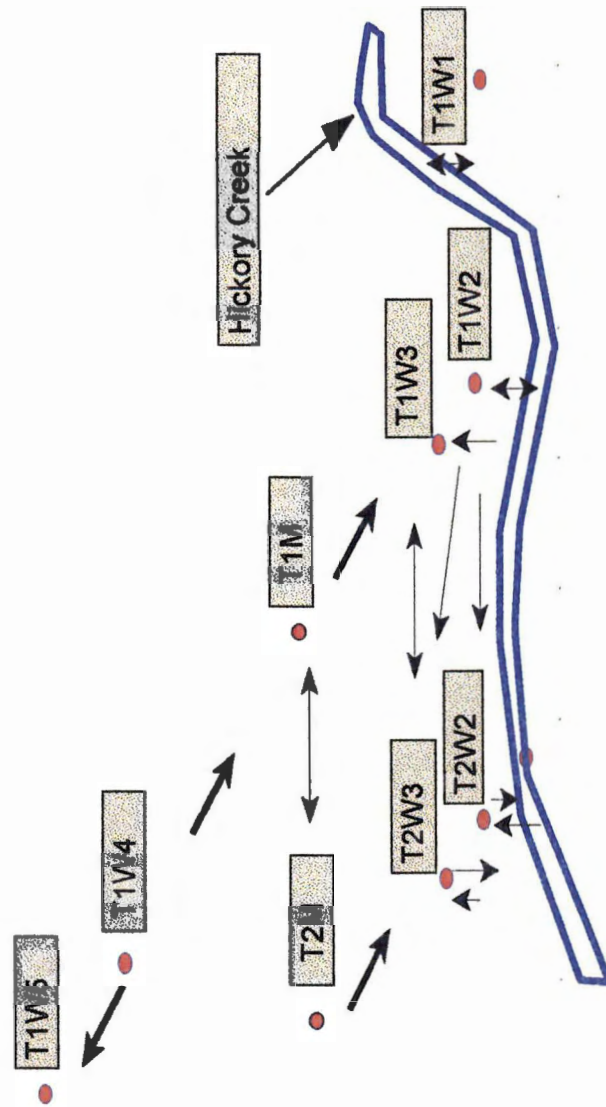


Figure 4.1. Groundwater flow patterns during the wet season using piezometer A at T1W2.

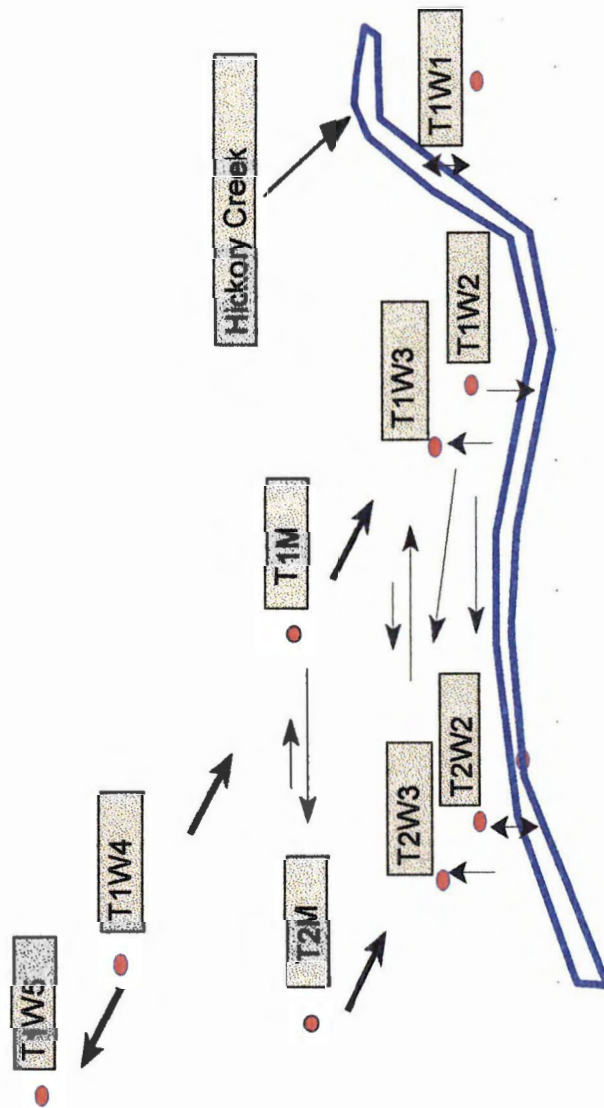


Figure 4.2. Groundwater flow patterns during the dry season using piezometer A at T1W2.

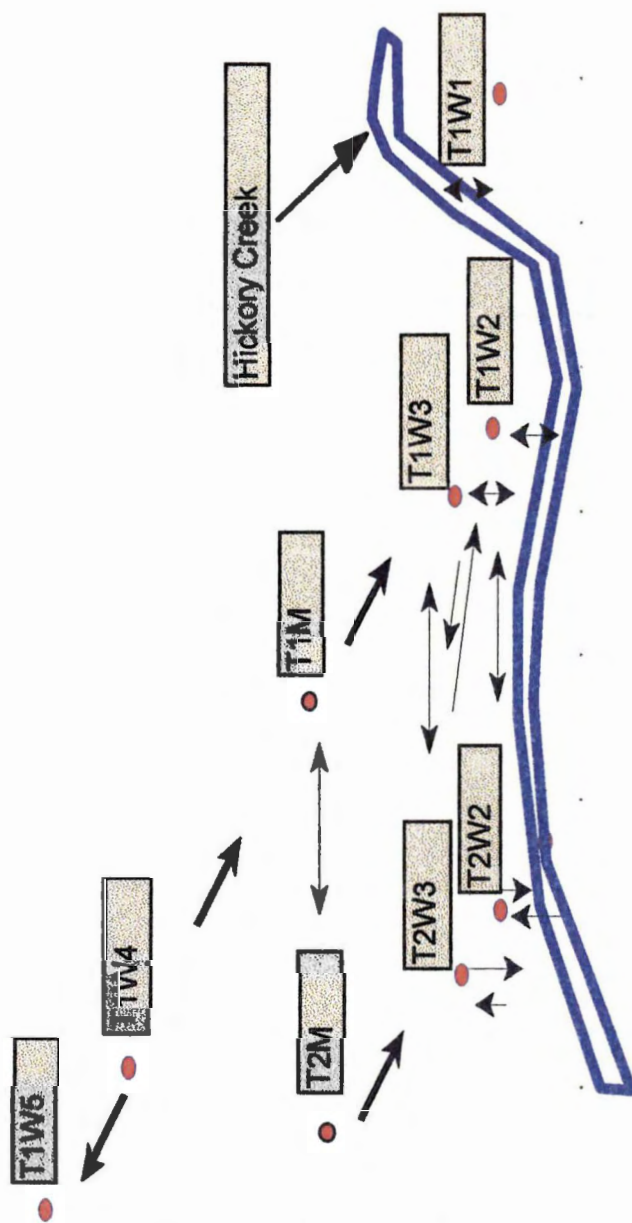


Figure 4.3. Groundwater flow patterns during the wet season using piezometer B at T1W2.

