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EFFECT OF MANAGEMENT PRACTICES ON DENITRIFICATION IN SOILS FERTILIZED WITH LIQUID SWINE WASTE

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ABSTRACT

North Carolina has experienced contemporary explosive growth in the number of large scale swine production facilities that utilize anaerobic lagoons and spray fields for waste storage and disposal, respectively. Liquid lagoon effluent is applied as an organic fertilizer to spray fields to meet the agronomic N requirement of the host crop. Denitrification is a soil microbial process that converts nitrate largely to inert N_2 gas, thus reducing undesirable offsite transport of biologically available N unassimilated by the cover crop. This one year study evaluated the effect of frequency of fertilization (weekly, biweekly or monthly) on denitrifying enzyme activity (DEA) and related microbial processes (nitrifying enzyme activity; N-mineralization) in agricultural fields previously fertilized with liquid swine waste for one and ten years. The overall aim was to identify waste management practices that promote denitrification. Denitrifying enzyme activity was not significantly influenced by fertilization frequency, but was significantly influenced by season and fertilization history. The older field showed values of DEA that were consistently 2 to 9-fold higher (100 to 300 ng N_2O-N g_{dw}^{-1} h^{-1}) than values in the younger field, while DEA averaged 2-fold higher in the summer than in the winter months in both fields. Nitrifying enzyme activity, net N-mineralization and net nitrification were also about 1.5-fold higher in the older field, ranging from 10 to 70 ng NO_2^--N g_{dw}^{-1} h^{-1}, 0 to 1.0 □g NH_4^+-N g_{dw} d^{-1} and 0 to 1.0 □g NO_3^--N g_{dw} d^{-1}, respectively. Strong correlations between net N-mineralization and net nitrification in both fields in concert with high summer DEA suggest that denitrification in anaerobic soil microzones is a potentially significant pathway for removal of effluent-N in a brief post-spray period when anaerobic soil microzones are likely to occur.

(liquid swine waste, organic fertilizer, nitrogen cycling, denitrification, nitrification)

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SUMMARY AND CONCLUSIONS

No effects of fertilization frequency on denitrifier enzyme activity (DEA), nitrifying enzyme activity (NA), net nitrification and net N-mineralization were found in this study. The prescribed fertilization scheme was deviated from too often, and effluent loads delivered to the study plots were too uneven to assign any meaning to the significant differences found among treatments. Better control of fertilization frequency in the field would be most desirable; however, factors such as adverse weather conditions and equipment malfunction are unavoidable.

Season had a significant effect on DEA. DEA was highest in summer when soil temperatures were high, substrate (NO_3^--N) availability was highest, and water-filled pore space (%WFPS) was low. These results suggest that denitrifying enzymes were preserved in soil under desiccated conditions.

Season had a significant effect on NA. Unlike DEA, NA was highest in winter. The results of this study do not provide conclusive evidence to explain this trend, although correlation of NA with pH at both sites could be partially responsible.

Season appeared to have an effect on net nitrification and net N-mineralization at Site B. Rates of both of these processes were qualitatively highest in summer.

All soil microbial processes were higher at Site B, which had a ten year fertilization history, as opposed to the one year fertilization history of Site A. This trend was most apparent with DEA, which was significantly higher at Site B for every month during the study. These results indicate that it takes several years to develop a large population of soil bacteria, which increase as soil organic matter, total-C and total-N increase.

Strong correlation of net N-mineralization and net nitrification at both sites, plus high summer DEA at both sites, suggest that denitrification in anaerobic soil microzones could have been a significant pathway of effluent N removal for a brief post-spray period, when saturation of soil microzones is likely to occur.

RECOMMENDATIONS

Results of this study were inconclusive with respect to the overall aim; namely, an evaluation of the potential for denitrification as a function of spray regime. High spatial variability in denitrifying enzyme activity and nitrifying enzyme activity suggest that even if the spray schedule were strictly observed, a prohibitively large number of samples would be necessary to statistically demonstrate a signinficant treatment effect. Further, mechanical malfunction and unpredictable weather render it impossible to adhere to a planned spray schedule. Accordingly, we do not recommend that the study be repeated.

This study did, however, demonstrate a high potential for denitrification in spray fields and strongly indicated that age of the spray field may be an important factor in determining the denitrification potential. This could have significant ramifications for offsite transport of applied waste from the perspective that an aged field with an established N cycling microbial consortium may be more efficient at denitrification, thereby minimizing N loss from the application site via leaching to ground water or runoff to surface waters. Consequently, we recommend a survey study of the potential for denitrification along a continuum of spray fields of known age to determine if age is positively correlated with the denitrification potential. An investigation of this nature would indicate whether age of the spray field should be considered in conjunction with the agronomic rate in determining an allowable N load to a receiving field.

1. INTRODUCTION

Agricultural Influence on Surface Water Quality

At levels ranging from national to local, attention of public and regulatory agencies has increasingly focused on identification and mitigation of nonpoint pollution. Agriculture is a major nonpoint polluter of aquatic ecosystems in the U.S. through discharge of suspended sediments, pesticides, commercial fertilizers and organic fertilizers and wastes. Nationwide, agricultural stresses from these pollutants affect 58% of surveyed lake area, 55% of surveyed river length, and 21% of estuarine systems (USEPA 1990). Agricultural nonpoint source pollutants are a particular problem in North Carolina. Citing North Carolina Department of Environmental Management statitistics, Lilly (1990) estimates that 96% of stream degradation in the state is caused by nonpoint source pollutants and that agriculture is responsible for 67% of that total.

Nitrogen fertilizers are of particular concern as agricultural nonpoint pollutants because N is a primary limiting nutrient to terrestrial and aquatic ecosystems (Vitousek and Howarth 1991) and because use of N fertilizers has accelerated markedly in recent years. Roughly half of the nitrogen fertilizer used worldwide in human history through 1992 has been applied since 1982 (Vitousek 1994). The U.S. has contributed substantially to this global increase in fertilizer consumption. In 1993, 10.3 Tg yr^{-1} of N-fertilizer was applied to U.S. crops, a figure that represents a 20-fold increase over the 1945 application rate (Puckett 1995). Nitrogenous fertilizer consumption will continue to increase worldwide in the near-term. The 1989 rate of application is projected to double by 2020 (Galloway et al. 1995).

