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Nitrogen transformations in a mitigated wetland in the Green Bottom Wildlife Management Area

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by

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ABSTRACT

Soil nitrogen (N) dynamics have been shown to be quite sensitive to the soil hydrology. Creation of a mitigation freshwater wetland from an old field provided an opportunity to examine changes in N dynamics in terrestrial, aquatic (but formerly terrestrial), and transition (seasonally inundated) soils.

This thesis determined N availability using *in situ* incubations of soils taken from three distinct habitat conditions based on the degree of inundation following wetland creation: (1) old field (no inundation), (2) transition (seasonally inundated, initially beneath 10-20 cm of water), and (3) mitigation wetland (permanently inundated). Sample plots were located along five parallel transects with one plot of each transect located in each of these three site types for a total of 15 plots in the study. Mineral soil was incubated within polyethylene bags and buried in the ground for 28 d. Nitrogen dynamics were assessed by comparing pre- versus post-incubation values of extractable NO_3^- and NH_4^+. In addition, soils were analyzed for organic matter, texture (% sand, silt, and clay), and moisture content. Climatic information was observed on site on sample days and daily information was provided by the local National Weather Service office.

Soils in the old field and transition sites had similar textures; whereas, mitigation wetland soil was different with significantly higher clay and silt content and lower sand content. It was not clear whether this resulted from extended inundation or as an artifact of sampling these soils when waterlogged. Soil moisture content increased from old field to transition to mitigation wetland sites as was expected with increased levels and period of inundation. Influences on moisture content for the old field and transition soils appeared to

differ. Old field soil seemed to respond to variations in precipitation in conjunction with other environmental factors, and transition soil responded to period of exposure. Soil organic matter was similar for all three site types.

Although sites were quite similar with respect to soil organic matter (the main source of N for these soils), sites differed substantially in the predominant form of available N, with NO_3^- dominating in the old field and late season transitional soils and NH_4^+ dominating in the mitigation wetland and early season transition soils. Similar patterns of contrast were found for net N mineralization and nitrification. Nitrate pools decreased significantly in old field soils in May, likely related to plant uptake, and increased in September, possibly related to plant senescence. Inundated soil in April and May showed no change in NO_3^- pools, but instead showed substantial increases in NH_4^+ pools. Over the length of the study mitigation wetland soil had fluctuating NO_3^- pools, but ended with an 87% increase in pool level, and the NH_4^+ pool in the mitigation wetland experienced a huge increase (>200%). Seasonal draw-down of the water table exposed the transition area in June and by July N dynamics of the transition soils were similar to those of the old field sites. These results support earlier work showing that N dynamics of these alluvial soils change rapidly toward those of typical hydromorphic soils following inundation, but also demonstrate that this change is reversible.

Principal component analysis of each site type for applicable climatic, physical, and N data was used and provided a distinct separation of months. The pattern was similar for all three site types; however, some of the specific variables of influence differed. Old field sites were positively correlated to daily high temperature, average daily temperature, precipitation between samples and precipitation within 5d of sample, and negatively to precipitation within

48h of sample, extractable NO_3^- and extractable NH_4^+. Transition sites positively correlated to daily high temperature, soil temperature, average temperature between samples, and precipitation within 5d of sample and negatively to precipitation within 48h of sample, extractable NO_3^- and extractable NH_4^+. Mitigation wetland sites correlated positively to daily high temperature, average daily temperature, precipitation between samples, and net N mineralization and negatively to net nitrification, extractable NO_3^-, extractable NH_4^+, and sample time (of day). The results of principal component analysis suggest these sites had trends in response to variations in temperature, precipitation, and soil N transformations.

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I dedicate this thesis, with all my love, to my wife, Judith Ann, and my daughter, Nicole Brooke.

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I: INTRODUCTION

This thesis is from a group of studies funded by the United States Army Corps of Engineers to examine the ecology of the Green Bottom Wildlife Management Area (GBWMA) and focuses on the effects of wetland creation on soil nitrogen (N) processes. Wetland creation offers an opportunity to study the dynamics of N transformations in and around the new wetland. Reddy and Patrick (1993) contended that soil-related research in wetlands has significant gaps, and that there is a need for information to understand these systems. Reddy and Graetz (1988) specifically mentioned the limited amount of data available on N and also carbon (C) dynamics in natural and altered wetlands. Given that few data exist on sediment characteristics of human-made wetlands, D'Avanzo (1990) stated that analysis of mitigation wetland soils is a useful indicator of project success. A previous study at GBWMA concluded that although the reconfiguration to wetland soil nutrient parameters was progressing, the 8 mo period of the study was insufficient for a complete change to occur (Gilliam 1995). Research at other sites has suggested that, whereas, macroorganic matter (living and dead root material >2 mm diameter) N pools may develop in 15-30 yr in created wetlands, soil N pools may take considerably longer (Craft et al. 1988 a,b). Other research has suggested 2-5 yr for total N and ammonium (NH_4^+) levels to match natural systems, but studies have not been conducted long enough to make reliable predictions (D'Avanzo 1990).

GBWMA mitigation plan

GBWMA was purchased by the federal government through the United States Army Corps of Engineers to alleviate the environmental and recreational impacts of the Gallipolis

Locks and Dam Replacement Project as the major portion of the mitigation settlement. There were also some mitigation activities at the project site.

At the time of purchase, the approximately 340 ha GBWMA included about 60 ha of native wetland known as Green Bottom Swamp (Fig. 1). The presence of this wetland was a major factor in the choice of the GBWMA for the majority of the mitigation for the Gallipolis Locks and Dam Replacement Project. The swamp is a native wetland which was drained for agricultural use at the end of the 19th century. Abandonment of agricultural practices in about 1950 provided the opportunity through which natural processes began to reestablish the swamp.

The wetland replacement for the destruction of the approximately 16 ha of bottomland hardwood wetlands at the Gallipolis Locks and Dam Replacement Project involved the creation of about a 40 ha wetland within the GBWMA, and a 4-10 ha wetland at the project site. In addition to the creation of new wetland areas, the mitigation plan involved adding a preservation weir to allow a maintainable water level in the Green Bottom swamp at 165.8 m above mean sea level (Fig. 2).

The Green Bottom Mitigation Area (GBMA) wetland was created by impounding an area using natural topography and hydrology enhanced with a series of dikes. Water levels were planned to be established and primarily maintained by pumping Ohio River water into the area. The projected water levels were to vary between 165.5 and 166.1 m above mean sea level. A water control structure feeding into an intermittent stream flowing into the river would also be used to control water levels. The pump station on the Ohio River was damaged by flooding before the GBMA could be inundated and is scheduled for replacement.

Figure 1. Green Bottom Wildlife Management Area, pre-mitigation 1990 (Evans and Allen 1995).

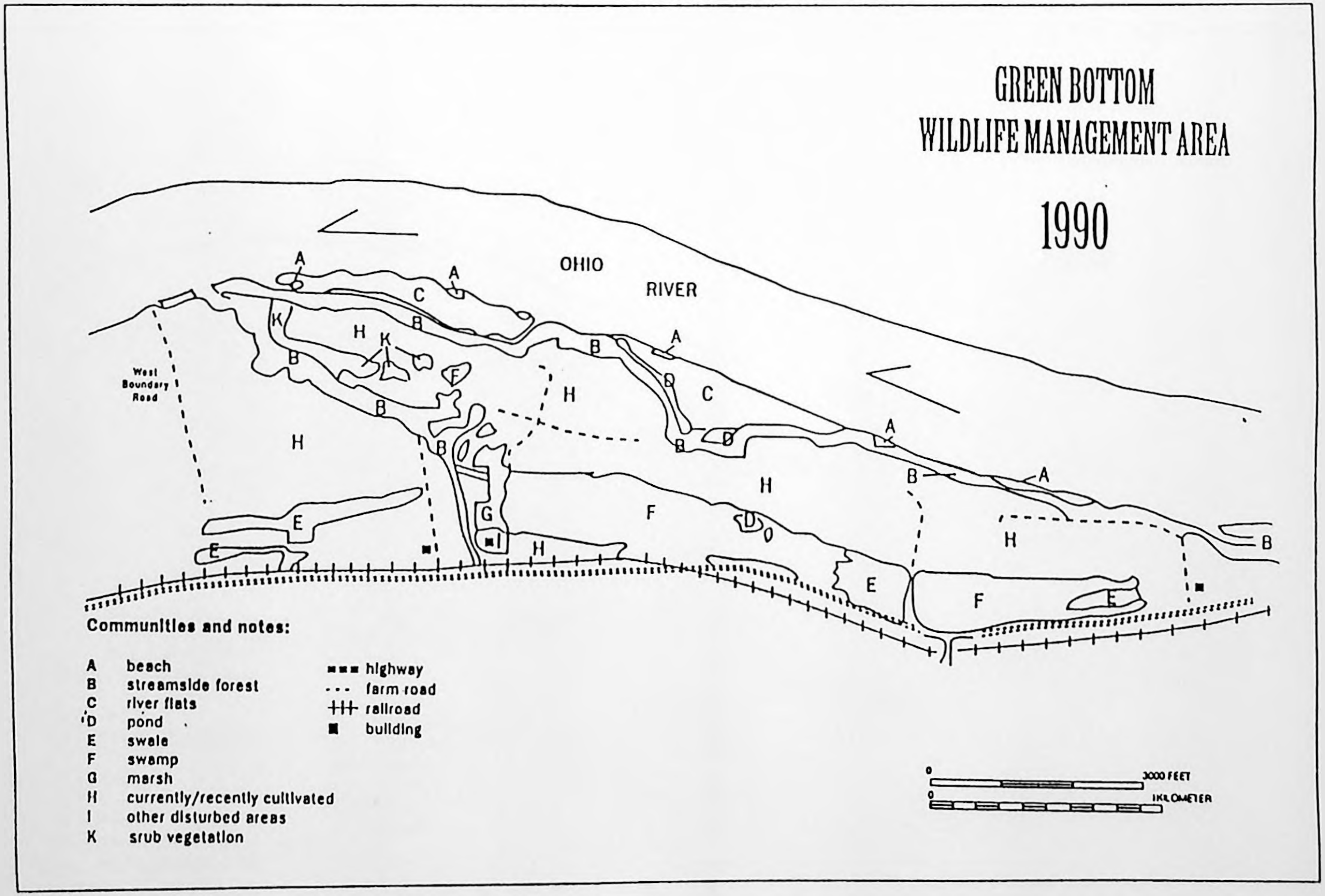
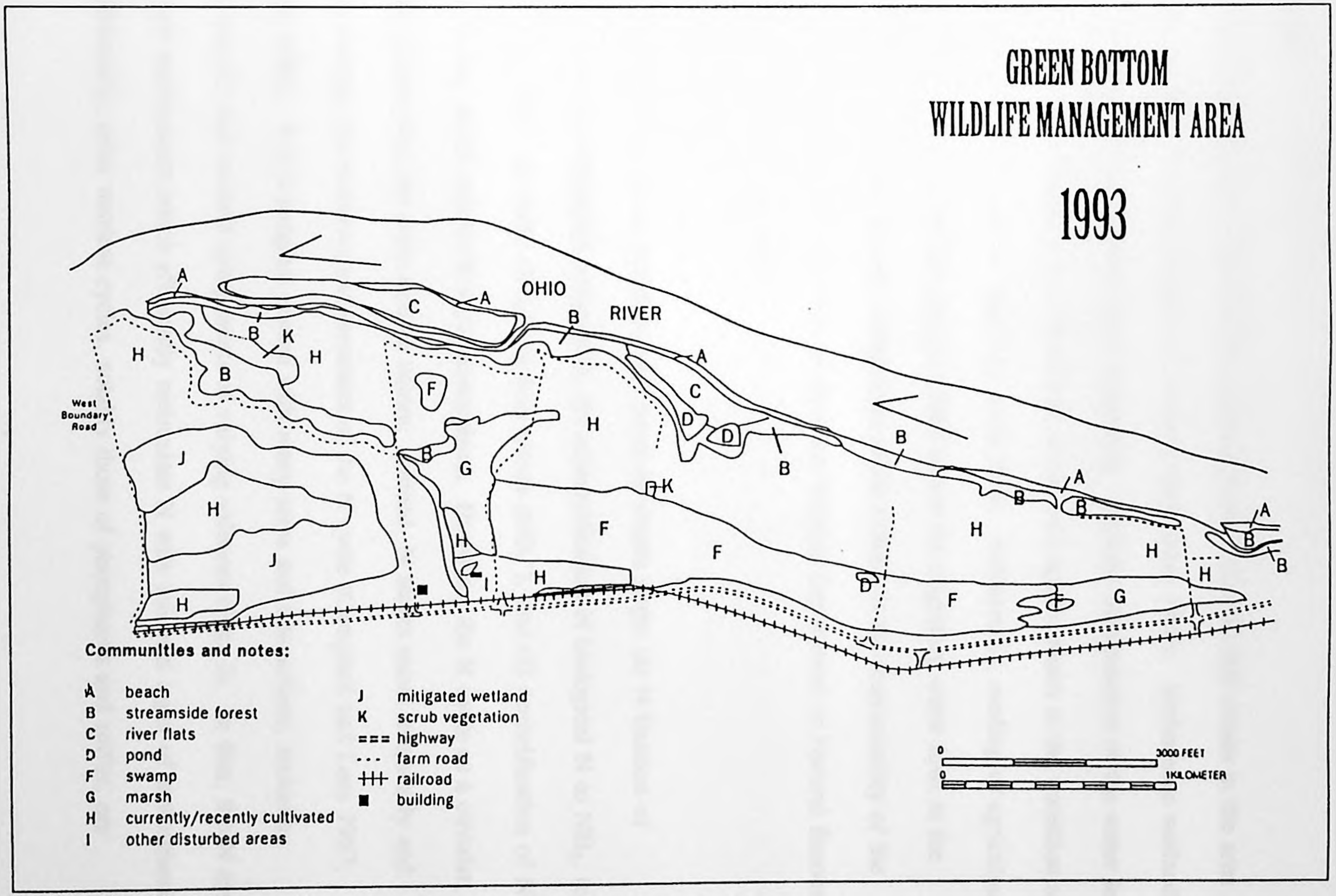

GREEN BOTTOM
WILDLIFE MANAGEMENT AREA
1990
OHIO
RIVER
West Boundary Road
Communities and notes:
A beach
B streamside forest
C river flats
D pond
E swale
F swamp
G marsh
H currently/recently cultivated
I other disturbed areas
K srub vegetation
highway
farm road
railroad
building
0
3000 FEET
0
1KILOMETER

Figure 2. Green Bottom Wildlife Management Area, post-inundation 1993 (Evans and Allen 1995).


GREEN BOTTOM
WILDLIFE MANAGEMENT AREA
1993
OHIO
RIVER
West Boundary Road
Communities and notes:
A beach
B streamside forest
C river flats
D pond
F swamp
G marsh
H currently/recently cultivated
I other disturbed areas
J mitigated wetland
K scrub vegetation
=== highway
--- farm road
railroad
building
0 3000 FEET
0 1KILOMETER

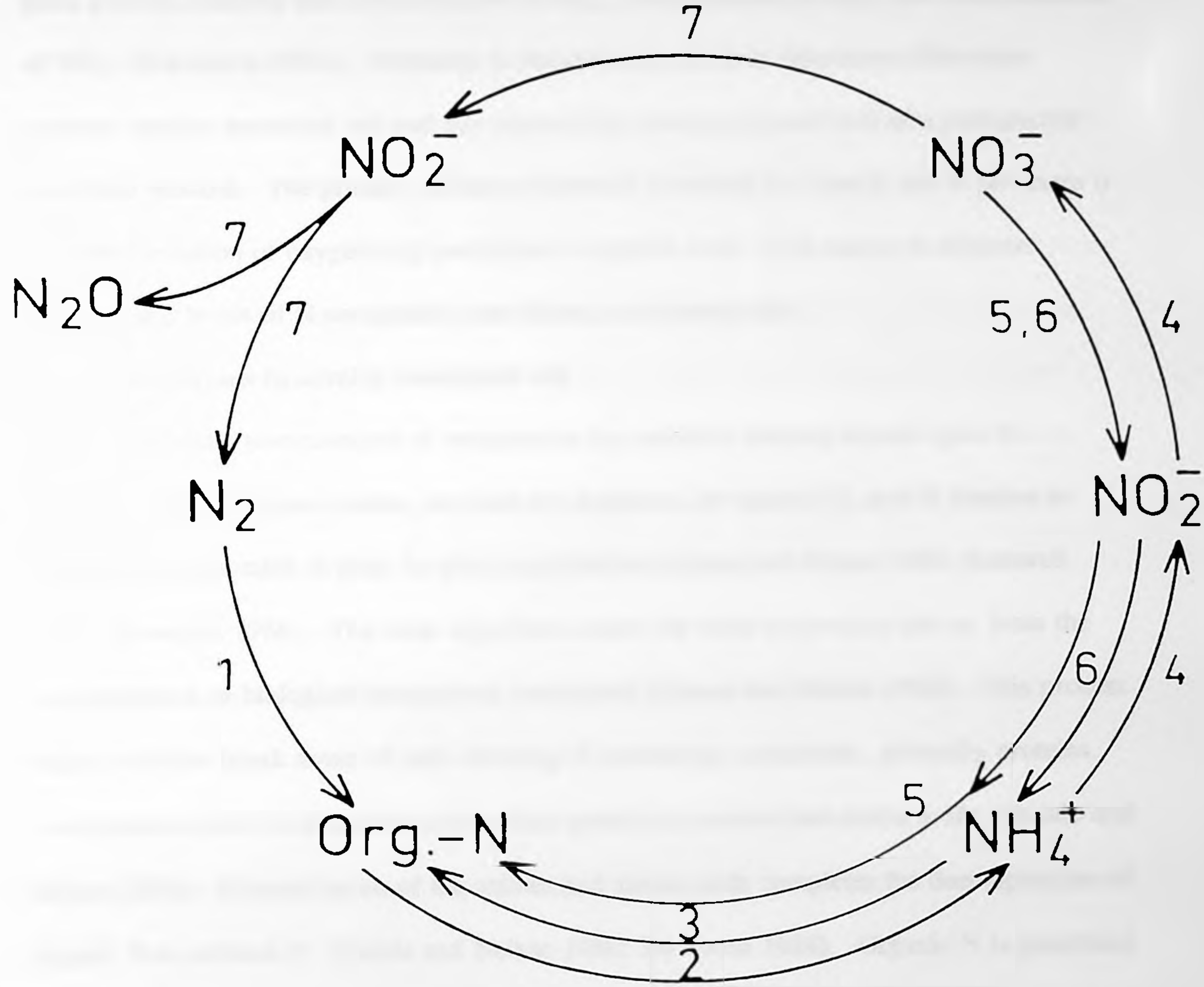
Until that time, the GBMA will rely on natural inputs of water to maintain the wetland's water levels.

The mitigation plan called for the planting of more than 50,000 shrubs in the area of the GBMA to begin the establishment of wetland plants (May 1995). Herbaceous wetland vegetation is expected to establish itself voluntarily. Periodic manipulation of the water level has been planned to enhance the establishment of wet-soil aquatic plants in the transition area and to provide feeding areas for migratory shore birds. Additionally, seeding of agricultural crops may be used in the GBMA area as a food source for migratory water fowl in the spring and fall. This high energy management of the GBMA is the responsibility of the GBWMA manager under the direction of the West Virginia Department of Natural Resources (Wildlife Resource Section 1991).

The Nitrogen Cycle

The N cycle could be described as a series of simple steps: (a) N fixation of atmospheric N_2 into biological compounds, (b) ammonification of biological N to NH_3, (c) nitrification of NH_4^+ to nitrite (NO_2) and then nitrate (NO_3^-), and (d) denitrification of NO_3^- into N_2O or N_2, which returns N to the atmosphere. However, the N cycle as a circular, sequential system does not truly exist in nature. Instead, N atoms move irregularly and randomly through the reactions and processes of the N cycle (Campbell and Lees 1967; Stevenson 1986). It is a complex system with many steps and interactions, including gaseous, organic and mineral components of varying valences (Fig. 3). In fact, the N cycle has internal mechanisms which efficiently recirculate N with minimal input of atmospheric N_2. Additionally, other nutrient cycles, notably those of phosphorus and sulfur, are

Figure 3. Schematic view of the biogeochemical nitrogen cycle (Rosswall 1982). 1. Nitrogen fixation, 2. ammonification, 3. assimilation, 4. nitrification, 5. nitrate assimilation, 6. dissimilatory nitrate reduction, 7. denitrification.



7
NO_2^-
NO_3^-
7
N_2O
7
5,6
4
N_2
NO_2^-
1
6
4
5
Org.-N
NH_4^+
3
2

integrally associated with biogeochemical transformations of N (Stevenson 1986).

The most important internal N cycle is between plants and soil. The levels of various forms of N in soil are dictated by many factors, including temperature, moisture, C/N ratio, plant growth, leaching and denitrification of NO_3^-, volatilization of NH_3, and immobilization of NH_4^+ (Stevenson 1986). Variations in these factors result in distinctive differences between aerobic terrestrial soil and the substantially anaerobic hydric soil of a permanently inundated wetland. The primary difference between terrestrial and hydric soil N processes is a severe limitation of oxygen (O_2) availability in hydric soils. This results in different balances and levels of N compounds than found in terrestrial soils.