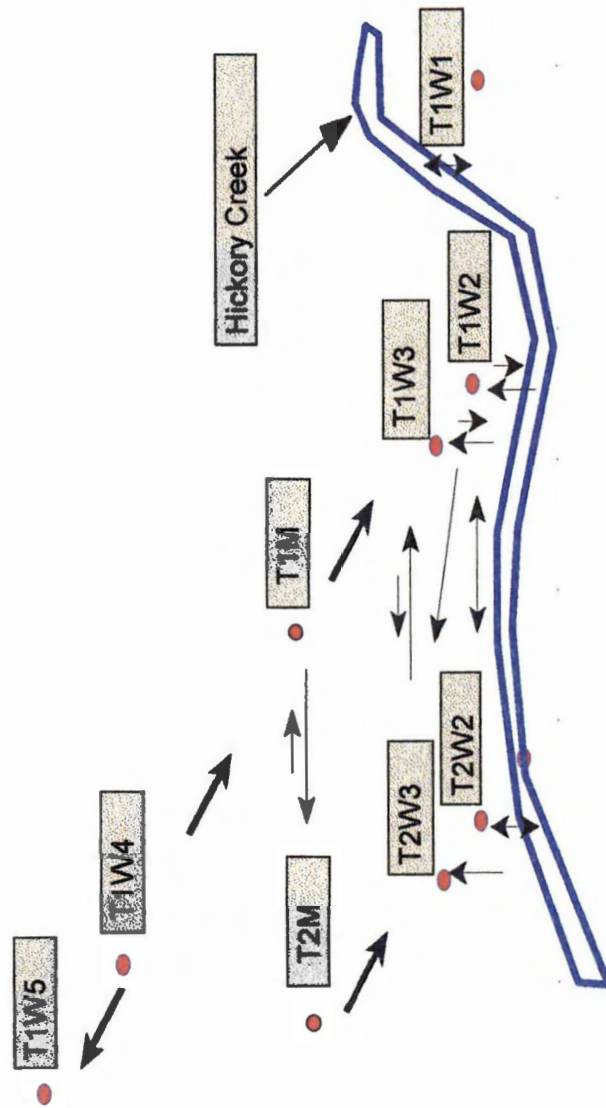


Figure 4.3. Groundwater flow patterns during the dry season using piezometer B at T1W2.

1. if the flow at Transect 1 Well 2 represented a groundwater discharge point (i.e., piezometer A represented the appropriate hydraulic head), then many of the constituents at Transect 1 Well 2 should significantly differ from those at Transect 2 Well 2, but
2. if the flow at Transect 1 Well 2 was bi-directional between the creek and the well (i.e., piezometer B represented appropriate hydraulic head), then many of the constituents at Transect 1 Well 2 should resemble those at Transect 2 Well 2.

As seen in Table 4.1, Transect 1 Well 2 only differed from Transect 2 Well 2 for one constituent during the dry season: total solids. During the wet season, the wells differed only in sulfate-S concentrations and pH. Based upon the mean separation technique, the results indicate that the groundwater flow at Transect 1 Well 2 resembled that of Transect 2 Well 2, since virtually all of the constituents were statistically the same. Since the flow pattern at Transect 2 Well 2 revealed bi-directional trends, piezometer B must have given the appropriate hydraulic head at Transect 1 Well 2, thus indicating that the flow pattern at this well was also bi-directional. Figures 4.3 and 4.4 represent the correct flow patterns (the ones used for the remainder of the analyses). The lengths of the arrows in Figures 4.3 and 4.4 represent the relative magnitude of flow. Note that Transect 2 Well 1 was not included in the analytical portion of this study, since no water was ever detected in the applicable piezometers. The surface water elevation in Hickory Creek never reached the bottom of the piezometers or Transect 2 Well 1

Table 4.1. Mean separation results for Transect 1 Well 2 and Transect 2 Well 2.

Season	Constituent	Relationship
Wet	NO ₃ -N	same
Wet	Redox	same
Wet	NH ₃ -N	same
Wet	SO ₄ -S	different
Wet	Cl	same
Wet	TOC	same
Wet	BOD ₅	same
Wet	pH	different
Wet	Fe ²⁺	same
Wet	Fe ³⁺	same
Wet	FC	same
Wet	FS	same
Wet	S ²⁻	same
Wet	COD	same
Wet	TS	same

Table 4.1. continued

Season	Constituent	Relationship
Dry	NO ₃ -N	same
Dry	Redox	same
Dry	NH ₃ -N	same
Dry	SO ₄ -S	same
Dry	Cl	same
Dry	TOC	same
Dry	BOD ₅	same
Dry	pH	same
Dry	Fe ²⁺	same
Dry	Fe ³⁺	same
Dry	FC	same
Dry	FS	same
Dry	S ²⁻	same
Dry	COD	same
Dry	TS	different

during the days of sampling. Compared to the other piezometers and wells, the instrumentation at Transect 2 Well 1 was not as deeply installed due to the presence of bedrock at this location.

A well test was performed after sampling from the shallow groundwater monitoring wells had ceased (refer to Chapter 3 for specific test details). The initial purpose of the well test was to determine if not purging any well volumes prior to sampling greatly affected the constituent concentrations. Appendix J contains the data from the well test. However, upon examination of the test results, an interesting point became apparent that reconfirmed the earlier conclusions concerning the flow direction at Transect 1 Well 2. The assumption was made that stream water significantly influencing the hyporheic zone would be detected in wells after the removal of each well volume (i.e., by removing water from a well influenced by the stream, additional stream water should flow into the well provided that the well lies in the hyporheic zone). Dissolved organic carbon levels were used to analyze the influence of the stream based upon previous research conclusions. Wallis et al. (1981) noted that groundwater offered minimal contribution to dissolved organic carbon (DOC) levels. Hendricks and White (1991) measured surface water DOC levels almost six times that of groundwater. They also noted that DOC levels in the hyporheic zone were greater than groundwater or surface water values. Based on previous research the following conclusions were drawn: 1.) areas with a greater contribution of water from the stream should exhibit higher DOC values than those mainly from

groundwater, and 2.) hyporheic DOC values should be elevated in comparison to DOC measurements from Hickory Creek.

The wells Transect 1 Well 2 and Transect 2 Well 2 were compared to the group Transect 1 Well 3 and Transect 2 Well 3. In all four wells, the concentration of DOC initially decreased from the time of zero minutes to the time of 15 minutes. At Transect 1 Well 2, an increase in the DOC concentration was detected at the time of 30 minutes, and a similar increase was detected in the DOC level at Transect 2 Well 2 for the 45 minute sampling period (Figures 4.5 to 4.6). However, the DOC concentrations at Transect 1 Well 3 and Transect 2 Well 3 decreased steadily over time with no rebound (Figures 4.7 to 4.8). The increase in DOC concentrations with time at Transect 1 Well 2 and Transect 2 Well 2 indicated that these wells had strong hydrological connections to the stream while the hydrological connection between the stream and the wells Transect 1 Well 3 and Transect 2 Well 3 was much weaker. Hence, the results from the well study confirmed the bi-directional flow at Transect 1 Well 2.

A general summarization of the afore mentioned data indicated that the general flow paths were as follow:

Wet Season

- water flow between the stream and each stream-side well (Transect 1 Well 1, Transect 1 Well 2, and Transect 2 Well 2) was bi-directional,

DOC Concentration Transect 1 Well 2

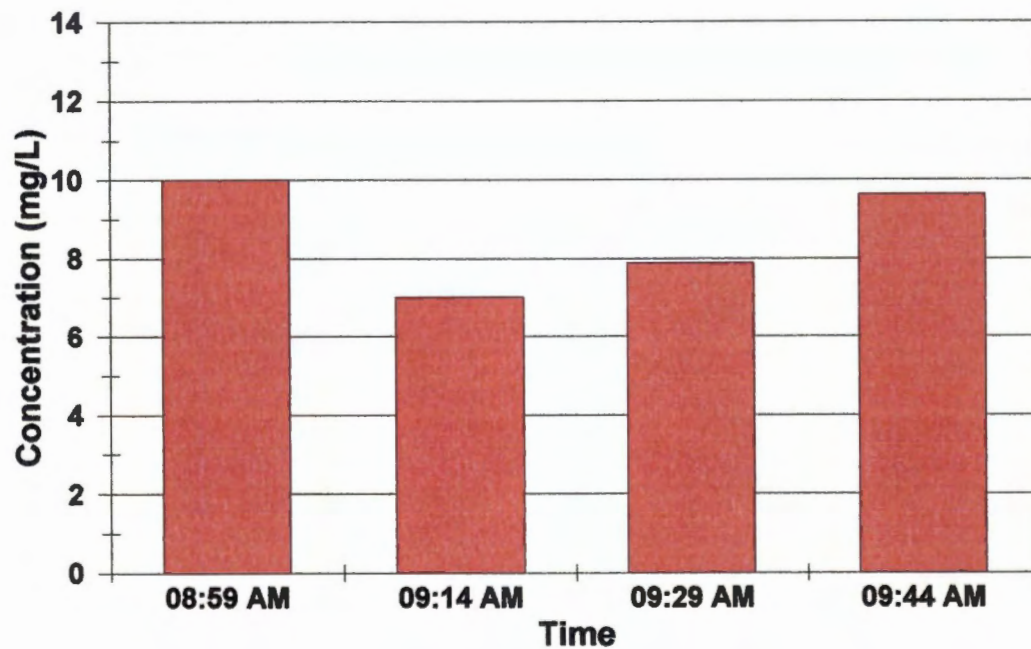


Figure 4.5. DOC concentrations increased after the initial sample was collected.