Surface water loss to estuarine and coastal waters is the most well-documented fate of unassimilated fertilizer N for exorheic regions (Correll and Ford 1982; Fleischer et al. 1987; Brockmann et al. 1988; Smetacek 1991; Billen et al. 1991), although aeolian transport of volatilized NH_3 and other reactive N compounds is increasingly recognized as an important external source of aquatic N loading (Fisher and Oppenheimer 1991; Hinga et al. 1991; Paerl and Fogel 1994; Paerl 1995; Paerl et al. 1995, 1998). Agricultural N input has been identified as a major cause of accelerated eutrophication of estuarine and coastal waters worldwide (Nixon 1995). In North Carolina, agricultural activities have been implicated in the general deterioration of water quality in the Neuse River Estuary (Copeland and Gray 1989; Paerl et al. 1995, 1998; Burkholder et al. 1997; Mallin et al. 1997; Mallin 2000) and the Albemarle-Pamlico watershed in general (Stanley 1992).

Swine Production and Waste Management in North Carolina

North Carolina Department of Agriculture (2000) statistics indicate that North Carolina ranks second among swine producing states and further indicate that production and inventory increases have followed a national trend toward consolidation and specialization of farms. This growth is geographically localized in the Coastal Plain and Piedmont, largely in Duplin, Sampson, Wayne and Bladen Counties. Water quality nondischarge rules for livestock farms in North Carolina (2H .0200 rule) require that swine farms with waste management systems

designed to serve 250 or more animals follow registration, approval and certification procedures which generally include a plan for storage of swine wastes in anaerobic lagoons and land application of the waste (Barker et al. 1994). Organic animal wastes have fertilizer value. However, contents of swine waste lagoons are viewed more as a pollutant-disposal problem than as a fertilizer, as host crops grown on land-applied effluent are not agressively marketed (Dr. James Barker, Professor and Extension Specialist, NCSU, pers. com. 1996). Although reliable statewide statistics for the number of swine waste lagoons and total land area to which lagoon effluent is applied are unavailable, "best guess" estimates are impressive; roughly 3500 swine producing operations encompassing 101,000 ha are registered with the North Carolina Department of Environment and Natural Resources Management (Lou Polletta, DEM, pers. com. 1996). Inasmuch as swine growth occurs in confined facilities, it is reasonable to infer that much of the total land area dedicated to production is occupied by lagoons and field crops fertilized with lagoonal effluent.

The explosive growth in the swine producing industry poses a formidable challenge with regard to disposal of manure. Statewide, the current rate of swine waste production is 8 Tg yr^{-1}, which includes 48 Gg N (Crouse 1995). Uninformed or improper waste management may encourage offsite transport, leading to enhanced N loading in adjoining ecosystems, particularly rivers, estuaries and coastal waterways such as the Neuse. Clearly, agricultural water quality priorities relative to disposal of swine lagoon effluent must include development and implementation of best management practices (BMPs) and land use policies that both minimize loss of plant-available N from the site of land application and meet the nutritional N requirements of the host crop.

Fate of Nitrogenous Material in Land-Applied Liquid Swine Waste

Nitrogenous material in anaerobic lagoonal swine waste consists entirely of organic-N (dissolved and particulate) and NH_4^+-N (Kirchmann 1994). This nutrient has several potential fates following land application (Figure 1.1). Briefly, organic-N can be microbially decomposed to NH_4^+. Ammonium can be immobilized in the soil cation exchange complex, lost to the atmosphere via volatilization (NH_3) or oxidized to NO_3^--N by nitrifying bacteria in aerobic soil zones. Nitrate can be reduced to N_2 (and to a much lesser extent N_2O) by denitrifying bacteria in anaerobic microzones, with the gaseous end products again lost to the atmosphere. Dinitrogen gas is availabe as a plant and microbial nutrient only to a few specialized groups of N-fixers and symbionts. Among other factors, rates of denitrification are dependent microbial release of NH_4^+-N from decomposition of organic materials and subsequent nitrification of NH_4^+-N to NO_3^--N. Inorganic-N (NO_3^- and NH_4^+) is available for plant consumption and all organic and inorganic species are subject to export from the ecosystem via surface and ground waters, although NO_3^- shows the most mobility.

Microbial Denitrification as an Ecosystem-Level Sink for External Nitrogen Inputs

Microbial denitrification is an important N sink (pathway of export) in all ecosystems. It is

a respiratory process where electron transport phosphorylation is coupled to the sequential reduction of nitrogenous oxides ($NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2$) by facultative anaerobes. Globally, an estimated 65% of total N input to terrestrial environments is denitrified (Berner and Berner 1987), while studies of lacustrine and estuarine ecosystems (summarized by Seitzinger 1988) show denitrification rates equivalent to 1 to 36% and 20 to 50%, respectively, of N input rates.

Environmental controls on denitrification have been reviewed in depth by Tiedje (1988). Inasmuch as denitrifiers are facultative heterotrophs, soil atmosphere O_2 concentration is the major control on denitrification in terrestrial environments. Soil moisture content is often considered a proxy for soil atmosphere O_2, because it promotes consumption and retards gas diffusion. Denitrification is also regulated by the availability of NO_3^- and metabolizable organic-C. Nitrate supply is frequently determined by rates of aerobic processes, namely N mineralization (org-N $\rightarrow NH_4^+$) and nitrification ($NH_4^+ \rightarrow NO_3^-$). The latter is an energy-yielding activity for specialized chemoautotrophs (nitrifying bacteria) and microbial nitrification and denitrification are coupled at oxic-anoxic interfaces. Seasonal changes in quality and quantity of organic matter and decomposition rates can effect shifts between NO_3^--N and C limitation of denitrification (Aulakh et al. 1992). In summary, regulating factors interact to create "hotspots", anaerobic soil microzones where substrate (NO_3^- and metabolizable C) supply is adequate to support denitrification in what is otherwise oxic soils.