N transformations in aerobic terrestrial soil

Terrestrial environments of moderate to low moisture content depend upon N mineralization of organic matter, wet and dry deposition of mineral N, and N fixation to provide the extractable N pool for plant assimilation (Franco and Munns 1982; Rosswall 1982; Stevenson 1986). The most significant source for these N pools in soil is from the decomposition of biological nitrogenous compounds (Franco and Munns 1982). This process begins with the break down of cells releasing N containing compounds, primarily proteins. Aminization hydrolytically decomposes these proteins to amines and amino acids (Tisdale and Nelson 1968). Mineralization of the amines and amino acids completes the decomposition of organic N to mineral N (Tisdale and Nelson 1968; Stevenson 1986). Organic N is processed to mineral form by ammonification, which is sometimes followed by nitrification (Franco and Munns 1982; Rosswall 1982).

Ammonification is the reduction of organic N compounds to ammonia (NH_3) (Reddy

1982). Several species of heterotrophic bacteria are capable of metabolic ammonification. They satisfy their energy needs by reducing amino acids, amines, and aminosaccharides, releasing NH_3 as a by-product. Most soils protonate the NH_3 from ammonification to NH_4^+. Direct assimilation by plants, immobilization by certain expanding clay minerals and utilization by soil heterotrophs to further decompose C residues are some pathways of ammonium, but in aerobic terrestrial soils, NH_4^+ may be nitrified (Tisdale and Nelson 1968; Rosswall 1982; Stevenson 1986; Kralova, et al. 1992).

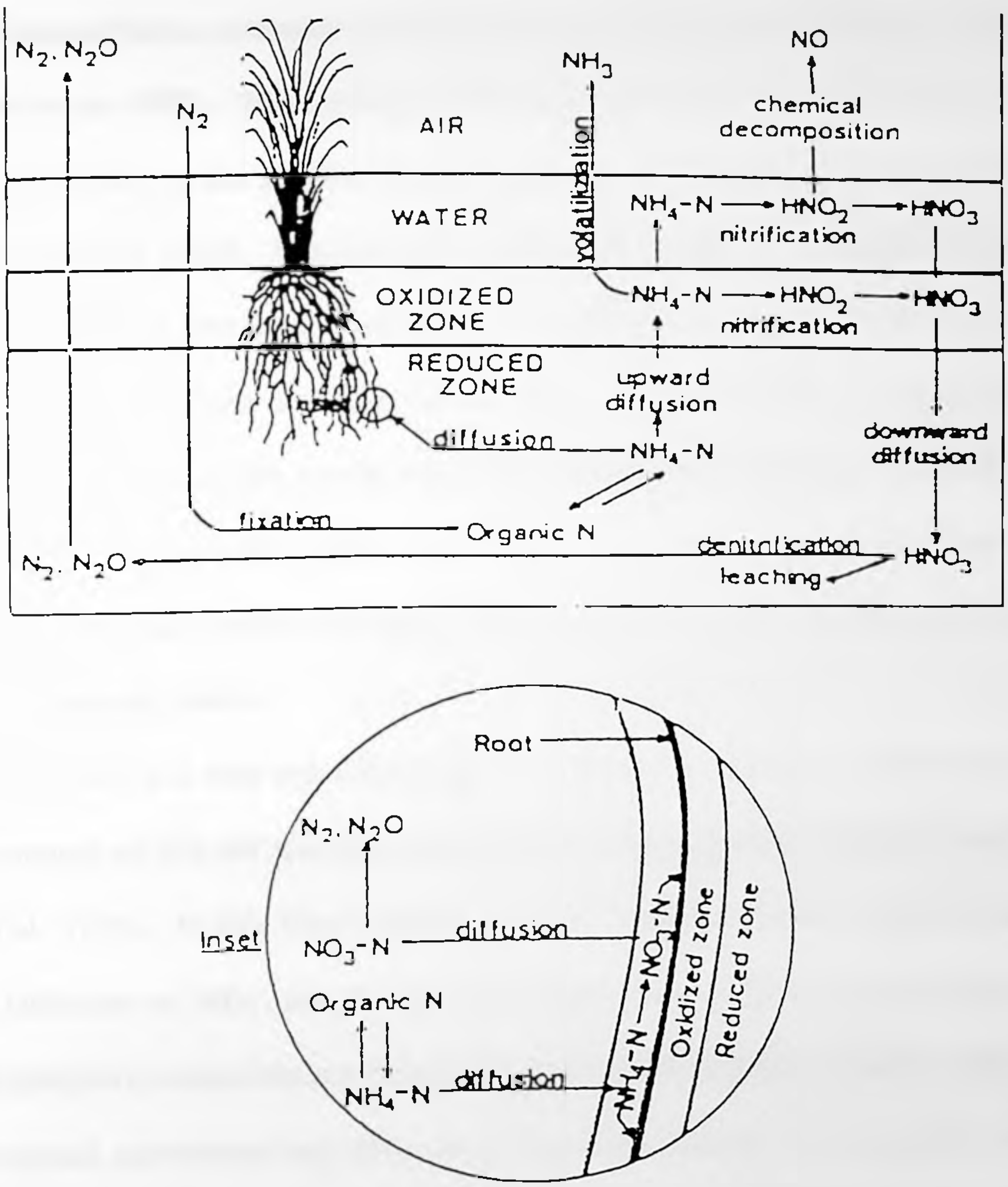
Nitrification is the biological oxidation of NH_4^+ to NO_2 and then to NO_3^-. These are aerobic reactions and require free O_2. Although several organisms are nitrifiers, including species of actinomycetes, fungi, and algae, bacteria appear to be the largest contributors to nitrification. Most nitrifying bacteria are members of the family Nitrobacteraceae. Two species from this family are considered the sources of most nitrification: *Nitrosomonas europaea*, which oxidizes NH_4^+ to NO_2^-, and *Nitrobacter winogradskyi*, which oxidizes NO_2^- to NO_3^- (Stevenson 1986). *Nitrobacter* oxidizes NO_2^- more quickly than *Nitrosomonas* can oxidize NH_4^+; consequently, NO_2^- is usually not found above trace amounts in soil (Franco and Munns 1982).

In undisturbed terrestrial systems that are not N saturated, assimilation and immobilization of N are the most significant processes for the removal of extractable mineral N from its pool in soil. Plants readily absorb N in the form of NO_3^- or NH_4^+ (Dørge 1994; Franco and Munns 1982; Rosswall 1982). High C/N ratios result in microbial immobilization incorporating mineral N to decompose organic material. Litter fall, excretion of nitrogenous wastes, and organismal death return biological N to the soil for decomposition. Clay and organic colloids in soil may immobilize NH_4^+ through ionic attraction. Ammonium captured in this manner may be released by cation exchange with Ca^{++}, Mg^{++}, Na^+ or H^+. These processes of N mineralization and assimilation/immobilization provide a continuous turnover of N in aerobic terrestrial soil.

N transformations in inundated soils

Under inundated conditions there is a rapid depletion of O_2 in soils. Diffusion is the only transport method of O_2 into inundated soils; whereas, most terrestrial soils can utilize partial pressure gradients and mass transfers (Stepniewski and Glinski 1988). In conjunction with lower O_2 availability is a substantial increase in biochemical O_2 demand (Patrick, et al. 1976; Stepniewski and Glinski 1988). The imbalance between O_2 supply and demand can result in a near total loss of available O_2 within hours of inundation, producing the reducing conditions typical of these soils (Patrick, et al. 1976; Reddy and Patrick 1984; Stepniewski and Glinski 1988). Reduced gas exchange capabilities of an inundated soil affect the physical, chemical, and biological properties of the soil and alter the dynamic interactions of mineralization and assimilation/immobilization. Inundated systems tend to be more susceptible to N losses than terrestrial systems. The other parts of the N cycle including denitrification, NH_3 volatilization, leaching, N fixation, and diffusion may influence the flow and balance of N in the soil. Additionally, as inundated soils mature, they frequently become bilayered in O_2 availability, with a thin aerobic layer over an anaerobic layer (Fig. 4) (Reddy 1982). An aerobic soil layer occurs when O_2 from the overlying water diffuses into the soil following the concentration gradient. The thickness of this layer has an inverse relationship with the rate of biochemical activity, varying from 0 - 2 cm (Patrick et al.

Figure 4. Nitrogen cycle in a flooded soil ecosystem (Reddy 1982).


N2, N2O
N2
AIR
NH3
volatilization
NO
chemical
decomposition
WATER
NH4-N
HNO2
HNO3
nitrification
OXIDIZED
ZONE
NH4-N
HNO2
HNO3
nitrification
REDUCED
ZONE
upward
diffusion
diffusion
NH4-N
downward
diffusion
fixation
Organic N
denitrification
HNO3
N2, N2O
leaching
Root
N2, N2O
Inset
NO3-N
diffusion
Organic N
NH4-N
diffusion
NH4-N
NO3-N
Oxidized zone
Reduced zone

1976). In submerged soils with an aerobic layer of soil overlying an anaerobic layer of soil certain N transformations may be segregated into a specific layer, but gradient diffusion into an adjacent layer or the water can result in further N transformations (Fig. 4).

Ammonification in inundated soils is mostly confined to the anaerobic layer. Anaerobic ammonification proceeds at a slower rate than aerobic ammonification (Reddy 1982; Moore et al. 1992). Without free O_2, NH_4^+ produced cannot be nitrified and may accumulate, especially in the absence of plant uptake (Bowden 1984; Moore et al. 1992; Mitsch and Gosselink 1993). Microbial assimilation and mineral immobilization become the major sources of NH_4^+ loss in the anaerobic soil. Additionally, if the concentration gradient causes diffusion of NH_4^+ into the water column with > pH 8, the NH_4^+ can disassociate, losing a proton, becoming NH_3 and be lost through volatilization (Reddy et al. 1976; Reddy 1982; Reddy and Patrick 1984; Reddy et al. 1986). If an aerobic soil layer is present, NH_4^+ can diffuse into this layer and be nitrified. Aerobic nitrifying zones can also exist in the rhizosphere of aquatic plants.

Nitrification will only occur if enough O_2 is present (> 0.3 ppm, Moore et al. 1992). A redox potential of 200 mV has been used as the minimum level for microbial nitrification (Patrick et al. 1976). Within these aerobic zones NH_4^+ diffusing from anaerobic soil can be nitrified. Diffusion of NO_3^- into the soil from nitrification within the water column, run-off, and atmospheric deposition can be additional sources of soil NO_3^- (Reddy 1982).

Biological assimilation and diffusion into the water column can cause NO_3^- loss, but denitrification of NO_3^- diffusing into the anaerobic soil has been claimed to be the major source of NO_3^- loss in inundated soils. In natural systems, denitrification rates are much higher in areas with aquatic plant populations. The aerobic zones associated with their rhizospheres can produce more NO_3^- than areas lacking plants. The quantity and quality of labile C, the C/N ratio, temperature, pH, and the size of the microbial population capable of denitrfication also influence denitrification rates (Reddy 1982). A final source of NO_3^- loss is through dissimilatory reduction to NH_4^+. Certain members of the bacterial genus *Clostridium* have been found capable of this NO_3^- transformation (Buresh and Patrick 1981). Anaerobic and highly reducing conditions (< -200 mV, Buresh and Patrick 1981) are necessary for dissimilation, and the NO_3^- must diffuse into these areas before it is denitrified. Nitrogen fixation in inundated systems can be an important N source, if the epiphytic, substrate, or planktonic N fixing populations are large enough and conditions permit diffusion of NH_4^+ produced into the substrate (Reddy 1982).

Seasonal Fluxes of Extractable N

Seasonal variation of extractable soil N is dependent upon climatic influences, abiotic factors within the soil, and most importantly, biological assimilation (Stevenson 1986; Bowden 1987). Plant uptake is a major sink for N and may be the most important factor in causing seasonal fluxes in N pools (Nadelhoffer et al. 1984; Bowden 1987; Caffery and Kemp 1990; Levine and Willard 1990; Langis et al. 1991). Nitrogen uptake is highest in immature plants and during leaf emergence (Sasser et al. 1991). Assimilation continues until plant senescence at the end of the growing season. As the growing season ends and plant uptake decreases, the pool of extractable N will begin to rise again (Caffery and Kemp 1990).

Objectives

This thesis was designed to investigate soil N dynamics in the pools, nitrification and N mineralization of extractable nitrogen from three habitat types: (1) an old field (OF), an area of former cultivation experiencing secondary succession and permanently above the water level, (2) a transition (TR), an area at the perimeter of the mitigation area, which was expected to experience seasonal inundation and exposure, and (3) a mitigation wetland (MW), an area of permanent inundation. The objectives of this study were:

(1) to observe and quantify a possible shift in extractable N from a balance of NO_3^- and NH_4^+ to an NH_4^+dominated system in the inundated mitigation soils,

(2) to monitor the transition zone for plasticity in N processes as draw-down occurs, and observe the ability of the soils to revert from inundated to terrestrial N cycle patterns, and

(3) to observe the seasonal fluxes in N levels at the 3 site types and establish trends for their patterns.

II: MATERIALS and METHODS

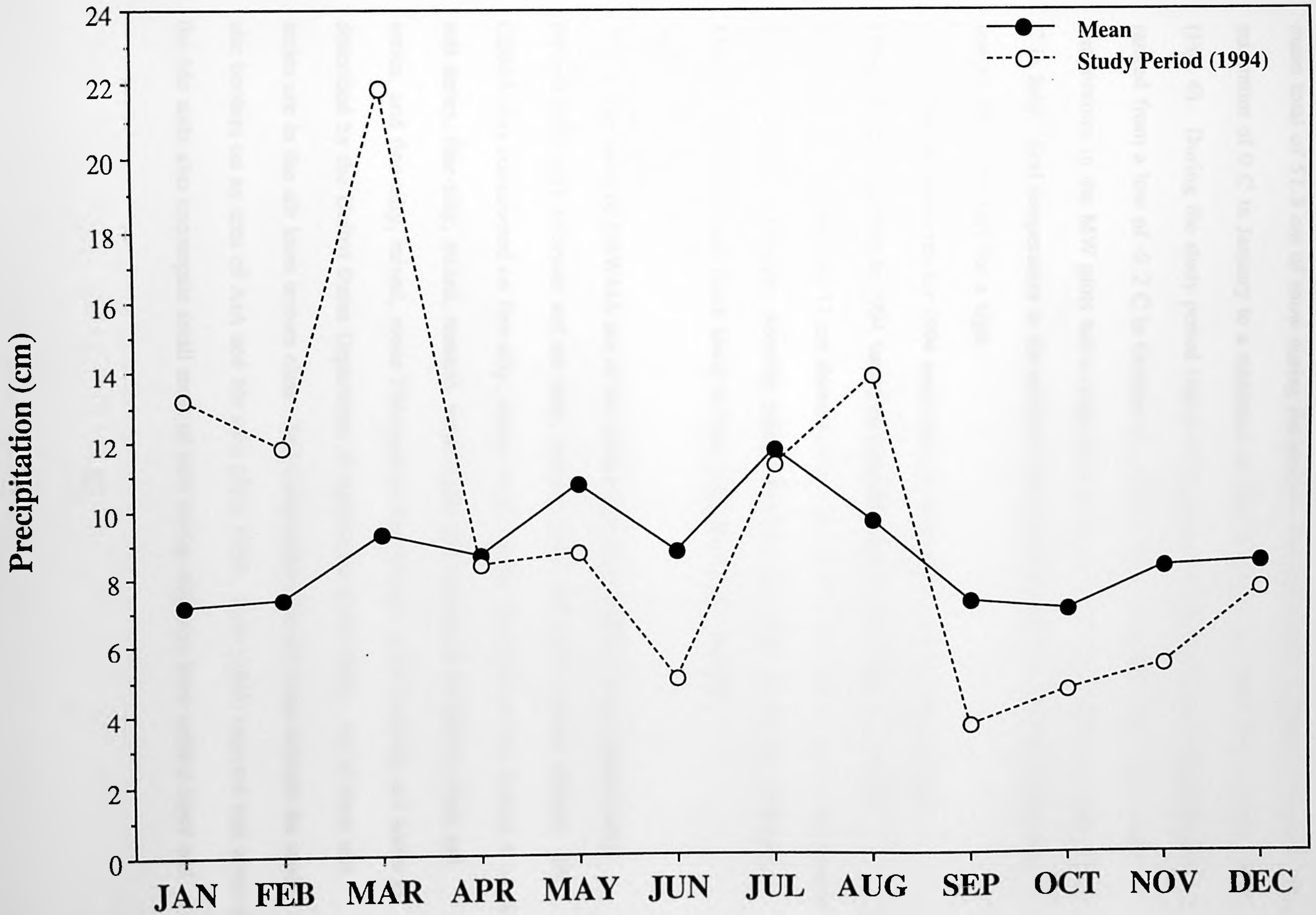
Study Site

The Green Bottom Wildlife Management Area, Cabell County, West Virginia is a 338 ha area of riparian floodplain supporting terrestrial and wetland habitats along the Ohio River (35°00'N, 82°14'W). The most predominant habitat feature of GBWMA is the Green Bottom swamp, consisting of approximately 60 ha of native freshwater wetland which experienced several changes related to past land-use practices, but is now primarily a buttonbush swamp. Adjacent to the Green Bottom swamp are fields, some cultivated, some maintained in early stages of old field succession. The study area involves a 40 ha mitigation wetland created in the GBWMA by the United States Army Corps of Engineers in 1992, as part of the mitigation for approximately 16 ha of bottomland hardwood wetland destroyed and other environmental and recreational damage caused during the construction of the Robert C. Byrd Locks and Dam project on the Ohio River near Gallipolis, Ohio. This area was created by building a series of dikes from native soil around a natural depression, west of the original swamp. It was intended to be flooded with Ohio River water via a pump station, but natural events of snow melt, spring rain, and run-off inundated the area before this occurred.

Climate

GBWMA has a temperate continental climate. Mean annual precipitation is 105.4 cm, somewhat evenly distributed throughout the year, with the maximum monthly mean of 11.8 cm in July and a minimum monthly mean of 7.2 cm in January and October (Cole 1989; NOAA 1993, 1994) (Fig. 5). The heaviest snows are from January to March with a

Figure 5. Annual precipitation estimate for study area. Data from National Weather Service, Huntington, West Virginia, Tri-State Airport office.


Mean
Study Period (1994)
Precipitation (cm)
0
2
4
6
8
10
12
14
16
18
20
22
24
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

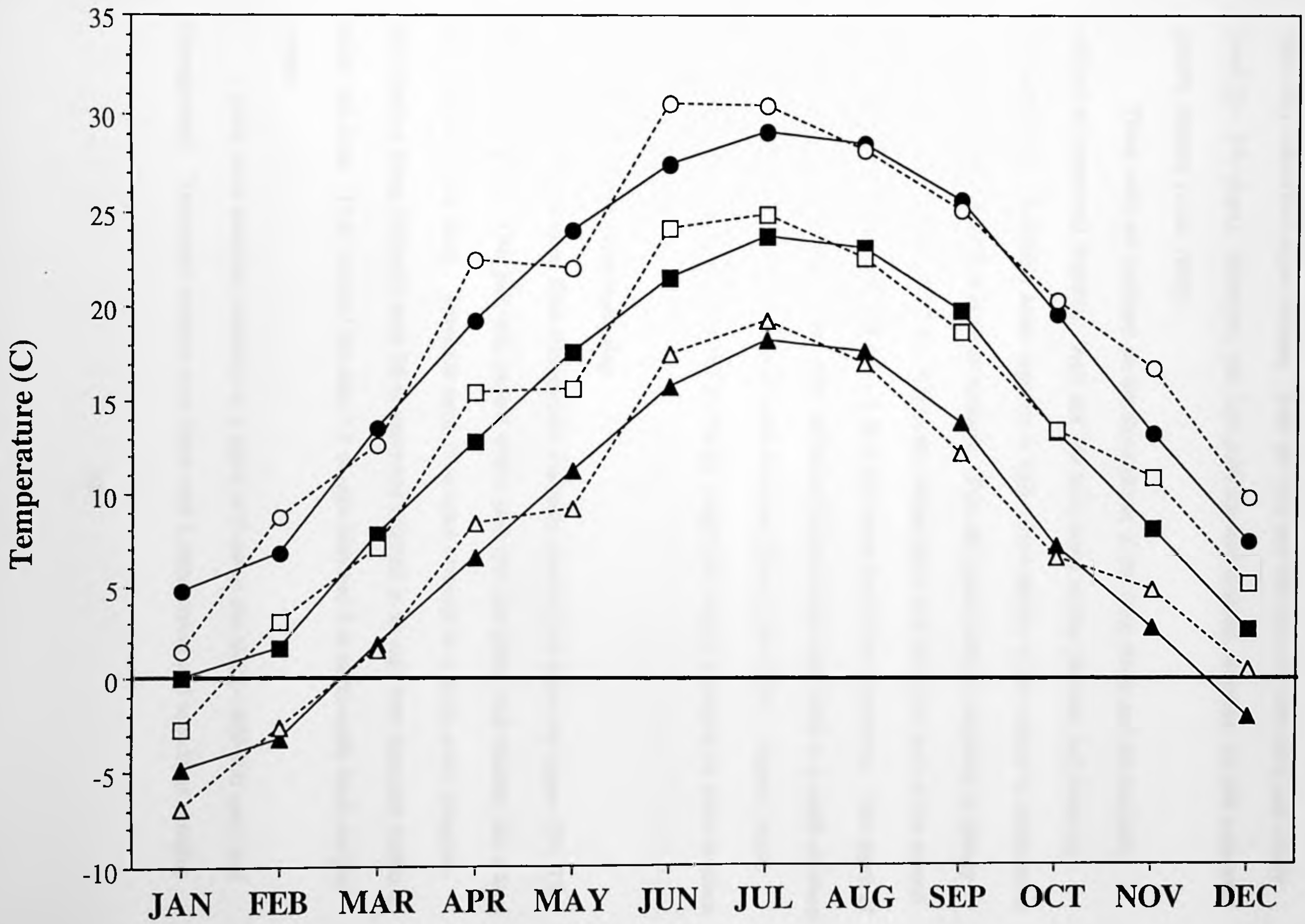
mean total of 51.8 cm of snow during the period. The mean daily temperatures range from a minimum of 0 C in January to a maximum of 24 C in July (Cole 1989; NOAA 1993, 1994) (Fig. 6). During the study period (April to November 1994) temperature measurements ranged from a low of -2.2 C in October to a high of 36.7 C in June. The surface water temperature in the MW plots had a range from a low 14.2 C in November to a high of 33.1 C in July. Soil temperature in the terrestrial plots varied from 13.0 C in November for a low to 30.1 C in July for a high.