DOC Concentration Transect 2 Well 2

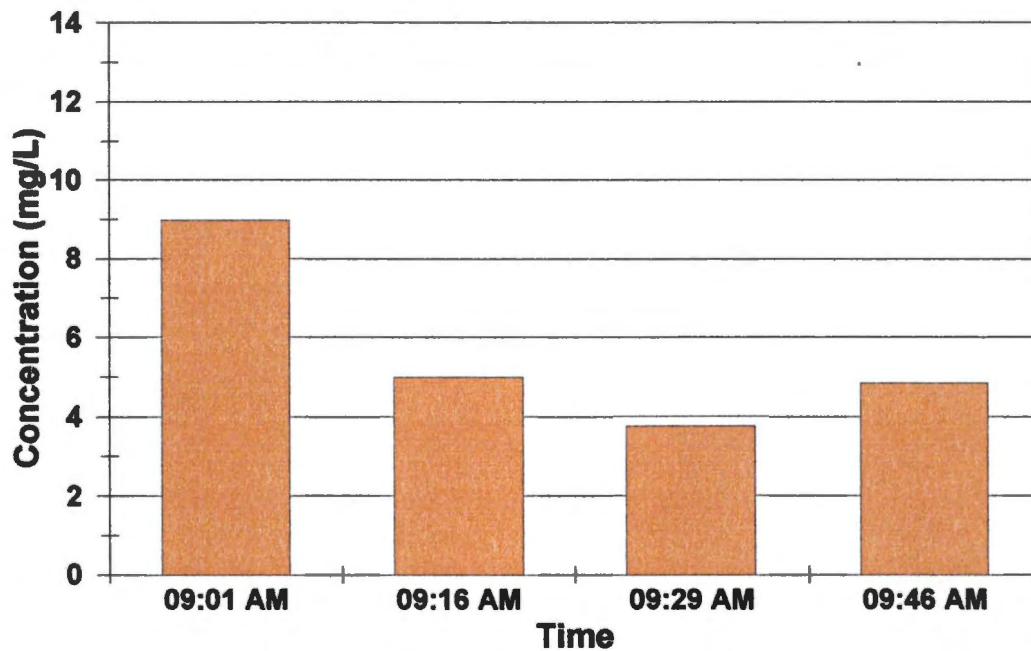


Figure 4.6. This well exhibited a similar trend to Transect 1 Well 2, but at a slower rate.

DOC Concentration Transect 1 Well 3

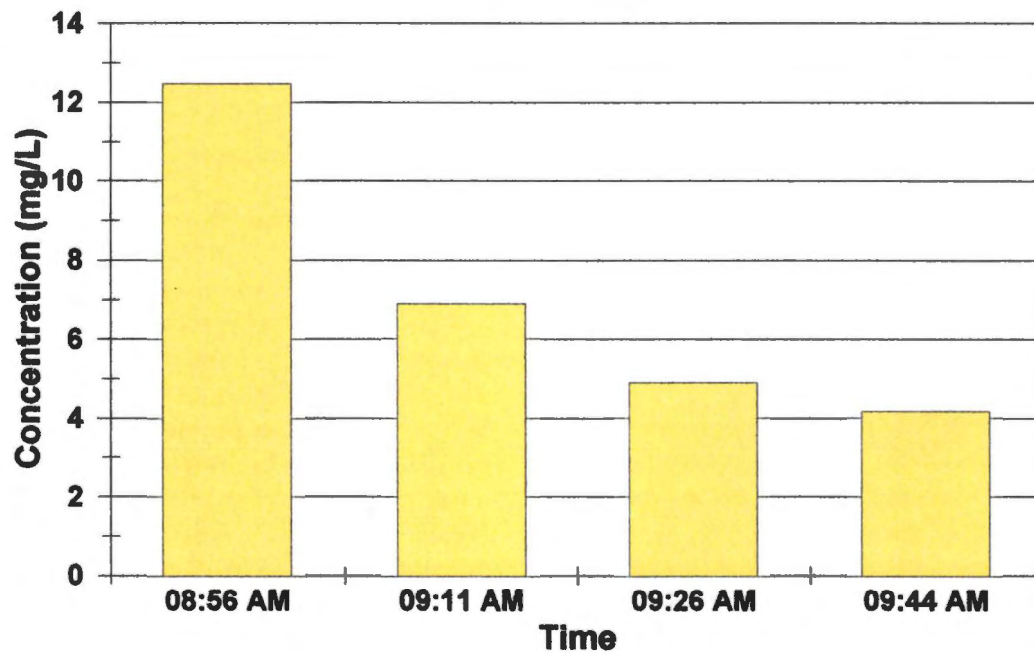


Figure 4.7. The largest changes in DOC concentrations occurred at Transect 1 Well 3.

DOC Concentration Transect 2 Well 3

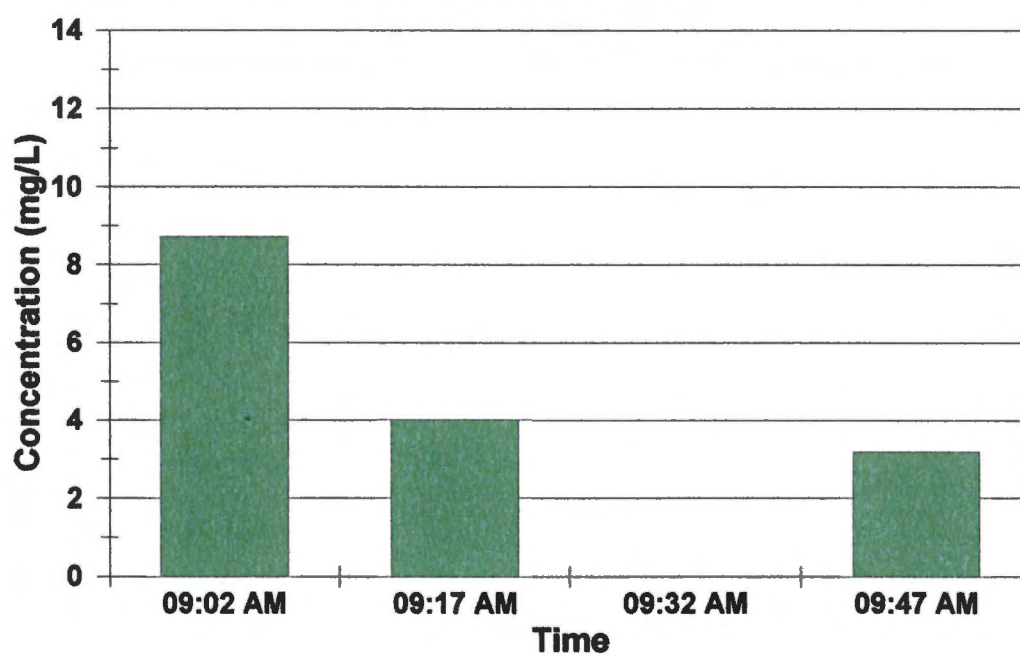


Figure 4.8. Equilibrium was quickly reached at this well.

- water flow between Transect 1 Well 2 and Transect 1 Well 3 was bi-directional,
- water flowed from Transect 1 Well 4 to Transect 1 Well 3,
- water flowed from Transect 1 Well 4 to Transect 1 Well 5,
- water flow from Transect 2 Well 2 to Transect 2 Well 3 was somewhat bi-directional with the majority of the flow traveling from Transect 2 Well 3 to Transect 2 Well 2,
- water flowed from Transect 2 Middle to Transect 2 Well 3,
- the direction of flow between Transect 1 Middle and Transect 2 Middle was bi-directional along with that between Transect 1 Well 3 and Transect 2 Well 3, and
- water flow between Transect 1 Well 2 and Transect 2 Well 3 was somewhat bi-directional with most of the flow originating at Transect 2 Well 3.