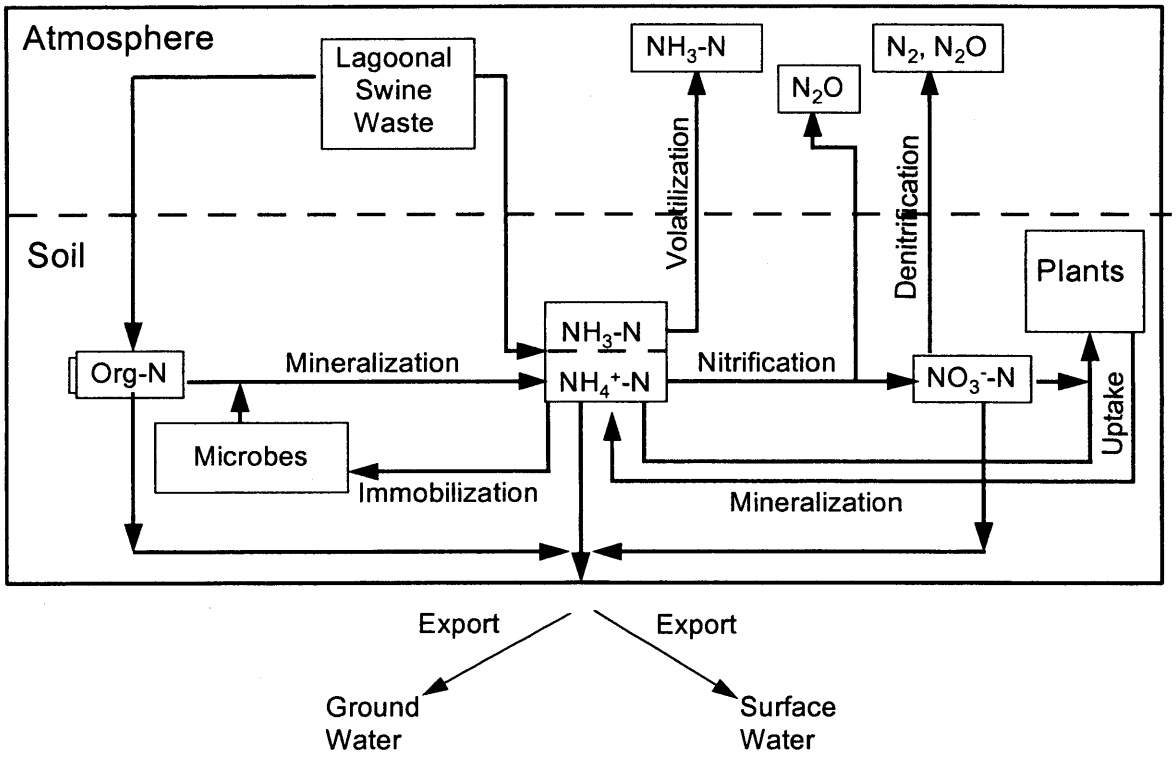


Figure 1.1. Fate of nitrogen applied to agricultural crops as lagoonal swine waste.

Denitrification as a Sink for Fertilizer-N in Agroecosystems

Agricultural crops are inefficient at using fertilizer. On average, 36% of N applied in commercial fertilizers, crop residues, manure and other organic wastes is recovered by crops (Power 1985) and the balance is potentially available for transport from the site of application. Efficiency of plant utilization is lower for organic animal wastes than for commercial fertilizers. In a review of the few N mass balance studies involving land application of animal wastes, Kirchmann (1994) reported that 15-30% of the N was assimilated into crops, 15-45% remained in the soil and 40-60% was lost from the soil.

Denitrification is a potentially important sink for unassimilated fertilizer-N in agroecosystems. Losses for commercial fertilizers range from 2.5 to 50% of applied N (summarized by Nieder et al. 1989). There are fewer data for manures and organic wastes, but limited extant information suggests that percentage losses of applied N to denitrification are similar for animal wastes and commercial fertilizers. Thompson et al. (1987) found losses of 2 to 21% for N applied in cattle waste slurries, depending on season and method of application, while Maag (1989) and van den Abeel et al. (1989) showed losses as high as 50% and 30%, respectively, for animal waste slurries.

Despite the scarcity of information for percent loss of N in animal waste fertilizers to denitrification, several lines of evidence indicate that denitrification can be a significant N sink for swine lagoon effluent applied to fields. First, numerous studies (Burford 1976; Guenzi et al. 1978; Reddy et al. 1980; Christensen 1983, 1985; Thompson et al. 1987; Rice et al. 1988; Paul and Beauchamp 1989; Coyne et al. 1995) report that animal waste additions to soil stimulate denitrification. Second, the roughly equal quantities of NH_4^+-N and organic-N in anaerobically stored lagoonal swine waste (Kirchmann 1994) provide nitrogenous nutrient to immediately stimulate coupled nitrification-denitrification (NH_4^+-N) and to resupply NH_4^+-N (mineralization of organic-N) for continued activity. Third, anaerobically stored animal waste is high in energy-rich and easily decomposable carbon compounds such as volatile fatty acids (Guenzi and Beard 1981), which may compose as much as 30% of total-C in swine waste (Cooper and Cornforth 1978). Volatile fatty acids in animal wastes serve directly as a C source for denitrifiers (Paul et al. 1989), while aerobic decomposition of such readily mineralizable organic compounds enhances denitrification by creation of anaerobic microsites (Burford and Bremner 1975; Paul and Beauchamp 1989). Finally, liquid waste will increase soil water-filled pore space at the expense of air-filled pore space, further favoring development of anaerobic microzones.

BMPs for disposal of lagoonal effluent in North Carolina require application at no more than the agronomic N requirement of the host crop, that is in a defined quantity that only ensures plant nutritional needs are met. Within these guidelines, swine operation managers have flexibility regarding selection of host crop and frequency of waste application. The major end product of denitrification is N_2, an inert gas that is the primary constituent (~79%) of the atmosphere. Thus, management practices that simultaneously promote denitrification and meet the N requirements of the cover crop are beneficial in the respect that they remove from the soil as N_2 excess plant-available nitrogenous nutrient that may otherwise be available for export from the ecosystem via ground or surface waters.