Air temperatures for 1994 were close to mean annual temperatures (Fig 6). However, precipitation in 1994 varied considerably from means (Fig 5). Annual precipitation for 1994 was 11 cm above average, but for the study period, total accumulation was 8.4 cm below average. Monthly precipitation was very high for January, February, March, and August and much lower in June, September, and October.

Soils

The soils of GBWMA are of the Ashton-Huntington-Melvin unit predominantly formed from silty alluvium and are deep, typically level, and well to poorly drained. The GBMA was constructed on fine-silty, mixed, mesic Mollic Hapludalfs of the Ashton A (AsA) soil series, fine-silty, mixed, nonacid, mesic Typic Fluvaquents of the Melvin (Me) soil series, and fine-silty, mixed, mesic Fluvaquentic Eutrochrepts of the Lindside soil series as described by the United States Department of Agriculture (Cole 1989). All of these soil series are in the silt loam texture class. Soil Conservation Service maps indicate the study site borders on an area of AsA and Me soils (Cole 1989). Cole (1989) reported that some of the Me soils also encompass small areas of soils having silty clay loam surface layer and

Figure 6. Annual temperature estimate for study area. Data from National Weather Service, Huntington, West Virginia, Tri-State Airport office. Solid symbols denote annual means, open symbols denote 1994 data. Δ - mean maximum, □ - mean, ○ - mean minimum.


Temperature (C)
35
30
25
20
15
10
5
0
-5
-10
JAN
FEB
MAR
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV
DEC

silty clay subsurface layer textures. Both the AsA and Me soils are very deep and nearly level (0 - 3% slope). However, the AsA soils are well drained, whereas, the Me soils are poorly drained (Cole 1989).

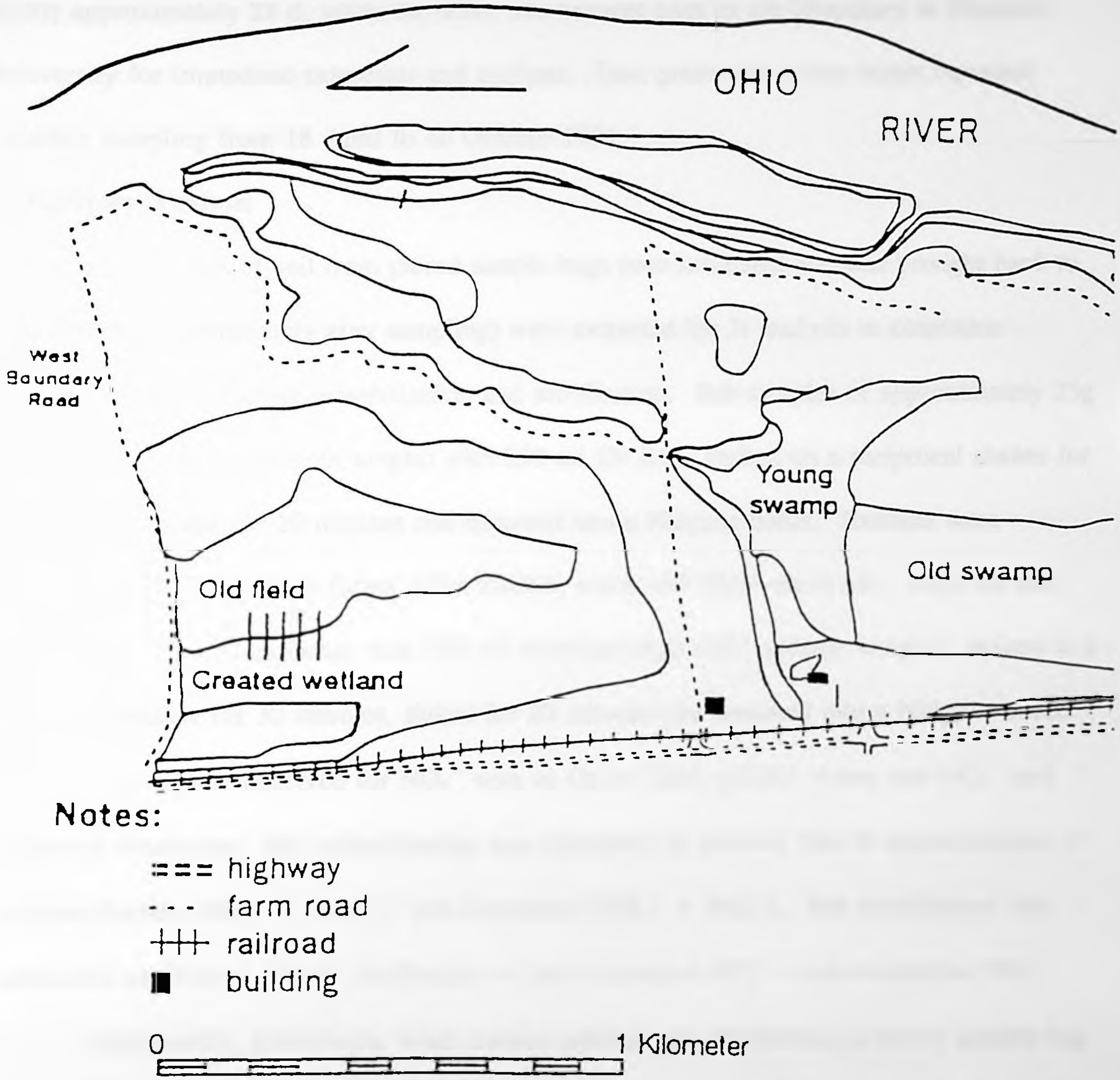
These soils are common on the flood plains of the Ohio River and are naturally subject to occasional flooding. AsA and Me have many similar physical and chemical characteristics. Available water capacity is high. Permeability of the subsoil is moderate to moderate slow. Runoff is slow or medium. This soil under natural conditions is usually medium acid to neutral (pH 5.6 - 7.3) in the surface layer and the upper part of the subsoil and slightly acid to neutral (pH 6.1 - 7.3) in the lower part of the substratum. The depth of the bedrock is more than 1.5 m. The soil has a seasonal high water table at a depth of about 0.5 m to 1.0 m that restricts the root zone for some plants (Cole 1989). Organic matter (OM) of these soils varies from 0.5 to 4% by weight but may be increased by plant residues (Cole 1989).

Plot Location and Field Sampling

Soil was sampled from five parallel transects spanning the three site types: OF, TR, and MW (Fig. 7). One plot was located within each site type along each transect for a total of 15 plots for the study. Transects were 15 m apart oriented in a north-south direction. Plot centers along transects were 16 m apart and oriented in an east-west direction within a given site type. Plots covered the area 7.5 m east-west and 8 m north-south from the plot center.

Soils were sampled randomly to a depth of 5 cm in the plot (~600-800 cm^3) and homogenized. Terrestrial samples were taken with a hand shovel and inundated samples

Figure 7. Transect location for sample plots (verticle lines between Old field and Created wetland ledgends) within the Green Bottom Mitigation Area of the Green Bottom Wildlife Management Area (Evans and Hollis 1995).


OHIO
RIVER
West Boundary Road
Young swamp
Old swamp
Old field
Created wetland
Notes:
highway
farm road
railroad
building
0
1 Kilometer

were taken with a 5 cm sediment corer. Mineral soil from each plot was divided into two sub-samples and each sub-sample was placed in a polyethylene bag. One sub-sample was incubated *in situ* being buried 5 cm beneath the soil surface (the "buried bag" technique, Eno 1960) approximately 28 d, while the other was brought back to the laboratory at Marshall University for immediate extraction and analysis. Data presented in this report represent monthly sampling from 18 April to 10 October 1994.

Laboratory Analysis

Sub-samples of soil from paired sample bags (one incubated and one brought back to the laboratory immediately after sampling) were extracted for N analysis to determine available N pools and net mineralization and nitrification. Sub-samples of approximately 25g were extracted (10:1 volume:weight) with 250 ml 1N KCl, shaken on a reciprocal shaker for 30 minutes, slaked for 20 minutes and decanted into a Nalgene bottle. Extracts were analyzed for NH_4^+ with an Orion 720A pH/ISA meter and NH_4^+ electrode. Separate sub-samples (25 g) were extracted with 250 ml deionized H_2O (10:1 volume:weight), shaken in a reciprocal shaker for 30 minutes, slaked for 20 minutes and decanted into a Nalgene bottle. These extracts were analyzed for NO_3^- with an Orion 720A pH/ISA meter and NO_3^- and reference electrodes. Net mineralization was calculated as follows: Net N mineralization = post-incubation (NH_4^+ + NO_3^-) - pre-incubation (NH_4^+ + NO_3^-). Net nitrification was calculated as follows: Net N nitrification = post-incubation NO_3^- - pre-incubation NO_3^-.

Additionally, gravimetric water content analysis was performed on every sample bag by placing 20 g subsamples in 50 ml beakers for drying at 105 C for 24 hr. Percent gravimetric water content was calculated as follows: Percent gravimetric water content =

(moist soil - dried soil)/moist soil * 100.

Other sub-samples from the initial sample (18 April 1994) were used for texture analysis. Soil was dried, ground, sieved to pass through a 2 mm screen, and analyzed using the hydrometer method (Bouyoucos 1951). Soil organic matter was measured as percent loss-on-ignition by placing oven-dried sub-samples in a muffle furnace at 550 C for 5 hr (Gilliam and Turrill 1993).

Data Analysis

Means of all measured variables were compared among the three site types (OF, TR, MW) using analysis of variance and Duncan's multiple range testing (Proc ANOVA; SAS 1992). Multivariate comparisons of extractable NO_3^- and extractable NH_4^+ were made among sites on a monthly basis (Zar 1984). Principal component analysis was performed on 15 to 17 variables for each site type. Unless otherwise stated, significant differences were determined at the $P<0.05$ level.

III: PHYSICAL DATA, RESULTS and DISCUSSION

Old Field

Texture analysis of the OF soils resulted in a clay loam texture classification (27 - 40 % clay and 20 - 45 % sand, Soil Survey Staff 1976) (Table 1) having higher sand and slightly higher clay content than previously reported (Cole 1989; Gilliam 1995) for the alluvial silt loams and silty clay loams typical of this area. However, the possibility of tightly-bound clay aggregates behaving as sand particles during hydrometric texture analysis may have artificially inflated the percentage of sand actually present and result in datum suggesting a texture class atypical for the area. Soil pH (H_2O) (Table 1) was in the upper range (pH 5.3 - 6.1, mean 5.67) reported by Gilliam (1995), and well within the pH 5.6 - 7.3 range ascribed to the AsA and Me soils (Cole 1989). The OM (Table 1) was similar to those of the previous study (Gilliam 1995), but above the 0.5 - 4 % reported by Cole (1989) for the AsA and Me soils, although he suggested plant residue deposition could elevate these values. Soil moisture of the pre-incubation samples seemed to respond to the quantity of precipitation between sample periods, especially extreme changes (Fig. 8).

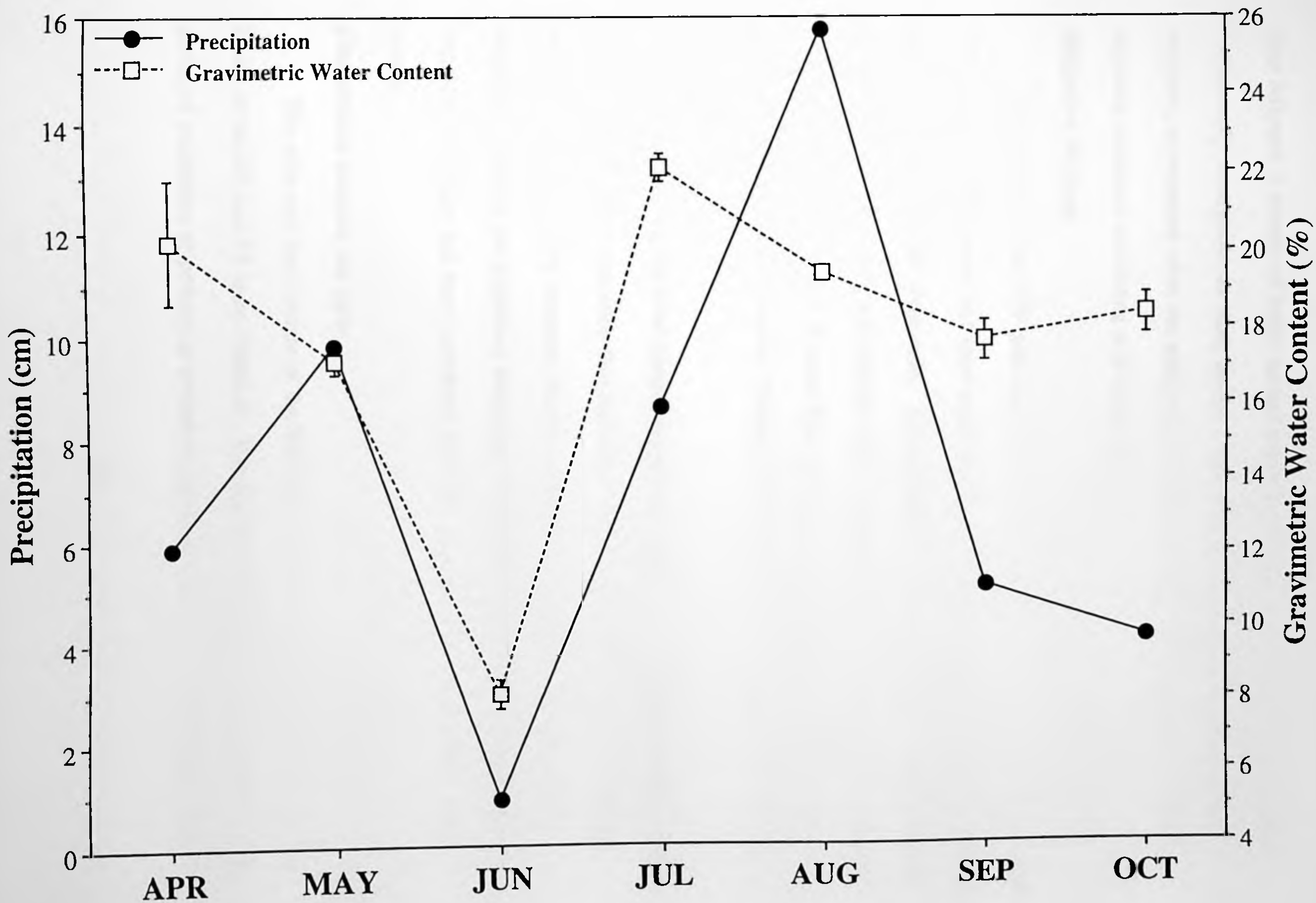
Transition

Transition soils were significantly similar to the OF soils in texture and OM (Table 1). The OM levels were somewhat lower than the 13.0 % reported by Gilliam (1995) under inundated conditions. This variation may be a result of differences in the sampling date (09/1993), site (300 m East), method (2 cm corer), and depth (0 - 10 cm) of the two studies. Soil pH (H_2O) was within the range determined by Cole (1989) for AsA and Me soil, but was again slightly higher than levels found by Gilliam (1995). Exposure of the TR soils in

Table 1. Texture (sand, silt, and clay), organic matter (OM), and pH (H_2O- and KCl-extractable) for soils from old-field (OF), transition (TR), and mitigation wetland (MW) sites. Values shown are means of five transect ± one standard error of the mean. Means followed by the same superscript are not significantly different between sites at $P<0.05$.

Site	Sand	Silt	Clay	OM	$pH_{(H_2O)}$	$pH_{(KCl)}$
	---- % ----					
OF	26.0 ± 1.3^a	44.5 ± 1.0^b	29.6 ± 1.3^b	10.0 ± 0.0^a	6.11 ± 0.05^b	4.67 ± 0.07^b
TR	25.8 ± 2.2^a	45.0 ± 0.8^b	29.7 ± 1.8^b	9.9 ± 0.1^a	6.33 ± 0.07^a	4.98 ± 0.06^a
MW	8.0 ± 2.5^b	52.3 ± 1.4^a	39.7 ± 1.2^a	10.4 ± 0.2^a	6.40 ± 0.06^a	4.75 ± 0.02^b

Figure 8. Total accumulation of precipitation between sample dates, and the soil percent gravimetric water content of the pre-incubation samples in the old field plots of the Green Bottom Wildlife Management Area. Gravimetric water content values shown are means of the five plots $\pm$ one standard error of the mean.


Precipitation
Gravimetric Water Content
Precipitation (cm)
Gravimetric Water Content (%)
APR
MAY
JUN
JUL
AUG
SEP
OCT
0
2
4
6
8
10
12
14
16
4
6
8
10
12
14
16
18
20
22
24
26

June followed 3 months of below normal precipitation (Fig. 5), and water levels dropped continuously throughout the study period. Soil moisture content declined sharply upon exposure, as expected when the soils were no longer saturated, and continued to decline as exposure continued correlating at $P<0.01$ (Fig. 9).

Mitigation Wetland

Soil texture in the MW sites was of the silty clay loam texture class (Soil Survey Staff 1976). Cole (1989) stated that some small areas with this texture are encompassed within the zones mapped by the Me series soils. The pH (H_2O) levels were normal for those described by Cole (1989), but higher than Gilliam (1995). Organic matter was above levels suggested by Cole (1989), but almost 3 % lower than the values found by Gilliam (1995) in the mitigation wetland soils. However, Gilliam (1995) suggested OM levels should decrease with time.

Water levels in the MW transect dropped throughout the study, approximately 60 cm, as a result of higher evaporation than input (Fig. 10). When pump repairs are complete, water fluctuations will be managed, thereby, reducing the influence of natural seasonal variations. Despite the significant water loss, the MW soils remained under conditions of complete inundation and were completely saturated, averaging 40.5% gravimetric water content.

Comparisons between site types

The silty clay loam texture of the MW soil was significantly different than the clay loams of the OF and TR areas (Table 1). It is not clear whether this is a result of > 1-yr period of inundation or perhaps an artifact of sampling conditions of waterlogged soils.

Figure 9. Percent gravimetric water content of soil sample from the transition site type of the Green Bottom Wildlife Management Area. Percent gravimetric water content values shown are means of the five plots ± one stardard error of the mean.