Dry Season

- water flow between the stream and the stream-side wells (Transect 1 Well 2 and Transect 2 Well 2) was bi-directional,
- water flowed from the creek to Transect 1 Well 1,
- water flow between Transect 1 Well 2 and Transect 1 Well 3 was bi-directional with the majority of the flow traveling from Transect 1 Well 2 to Transect 1 Well 3,

- water flowed from Transect 1 Well 4 to Transect 1 Well 3,
- water flowed from Transect 1 Well 4 to Transect 1 Well 5,
- water flowed from Transect 2 Well 2 to Transect 2 Well 3,
- water flowed from Transect 2 Middle to Transect 2 Well 3,
- the direction of flow between Transect 1 Middle and Transect 2 Middle was bi-directional (greater flow from Transect 1 Middle to Transect 2 middle) along with that between Transect 1 Well 3 and Transect 2 Well 3 (greater flow from Transect 2 Well 3 to Transect 1 Well 3), and
- water flowed from Transect 1 Well 2 and Transect 2 Well 3.

As seen from the above summary, the flow within the hyporheic zone along Hickory Creek was not as simple as a straight path either to or fro, but one that was dynamic. This information is important to consider in light of Findlay's (1995) observations that rapid flow along short flow paths through the hyporheic zone would most likely not alter water chemistry for the system, but that slow flow coupled with a long flow path possessed the potential to make significant changes in the water chemistry. Hence, one would expect the majority of contaminant removal to occur along the flow paths depicted in Figures 4.3 and 4.4, with an overall reduction in contaminant concentrations from the wetland to the hyporheic zone and then to the stream. However, these reductions largely depend upon the location of the highest contaminant concentrations. For this study, the stream was considered "clean" by EPA standards, thus easily justifying the reasoning for contaminant reduction from the wetland to the stream

(Appendix K). However, in another system with a polluted stream, the reverse trend may occur with contaminant reduction happening as the water travels further inland away from the stream. In either case, the largest reductions would likely occur along the most extensive flow paths.

HORIZONTAL CONCENTRATION GRADIENTS

One of the primary objectives of the study was to determine the existence of any horizontal trends associated with the list of constituents identified in Chapter 1. A more clear understanding of horizontal concentration gradients may allow for improved stream management practices as the role of the hyporheic zone is better understood. A simple linear regression between the distance from the edge of the stream and the contaminant concentration was performed for both the wet and dry season using SAS (1999). Using the same program, a multiple linear regression was performed between the contaminant concentrations and three variables, distance from the edge of the stream, average daily ambient temperature, and average daily stream discharge for each predefined season. Significant results reflect a 95% confidence interval around the specified parameter (range of values where in the parameter was expected 95% of the time). Hence, a value of $P < 0.05$ was considered significant (difference) while a value of $P > 0.05$ was nonsignificant (no difference). The coefficient of determination (R^2) indicated how well the model explained the

variation in the data. Orthophosphate was not detected in any samples and the DO meter produced erroneous results; therefore these constituents will not be discussed further. Appendix L contains constituent data relative to the shallow groundwater monitoring wells and Appendix M contains the datalogger data.

NITROGEN

Both nitrate-N and ammonium-N concentrations were measured to monitor nitrogen cycling in the hyporheic zone. During the wet season, 12% of the differences in $\text{NH}_3\text{-N}$ were explained by distance from the edge of the stream ($P < 0.002$) with concentrations increasing with distance from the edge of the stream. The slope was -0.13 indicating that an increase in distance by 1 m from the stream edge resulted in a 0.13 mg/L decrease in $\text{NH}_3\text{-N}$ levels. A reverse trend was noted for the dry season where 24% of the differences in $\text{NH}_3\text{-N}$ concentrations were explained by distance from the stream edge. With a slope of 0.008, $\text{NH}_3\text{-N}$ concentrations increased by 0.008 mg/L for each additional meter from the stream edge. For nitrate-N levels, no significant difference was seen between $\text{NO}_3\text{-N}$ levels and distance from the stream. However, for each season, a negative slope (-0.0024 for wet and -0.0008 for dry) was observed indicating that $\text{NO}_3\text{-N}$ concentrations decreased with distance from the stream edge.

Results from the dry season nitrogen data support the occurrence of nitrification near the stream side while denitrification occurred further inland. The

higher ammonium-N levels along the stream side coupled with the oxygenated stream waters provided an ideal environment for the process of nitrification. As the water moved further inland, dissolved oxygen was consumed (failure of the DO meter prohibited confirmation). Upon the consumption of DO, the microbes required another terminal electron acceptor: nitrate-N. The increase in denitrification rates (decrease in $\text{NO}_3\text{-N}$ levels) with distance from the wetted channel was reflective of this process. Additionally, the increase in ammonium-N demonstrated the lack of a suitable environment for nitrification, thus the accumulation of ammonium-N occurred.

A similar such trend was seen during the wet season, although not as great. The wetter environment allowed for a greater accumulation of $\text{NH}_3\text{-N}$ along the stream side in comparison to the dry season. This point was apparent in the differences in intercepts (1.12 for the wet season and 0.53 for the dry season). Nitrate-N levels were lower along the stream side for the wet season as compared to the dry season (0.02 and 0.07, respectively). Hence, the process of denitrification was more significant at the stream side during the wet season than the dry season. Multiple regression analysis indicated that nitrate-N concentrations were significant ($R^2 = 0.16$) during the wet season when analyzed with ambient temperature and stream discharge in addition to distance from the stream edge ($P < 0.02$). Stream discharge explained 9% of the differences in nitrate-N concentrations. The R^2 for stream discharge during the wet season was orders of magnitude greater than that for the dry season. One reason for this difference was the fact that the higher flows of the Spring (part of the wet season)

purged the stream bed of decomposing matter. Additionally, the use of fertilizers was notably higher during this period as local farmers fertilized their pastures. Thus, while distance was the most significant factor in nitrate-N concentrations for the dry season, discharge played a greater role for the wet season. These results were reflective of other hyporheic studies. Triska et al. (1990) noted greater nitrification potential in sediments closely connected to the main channel, thus decreasing with distance from the stream. Duff and Triska (1990) noted increases in denitrification potential with distance from the wetted channel.

ORGANICS

The organics measured during the study included DOC, BOD₅, and COD. Simple linear regression revealed that all three constituents were statistically significant with distance from the edge of the stream during the wet season with DOC being the only one significant during the dry season. For the wet season, distance from the edge of the streams accounted for 27% of the difference in DOC concentrations ($P < 0.0001$), 10% of the difference in BOD₅ levels ($P < 0.01$), and 30% of the difference in COD concentrations ($P < 0.0001$). Examination of the slopes for each wet season constituent regression revealed that the concentration of DOC, BOD₅, and COD all increased with distance from the wetted channel (slopes = 0.076, 0.049, and 0.343). A similar trend was noted for DOC concentrations for the dry season (slope = 0.141) where distance explained 12% of the differences ($P < 0.01$). Research by Triska et al. (1993) noted that

DOC concentrations were significantly higher for areas with the greatest potential for denitrification. A similar trend was observed with this study; higher DOC levels were coupled with lower nitrate-N and higher ammonium concentrations. The greater denitrification potential witnessed during the wet season was likely a result of a more readily available carbon supply, since denitrification rates are partially determined by carbon availability (Myrold, 1998). Examination of the groundwater flow paths indicated that a larger amount of flow occurred from the upland to the stream during the wet season as compared to the dry season. Water flow from the organically rich wetland may have aided in elevating carbon levels at the wells. Currently, no other hyporheic study documents BOD₅ and COD trends with distance from the stream.