Scope and Objectives of Research

This study focused on microbial denitrification as a beneficial mechanism of agroecosystem N export, because the end product of denitrification is largely the chemically and radiatively inert gas N_2. The purpose of the project was to assess the influence of management practices (frequency of fertilization) on denitrifying enzyme activity (DEA) and associated microbial processes (N-mineralization and nitrification) that supply NO_3^--N for denitrification. Since rates of nitrification and especially denitrification show high spatiotemporal heterogeneity (Folorunso and Rolston 1984) on meter and hour scales, it is impossible to accurately estimate *in situ* rates of these processes at field and annual scales. Therefore, we focused on *potential* rates of denitrification (DEA) and nitrification (nitrifier activity, or NA). DEA and NA estimate the potential *in situ* denitrification and nitrification rates when microbial populations are not limited by substrate supply or other environmental factors. Our rationale was that if DEA and NA were maximized, then the percentage of applied N that is denitrified *in situ* would, in general, increase. Accordingly, N available for environmentally harmful fates would be reduced.

Three fertilization frequencies (weekly, biweekly, monthly) delivering the same amount of N on an annual basis were evaluated to determine their influences on DEA, NA and net nitrification and net N-mineralization. The overall objective was to identify the most beneficial fertilization scheme in terms of maximizing the potential for denitrification, thereby providing information that would be useful for modifying and developing BMPs.

2. MATERIALS AND METHODS

Study Site Description

The study site was a farrow to half-finish facility in Sampson County operated by a major North Carolina swine producer (Figure 2.1). The soil type is a well-drained Blanton sand (Brandon 1985). The waste management practice employed is representative of the industry. Swine waste is stored in an anaerobic lagoon and applied to host crops throughout the year with a solid set sprinkler system. Soils are planted with a summer crop of hybrid coastal Bermuda grass (*Cynoden* sp.) during May through October, and are sometimes overseeded with tall fescue (*Festuca* sp.) in the winter. Both crops are fertilized with lagoonal waste according to the agronomic N requirement of these species, 20 to 22 kg (dry tonne)$^{-1}$ yr^{-1} (Zublena et al. 1993). Application frequency and biannual analysis of plant-available N in lagoon effluent determine the volume of application. During the growing season, host crops are most commonly fertilized on a monthly basis. The volume of application may be adjusted for biweekly or weekly fertilization to give the same monthly N loading rate.

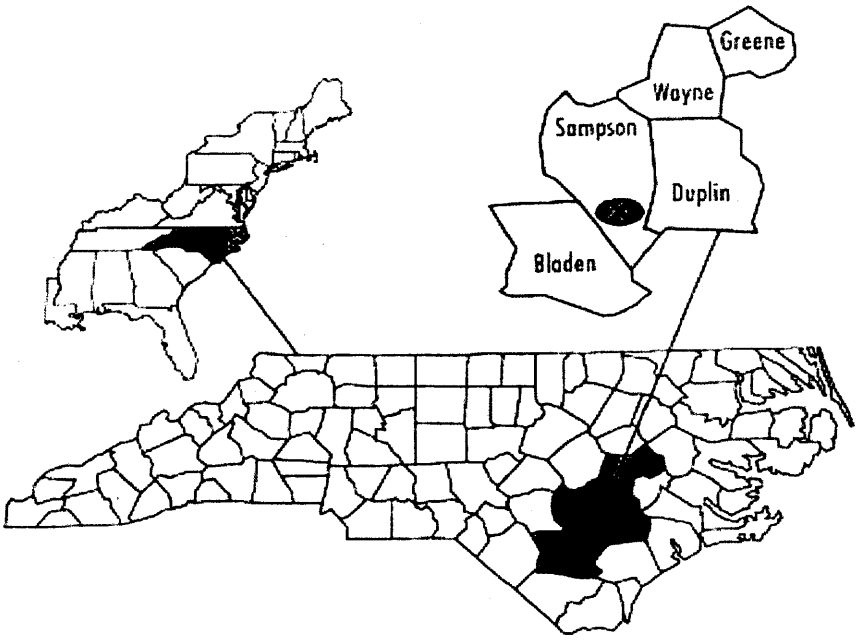


Figure 2.1 Location of the study site in eastern North Carolina.

Two spray fields were selected at the study site, one having been fertilized approximately one year (Site A), the other having been fertilized for approximately 10 years (Site B). Both sites were fertilized from a common lagoon. The two sites were divided into sprinkler zones that allowed for within-site manipulations of frequency and volume of application (Figure 2.2). Each field was randomly separated into monthly (M), biweekly (B) and weekly (W) fertilization zones. This yielded a total of six treatments (AW, AB, AM, BW, BB, BM), where the first and second letters designate the field and frequency, respectively.

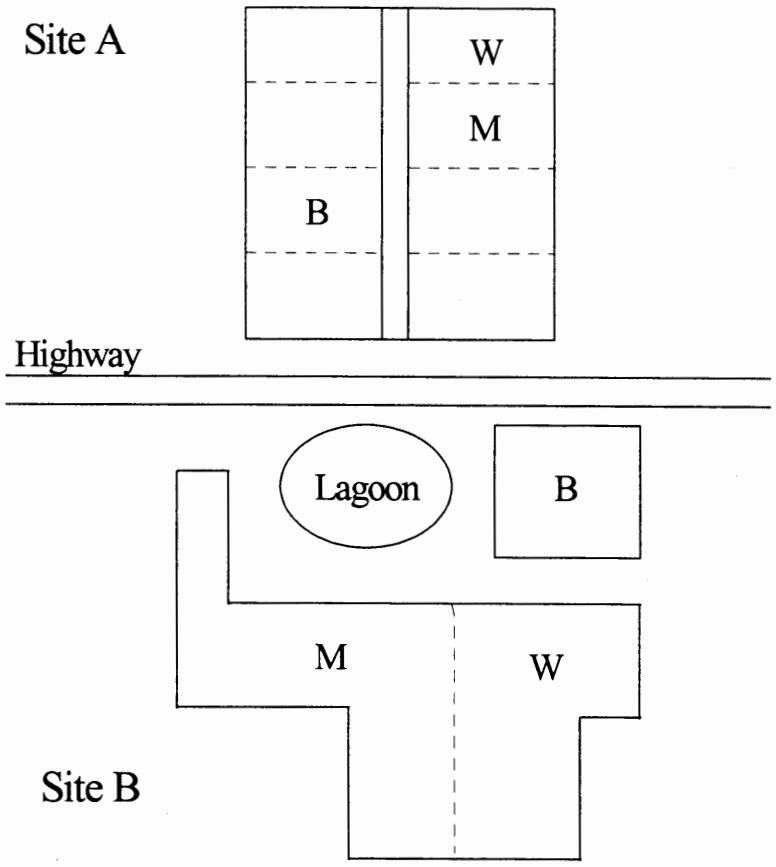


Figure 2.2. Layout of the study site. Sprinkler zones are separated by dashed lines.