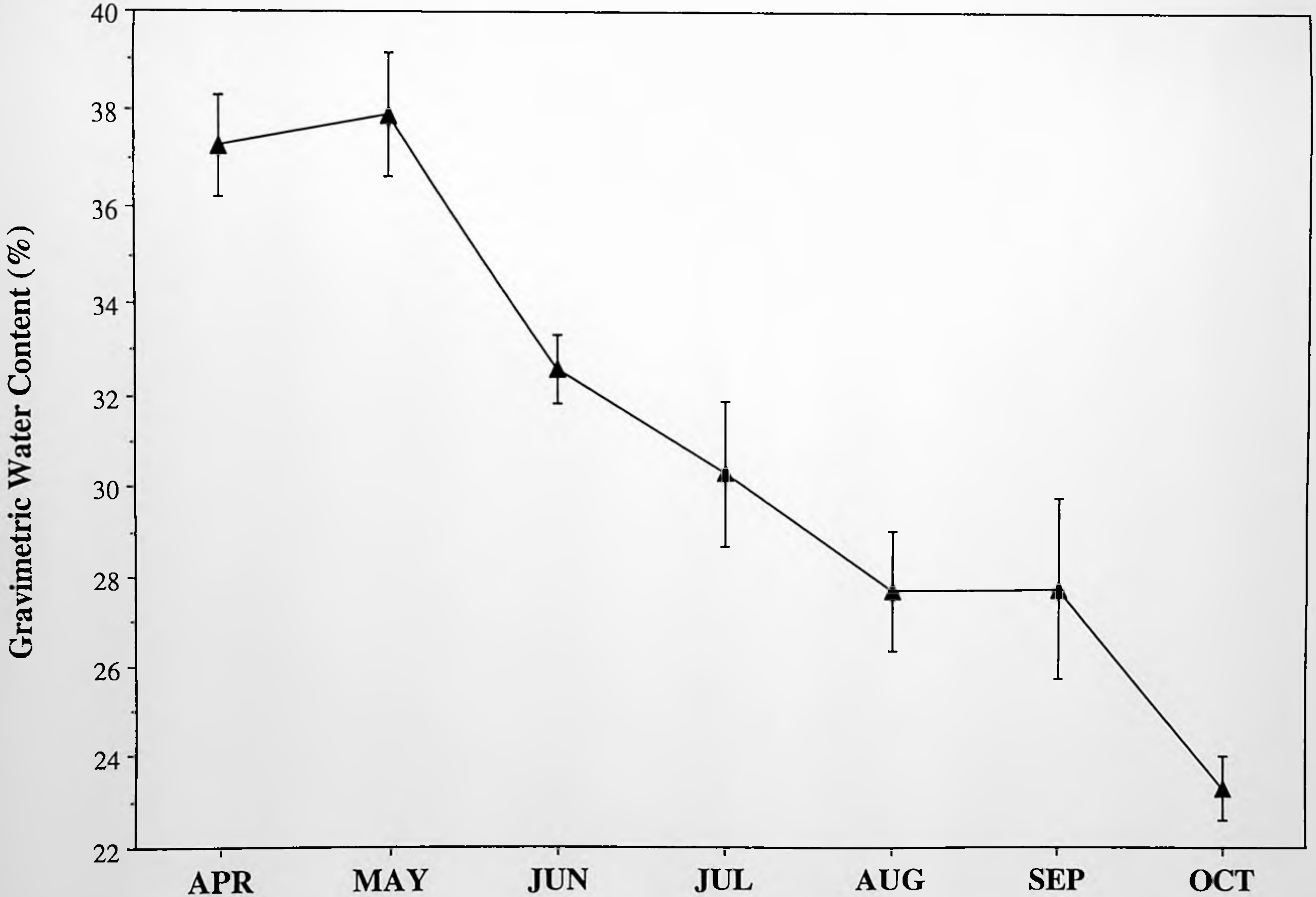
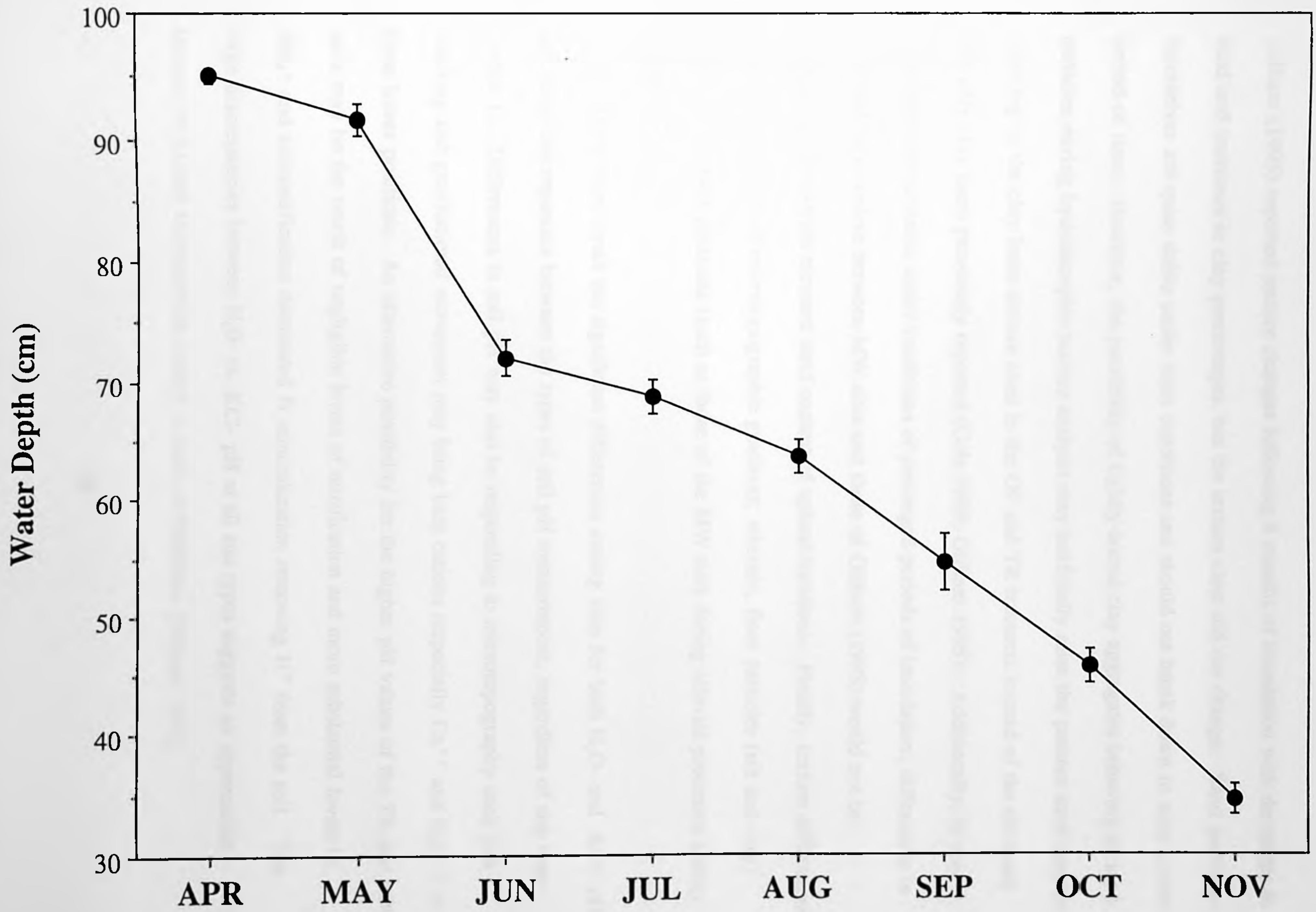

Gravimetric Water Content (%)
40
38
36
34
32
30
28
26
24
22
APR
MAY
JUN
JUL
AUG
SEP
OCT

Figure 10. Water depth at the site marker in the mitigation wetland plots of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ on standard error of the mean.


Water Depth (cm)
100
90
80
70
60
50
40
30
APR
MAY
JUN
JUL
AUG
SEP
OCT
NOV

Gilliam (1995) reported texture changes following 8 months of inundation with decreases in sand and increases in clay percentages, but the texture class did not change. Sand particles themselves are quite stable under most conditions and should not break down in such a short period of time. However, the possibility of tightly-bound clay aggregates behaving as sand particles during hydromorphic texture analysis may artificially raise the percent sand content resulting in the clay loam texture class in the OF and TR transects instead of the silt loam and silty clay loam previously reported (Cole 1989; Gilliam 1995). Additionally, if clay aggregates are unstable under conditions of prolonged periods of inundation, differences in sand and clay content between MW sites and those of Gilliam (1995) would not be unexpected nor would elevated sand content of upland locations. Finally, texture differences could be the result of microtopographic gradients; wherein, finer particles (silt and clay) settle out at lower positions (such as those of the MW site) during alluvial processes (Jenny 1980).

There were small but significant differences among sites for both H_2O- and KCl- pH and large discrepancies between the types of soil pH measurement, regardless of site type (Table 1). Differences in soil pH may also be responding to microtopography such that leaching and gravitational movement may bring base cations (especially Ca^{++} and Mg^{++}) to these lower positions. An alternative possibility for the higher pH values of the TR and MW soils may be the result of negligible levels of nitrification and more substantial levels of NH_4^+ and ammonification dominated N mineralization removing H^+ from the soil. The large discrepancies between H_20- vs. KCl- pH at all site types suggests an appreciable amount of Al and exchangeable acidity in soils of GBWMA (Gilliam 1991).

There were no significant differences in OM content between the three site types (Table 1), although all were above the 0.5 - 4 % range reported by Cole (1989). The lack of significant difference between the sites is inconsistent with the results of Gilliam (1995). However, because OM content was examined from only one set of samples at the beginning of this study, it is difficult to establish if a trend, seasonal or otherwise, exists in these soils.

Soil moisture content increased predictably from the terrestrial to the inundated site types, OF < TR < MW. What influenced the moisture content, however, appeared to be different in each site. The OF soils seemed to respond to variations in precipitation in conjunction with other environmental factors; whereas, the TR soils reacted to length of exposure, and the inundated MW soils were permanently water-logged.

IV: NITROGEN TRANSFORMATIONS, RESULTS and DISCUSSION

N pools

Old field

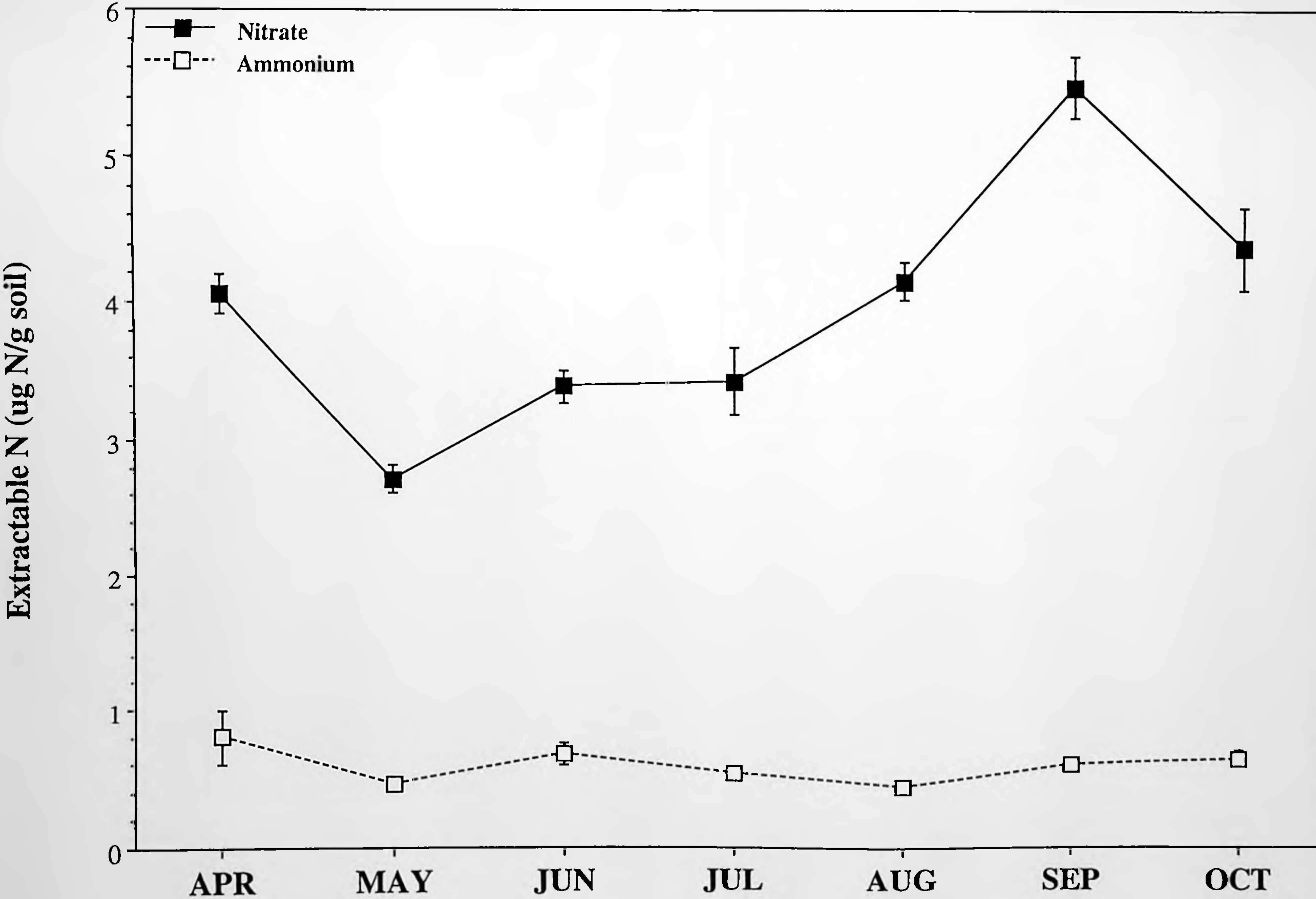
Extractable NO_3^- pools were relatively low during the study period in the old field site, ranging from a low of 2.7 μg N/kg soil in May and a high of 5.5 μg N/kg soil in September (Fig. 11). Slight variations in the NO_3^- pools during the study period suggested a seasonal variation influenced by plant growth (Fig. 11). High levels of N uptake during the period of early plant growth can reduce the N pool. As the plants mature, N uptake decreases, and N pool levels may increase (Stevenson 1986). Additionally, N pools frequently increase at the end of the growing season as plant metabolism decreases (Caffery and Kemp 1990).

Available pools of NH_4^+ in the OF were very small (Fig. 11), typical of aerobic terrestrial systems where NH_4^+ is rapidly nitrified (Franco and Munns 1982; Stevenson 1986; Kralova et al. 1992). This microbial assimilation of NH_4^+, combined with plant uptake, (Cassman and Munns 1980; Dørge 1994) could lead to the negligible pools of NH_4^+ found for the old field sites. Additionally, NH_4^+ pools correlated negatively to precipitation between samples (Fig. 12), suggesting higher levels of precipitation in the old field may induce microbial immobilization of NH_4^+, or possibly increase its availability for plant uptake.

Transition

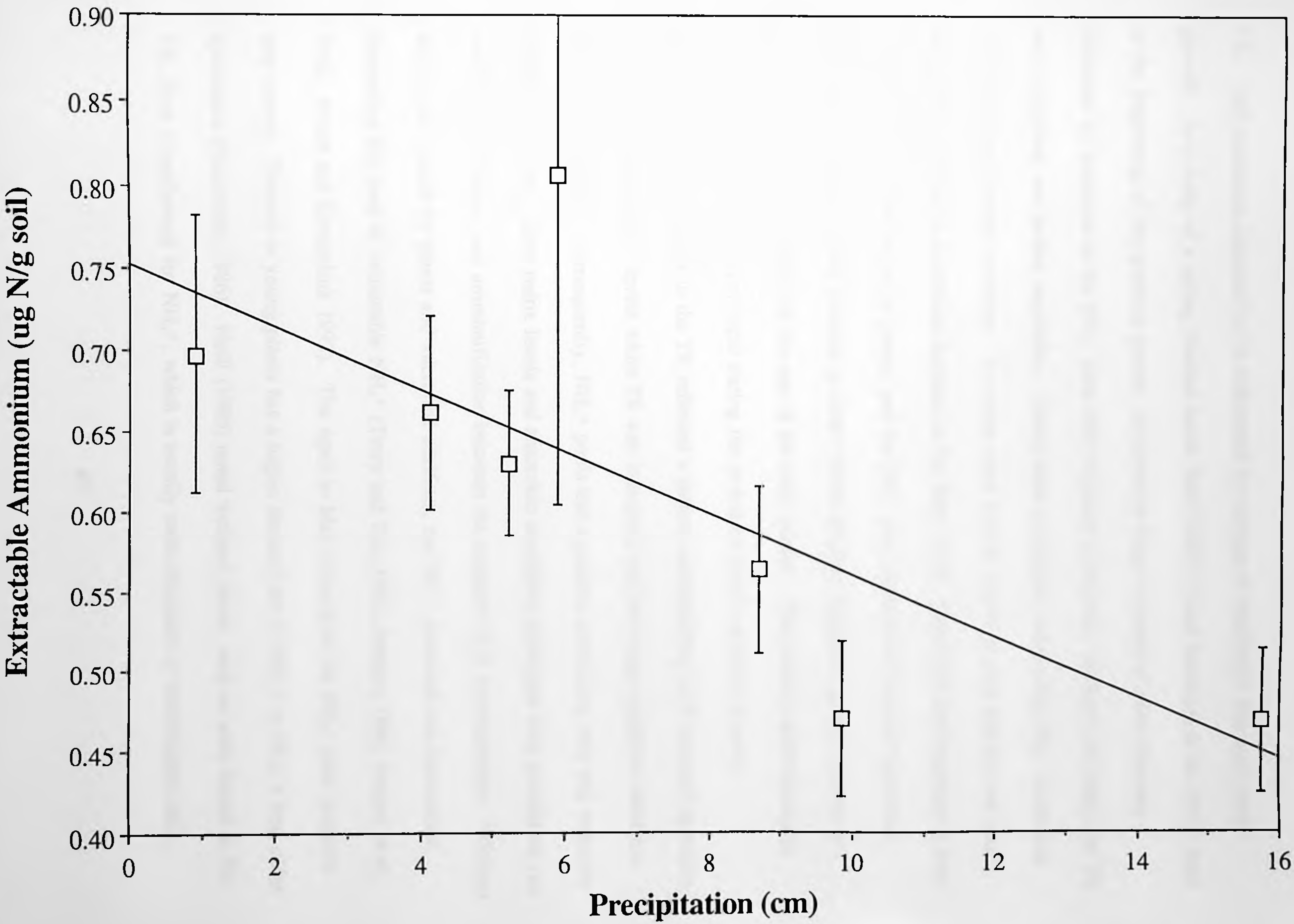
Soil NO_3^- pools were consistently low throughout the study in the transition site, with a low level of 3.04 μg N/g soil in July and a high level of 5.63 μg N/g soil in April (Fig.

Figure 11. Extractable nitrate and ammonium from the old-field soils of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ one standard error of the mean.



Nitrate
Ammonium
Extractable N (ug N/g soil)
0
1
2
3
4
5
6
APR
MAY
JUN
JUL
AUG
SEP
OCT

Figure 12. Correlation of extractable ammonium with total accumulation of precipitation between sample dates from the old field site type of the Green Bottom Wildlife Management Area. Extractable ammonium values shown are means of the five plots $\pm$ one standard error of the mean. Regression line $y = -0.02x + 0.75$, $r^2 = 0.58$, $P<0.01$.


Extractable Ammonium (ug N/g soil)
0.90
0.85
0.80
0.75
0.70
0.65
0.60
0.55
0.50
0.45
0.40
0
2
4
6
8
10
12
14
16
Precipitation (cm)

13). The variations appeared to be influenced by changes in inundation and early plant growth. In a study of a spring flooded marsh Neill (1995) found decreases in the NO_3^- pool at the beginning of the growing season, indicated by larger amounts of plant biomass, followed by increases in the NO_3^- pool after exposure of the soil. In April and May, the TR was inundated and lacked vegetation. Under these conditions, extractable NO_3^- levels had no significant monthly variation. As young plant growth started in June and the soil was exposed, there was a significant decrease in the NO_3^- pool. Continued soil exposure in July fostered rapid colonization by plants, and the NO_3^- pool experienced another significant decrease. The NO_3^- pool returned to June's levels as plant vegetative growth slowed in August and remained there for the rest of the study period. This concurs with Stevenson (1986) that N uptake is very rapid during the period of rapid vegetative growth.

Ammonium pools in the TR reflected a pattern corresponding with seasonal exposure, being higher than NO_3^- levels while TR was inundated and becoming negligible when the soils were exposed. Consequently, NH_4^+ pools had a positive correlation with soil moisture content (Fig. 14). Low redox levels and anaerobic conditions associated with inundation can restrict nitrification, and ammonification becomes the endpoint of N mineralization. Without significant uptake by plants and microbial nitrifiers, the NH_4^+ produced can accumulate, increasing the pool of extractable NH_4^+ (Terry and Tate 1980, Bowden 1984; Moore et al. 1992; Mitch and Grosselink 1993). The April to May increase in the NH_4^+ pool supports this theory. Growth in young plants has a higher demand for N (NH_4^+ or NO_3^-) than older specimens (Stevenson 1986). Neill (1995) noted wetland plants, such as were found in the TR, have a preference for NH_4^+, which is usually more abundant in waterlogged soils,

Figure 13. Extractable nitrate and ammonium from the transition soils of the Green Bottom Wildlife Management Area. Values shown are means of the five plots ± one standard error of the mean.