Examination of the results from the multiple linear regression analysis indicated that along with distance, temperature was a major influence. By including temperature and discharge with distance from the stream, BOD₅ concentrations were statistically significant during the dry season. Results from the multiple regression analysis indicated that for the dry season, 66% of the differences in BOD₅ could be explained ($P < 0.0001$) with temperature and discharge accounting for 28% and 64%, partially. For the wet season, temperature was the largest influence on BOD₅ levels partially explaining 20% of the differences. Temperature was the second most significant influence on DOC (partial $R^2 = 0.9, 0.15$) and COD (partial $R^2 = 0.4, 0.5$) concentrations during the wet and dry seasons. Analyses of the organics revealed a seasonality trend in that temperature played a significant role in the constituent concentrations.

Positive slopes for each regression line indicated that increases in temperature resulted in increased concentrations of DOC, BOD₅, and COD. Currently, no other hyporheic study documents a seasonality trend with the afore mentioned contaminants.

PHYSICAL PARAMETERS

The physical parameters examined during the study included the oxidation reduction (redox) potential and pH. Results from a simple linear regression indicated that distance could explain 9% of the difference in redox potential during the dry season ($P < 0.05$), but was not significant during the wet season. During both seasons, the slope was negative (-0.64 and -0.39) indicating that redox potential decreased with distance from the edge of the stream. A multiple regression of redox potential with distance, temperature, and discharge revealed a significant trend only during the wet season with a total $R^2 = 12\%$.

Further examination of the redox results for the wet season revealed that discharge was a significant factor partially accounting for 6% of the differences in the redox potential. Increases in discharge resulted in a decrease in the redox potential (slope = -0.004). This trend was as expected, for higher discharge levels resulted in a greater volume of water flowing into the hyporheic zone. As water entered the hyporheic zone, microbes consumed the dissolved oxygen carried in the water, hence reducing the redox potential. Hendricks and White (1991) found that the redox potential was also indicative of the source of the

water in the hyporheic zone. They noted lower redox values with groundwater as compared to surface waters. Hence, as the redox potential decreased with distance from the stream edge, the percentage of stream water decreased while that of groundwater increased; an expected trend. The R^2 during the dry season was 13% , but was not considered statistically significant. Temperature was the second largest contributor to the differences in the redox potential (after distance) for the dry season with a partial $R^2 = 4\%$.

Simple linear regression analysis revealed that distance was a significant factor in the pH levels during both the wet and dry seasons. During the wet season, distance explained 8% of the variation in pH ($P < 0.01$) while 10% of the variation in pH was the result of distance for the dry season ($P < 0.02$). As the distance from the edge of the stream increased, the pH decreased for both the wet and dry seasons (slope = -0.002 and slope = -0.0015). A multiple regression analysis of pH with distance, temperature, and discharge revealed significant trends for both seasons. Seventeen percent of the variation in pH was explained by these variables during the wet season while 33% was accounted for during the dry season. Both distance and temperature were significant contributors to pH variation during the wet season with partial R^2 of 9% and 8%. A similar trend was noted during the dry season; however, discharge played a larger role than in the wet season.

For the dry season, the partial R^2 were 17% for distance, 14% for temperature, and 4% for discharge. Again, pH decreased with increasing distance for both seasons; and as expected, pH also decreased with temperature

increases for both seasons. Rises in temperature brought about a greater level of microbial activity (i.e., nitrification), and hence, the release of more H^+ ions. For the dry season, pH increased with increasing discharge levels. This result seemed appropriate, for higher discharge rates resulted in a greater influx of the more basic stream water into the hyporheic zone. The average pH of Hickory Creek was 7.8 while that of the hyporheic zone ranged between 6.8 and 7.2; values approximately 0.6 to 1 pH unit less than that of the stream. Similar results were reported by Hendricks and White (1991) who noted that surface waters averaged 0.5 to 1 pH unit higher than those of the hyporheic waters (7.5 to 8.5 for surface waters and 6.5 to 7.0 for hyporheic waters). However, the research performed by Hendricks and White (1991) did not explore pH concentration gradients with distance from the stream. While the pH ranges appear similar, a generality in trends between the two systems was difficult to define.

IRON

Both ferrous and ferric iron concentrations were measured during the study. The simple linear regression model used to relate distance with iron concentrations revealed only one significant trend. During the wet season, 18% of the variation in ferrous iron was explained by distance ($P < 0.001$). As distance from the edge of the stream increased, the concentration of Fe^{2+} also increased (slope = 0.0161). Based upon the previously mentioned results for redox potential, this trend was appropriate, for ferrous irons are produced under

anaerobic or reduced conditions. A multiple regression analysis indicated that all three components (distance, temperature, and discharge) were important contributors to ferrous iron variations during the wet season with distance accounting for 18% of the variation, temperature causing 11% of the variation, and discharge producing 5%, each partially. Again, the concentration of ferrous iron increased with distance, and as expected, decreased with increasing discharge (higher discharge levels introduce a greater volume of oxygenated waters into the hyporheic zone). Surprisingly, the concentration of ferrous iron increased with rising temperatures. Mullen (1998) noted that the process of iron oxidation and reduction can occur both biotically and abiotically, but researchers often have a difficult time quantifying the cause. Based upon the ferrous iron trend with temperature, it appeared that a significant portion of ferrous iron reduction occurred biotically in the hyporheic zone.

Ferric iron concentrations did not exhibit a significant trend under simple linear regression analysis. The only significant trend associated with ferric iron resulted from a multiple regression analysis with distance, temperature, and discharge. During the wet season, the afore mentioned variables accounted for 16% of the variation in ferric iron concentrations ($P < 0.05$). Partially, temperature accounted for the majority of the variation ($R^2 = 0.11$) followed by distance ($R^2 = 0.7$) and discharge ($R^2 = 0.5$). Again, a seasonality trend was associated with iron concentrations; ferric iron levels decreased with temperature. This relationship agreed with that of ferrous iron, for as ferric iron was consumed, ferrous iron was produced.

SULFUR

Both sulfate-S and sulfide were examined using simple and multiple linear regression analysis. The only significant trend with sulfide occurred during the wet season. Using multiple linear regression, 13% of the variation in sulfide concentration was explained by distance, temperature, and discharge ($P < 0.05$) with temperature accounting for the majority of the variation (partial $R^2 = 11\%$) followed by discharge (partial $R^2 = 0.5$) and distance (partial $R^2 = \text{less than } 1\%$). Surprisingly, sulfide concentrations decreased with increases in temperature (slope = -0.02), since warmer temperatures are typically indicative of greater levels of microbial activity. However, further examination of sulfide trends with the redox potential gradient proved helpful. Both sulfide concentration and the redox potential decreased with increasing temperatures. Hence, the greatest concentrations of sulfide were detected under the most reduced conditions. This trend was appropriate, for upon oxygen depletion, microbes utilize other terminal electron acceptors in the order of nitrate, ferrous iron, and sulfate (Brady and Weil, 1999). The lowest temperatures were recorded in the early Spring which also coincides with the period of greatest rainfall, and thus the most reduced conditions (recalling that the redox potential decreased with increasing discharge).

For sulfate-S levels, multiple regression analysis detected a significant trend during the dry season between the constituent concentration and distance, temperature, and discharge with the variables accounting for 17% of the total

variation ($P < 0.03$). Discharge was the most significant factor resulting in 8% of the variation followed by temperature (partial $R^2 = 0.3$) and distance (partial $R^2 = 0.3$). Noteworthy was the season that a significant variation in sulfate-S concentration was detected. During the dry period, more oxidized conditions were present, hence resulting in a greater production of sulfate-S. The importance of discharge was reflective of the oxidation and reduction processes of sulfur. As the discharge increased, the sulfate-S concentration decreased. Therefore, the larger volume of water introduced into the hyporheic zones (upon oxygen depletion) produced more anaerobic zone resulting in sulfate-S reduction.

OTHER CONSTITUENTS

Both simple and multiple linear regression analyses were performed on the remaining constituents listed in Chapter 1 (Cl, FC, FS, and TS) between distance (simple linear regression analyses) and distance, temperature, and discharge (multiple linear regression analyses), but no significant trends were detected.