Pretreatment data were collected in December 1996 to determine which soil layers were biologically active in terms of DEA and NA, and to identify spray zones showing equivalent DEA and NA. Post-treatment data were collected January through December 1997. On a monthly basis, six 5.5-cm diameter cores were collected randomly to a depth of 20 cm within each treatment for a total of 36 cores. Cores were transported on ice to the laboratory where each was sieved (2-mm mesh) and homogenized. Two composites were made with aliquots of soil for each treatment (3 cores per composite), yielding 12 total composites. Each was analyzed for soil physicochemical properties.

Soil Physical Properties

Soil texture and particle density (D_p) were determined hydrometrically (Gee and Bauder 1986) and pycnometrically (Blake and Hartge 1986). Gravimetric moisture content was determined by oven-drying approximately 200 g of each homogenized soil core at 105°C for 24 hours (Gardner 1982). Soil bulk density (D_b) was computed as the quotient, oven-dried mass divided by field volume. Percent water-filled pore space (%WFPS) was determined for each soil core as the ratio

of volumetric soil water content to total soil porosity. Total porosity was computed from the bulk and particle densities as $(1 - D_b/D_p)100$. Soil temperature at a depth of 5 cm was measured monthly at Site A using a thermistor probe and an Omega Model 866 digital display unit.

Soil Chemical Properties

Field moist subsamples (10 g) of each composite were extracted with 2 M KCl (5 or 10:1 volume/solid weight) and analyzed colorimetrically (Shimadzu Model UV-1201V spectrophotometer) for NO_3^--N (Cu-Cd reduction method) and NH_4^+-N (indophenol blue method) according to Keeney and Nelson (1982). Concentrations are expressed per g dry soil mass (g_{dw}^{-1}). Soil pH was determined on a 1:2 soil/water solution, equilibrated overnight. Total-N and total-C were determined by dry combustion (Carlo Erba NA 1500 Elemental Analyzer). Organic content was determined by loss-on-ignition from oven-dried soil (Ben-Dor and Banin 1989).

Denitrifying Enzyme Activity

Denitrifying enzyme activity (DEA) was determined according to Tiedje's (1994) short-term anaerobic assay procedure. A field moist soil sample (25 g) from each homogenized core (6 soil cores per treatment) was placed in a 133-cc jelly jar (nominal volume) equipped with an O-seal fitting to permit introduction and withdrawal of gases. Samples were amended with 25 ml of a nutrient medium consisting of 1 mM glucose, 1 mM KNO_3, and 1 g L^{-1} chloramphenicol. Jars were made anaerobic by repeatedly evacuating/filling with N_2 and vigorously shaking. Headspaces were then adjusted to 10 kPa C_2H_2 with welding grade C_2H_2 that had been scrubbed of contaminants in a purifying apparatus consisting of two concentrated sulfuric acid traps, a dryer (drierite, $CaSO_4$) and a collection vessel (Hyman and Arp 1987). Acetylene was equilibrated in the aqueous phase by shaking the jars vigorously by hand. Soils were incubated on a rotary shaker (150 rpm) at room temperature, and gas samples were withdrawn at 0.5 and 1.5 hours. The denitrification rate was determined to be linear to at least 1.5 hours. Gas samples were collected in 5-ml PP/PE or nylon syringes and immediately analyzed for N_2O with an electron capture gas chromatograph (Shimadzu GC-14A) under the following operating conditions: carrier gas 5% CH_4/95% Ar; flow rate 25 mL min^{-1}; 1-m Porapak Q precolumn (50/80); 3-m Porapak Q analytical column (50/80); column temperature 40°C; injector/detector temperature 325°C; current 2 nA. Acetylene at 10 kPa inhibits N_2O reduction to N_2 by denitrifiers, while chloramphenicol inhibits de novo enzyme synthesis. A relative measure of DEA, or potential denitrification, was provided by the rate of total N_2O accumulation (aqueous and gas phase) under the optimal conditions supplied by slurry amendments. Aqueous phase N_2O accumulation was determined using the Bunsen solubility coefficient (Weiss and Price 1980). Data are expressed as total N_2O accumulation on a dry soil mass basis.

This assay does not give an estimate of the instantaneous denitrification rate in the field. Rather, it gives an estimate of soil denitrifying enzyme concentration at the time of sampling. The principle is that the rate of accumulation of N_2O (potential denitrification) in the incubation vessel is proportional to the soil enzyme concentration when no other factors are limiting. The *in*

situ denitrification rate of a soil is difficult to measure due to high spatial and temporal heterogeneity (Parkin 1987).

Short-term Nitrifier Activity

Short-term nitrifier activity (NA) was determined by the ClO_3^- inhibition method (Schmidt and Belser 1994). This assay provides optimum conditions of O_2 and substrate for nitrifiers, and the incubation time is short enough to avoid increases in the standing population during experimentation. Field moist soil samples (20 g) were placed in 250-mL flasks, amended with 90 mL phosphate buffer, 0.2 ml 0.25 M $(NH_4)_2SO_4$ and 2 mL 1 M $KClO_3$. Flasks were placed on a rotary shaker at room temperature and 3.5 ml samples were withdrawn at 12 and 24 hours, filtered (0.8 μm Metricel filters) and analyzed colorimetrically for NO_2^--N according to Keeney and Nelson (1982). NA (dry soil mass basis) was calculated from the increase in NO_2^--N, which was determined to be linear over 24 hours.