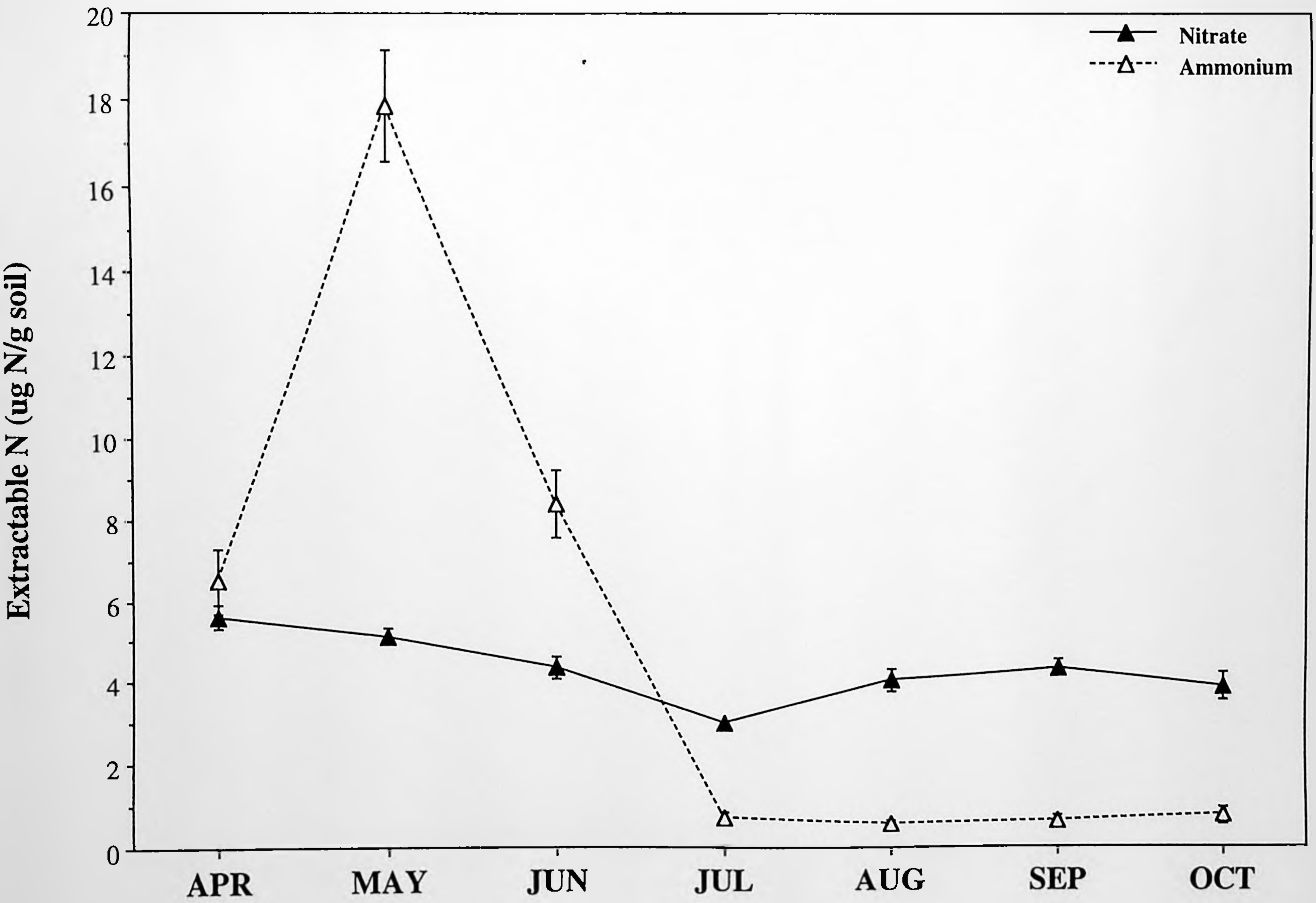
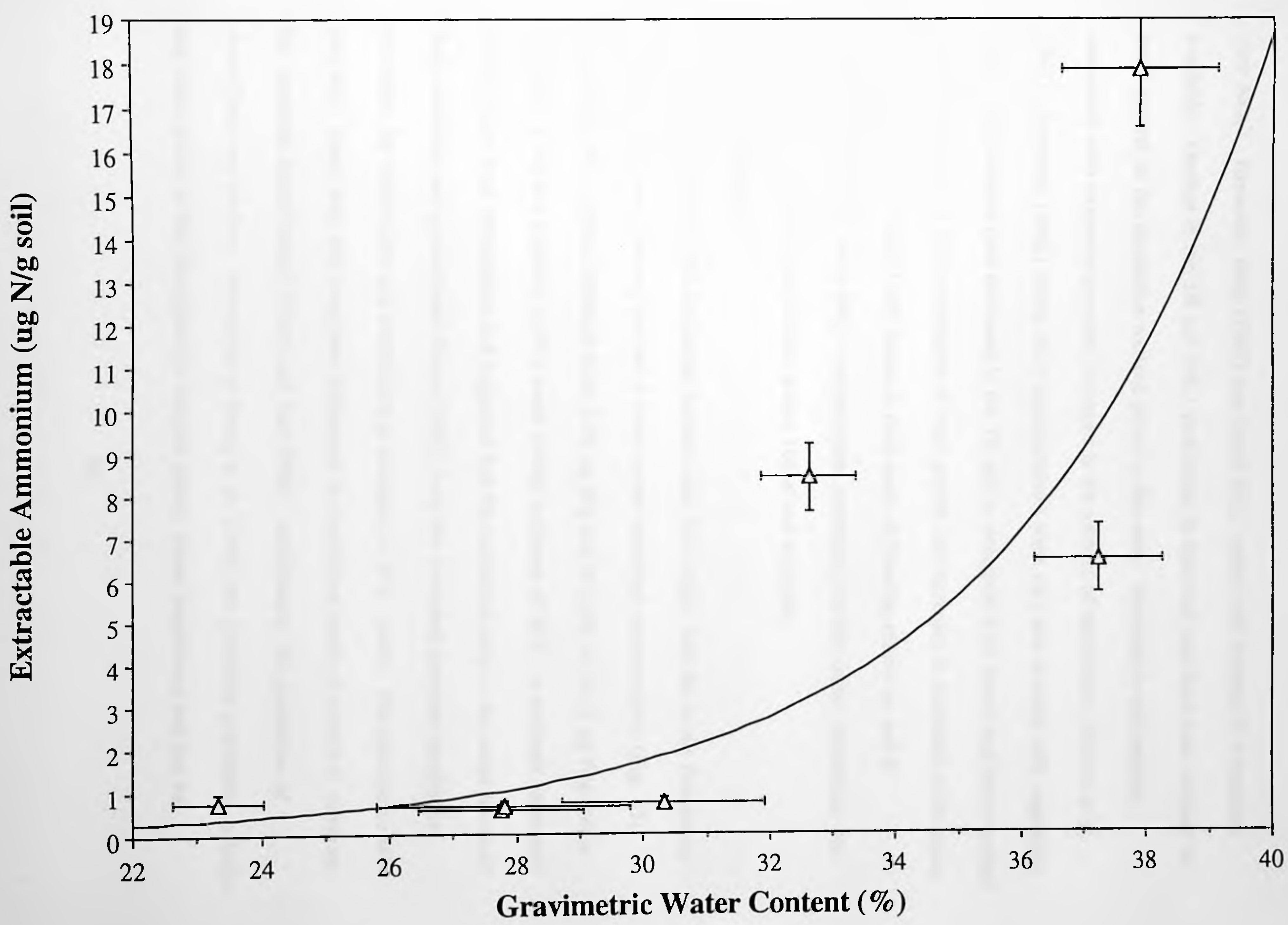

Nitrate
Ammonium
Extractable N (ug N/g soil)
0
2
4
6
8
10
12
14
16
18
20
APR
MAY
JUN
JUL
AUG
SEP
OCT

Figure 14. Correlation of extractable ammonium with percent gravimetric water content of soil sample for the transition site type of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ one standard error of the mean. Regression line $y = -3.0 \times 10^{0.10x} + 1.25e$, $r^2 = 0.75$, $P < 0.01$.

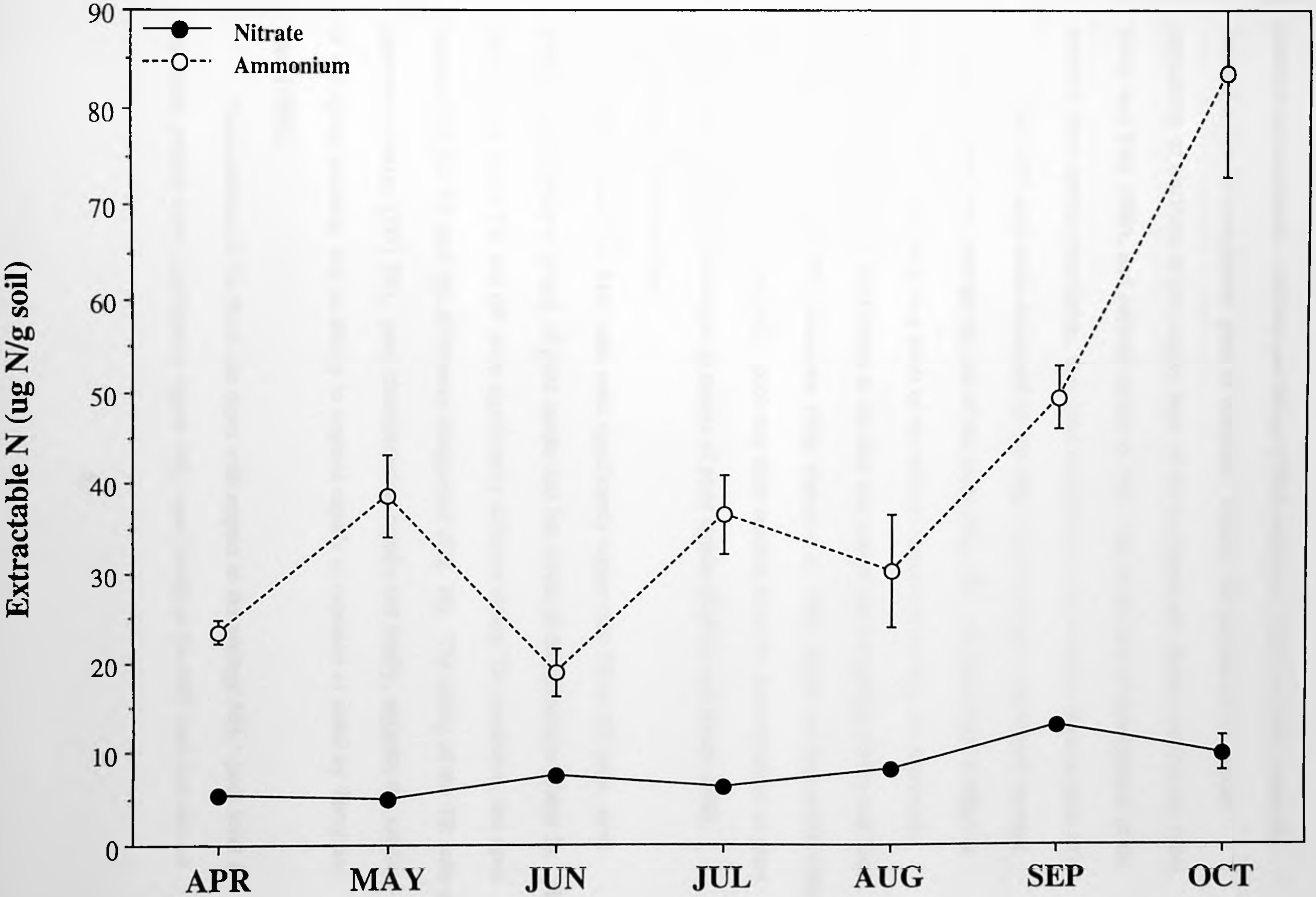

Extractable Ammonium (ug N/g soil)
Gravimetric Water Content (%)
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
17
18
19
22
24
26
28
30
32
34
36
38
40

over NO_3^-. However, Neill (1995) also found NO_3^- uptake will increase if it becomes available. Decline in the TR soil NH_4^+ pool began in June and may have been initiated by early growth in the facultative wetland plants in this area. Increases in soil aeration associated with exposure permitted increases in the amount of nitrification (Moore et al. 1992). Rosswall (1982) found NH_4^+ concentrations were very low in areas with vegetative cover. Ammonium pool decreases in the TR soil to terrestrial (OF) levels may have resulted from its removal by the combination of plant growth and increases in microbial nitrification. Finally, Terry and Tate (1980) found in their study of flooding effects on soil N transformations that, while NH_4^+ concentrations increased five-fold under inundation, pre-inundation levels were reestablished within 10d of soil exposure.

Mitigation Wetland

Nitrate pools in the mitigation wetland were fairly high, with the levels fluctuating throughout the study period, but had a trend toward increased concentrations (Fig. 15). Extractable NO_3^- levels increased form 5.40 μg N/g soil in April to 10.11 μg N/g soil in October. Cole and Lefebvre (1991) noted similar increases of NO_3^- in wetlands associated with surface mine reclamation and suggested that the substantial drop in the water levels of these wetlands and groundwater flow of NH_4^+ from the watershed provided conditions favorable for nitrification and contributed to increases in NO_3^- pools. The increase in MW soil NO_3^- pools may also have been influenced by insufficient levels of soluble C substrate for complete denitrification (Terry and Tate 1980). Additionally, the population of denitrifiers may be low. According to Reddy et al. (1989) the denitrifier population is larger and more active in the rhizosphere of wetland plants, whose populations had not yet

Figure 15. Extractable nitrate and ammonium from the mitigation wetland soils of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ one standard error of the mean.


Nitrate
Ammonium
Extractable N (ug N/g soil)
0
10
20
30
40
50
60
70
80
90
APR
MAY
JUN
JUL
AUG
SEP
OCT

established themselves. Caffery and Kemp (1990) attributed <10% of NO_3^- losses to denitrification in unvegetated areas of wetlands. Finally, the persistence of a small population of nitrifiers in the aerobic layer of the inundated soil (Reddy and Patrick 1984; Terry and Tate 1980), and reduced uptake of NO_3^- due to the lack of development of the wetland plant species population, may have contributed to the increase of extractable NO_3^-.

The MW soils were dominated by the NH_4^+ pools and had a significant increase (>300%) from June through the end of the study (Fig. 15). An abundance of NH_4^+ is typical for inundated soils as a result of the reduced conditions limiting the aerobically dependent processes of nitrification to the first few mm of the soil profile (Terry and Tate 1980; Bowden 1984; 1987; Stevenson 1986; Moore et al. 1992, Mitch and Grosselink 1993). Further contributions to the NH_4^+ pool may have resulted from the decomposition of plant residues produced by inundation in excess of plant uptake (Caffery and Kemp 1990).

Comparisons between sites

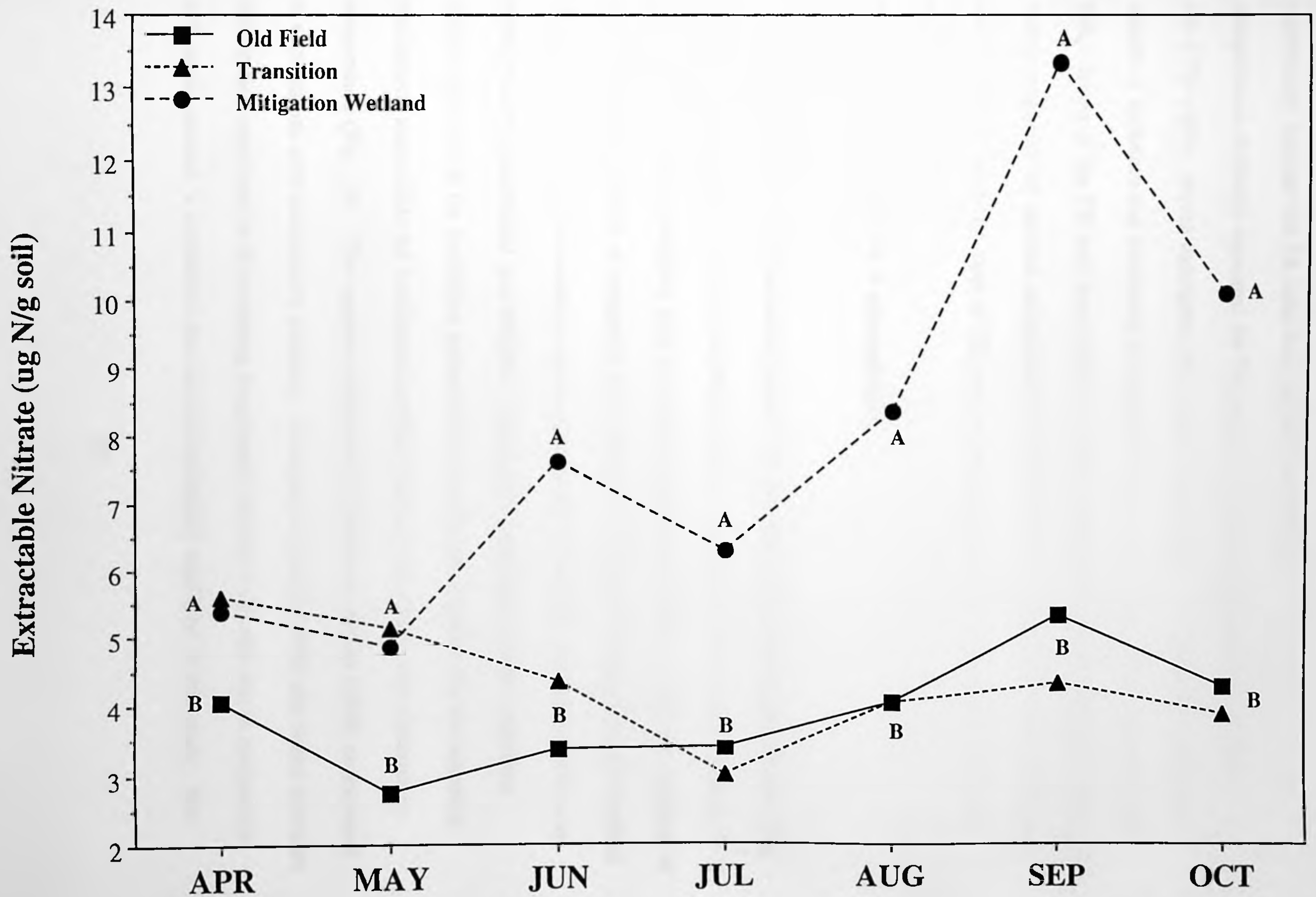
Nitrate pools for MW soils were significantly higher than TR or OF soils, most probably as a result of a lack of plant uptake and low levels of denitrification (Table 2). The NO_3^- pools in the TR and OF were significantly different during TR inundation, but upon exposure of the TR plot the differences disappeared (Fig. 16). The ability of the TR soils to assume terrestrial (OF) NO_3^- pool characteristics, rapidly and totally, suggests the survival of nitrifying bacteria, and an ability to respond rapidly to exposure as noted by Terry and Tate (1980).

Comparisons of the three site types with respect to the average NH_4^+ pool level for the study period show significantly higher NH_4^+ pool levels in the MW soils but did not

Table 2. Nitrogen data for soils from old-field (OF), transition (TR), and mitigation (MW) sites. "Pre" and "post" refer to pre-incubation and post-incubation N values, respectively. Values shown are means of five transects $\pm$ one standard error of the mean. Means followed by the same superscript are not significantly different between sites at $P<0.05$.

Site	NO_3		NH_4	
	pre	post	pre	post
	μg N/g soil			
OF	3.94 ± 0.15^{b}	8.35 ± 1.24^{a}	0.61 ± 0.04^{b}	3.36 ± 1.12^{b}
TR	4.40 ± 0.16^{b}	5.54 ± 0.42^{b}	5.08 ± 1.06^{b}	8.39 ± 1.96^{b}
MW	7.71 ± 0.54^{a}	8.63 ± 0.80^{a}	39.58 ± 4.10^{a}	46.75 ± 3.09^{a}

Figure 16. Extractable nitrate for the soils from the three site types of the Green Bottom Wildlife Management Area. Values shown are means of the five plots within the site type. Different letters are significantly different.



Extractable Nitrate (ug N/g soil)
Old Field
Transition
Mitigation Wetland
APR
MAY
JUN
JUL
AUG
SEP
OCT

statistically separate the TR soils from the OF soils (Table 2). Conversely, monthly comparisons distinctly separated the TR soils to an intermediate pool level of NH_4^+, OF<TR<MW, during inundation from April to June (Fig. 17). However, within one month of exposure and continuing throughout the rest of the study period, the extractable NH_4^+ levels of the TR soils were statistically indistinguishable from the OF soils. This rapid change suggests the aeration accompanying exposure has the potential to induce conditions, which reduced the NH_4^+ pool of TR soils to the negligible aerobic terrestrial (OF) levels within one month.

Net Nitrification and Net N mineralization

Old Field

Net nitrification accounted for most of the net N mineralization in the OF soils (Fig. 18). Franco and Munns (1982) concluded there is a continual net conversion of organic N to NO_3^- under aerobic conditions with very little accumulation of NH_4^+. With the exception of May and June, a period of substantial plant growth, virtually all N mineralized was nitrified (Fig. 18). In a study of secondary successional gradients, Barford and Lajtha (1992) also found most N mineralized was nitrified. The positive correlation between maximum temperature during the incubation period and net nitrification suggests that the microbial populations responsible for nitrification exhibit an increase in activity with increases in temperature (Fig. 19). This supports references by Tisdale and Nelson (1968) on increases in nitrification with temperature increases. Cassman and Munns (1980) also found increased nitrification associated with increasing temperature, but found that this was in conjunction with water content, a correlation that was not statistically significant in this study. Net

Figure 17. Extractable ammonium for the soils from the three site types of the Green Bottom Wildlife Management Area. Values shown are means of the five plots within the site type. Different letters are significantly different.