ENZYMES

Enzymatic data were analyzed in two manners. The first was with a simple linear regression model that attempted to explain variations in the enzyme concentrations with distance from the stream, and the second analysis involved a

comparison between the enzymatic data and certain constituents, namely DOC, NO₃-N, NH₃-N, SO₄-S, BOD₅, COD, oxidation reduction potential, pH, and Fe²⁺. Enzymatic data included microbial biomass, dehydrogenase activity, arylsulfatase, L-asparaginase, soil pH, and soil moisture content. The respective values at all three depths were averaged to provide the mean values for the appropriate wells. All three enzymatic values for each well (all three depths) were averaged to provide a representative total for the entire well, since the elevation of the water table with respect to the soil core samples was not known. Potentially, the enzymatic activity at any soil depth could influence the water quality as either the water table rises or leaching from the upper layers introduces microbes, their substrates, or their products into the water system. Appendix N contains the enzymatic data.

The simple linear regression model was divided into the same wet and dry seasons used in the previous analyses. Only microbial biomass and soil moisture content exhibited significant trends with distance. During the wet season, 74% of the variation in microbial biomass was explained by distance from the edge of the stream ($P < 0.02$). Microbial biomass levels increased with distance from the stream edge (slope = 2.36). This trend was similar to the ones displayed by DOC, BOD₅, and COD where all three constituents increased with distance from the wetted channel.

The relationship between the microbial biomass and the organics was noteworthy. Based upon the information from previous researchers that groundwater contributes minimal amounts of DOC to waters (Wallis et al., 1981)

coupled with information from Hendricks and White (1991) concerning the elevated DOC levels in the hyporheic zone in comparison to both the surface water and the groundwater, the expectation was for DOC values to decrease with distance from the stream (as the source of the hyporheic waters became predominately groundwater). However, the opposite trend was detected; DOC values increased with distance from the stream. The presence of the wetland was likely a main factor contributing to this trend; the karst environment of the study site likely indicates that the water source for the wetland was not from a deep aquifer, thus DOC levels were likely higher than those indicated for a "true" groundwater source. The question still remains as to whether the DOC trend was due to the presence of a larger microbial population (hence more carbon), or whether a larger carbon supply was able to support more microbes. Likely, the two were interdependent. Carbon was probably carried from the wetland to the stream via shallow groundwater flow. Additionally, carbon rich waters from the stream also aided hyporheic microbes. However, the DOC levels of Hickory Creek were initially low (6.11 mg/L), so the likelihood of this method largely contributing to the hyporheic carbon supply was slim. Probably, microbes present along the flow paths consumed the carbon, thus decreasing the carbon concentration while increasing their population.

The moisture content of the soil was also significant for the wet season with distance explaining 67% of the variation ($P < 0.04$). As distance from the stream increased, so did the moisture content of the soil. Examination of the flow pattern for the wet season corroborated such a trend. Water flow was

predominately from the wetland to the stream; hence, one would expect the more highly saturated soils to originate with the water flow. This trend of soil moisture content decreasing with distance towards the stream was probably typical of well-drained upland soils. The higher soil moisture levels at the inland sites agreed with the results from the redox, ferrous iron, and nitrate-N data.

A comparison between the enzymatic data and the pre-specified constituents revealed few significant trends. A relationship was noted between higher concentrations of ammonium-N and an increased soil moisture content ($P < 0.05$). This trend appeared sound, for at more anaerobic sites, the potential for nitrification was reduced, thus allowing ammonium-N to accumulate. An increase in pH was also seen with higher concentrations of ammonium-N ($P < 0.04$). Again, the process of denitrification played an important role, for the anaerobic processes resulted in the usage of hydrogen ions, thus elevating the pH.

Increased levels of COD occurred with larger microbial biomass numbers ($P < 0.03$). However, such a trend was not seen with BOD₅ or TOC. The occurrence of such a trend with COD likely relates to the test method. Of the three carbon measurements, COD measures all chemically oxidizable organic matter in a sample. Thus, the increased COD levels probably reflect a portion of the microbial population and not merely the carbon that the microbes would consume such as in BOD₅ and TOC. Increases in soil pH were significantly related to increases in BOD₅ ($P < 0.05$) but not with the other organics. The reason for this trend was not understood.

The final significant trend noted between the enzymatic data and the constituents was with ferrous iron and the soil pH. Increases in the ferrous iron concentrations were related to decreased soil pH. Based upon literature by Mullen (1998), this trend was appropriate, for under aerobic conditions with a pH greater than 3, the oxidation of ferrous iron to ferric iron occurs rapidly. Hence, a low pH should result in a higher ferrous iron concentration; the trend seen in this study. Based on all of the afore mentioned trends, it appears that microbially mediated mineralization was not a significant process, unlike microbial oxidation and reduction processes which appeared to govern constituent transformations.

VERTICAL CONCENTRATION GRADIENTS

The second main objective of the study was to determine the presence of any vertical trends associated with nitrate-N, sulfate-S, chloride, and total organic carbon, thereby supplementing the minimal amount of research that has been performed on the hyporheic zone in the vertical plane. Data obtained from the suction lysimeters were not used in the analyses because the majority of the data pertained to the vadose zone water quality rather than the saturated flow in the hyporheic zone (Appendix O). The occasional exception was the deepest suction lysimeters at the stream-side wells and those at the wetland wells (Transect 1 Well 4 and Transect 1 Well 5) during the wet season. Vertical component analyses were focused upon data obtained from the peepers (Appendix P). Data

pertaining to the streambed section of the hyporheic zone may assist in stream management practices that recognize the potential importance of this area in stream recovery following major storm events.

NITRATE-N

A simple linear regression was performed on nitrate-N concentrations and the chamber depth with chamber one representing the deepest depth and chamber ten the most shallow (i.e., the y-axis began with the deepest and proceeded to the most shallow). Depth explained 14% of the variation in nitrate-N concentrations ($P < 0.01$) as levels decreased with depth (slope = 1.2). This trend seemed appropriate, for as the depth into the hyporheic zone increased, the percentage of oxygenated waters reaching the chambers likely decreased. Hence, the reduction in nitrate-N was the result of the process of denitrification that ensued under anaerobic conditions; denitrification potential increased with depth. Hendricks and White (1991) also noted nitrate reductions with depth in the hyporheic zone of a Michigan stream. A similar decline in nitrate concentration with depth was observed by Hill and Lymburner (1998) in a Canadian stream.

SULFATE-S

Similar to nitrate-N trends, the concentration of sulfate-S in the chambers decreased with depth when a simple linear regression was performed. Depth accounted for 17% of the variation in sulfate-S concentration. As depth increased, the level of sulfate-S detected in the chambers decreased (slope = 0.98). Parallel to the nitrogen analysis, the more reduced conditions produced at the lower chamber depths allowed for the reduction of sulfate-S. Currently, no hyporheic research has examined changes in sulfate-S levels along the vertical plane.

CHLORIDE

Using a simple linear regression analysis, chloride trends were found to be nonsignificant, although the chloride levels did decrease with depth (slope=0.06). This trend suggests that a larger groundwater contribution to the hyporheic zone occurred at the deepest chambers. Typically, a 1 mg/L decrease in chloride concentrations was detected between the uppermost chamber and the deepest chamber. The relative chloride concentration of Hickory Creek was 3.9 mg/L; a value that was equivalent to the average values detected at the urban sampling site of Sweetwater Creek (another eastern Tennessee stream).

DISSOLVED ORGANIC CARBON

As with the previously discussed constituents, a simple linear regression was performed on DOC concentration and depth. The results of the analysis indicated that 11% of the variation in DOC concentrations was due to depth ($P < 0.03$). However, unlike the other constituents, DOC levels increased with depth. This trend supports the assertion that denitrification was the primary cause for nitrate-N reduction at the lower chambers. Grimm and Fisher (1984) and Triska et al. (1993) noted the importance of high organic matter content to greater denitrification rates. The DOC results for this study differ somewhat from those of Hendricks and White (1981) and Schindler and Krabbenhoft (1998) who measured DOC decreases with depth. However, these researchers measured DOC levels at greater depths than those done in this study. The presence of bedrock prevented any deeper installation of the peepers; so, a similar trend may have potentially been seen. Additionally, the high amount of leaf litter introduced into the stream over the years was a likely contributor to the DOC levels.