In unamended soil, nitrification can be limited by the rate of NH_4^+-N formation from mineralization of organic-N. In this assay, nitrifiers are not substrate-limited because NH_4^+-N is provided in excess by the medium. Chlorate blocks oxidation of NO_2^- to NO_3^-, hence this assay gives an indication of the soil NH_4^+-N oxidizer population and the potential for nitrification in the soil. NA and DEA bioassays compliment each other, since nitrifying bacteria provide substrate (NO_3^--N) for denitrifiers.

Net N-mineralization and Net Nitrification Rates

Net N-mineralization and net nitrification were measured by the buried bag technique (Peterjohn et al. 1993). Fifteen soil cores from each treatment were collected, placed intact in gas permeable polyethylene bags of 20.3 μm thickness (Gordon et al. 1987), and incubated *in situ* for one month. On recovery, the 15 cores were homogenized into 5 composites (3 cores per composite). These composites were assayed for KCl-extractable NH_4^+-N and NO_3^--N. The change in NH_4^+-N plus NO_3^--N during the incubation gives the rate of net N-mineralization, while change in NO_3^--N concentration gives the rate of net nitrification. Both are expressed on a dry soil mass basis. Data for net N-mineralization and net nitrification provided an indication of rates of N supply to denitrifiers under the three fertilization frequencies.

Lagoon Samples

Monthly samples of lagoon effluent were analyzed for NH_4^+-N as described previously, and total-N (persulfate oxidation, followed by Cu-Cd reduction) according to Solorzano and Sharp (1980).

Statistical Analyses

Statistical analyses were performed using SAS (SAS Institute 1996). All statistical analyses were performed at $\alpha = 0.05$. In testing for seasonal differences, post-treatment months were divided into winter (January, February, November, December 1997), spring/fall (March, April, May, October 1997) and summer (June, July, August, September 1997) groups according to monthly mean soil temperature at a 4 cm depth from the Clinton Weather Station

(http://www2.ncsu.edu/unity/lockers/ftp/kbp/soilCLN7.html) (winter < 10°C, spring/fall 10 to 17°C, summer > 17°C). Analysis of mean seasonal data follows these temperature divisions.

Analysis of Variance (ANOVA) was performed using the General Linear Model Procedure (PROC GLM) for Analysis of Variance. ANOVA was used to evaluate effect of treatment on DEA and NA. Normal distribution of the data was verified using PROC UNIVARIATE. The t-test procedure was used to determine differences in annual means of soil physicochemical properties.

Homogeneity of variances was determined using Bartlett's test (Zar 1984). DEA and NA data were log-transformed (x^i =log x) before statistical analyses in SAS were performed. Precision of analyses of the analytical methods used in this study are given in Table 2.1.

Table 2.1. Analytical precision for repeated determinations of variables measured in this study. CV = coefficient of variation.

Analysis	**n**	**Mean**	**CV**
Total-N (%)	5	0.059	14.0
Total-C (%)	5	0.94	14.0
WFPS (%)	5	22.9	1.3
Organic Content (%)	5	3.32	4.0
DEA (ng N_2O-N g_{dw}^{-1} h^{-1})	5	30.6	10.9
NA (ng NO_2^--N g_{dw}^{-1} h^{-1})	5	30.6	7.6
Net Nitrification Rate (μg NO_3^--N g_{dw}^{-1} d^{-1})	5	13.9	4.4
Net N-mineralization Rate (μg NH_4^+-N g_{dw}^{-1} d^{-1})	5	1.3	14.4
Total-N, Lagoon (mg N L^{-1})	5	355	18.0
Soil NH_4^+-N (μg NH_4^+-N g_{dw}^{-1})	5	1.4	10.7
Soil NO_3^--N (μg NO_3^--N g_{dw}^{-1})	5	3.5	6.6

3. RESULTS

Preliminary Data

A sample survey conducted in November 1996 indicated that soils at Sites A and B had similar physical characteristics, showing a bulk density of 1.56 g cm^{-3}, an average particle density of 2.55 g cm^{-3}, a total porosity of 37% and a texture of 79% sand, 17% silt and 4% clay. Soils at the two sites did differ somewhat with respect to overall concentrations and depth distributions of organic material. In general, percent organic matter, total-C and total-N showed more homogenous depth distributions and lower concentrations throughout the soil profile at Site A than at Site B (Figure 3.1). Differences were most apparent in the 0 to 10 cm zone where Site B showed an organic content of 2.59%, compared to 1.66% for Site A. Similarly, values for percent C and percent N were about four-fold higher at Site B (1.99% and 0.16%, respectively) than at Site A (0.62% and 0.04%, respectively) in the surface 10 cm of soil. Differences likely stem from the land use history. Site B had been fertilized for 10 years prior to this study, while Site A had been in use for only one year.