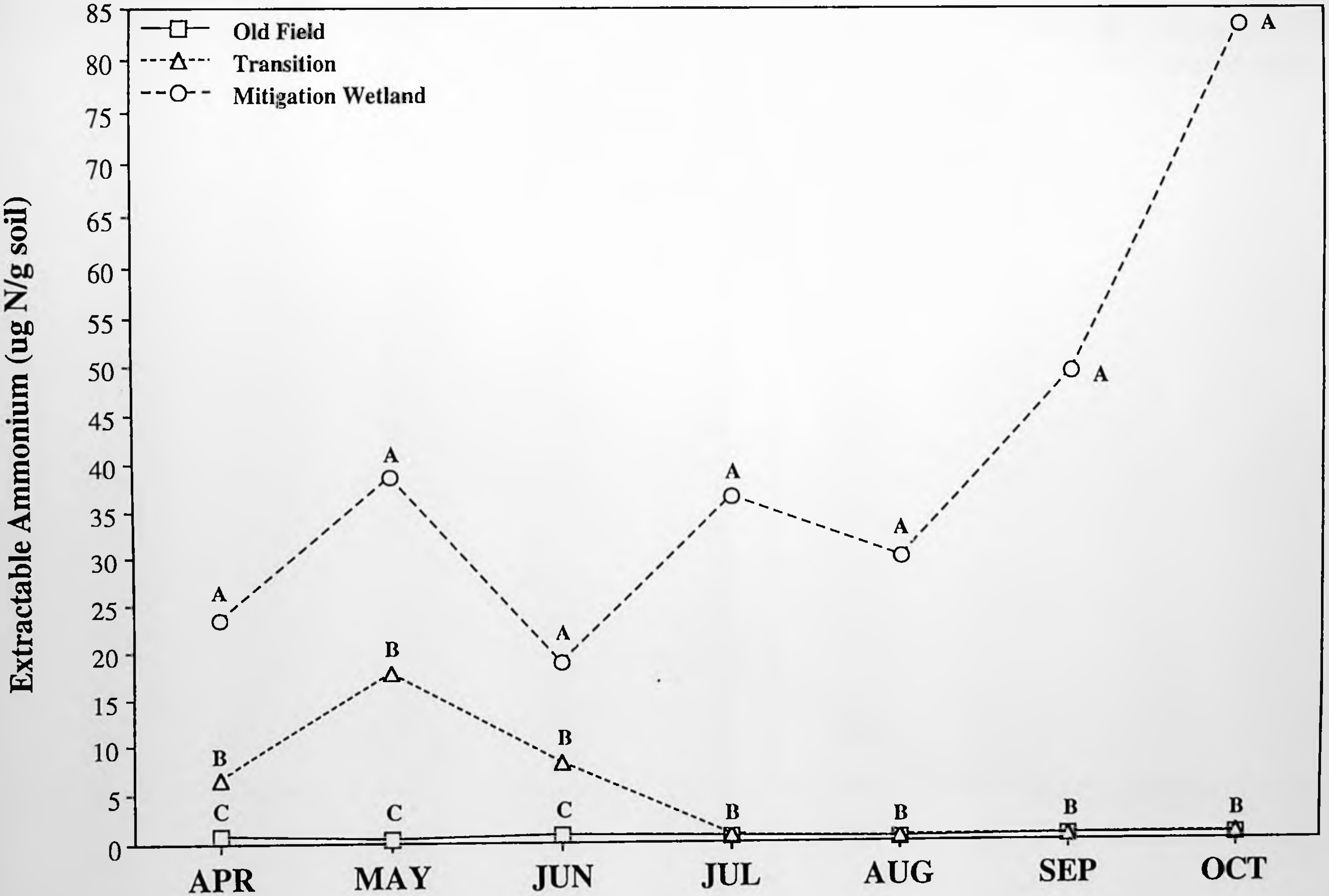

Old Field
Transition
Mitigation Wetland
Extractable Ammonium (ug N/g soil)
85
80
75
70
65
60
55
50
45
40
35
30
25
20
15
10
5
0
A
A
A
A
A
A
A
B
B
B
B
B
B
B
C
C
C
APR
MAY
JUN
JUL
AUG
SEP
OCT

Figure 18. Net nitrification and mineralization from the old field soils of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ one standard error of the mean.

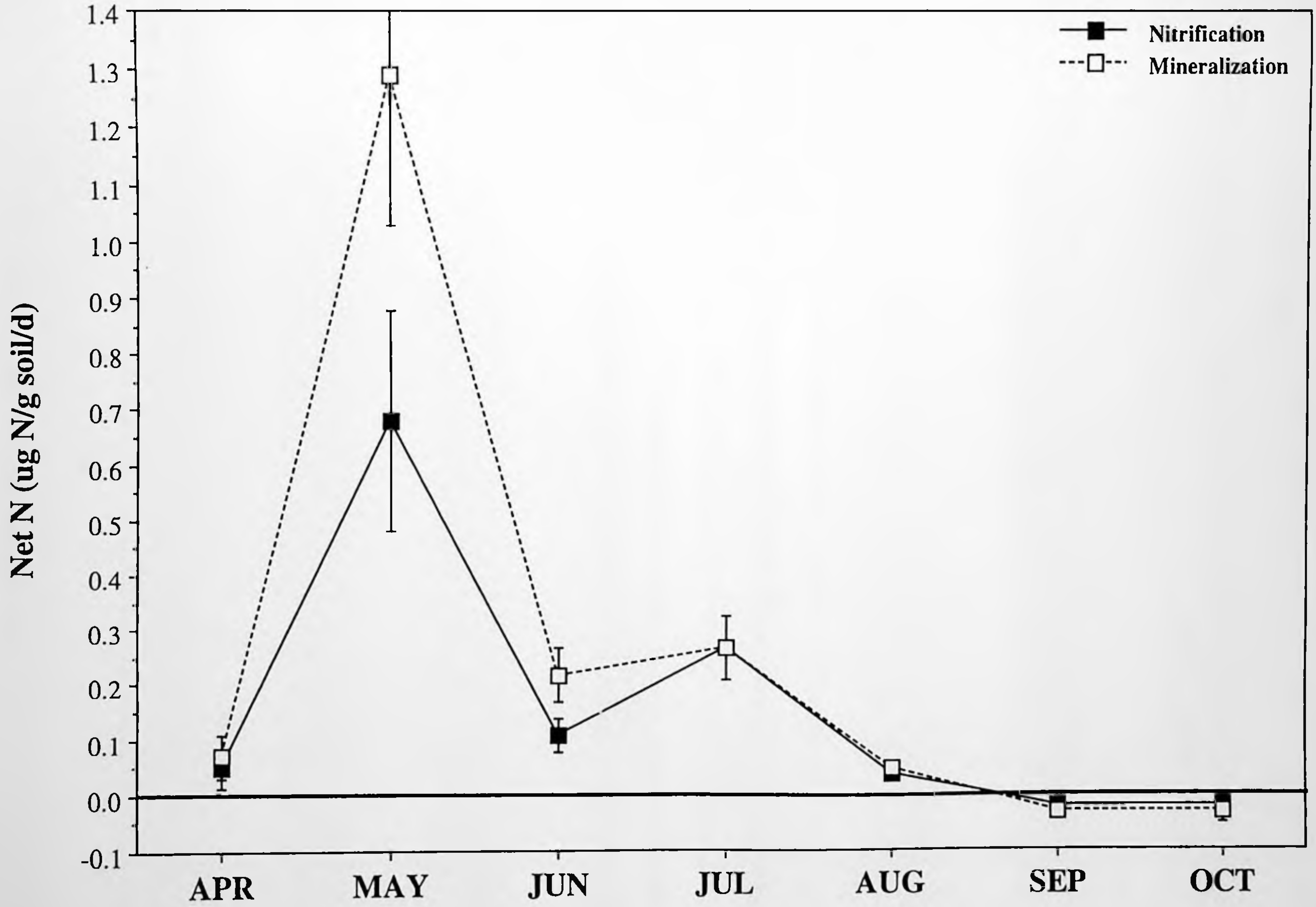
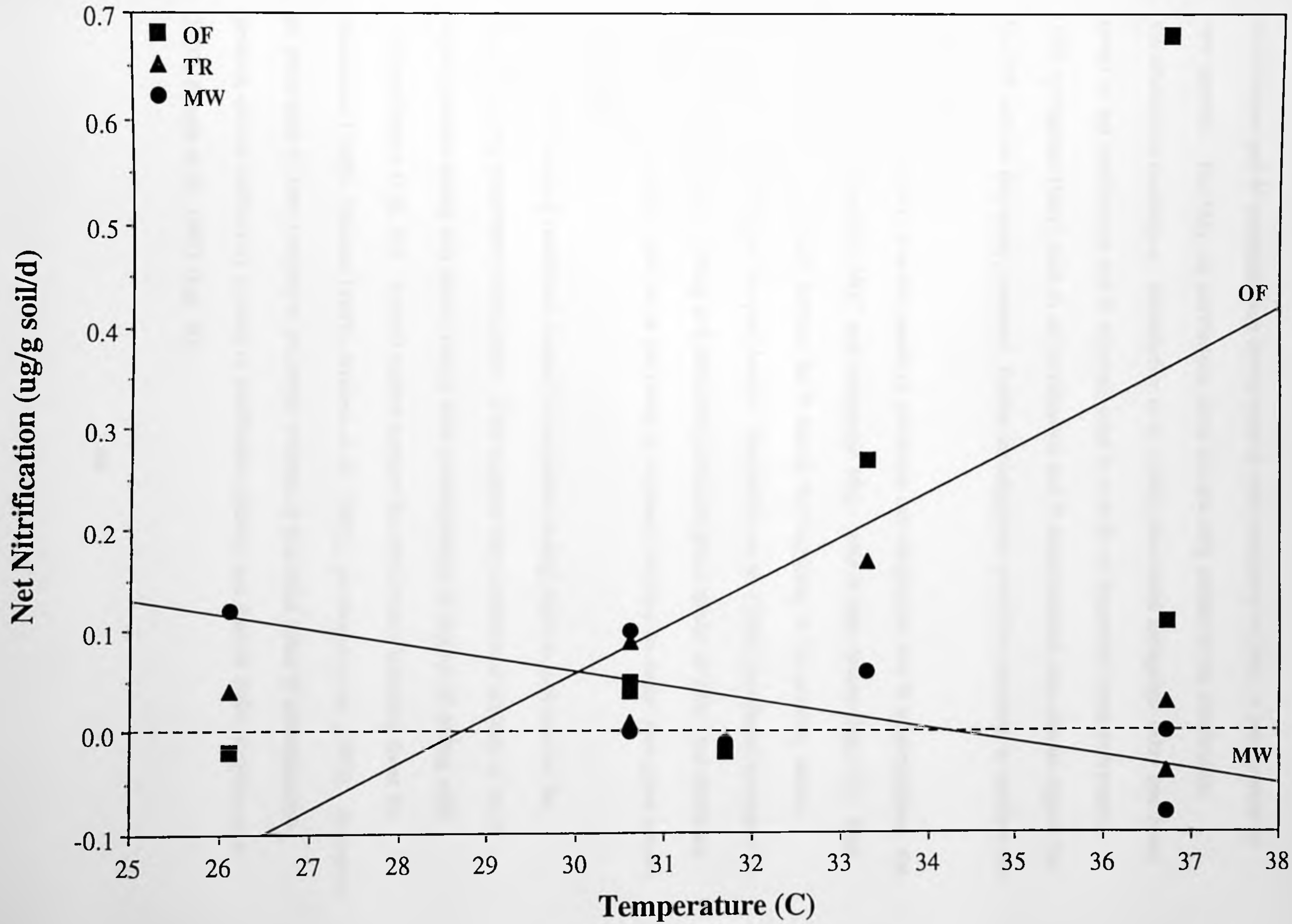

Net N (ug N/g soil/d)
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
APR
MAY
JUN
JUL
AUG
SEP
OCT
Nitrification
Mineralization

Figure 19. Correlation of net nitrification (μg N/g soil/d) with maximum incubation period temperature for soils from the three site types of the Green Bottom Wildlife Management Area: old-field (OF), transition (TR), and mitigation wetland (MW). Values shown are means of five plots within the site type. OF regression line $y = 0.04x - 1.29$; $r^2 = 0.45$; $P<0.05$, MW regression line $y = -0.01x + 0.48$; $r^2 = 0.57$; $P<0.05$.


OF
TR
MW
OF
MW
Net Nitrification (ug/g soil/d)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
25
26
27
28
29
30
31
32
33
34
35
36
37
38
Temperature (C)

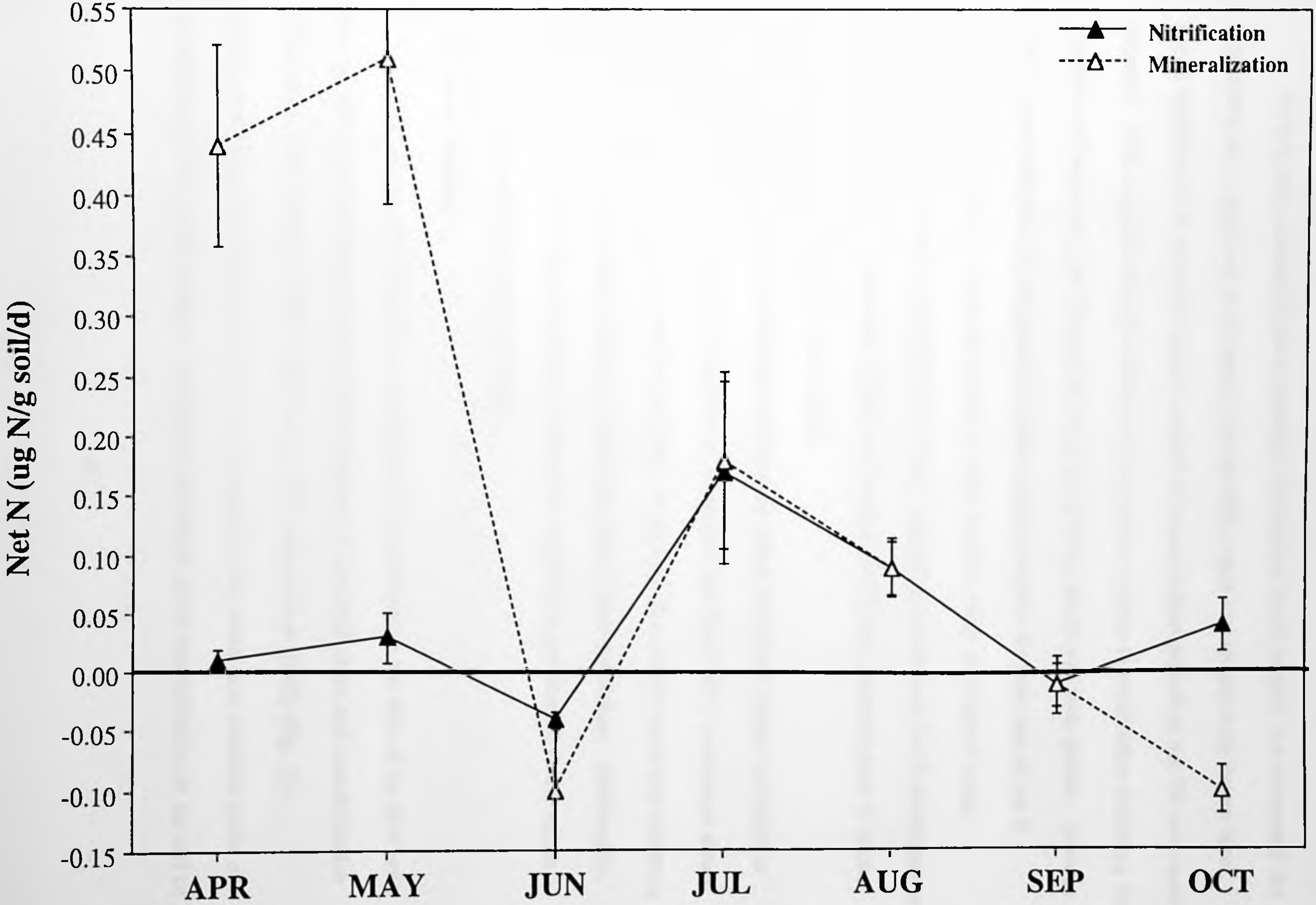
nitrification and N mineralization levels were at their maximum in May, a peak period of new growth. The May net nitrification level was the only outlier in the nitrification-mineralization correlation. Nadelhoffer et al. (1984) also noted springtime (May and June) peaks in net nitrification and N mineralization in a study of temperate forest ecosystems. This springtime (May) peak in net nitrification and N mineralization may also be typical for the OF soils in this study, however, further investigations would be necessary to confirm this hypothesis.

Although May was the month of maximum net nitrification and N mineralization, the pool levels of extractable NO_3^- and extractable NH_4^+ were at their lowest (Fig. 11). This suggests an especially high demand for N during the beginning of the growing season, resulting in a lowering of the pool levels. Nadelhoffer et al. (1984) also found decreases in the N pools during the spring and concluded increased plant uptake of NO_3^- but attributed decreases in the NH_4^+ pool to be the result of increased nitrification rather than plant uptake.

Transition

The reducing conditions caused by inundation during April to mid-June in the transition sites suppressed nitrification. This suggests that a substantial amount of net N mineralization during this period results from mineralization of organic N ending with ammonification (Fig. 20). Several studies support this observation including those by Stevenson (1986), Bowden (1987), Kralova et al. (1992), and Moore et al. (1992). Exposure of these sites in June resulted in an abrupt reversal of this trend when N mineralization became almost exclusively a result of nitrification (Reddy and Patrick 1984; Kralova et al. 1992; Moore et al. 1992) (Fig. 20).

Figure 20. Net nitrification and mineralization from the transition soils of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ one standard error of the mean.


Nitrification
Mineralization
Net N (ug N/g soil/d)
0.55
0.50
0.45
0.40
0.35
0.30
0.25
0.20
0.15
0.10
0.05
0.00
-0.05
-0.10
-0.15
APR
MAY
JUN
JUL
AUG
SEP
OCT

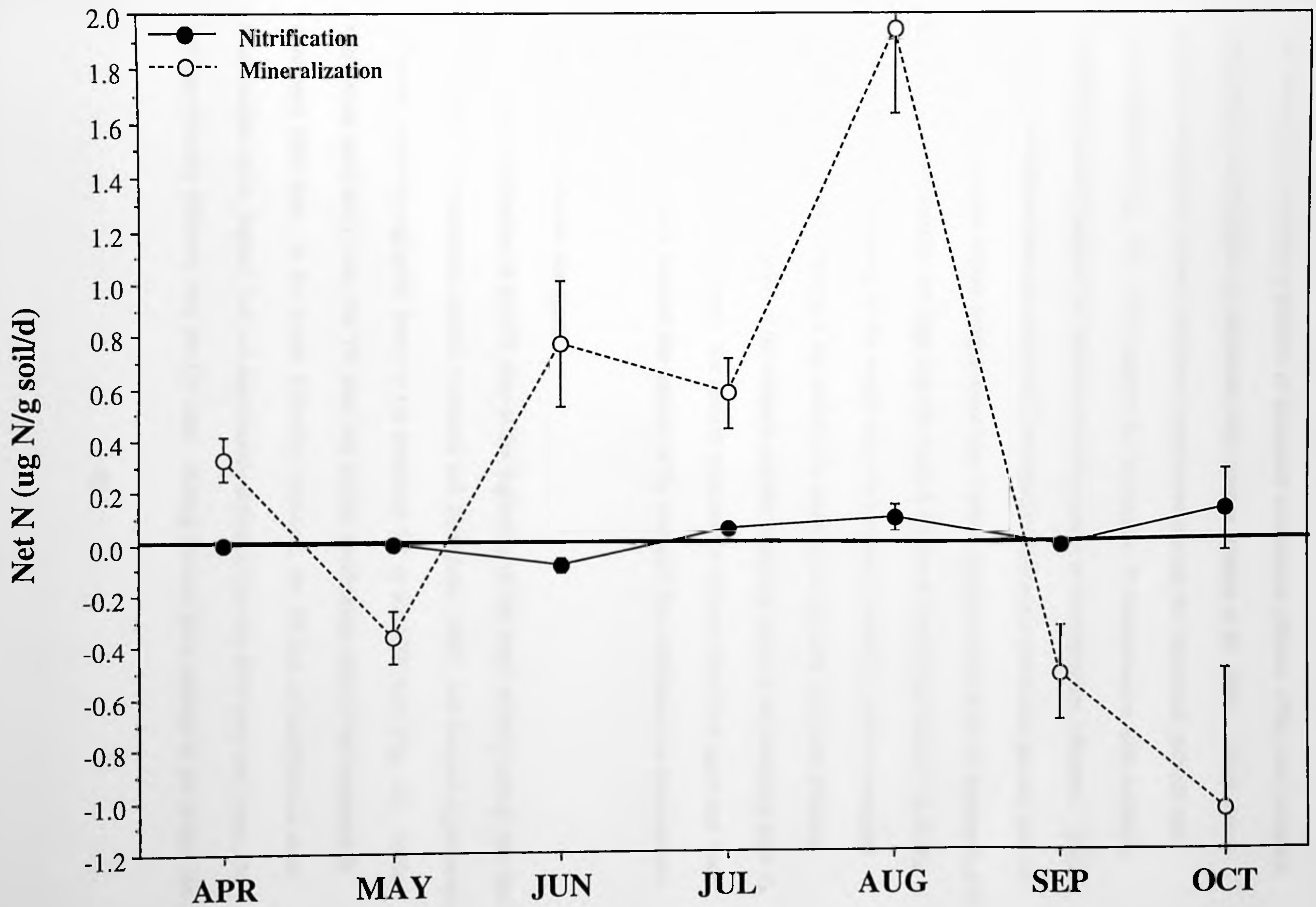
In July only one month after exposure, nitrification levels peaked, but conversely the extractable NO_3^- pool was at its lowest, and the NH_4^+ pool was almost at its lowest level. Rapid depletion of N occurred during a period of vigorous plant growth as the TR soils were exposed. This suggests although there was a significant increase in nitrification following the exposure of these soils, the demand for N by the growing plants was even greater. Bowden (1987) concluded that the peak period of plant uptake precedes the peak rate of net N production in wetlands. After this period of early growth, while nitrification levels decreased, the extractable N pools levels did not, suggesting the demand for N diminished as the season progressed. Stevenson (1986) and Bowden (1987) both reported plant N uptake decreases as vegetative growth rates decline.