WELL COMPARISONS

An ANOVA table was generated from a completely randomized design using the constituent data from the wells, and a Tukey's mean separation was performed (SAS, 1999). In accordance with the previously performed analyses,

the data were examined in both a wet and a dry season. Based upon the ANOVA table, only four constituents displayed statistically significant concentrations differences among the wells for the dry season: $\text{NH}_3\text{-N}$ ($P<0.0001$), $\text{SO}_4\text{-S}$ ($P<0.0001$), pH ($P<0.01$), and TS ($P<0.0001$). For the wet season, a larger number of constituents displayed concentration differences among the wells: $\text{NH}_3\text{-N}$ ($P<0.0001$), $\text{SO}_4\text{-S}$ ($P<0.001$), pH ($P<0.0001$), Fe^{2+} ($P<0.005$), Cl ($P<0.0001$), COD ($P<0.0001$), BOD_5 ($P<0.01$), and TOC ($P<0.0001$).

The reason for the notable difference in significant constituent variations among the wells may be related to the flows exhibited during the seasons (changes in the general flow direction was how the seasons were defined). During the wet season, flow from the creek into the hyporheic zone appeared to cease shortly beyond the stream-side wells. After the stream-side wells, the majority of the groundwater flow was from the wetland to the stream. However, the horizontal area of the hyporheic zone was extended during the dry season with flow from the stream traveling to the wells located approximately 9.1 m from the stream side; beyond that point, groundwater flow was from the wetland towards the stream. Table 4.2 contains a listing of the mean separation output indicating which wells differed significantly for the respective constituents and seasons. A general hypothesis can be made that the reason for the differences among seasons was related to the effectiveness of the hyporheic zone in providing treatment. During the dry season, water from the stream traveled further into the hyporheic zone, thus increasing the retention time as compared to that for the wet season (using the assumption that the water

Table 4.2. Mean separation results for all of the wells.

Season	Well	Constituent	Group
Dry	T1W1	Redox	A
Dry	T1W2	Redox	A
Dry	T1W3	Redox	A
Dry	T1W4	Redox	A
Dry	T1W5	Redox	A
Dry	T2W2	Redox	A
Dry	T2W3	Redox	A
Wet	T1W1	Redox	A
Wet	T1W2	Redox	A
Wet	T1W3	Redox	A
Wet	T1W4	Redox	A
Wet	T1W5	Redox	A
Wet	T2W2	Redox	A
Wet	T2W3	Redox	A
Dry	T1W1	NO ₃ -N	A
Dry	T1W2	NO ₃ -N	A
Dry	T1W3	NO ₃ -N	A
Dry	T1W4	NO ₃ -N	A
Dry	T1W5	NO ₃ -N	A
Dry	T2W2	NO ₃ -N	A
Dry	T2W3	NO ₃ -N	A
Wet	T1W1	NO ₃ -N	A
Wet	T1W2	NO ₃ -N	A
Wet	T1W3	NO ₃ -N	A
Wet	T1W4	NO ₃ -N	A

Table 4.2. continued

Season	Well	Constituent	Group
Wet	T1W5	NO ₃ -N	A
Wet	T2W2	NO ₃ -N	A
Wet	T2W3	NO ₃ -N	A
Dry	T1W1	NH ₃ -N	AB
Dry	T1W2	NH ₃ -N	C
Dry	T1W3	NH ₃ -N	ABC
Dry	T1W4	NH ₃ -N	BC
Dry	T1W5	NH ₃ -N	A
Dry	T2W2	NH ₃ -N	BC
Dry	T2W3	NH ₃ -N	C
Wet	T1W1	NH ₃ -N	AB
Wet	T1W2	NH ₃ -N	BC
Wet	T1W3	NH ₃ -N	BC
Wet	T1W4	NH ₃ -N	BC
Wet	T1W5	NH ₃ -N	A
Wet	T2W2	NH ₃ -N	BC
Wet	T2W3	NH ₃ -N	C
Dry	T1W1	SO ₄ -S	B
Dry	T1W2	SO ₄ -S	B
Dry	T1W3	SO ₄ -S	AB
Dry	T1W4	SO ₄ -S	B
Dry	T1W5	SO ₄ -S	B
Dry	T2W2	SO ₄ -S	B
Dry	T2W3	SO ₄ -S	A
Wet	T1W1	SO ₄ -S	AB

Table 4.2. continued

Season	Well	Constituent	Group
Wet	T1W2	SO ₄ -S	A
Wet	T1W3	SO ₄ -S	C
Wet	T1W4	SO ₄ -S	ABC
Wet	T1W5	SO ₄ -S	ABC
Wet	T2W2	SO ₄ -S	C
Wet	T2W3	SO ₄ -S	BC
Dry	T1W1	Cl	A
Dry	T1W2	Cl	A
Dry	T1W3	Cl	A
Dry	T1W4	Cl	A
Dry	T1W5	Cl	A
Dry	T2W2	Cl	A
Dry	T2W3	Cl	A
Wet	T1W1	Cl	A
Wet	T1W2	Cl	BC
Wet	T1W3	Cl	C
Wet	T1W4	Cl	BC
Wet	T1W5	Cl	C
Wet	T2W2	Cl	BC
Wet	T2W3	Cl	C
Dry	T1W1	TOC	AB
Dry	T1W2	TOC	AB
Dry	T1W3	TOC	AB
Dry	T1W4	TOC	AB
Dry	T1W45	TOC	A

Table 4.2. continued

Season	Well	Constituent	Group
Dry	T2W2	TOC	AB
Dry	T2W3	TOC	B
Wet	T1W1	TOC	AB
Wet	T1W2	TOC	AB
Wet	T1W3	TOC	B
Wet	T1W4	TOC	AB
Wet	T1W5	TOC	A
Wet	T2W2	TOC	B
Wet	T2W3	TOC	B
Dry	T1W1	BOD ₅	A
Dry	T1W2	BOD ₅	A
Dry	T1W3	BOD ₅	A
Dry	T1W4	BOD ₅	A
Dry	T1W5	BOD ₅	A
Dry	T2W2	BOD ₅	A
Dry	T2W3	BOD ₅	A
Wet	T1W1	BOD ₅	AB
Wet	T1W2	BOD ₅	AB
Wet	T1W3	BOD ₅	B
Wet	T1W4	BOD ₅	AB
Wet	T1W5	BOD ₅	A
Wet	T2W2	BOD ₅	B
Wet	T2W3	BOD ₅	B
Dry	T1W1	pH	AB
Dry	T1W2	pH	A

Table 4.2. continued

Season	Well	Constituent	Group
Dry	T1W3	pH	AB
Dry	T1W4	pH	B
Dry	T1W5	pH	AB
Dry	T2W2	pH	AB
Dry	T2W3	pH	AB
Wet	T1W1	pH	A
Wet	T1W2	pH	AB
Wet	T1W3	pH	C
Wet	T1W4	pH	C
Wet	T1W5	pH	C
Wet	T2W2	pH	C
Wet	T2W3	pH	BC
Dry	T1W1	Fe ²⁺	A
Dry	T1W2	Fe ²⁺	A
Dry	T1W3	Fe ²⁺	A
Dry	T1W4	Fe ²⁺	A
Dry	T1W5	Fe ²⁺	A
Dry	T2W2	Fe ²⁺	A
Dry	T2W3	Fe ²⁺	A
Wet	T1W1	Fe ²⁺	B
Wet	T1W2	Fe ²⁺	B
Wet	T1W3	Fe ²⁺	AB
Wet	T1W4	Fe ²⁺	AB
Wet	T1W5	Fe ²⁺	A
Wet	T2W2	Fe ²⁺	B

Table 4.2. continued

Season	Well	Constituent	Group
Wet	T2W3	Fe ²⁺	B
Dry	T1W1	Fe ³⁺	A
Dry	T1W2	Fe ³⁺	A
Dry	T1W3	Fe ³⁺	A
Dry	T1W4	Fe ³⁺	A
Dry	T1W5	Fe ³⁺	A
Dry	T2W2	Fe ³⁺	A
Dry	T2W3	Fe ³⁺	A
Wet	T1W1	Fe ³⁺	A
Wet	T1W2	Fe ³⁺	A
Wet	T1W3	Fe ³⁺	A
Wet	T1W4	Fe ³⁺	A
Wet	T1W5	Fe ³⁺	A
Wet	T2W2	Fe ³⁺	A
Wet	T2W3	Fe ³⁺	A
Dry	T1W1	FC	A
Dry	T1W2	FC	A
Dry	T1W3	FC	A
Dry	T1W4	FC	A
Dry	T1W5	FC	A
Dry	T2W2	FC	A
Dry	T2W3	FC	A
Wet	T1W1	FC	B
Wet	T1W2	FC	B
Wet	T1W3	FC	A