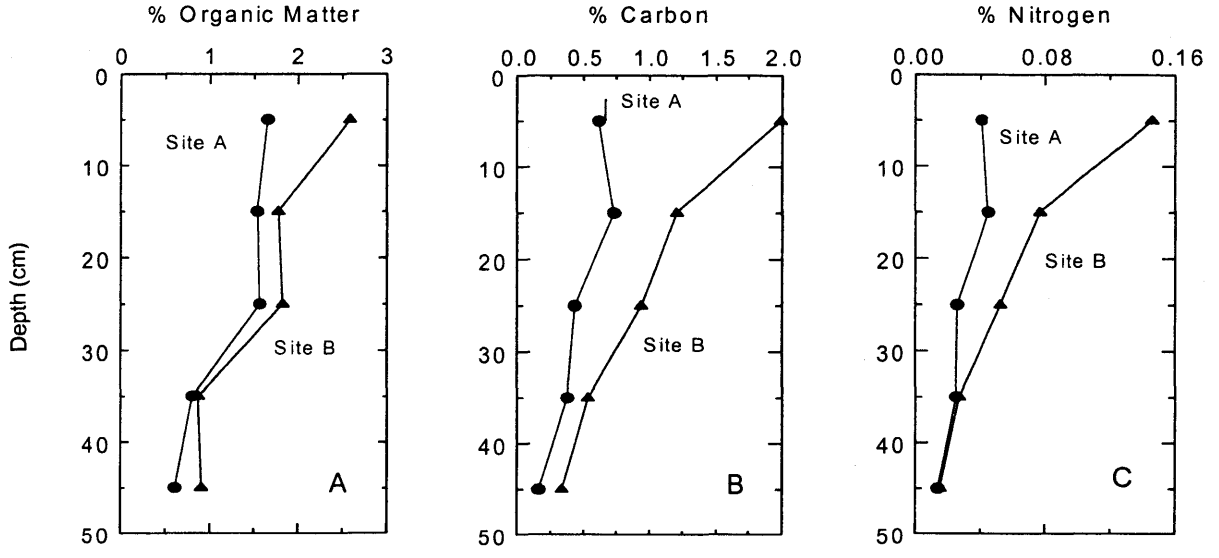


Figure 3.1. Depth profiles of percent organic matter, total-C and total-N, November 1996. Data for duplicate cores for each site are plotted at the midpoint of each 10 cm core section.

Depth profiles for DEA (Figure 3.2) and NA (Figure 3.3) indicate that most denitrifier and nitrifier activity at both sites was localized in the 0 to 20 cm soil zone, and decreased sharply with depth to 50 cm. Eighty-two percent of DEA and 86% of NA occurred in the top 20 cm of soil at Site A, compared to 96% (DEA) and 86% (NA) at Site B. NA and DEA at Site B were about 2 and 10-fold higher, respectively, than at Site A, indicating that Site B was potentially more active with regard to nitrification and denitrification. Accordingly, determinations of soil physicochemical and biological properties during the study year focused on homogenized samples from the 0 to 20 cm zone ("active zone").

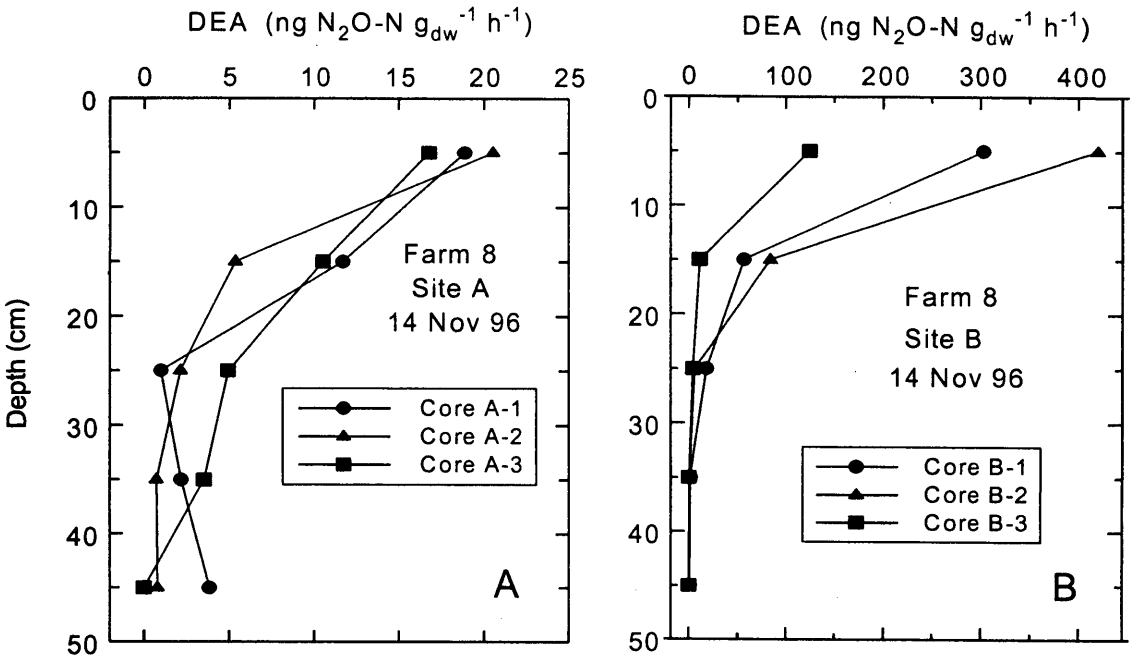


Figure 3.2. Depth profiles for DEA prior to the intensive one-year study. Data for triplicate cores are plotted at the midpoint of each 10 cm core section.

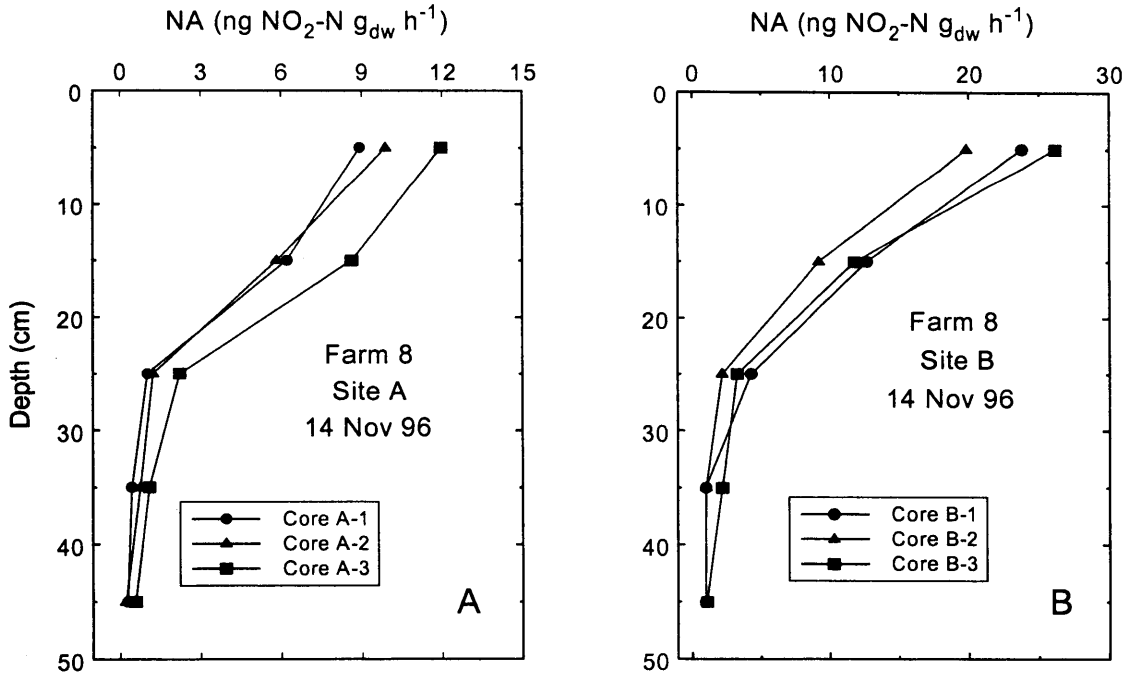


Figure 3.3. Depth profile for NA prior to the intensive one-year study. Data for triplicate cores are plotted at the midpoint of each 10 cm core section.