This rapid response of nitrifiers to change in redox conditions implies an ability to survive the temporarily reduced conditions of inundation and begin NO_3^- production almost immediately as sufficient O_2 becomes available. Terry and Tate (1980) found that nitrifying bacteria can survive flooding and even maintain the size of their population. Additionally, they showed the NO_3^- pool, indicating nitrification, returned to pre-flooded levels within 15 d of soil exposure (Terry and Tate 1980).

Mitigation Wetland

Small levels of nitrification were expected and displayed in the data of the MW soil, but the net N mineralization seems to be dominated by ammonification and immobilization (Stevenson 1986; Bowden 1987; Langis et al. 1991; Moore et al. 1992) (Fig. 21). Macrophytic aquatic vegetation had not yet developed to any extent and therefore could not influence MW N transformations. Significant declines in net N mineralization at the end of

Figure 21. Net nitrification and mineralization from the mitigation wetland soils of the Green Bottom Wildlife Management Area. Values shown are means of the five plots $\pm$ one standard error of the mean.



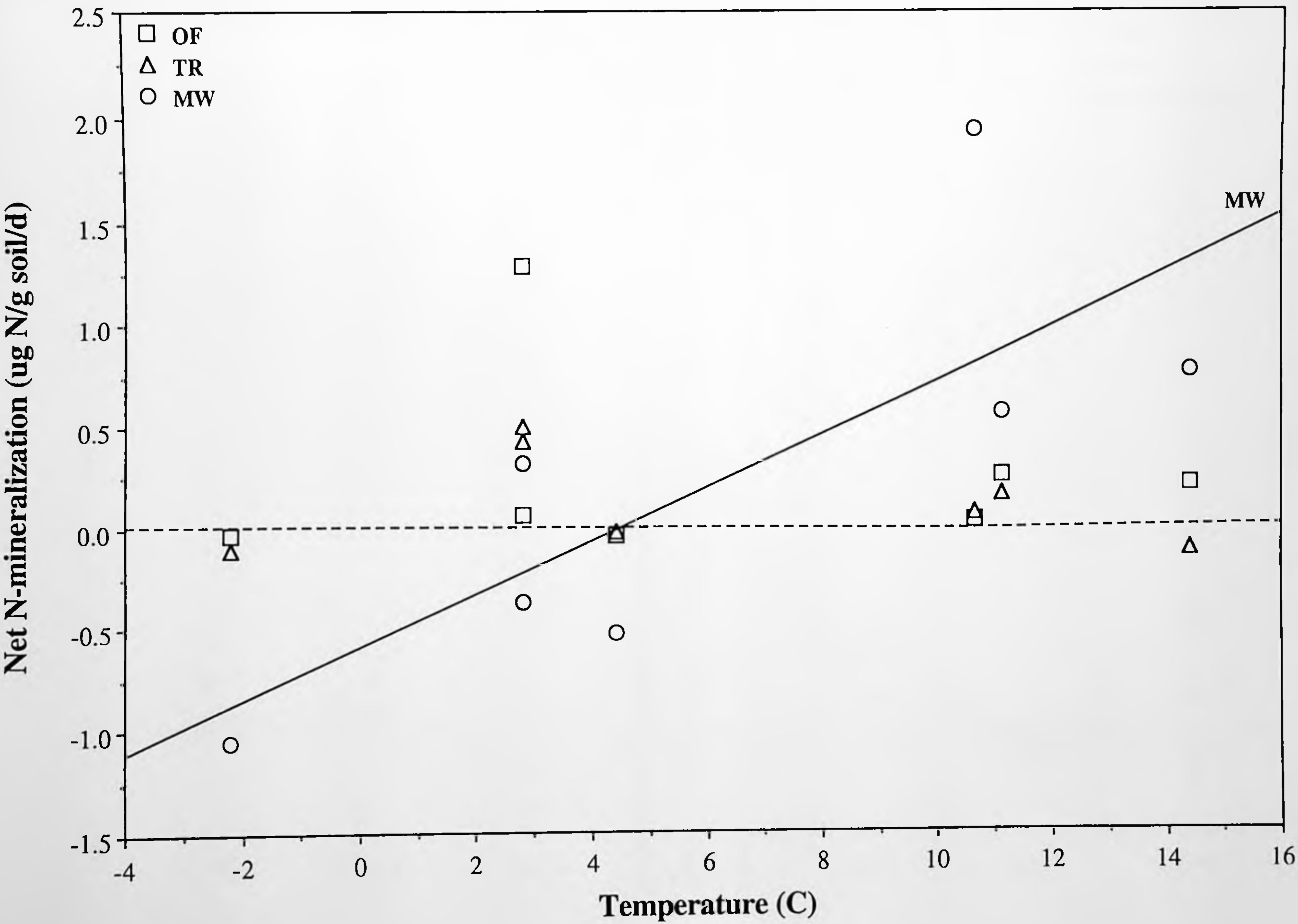
Nitrification
Mineralization
Net N (ug N/g soil/d)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.8
-1.0
-1.2
APR
MAY
JUN
JUL
AUG
SEP
OCT

the study may have been a product of decreased temperature (Nishio 1994) and increased microbial immobilization in September and October (Garten et al. 1994). There was a positive correlation between minimum temperature (during the incubation period) and mineralization (Fig. 22). This supports the decrease in N mineralization with declining temperatures and suggests the extreme low temperature as the important influence. The negative correlation between maximum temperature (during the incubation period) and net nitrification suggests higher temperatures may further suppress nitrification in inundated soils (Fig. 19). Additionally, the high specific heat of the water (averaging almost 1 m in the MW sites at the beginning of the study) may have caused a buffering effect to seasonal temperature increases causing a lag time in the warming of the MW soils and affecting nitrification levels. Finally, if the obligate aerobic nitrifying bacteria are receiving their O_2 via diffusion through the water, the inverse relationship between dissolved gases and water temperature may have lowered the amount of O_2 available for nitrification as temperatures increased.

Comparisons between site types

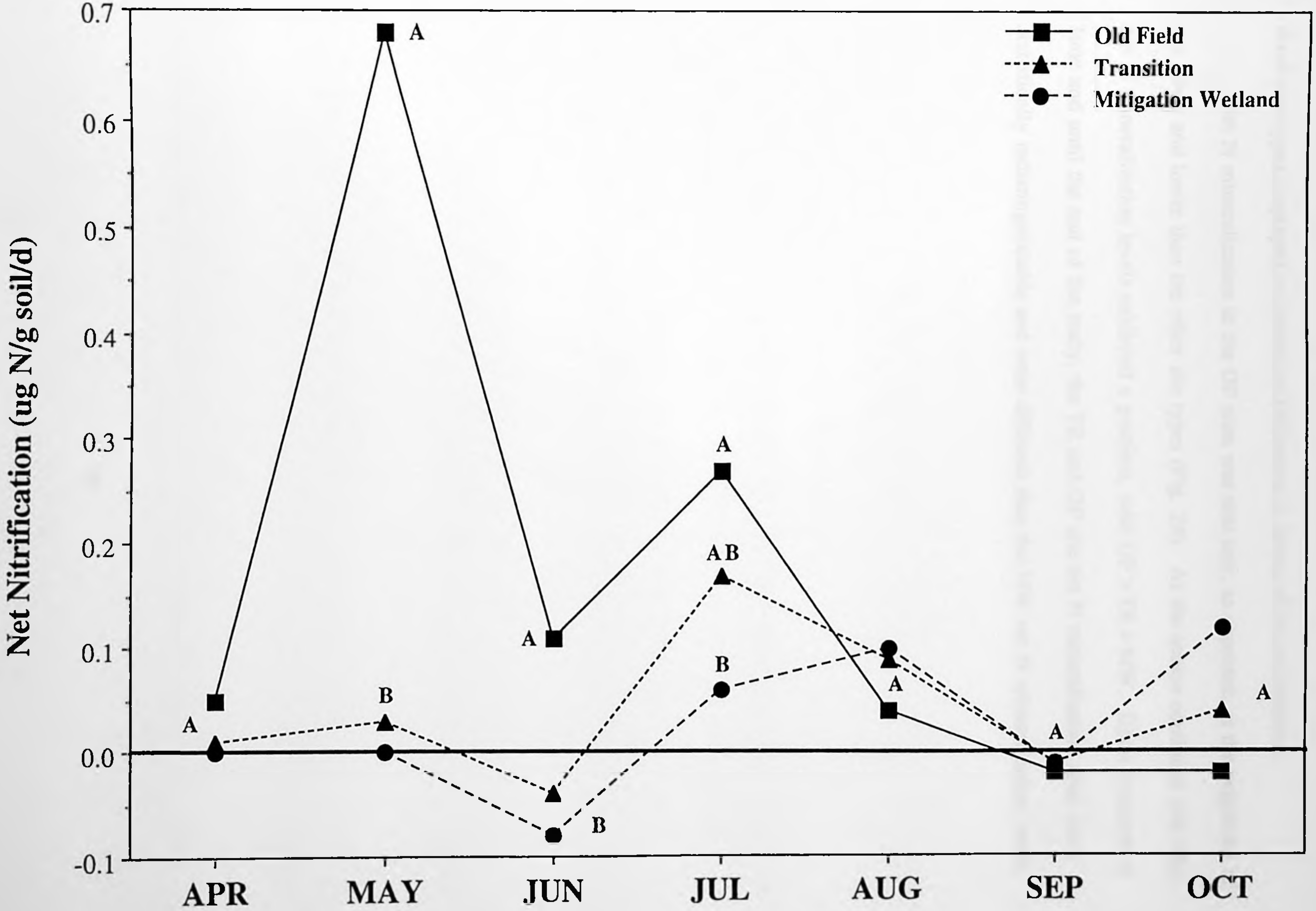
Net nitrification in the OF sites at the beginning of the study in early spring was low, as expected in a temperate aerobic terrestrial soil (Stevenson 1986), and was not significantly different from the negligible levels in the inundated TR or the MW sites (Fig. 23). Under inundation until early June, the TR sites had similar nitrification rates to the permanently inundated MW sites. In the month following exposure, the TR had net nitrification at an intermediate level, higher, but not significantly different than the MW sites and lower, but not significantly different than the OF sites. During the last three months of the study, the

Figure 22. Correlation of net mineralization (μg N/g soil/d) with minimum incubation period temperature for soils from the three site types of the Green Bottom Wildlife Management Area: old-field (OF), transition (TR), and mitigation wetland (MW). Values shown are means of five plots. MW regression line $y = 0.13x - 0.59$; $r^2 = 0.62$; $P<0.01$.



OF
TR
MW
MW
Net N-mineralization (ug N/g soil/d)
Temperature (C)
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-4
-2
0
2
4
6
8
10
12
14
16

Figure 23. Net nitrification for the soils from the three site types of the Green Bottom Wildlife Management Area. Values shown are means of the five plots within the site type. Different letters are significantly different.

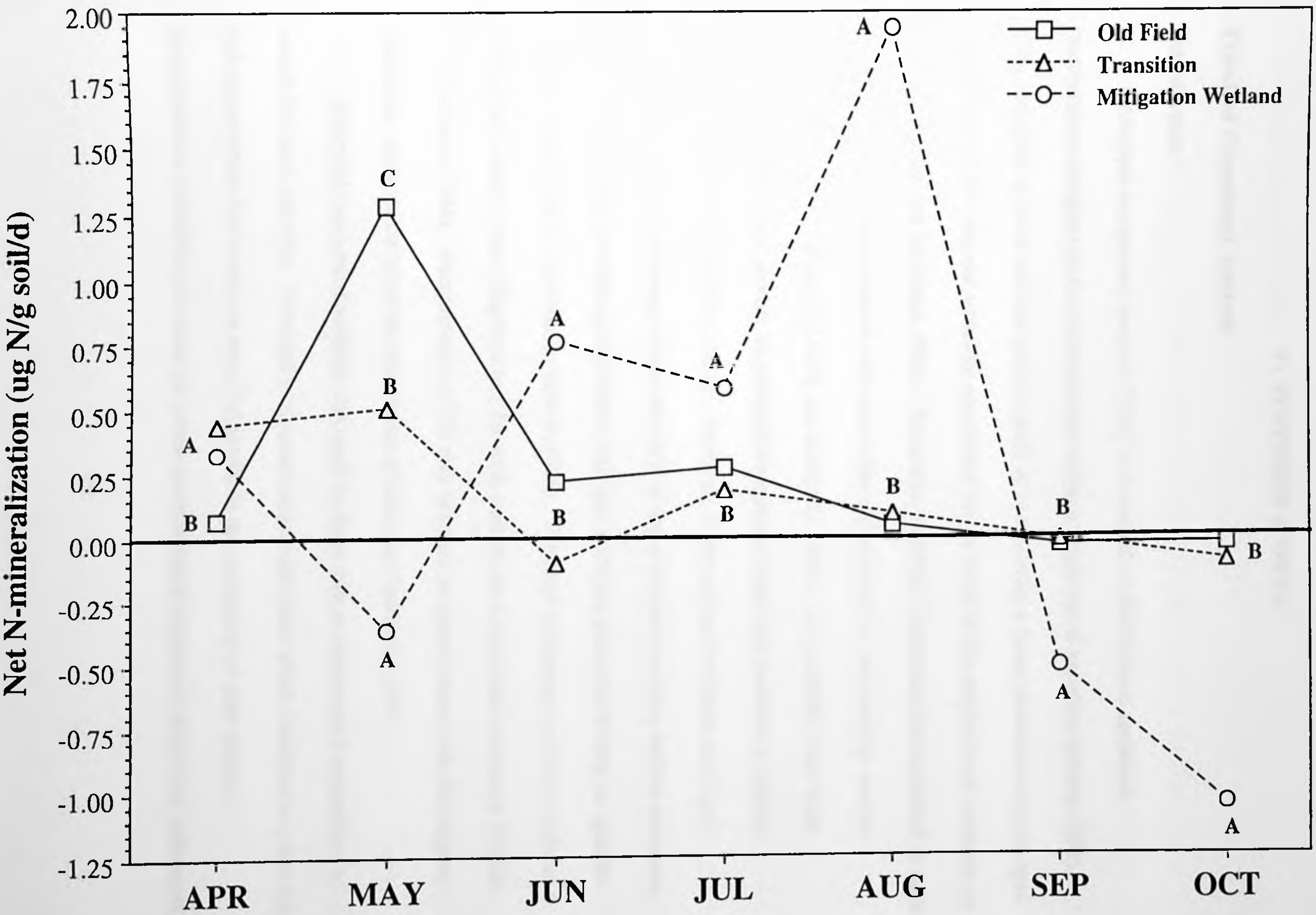


Net Nitrification (ug N/g soil/d)
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
APR
MAY
JUN
JUL
AUG
SEP
OCT
Old Field
Transition
Mitigation Wetland
A
B
A
B
A
AB
B
A
A
A

three site types displayed no statistical difference in levels of net nitrification.

Net N mineralization in the OF sites was also low, as expected, at the beginning of the study and lower than the other site types (Fig. 24). As the season continued into May, net N mineralization levels exhibited a gradient, with OF>TR>MW. Upon exposure in June and until the end of the study, the TR and OF site net N mineralization rates were statistically indistinguishable and were different than the MW net N mineralization levels.

Figure 24. Net nitrogen mineralization for the soils from the three site types of the Green Bottom Wildlife Management Area. Values shown are means of the five plots within the site type. For each month, different letters are significantly different.



Old Field
Transition
Mitigation Wetland
Net N-mineralization (ug N/g soil/d)
2.00
1.75
1.50
1.25
1.00
0.75
0.50
0.25
0.00
-0.25
-0.50
-0.75
-1.00
-1.25
APR
MAY
JUN
JUL
AUG
SEP
OCT

V: SYNTHESIS of DATA

Principal Component Analysis

Introduction

Principal component analysis (PCA) is a method of multivariate statistical manipulation designed to find relationships within a single set of variables (Harris 1975). Data reduction is used with the primary goal of constructing a linear combination (principal component) of the original variables accounting for as much of the original total variation as possible (Dillon and Goldstein 1984). Successive principal components are extracted in such a way that they are uncorrelated with each other and account for sequentially smaller amounts of the total variation (Dillon and Goldstein 1984). It is possible from these extractions to have the number of principal components equal the number of original variables (Dillon and Goldstein 1984). However, if the original variables are highly interrelated, a high percentage of the variation of these original variables will be accounted for on the first few principal components, and later principal components may be ignored with very little loss of information (Harris 1975). Principal component analysis produces a component loading value (eigenvector) for each variable on a principal component (Dillon and Goldstein 1984). Interpretation of the data is based on eigenvectors with the highest (absolute) value on the principal component (Dillon and Goldstein 1984).

Principal component analysis was used on study data to determine a separation by month for each site type. Principal component axes established which variables in a site type had eigenvectors that would be most influential in the separation of each month. Interpretations could then be made on which environmental parameters may have influenced the seasonal patterns observed.

Old Field

Principal component analysis of 16 variables from climatological, physical, and N data in the OF resulted in a distinct separation of months (Fig. 25). The first axis (PRIN 1) accounted for 42% of the variation with the major positive correlation from the vectors for maximum and daily air temperatures and negative weight from precipitation within 48 h and extractable NO_3^- (Table 3). The second axis (PRIN 2), accounting for 20% of the variation, was positively correlated to precipitation between sampling and precipitation within 5 d of sampling, and negatively correlated with extractable NH_4^+ (Table 3).

PRIN 1 suggests a link between temperature and extractable NO_3^-, and PRIN 2 supports the negative correlation between precipitation and extractable NH_4^+. Monthly responses to the rise and fall of these relationships was supported by the orientation of the monthly means (Fig. 25). Principal component analysis in the OF supports the conclusions of Cassman and Munns (1980) and Tietema et al. (1992) on the interrelationships between temperature, moisture, and N transformations.

Transition

Principal component analysis of 17 variables in the TR provided a distinct separation of months (Fig. 26). The first axis (PRIN 1) accounted for 46% of the variation. Daily high temperature and soil temperature were the largest positive eigenvectors for PRIN 1 and precipitation within 48 h of sampling was the major negative value (Table 4). Twenty-six percent of the variation was accounted for on the second axis (PRIN 2). The primary positive factors for PRIN 2 were average temperature between sampling and precipitation

Figure 25. Principal component analysis for the old field site type of the Green Bottom Wildlife Management Area: first axis of variation (PRIN 1), second axis of variation (PRIN 2), ordination (4 - 10) represents sample month (April - October).