Table 4.2. continued

Season	Well	Constituent	Group
Wet	T1W4	FC	B
Wet	T1W5	FC	B
Wet	T2W2	FC	B
Wet	T2W3	FC	B
Dry	T1W1	FS	A
Dry	T1W2	FS	A
Dry	T1W3	FS	A
Dry	T1W4	FS	A
Dry	T1W5	FS	A
Dry	T2W2	FS	A
Dry	T2W3	FS	A
Wet	T1W1	FS	A
Wet	T1W2	FS	A
Wet	T1W3	FS	A
Wet	T1W4	FS	A
Wet	T1W5	FS	A
Wet	T2W2	FS	A
Wet	T2W3	FS	A
Dry	T1W1	S ²⁻	A
Dry	T1W2	S ²⁻	A
Dry	T1W3	S ²⁻	A
Dry	T1W4	S ²⁻	A
Dry	T1W5	S ²⁻	A
Dry	T2W2	S ²⁻	A
Dry	T2W3	S ²⁻	A

Table 4.2. continued

Season	Well	Constituent	Group
Wet	T1W1	S ²⁻	A
Wet	T1W2	S ²⁻	A
Wet	T1W3	S ²⁻	A
Wet	T1W4	S ²⁻	A
Wet	T1W5	S ²⁻	A
Wet	T2W2	S ²⁻	A
Wet	T2W3	S ²⁻	A
Dry	T1W1	COD	AB
Dry	T1W2	COD	C
Dry	T1W3	COD	C
Dry	T1W4	COD	C
Dry	T1W5	COD	BC
Dry	T2W2	COD	A
Dry	T2W3	COD	BC
Wet	T1W1	COD	A
Wet	T1W2	COD	A
Wet	T1W3	COD	A
Wet	T1W4	COD	A
Wet	T1W5	COD	A
Wet	T2W2	COD	A
Wet	T2W3	COD	A
Dry	T1W1	TS	A
Dry	T1W2	TS	A
Dry	T1W3	TS	A
Dry	T1W4	TS	A

Table 4.2. continued

Season	Well	Constituent	Group
Dry	T1W5	TS	A
Dry	T2W2	TS	A
Dry	T2W3	TS	A
Wet	T1W1	TS	BC
Wet	T1W2	TS	BC
Wet	T1W3	TS	BC
Wet	T1W4	TS	AB
Wet	T1W5	TS	A
Wet	T2W2	TS	BC
Wet	T2W3	TS	C

****Note: Wells with the same letter group were statistically the same.****

traveled at a similar rate for both seasons). Additionally, the hyporheic zone was able to treat a smaller volume of water during the dry season. The results from this analysis corroborate results from Findlay (1995) who stated that rapid flow along short flow paths through the hyporheic zone would most likely not alter water chemistry with the opposite being true for slow flow along long flow paths. The longer flow paths produced during the dry season allowed for better hyporheic treatment of waters not only originating from the stream, but from the upland wetland as well.

CHAPTER 5

SUMMARY AND CONCLUSIONS

SUMMARY

The hyporheic zone of an upland creek in an agricultural environment was studied to identify the hydrological, biological, and chemical processes that occurred and to determine the effects of these processes on hyporheic water quality. Specific examination of both horizontal and vertical trends was performed upon numerous constituents. Along the horizontal plane, the examined constituents were redox potential, pH, nitrate-N, ammonium-N, sulfate-S, sulfide, chloride, ferric iron, ferrous iron, total organic carbon, biochemical oxygen demand, chemical oxygen demand, fecal coliforms, fecal streptococci, total solids, microbial biomass, dehydrogenase, arylsulfatase, L-asparaginase, soil pH, and soil moisture content. Only nitrate-N, sulfate-S, chloride, and total organic carbon were measured in the vertical plane. Data collected from piezometers allowed for the determination of groundwater flow paths with two distinct seasonal patterns emerging because of a drought: a wet season and a dry season. Constituent concentrations along the horizontal plane coupled with the data from the piezometer nests provided insight into the importance of

hydrological connections to the hyporheic zone while microbial soil analyses garnered information concerning the biological parameters. Statistical methods allowed for the identification of significant trends with a number of variables (i.e., distance). The information gained from this research may assist in developing improved stream management practices through engineering practices designed to enhance hyporheic processes.

CONCLUSIONS

Based upon the results of the analyses performed in this study, several conclusions were made. Because this study examined many constituents not previously examined, a **C** or an **N** will follow each point. **C** denotes that the conclusion was confirmatory of other's research (expected results) while **N** indicates a new conclusion. Support for the new conclusions was gained through confirmatory measurements.

1. Water flow in the hyporheic zone was bi-directional. **C**
2. Nitrification potential decreased with increasing distance from the stream's edge. **C**
3. Denitrification potential increased with distance from the edge of the stream. **C**

4. Total organic carbon, biochemical oxygen demand, and chemical oxygen demand, and ferrous iron concentrations; microbial biomass numbers; and soil moisture content increased with distance from the edge of the stream.
N
5. The oxidation reduction potential decreased with distance from the edge of the stream. **N**
6. A decrease in pH was noted as distance from the edge of the stream increased. **N**
7. Oxidation and reduction processes were the primary means of constituent transformations and not microbial mineralization. **N**
8. Denitrification potential increased with depth. **C**
9. Sulfate-S concentrations decreased with depth. **C**
10. Total organic carbon concentrations increased with depth. **C** (results from this study contradict others)
11. Most significant differences among the wells occurred during the wet season as compared to the dry season, thus indicating that the lengths of the flow paths was an important factor in the treatment of water in the hyporheic zone. Hence the retention times were important factors in the ability of the hyporheic zone to provide treatment. **C**

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APPENDICES

APPENDICES

All Appendices are contained on the CD along with a Readme.txt file that contains an explanation of each Appendix. A copy of the Readme.txt file follows.

Readme.txt

Each Appendix is located in a separate folder (i.e., the folder labeled Appendix A contains the documentation relative to Appendix A). When possible, the word processing files are saved as both Corel Wordperfect .wpd files and Microsoft Word .doc files. Likewise, the computational files are saved as both Quattro Pro.wb3 files and Microsoft Excel .xls files when possible. Version 8 was used for all of the afore mentioned software. Additionally, ArcView was used to generate topographical maps. SAS version 8 was used in the statistical analyses.

Appendix A

- Field Notes for Soil Coring

Appendix B

- Field Notes from Well Installation

Appendix C

- Suction Lysimeter Notes

Appendix D

- Piezometer Notes

Appendix E

- Datalogger Notes

Appendix F

- Pt Electrode Notes

Appendix G

- Surveyed Data and Maps

Appendix H

- SAS program

Appendix I

- Piezometer Data

Appendix J

- Well Test

Appendix K

- Stream Sampling (ISCO) Data

Appendix L

- Shallow Groundwater Monitoring Well and Additional Surface Water Data

Appendix M

- Datalogger Data

Appendix N

- Enzymatic Data

Appendix O

- Suction Lysimeter Data

Appendix P

- Peeper Data

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Carmen T. Anderson was born in Knoxville, TN on August 16, 1975 and attended Karns High School in Knoxville, TN. She received her Bachelor of Science degree in Agricultural Engineering in May 1998 from the University of Tennessee, Knoxville. With the aide of a research assistantship from the Agricultural and Biosystems Engineering Department at the University of Tennessee and the Tennessee Agricultural Experiment Station, she received her Master of Science degree in Biosystems Engineering from the University of Tennessee, Knoxville in August 2000. After graduation, the author plans to attend the University of Kentucky, Lexington to obtain a Ph. D. in Agricultural and Biosystems Engineering.

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