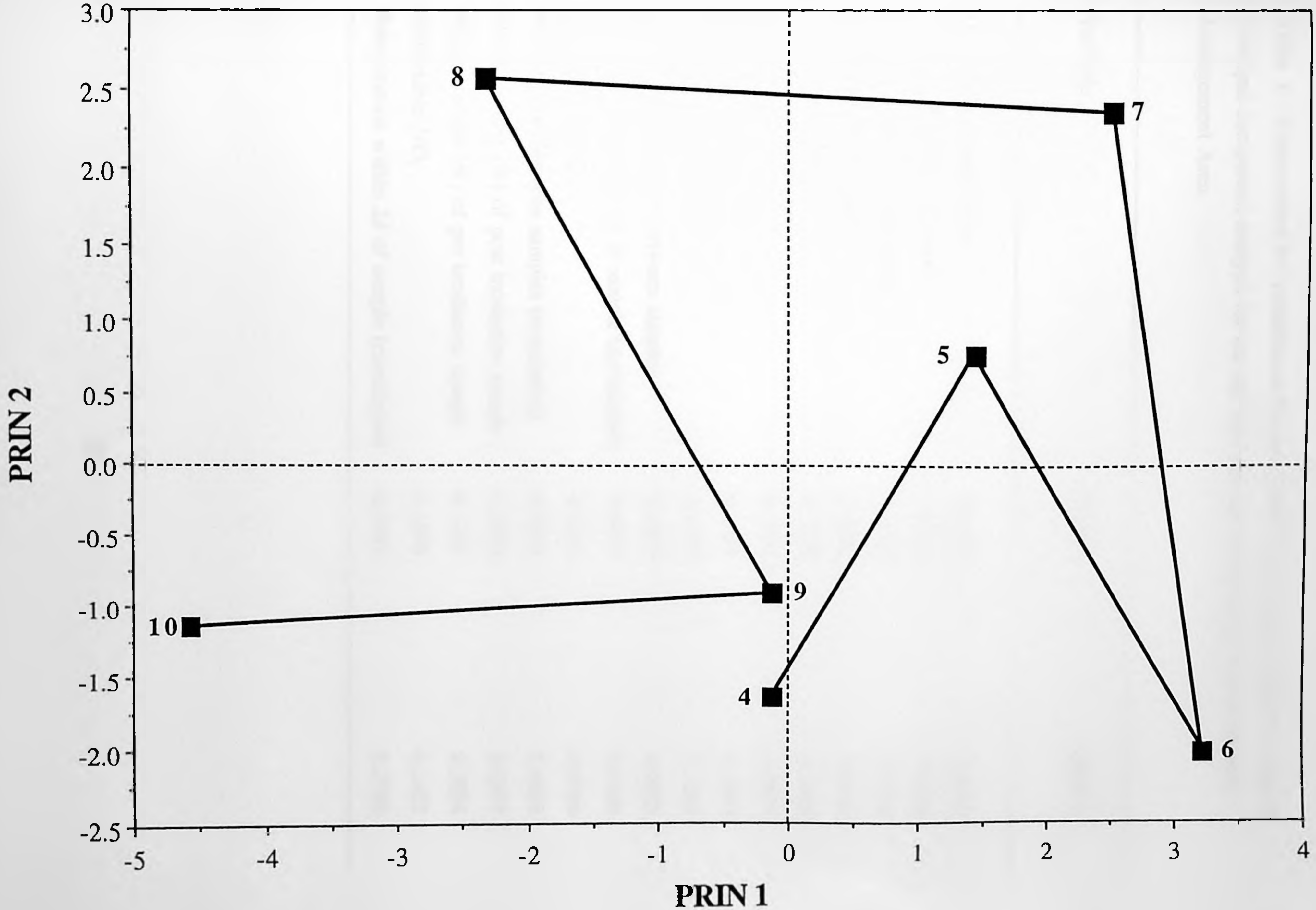

PRIN 2
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-5
-4
-3
-2
-1
0
1
2
3
4
PRIN 1
4
5
6
7
8
9
10

Table 3. Eigenvectors for variables on the first (PRIN 1) and second (PRIN 2) axes of principal component analysis for the old field site type of the Green Bottom Wildlife Management Area.

Variable	PRIN 1	PRIN 2
Daily high temperature	0.3591	-0.0112
Daily average temperature	0.3566	0.0434
Sample time air temperature	0.3466	-0.0758
Daily low temperature	0.3381	0.1415
Soil temperature	0.3158	0.1145
Sample time (of day)	0.2033	-0.0917
Net nitrification	0.1867	0.1943
Net N mineralization	0.1586	0.1292
Average temperature between samples	0.0923	0.2923
Precipitation within 5d of sample (cumulative)	0.0413	0.4169
Extractable NH_4	-0.0143	-0.4086
Precipitation between samples (cumulative)	-0.0843	0.4658
Water content (%) of post incubation sample	-0.1036	0.2477
Water content (%) of pre incubation sample	-0.1406	0.3024
Extractable NO_3	-0.1878	-0.1422
Precipitation within 2d of sample (cumulative)	-0.2189	0.2766

Figure 26. Principal component analysis for the transition site type of the Green Bottom Wildlife Management Area: first axis of variation (PRIN 1), second axis of variation (PRIN 2), ordination (4 - 10) represents sample month (April - October).

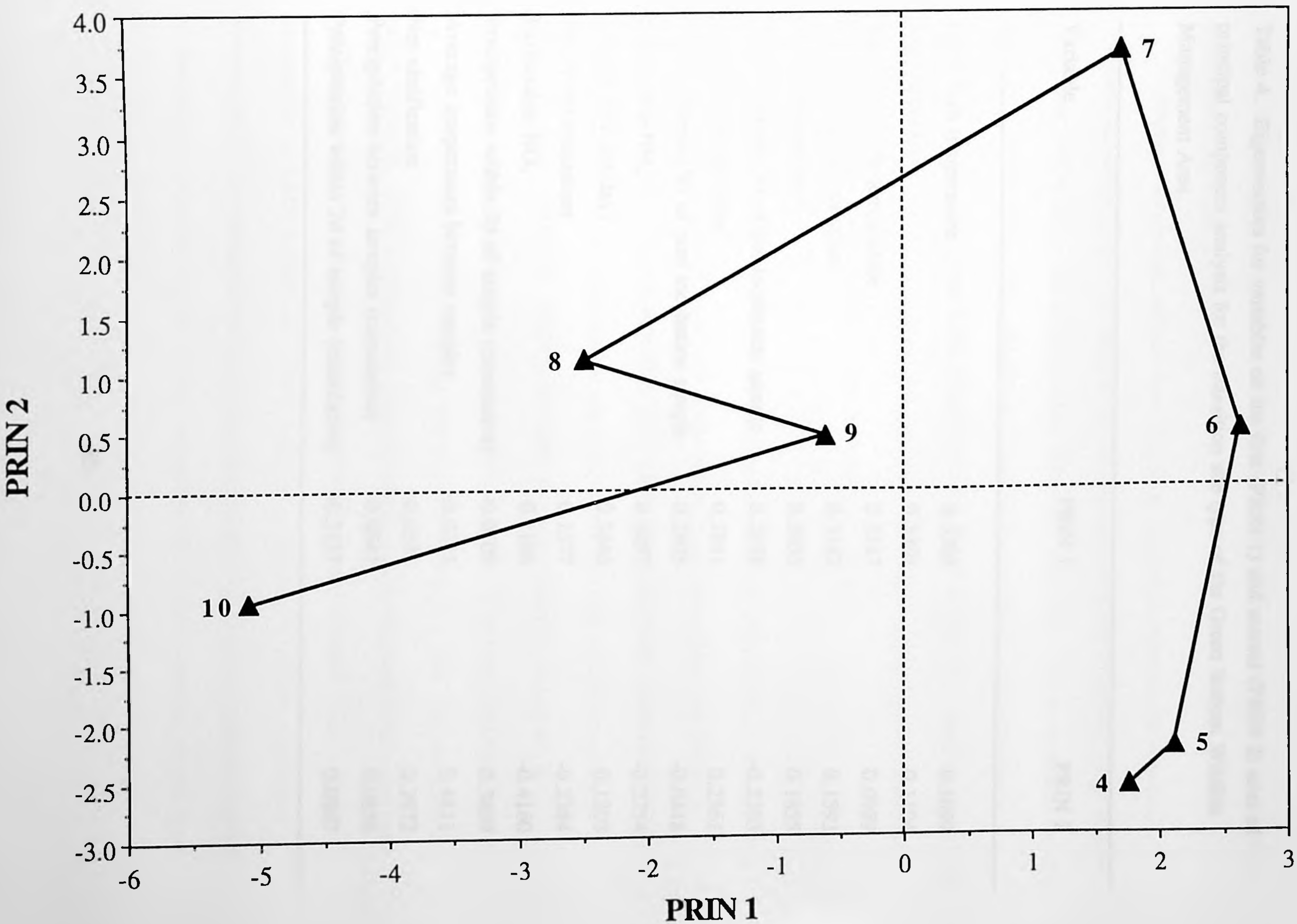

4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-3.0
-6
-5
-4
-3
-2
-1
0
1
2
3
PRIN 2
PRIN 1
4
5
6
7
8
9
10

Table 4. Eigenvectors for variables on the first (PRIN 1) and second (PRIN 2) axes of principal component analysis for the transition site type of the Green Bottom Wildlife Management Area.

Variable	PRIN 1	PRIN 2
Daily high temperature	0.3305	0.1099
Soil temperature	0.3301	0.1104
Sample time air temperature	0.3247	0.0989
Daily average temperature	0.3182	0.1593
Water temperature	0.3035	0.1855
Water content (%) of pre incubation sample	0.2938	-0.2103
Daily low temperature	0.2841	0.2561
Water content (%) of post incubation sample	0.2805	-0.0448
Extractable NH_4	0.2057	-0.2754
Sample time (of day)	0.1646	0.1208
Net N mineralization	0.1377	-0.2384
Extractable NO_3	0.1106	-0.4160
Precipitation within 5d of sample (cumulative)	-0.0105	0.3809
Average temperature between samples	-0.0115	0.4411
Net nitrification	-0.0351	0.2972
Precipitation between samples (cumulative)	-0.0641	0.0856
Precipitation within 2d of sample (cumulative)	-0.2157	0.0807

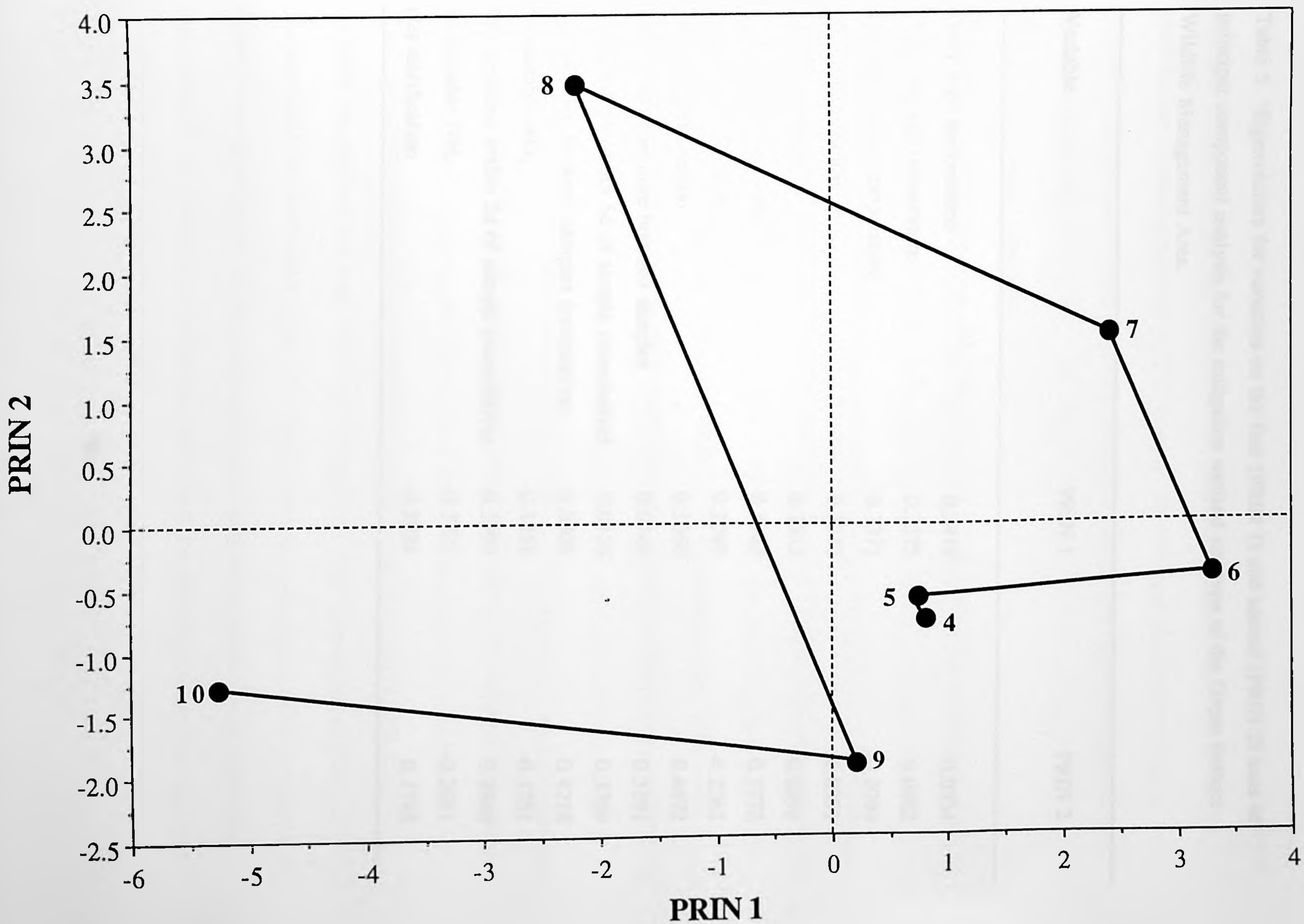
within 5 d of sampling. Extractable NO_3^- and extractable NH_4^+ were the greatest negative eigenvectors for PRIN 2 (Table 4).

Principal component analysis showed a close association between the months of complete inundation (April and May) at the TR sites as a result of a relatively large N pool (NO_3^- and NH_4^+) and a lack of temperature fluctuation (Fig. 26). The group means for June through September responded primarily to climatological variations, while October's placement showed a response to the N pool levels on PRIN 2 (Fig. 26). This suggests in the TR inundation in the spring insulates the soil from the weather, which become responsible for much of the seasonal variation when these soils are exposed.

Mitigation Wetland

Principal component analysis was performed on 15 variables for the MW site. The first axis (PRIN 1) accounted for 53% of the variance, and the second axis (PRIN 2) accounted for 22% of the variance (Fig. 27). PRIN 1 was positively correlated with high and average daily air temperatures and negatively correlated with net nitrification and extractable NH_4^+ (Table 5). PRIN 2 correlated positively primarily due to net N mineralization and precipitation between samples (Table 5). The most important negative eigenvectors for PRIN 2 were sampling time (of day), extractable NO_3^- and extractable NH_4^+ (Table 5). PCA confirmed the negative correlation between high temperature and nitrification and also suggested a similar connection between extractable NH_4^+ and temperature on PRIN 1 (Table 5). PRIN 2 showed evidence of a negative correlation between net N mineralization and extractable NH_4^+ (Table 5). April to June and September, all responded to positive correlations with temperature on PRIN 1; whereas, August and

Figure 27. Principal component analysis for the mitigation wetland site type of the Green Bottom Wildlife Management Area: first axis of variation (PRIN 1), second axis of variation (PRIN 2), ordination (4 - 10) represents sample month (April - October).



PRIN 2
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
-1.0
-1.5
-2.0
-2.5
-6
-5
-4
-3
-2
-1
0
1
2
3
4
PRIN 1
4
5
6
7
8
9
10

Table 5. Eigenvectors for variables on the first (PRIN 1) and second (PRIN 2) axes of principal component analysis for the mitigation wetland site type of the Green Bottom Wildlife Management Area.

Variable	PRIN 1	PRIN 2
Daily high temperature	0.3419	0.0334
Daily average temperature	0.3375	0.0882
Sample time air temperature	0.3371	-0.0799
Water temperature	0.3312	-0.0391
Soil temperature	0.3312	-0.0391
Daily low temperature	0.3183	0.1772
Sample time (of day)	0.2049	-0.2261
Net N mineralization	0.1060	0.4672
Average temperature between samples	0.0746	0.3191
Precipitation within 5d of sample (cumulative)	0.0316	0.3769
Precipitation between samples (cumulative)	-0.0805	0.4218
Extractable NO_3	-0.1254	-0.1751
Precipitation within 2d of sample (cumulative)	-0.1997	0.3946
Extractable NH_4	-0.2722	-0.2051
Net nitrification	-0.2934	0.1748

October were influenced by the eigenvector for net nitrification (Fig. 27). PRIN 2 clustered the April to June data, but July to October had definite responses to variations in the NH_4^+ pool and net N mineralization (Fig. 27). Overall, the MW sites seemed to respond to climatological changes in the spring and to N transformations and N pool fluctuations from summer into the fall.

Comparisons between site types

Except for precipitation within 48 h of sampling, climatological variables with the largest eigenvectors were positive for all of the site types. However, except for net N mineralization in the MW site, the influencing values for N were negative. The importance of the various N variables had distinct differences between the OF and MW sites with the TR sites falling in between. The extractable NO_3^- eigenvector was most important in the OF providing interpretation of the variance on PRIN 1. The extractable NO_3^- eigenvector decreased in magnitude from the TR to MW sites and was used for interpretation on PRIN 2, which explained substantially less of the total variance. As expected, extractable NH_4^+ had the greatest effects in the MW sites and was on PRIN 1. This eigenvector was of importance on PRIN 2 in the OF and TR sites explaining less of the variance, but because of the early (under inundation) large pools of NH_4^+ in the TR sites, it might be postulated that the magnitude of the extractable NH_4^+ eigenvector would be larger than in the OF sites, however, the opposite was true. This may be suggestive of the importance of NH_4^+ as limiting factor for nitrification in the OF. Net nitrification (negative on PRIN 1) and net N mineralization (positive on PRIN 2) were the most significant N vectors in the MW sites. Although different variables in the site types influenced the orientation of the plot centers for each month, the figures had similar patterns. Only the spring (April and May) values in the OF, when both of the TR and MW were inundated, did not follow the general pattern.

Conclusions

Results of this study generally support the findings of Gilliam (1995) concerning increases in the soil NH_4^+ pool in the MW site. Gilliam (1995) predicted that there would be an increase in pools of available N following inundation of terrestrial soils, predominately (>99%) in the form of NH_4^+. The N pools did increase substantially during the present study (>200%). However, although predominant, NH_4^+ averaged only 84% of the total extractable N pool. Gilliam's (1995) observation of changes between the sand and clay components of soil texture of hydromorphic, but formerly terrestrial, soils following inundation during mitigation wetland creation were supported by the differences in texture analysis of OF and MW soils in this study.

A notable difference in the field and analytical approach taken by Gilliam (1995) and the present study is that the earlier study analyzed air-dried soils; whereas, this study extracted and analyzed moist soils. Therefore, the similarities in conclusions of the two studies with different analytical conditions contradict the conclusions Gilliam and Richter (1985, 1988), who predicted that air-drying soils would have pronounced effects on extractable N and that this effect would be related to the oxidation-reduction (redox) status of the soil.

Results also support the idea that the mitigation wetland soils are developing towards true wetland (hydromorphic, *sensu* Buol and Rebertus 1988) soils after only ~1 yr of inundation. Conditions in these soils are distinctly reducing, causing a significant change in the N dynamics which are typical of hydromorphic soils (Mitch and Grosselink 1993). The net accumulation of NO_3^- in the extractable N pool in the MW was unexpected, but was also reported by Cole and Lefebvre (1991) in young surface mine wetlands that had experienced large decreases in water levels as had occurred in the MW during this study. Since N dynamics change rather rapidly following the creation of anaerobic conditions, substantial further changes in the MW soils are not expected. When the wetland plant population becomes established in the MW, it may cause minor fluctuations in the current N transformation rates and N pool levels.

Soils in the TR site, which have experienced seasonal fluctuations in inundation and exposure, have had fluctuations in redox conditions and exhibited a high degree of plasticity. Nitrogen transformations in the TR soils had distinct responses to inundation and exposure. Under inundation, nitrification was suppressed and resulted in a substantial amount of net N mineralization being provided by organic N mineralization ending with ammonification. Additionally, the soil NH_4^+ pool was larger than the NO_3^- pool.

These soils responded very rapidly to exposure and changed from hydromorphic to terrestrial N response characteristics in less than one month. Exposure resulted in the reversal of the dominant factor in N mineralization and the soil N pool proportions. Neill (1995) found similar changes in N transformations associated with exposure in a spring flooded prairie marsh and that these changes in N transformations associated with exposure seemed to increase plant growth as measured by increases in biomass. Likewise, plant growth in the TR had a rapid response to soil exposure. The N pool level declined drastically while plant growth was rapid and abundant. Plants in the TR appeared to have grown faster, had higher cover, and created more biomass than the OF plants during the same period.

The separation of the different months through PCA suggested seasonal trends in response to variations in temperature, precipitation, and N transformations in the soil. All three site types displayed a similar pattern of seasonality. Continued research is needed to establish if the patterns were unique to this study period or long term and if the importance of the different variables is consistent on an annual basis.

Future research at this site could give valuable information on the ability of these soils to maintain plasticity over long term cycles of inundation and draw-down periods. If further research is conducted on the N transformations of this site, it would probably prove useful to expand the data set to more fully appreciate the fluxes and trends of the different site types. This additional data collection might include splitting sample depth at 0 - 1 cm and 1 - 5 cm, daily (high and low) soil temperature in each site type, daily (high and low) surface and bottom water temperature as applicable, site collected weather data, and a more detailed investigation into plant species, abundance, and growth.

Many factors affect N transformations, and the changes evident from this thesis suggest a need for expanded and continued study of the mitigation area. Furthermore, similar data collection from the Green Bottom swamp could provide useful baseline data for comparison. This information would greatly further our understanding of the N biogeochemistry of terrestrial, transitional, and wetland soils, and the changes that occur in the process of wetland creation.

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