Individual spray zones within Sites A and B were surveyed for NA and DEA within the active soil zone. Three zones within each site showing no significant differences in DEA and NA were randomly assigned to weekly, biweekly or monthly fertilization treatments for the next year (Figure 2.2). Overall, these data for microbial activity in the active zone were consistent with the depth integrated data (Figures 3.2 and 3.3) in the respect that Site B was most active. Average NA was two-fold higher at Site B than Site A, 13 ng NO_2^--N g_{dw}^{-1} h^{-1} versus 6 ng NO_2^--N g_{dw}^{-1} h^{-1}. Similar data for DEA showed that the mean for Site B exceeded that for Site A by a factor of about five, 87 ng N_2O-N g_{dw}^{-1} h^{-1} versus 14 ng N_2O-N g_{dw}^{-1} h^{-1}.

Post-fertilization Time Course for NA and DEA

The experimental design involving three different fertilization frequencies made it logistically impossible to sample each site at a prescribed time following fertilization. Soil cores were collected on a mid-month sampling schedule throughout this year-long study. Consequently, it was necessary to determine whether time since fertilization had a significant effect on DEA and NA. This was tested with replicate soil cores collected at the Site B biweekly plot on the day before a spray event, and at periodic intervals for 11 days following fertilization (Table 3.1). DEA and NA were determined for each soil core. No significant differences were found among days for NA. One significant difference was found in the DEA experiment, between the pre-spray mean and the day 4 mean (124 and 210 ng N_2O g dw^{-1} h^{-1}, respectively). However, the day 4 data showed a large standard error of the mean with one extremely high value. This was likely a "hotspot" (see Parkin 1987). Elimination of this point gave a day 4 mean (mean $\pm$ SE) of 179 $\pm$ 23 that was not significantly different from other observations and it was concluded that time since fertilization had no influence on DEA or NA.

Table 3.1. Time course for changes in means and (standard error of the mean) for DEA and NA following fertilization of the biweekly plot at Site B. Day zero data are pre-fertilization.

	DEA (ng N_2O-N g_{dw}^{-1} h^{-1})		NA (ng NO_2^--N g_{dw}^{-1} h^{-1})	
Day	**n**	**Mean**	**n**	**Mean**
0	8	124(17)	8	46(5)
1	7	166(17)	7	42(6)
2	7	153(15)	8	59(6)
3	7	123(8)	8	69(13)
4	8	210(37)	7	39(8)
7	7	163(10)	8	45(9)
11	7	151(16)	8	41(9)

Lagoon Samples

Total-N measurements for monthly lagoon samples ranged from 172 to 469 mg L^{-1} (Table 3.2). Ammonium-N content of the lagoon samples was generally 80% or more of total-N concentrations.

Table 3.2. Total-N and NH_4^+-N concentrations for monthly lagoon samples.

Collection Date	Total-N (mg L^{-1})	NH_4^+-N (mg L^{-1})	NH_4^+-N (%)
12/11/96	171.8	143.0	83.2
1/14/97	221.4	176.1	79.5
2/12/97	253.2	226.7	89.5
3/15/97	292.0	238.3	81.6
4/16/97	357.6	314.5	87.9
5/12/97	288.8	246.9	85.5
6/16/97	226.0	117.0	51.8
7/17/97	326.0	310.6	95.3
8/16/97	309.8	248.1	80.1
9/23/97	261.6	161.6	61.8
10/21/97	238.6	210.4	88.2
11/20/97	290.2	263.7	90.9
12/15/97	469.4	462.7	98.6
Mean	285.1	240.0	82.6

Comparison of Planned and Realized Spray Events

The fertilization schedule was not strictly followed (Tables 3.3 and 3.4). Ideally, weekly treatments should have been sprayed 4 to 5 times per month, biweekly 2 to 3 times per month, and monthly once per month at evenly spaced intervals. In many cases, the actual fertilization schedule was altered due to unfavorable weather or equipment-related problems. Weekly treatment plots were sometimes sprayed just once a month, while monthly treatment plots were sometimes sprayed up to 3 times per month. Up until April of 1997, the prescribed fertilization scheme was more or less adhered to. Thereafter, fertilization deviated more from the prescribed schedule, especially at the weekly plot for Site B.

Table 3.3. Number of spray events, centimeters of effluent applied, and g N m^{-2} applied, January 1997 through December 1997, Site A.

Month	Weekly			Biweekly			Monthly		
	Events per month	cm	g N m^{-2}	Events per month	cm	g N m^{-2}	Events per month	cm	g N m^{-2}
Jan-97	3	1.1	2.4	2	1.4	3.0	1	1.8	4.0
Feb-97	4	1.4	3.6	2	1.4	3.5	1	1.8	4.6
Mar-97	3	1.1	3.1	2	1.4	4.2	1	1.8	5.3
Apr-97	4	2.1	7.6	2	2.5	8.8	3	4.5	16.1
May-97	1	0.7	2.1	2	2.5	7.1	3	4.5	12.9
Jun-97	1	0.4	0.8	0	0.0	0.0	2	4.5	10.1
Jul-97	4	3.2	10.4	5	3.5	11.5	1	1.8	5.9
Aug-97	3	2.1	6.6	3	3.2	9.8	1	2.2	6.9
Sep-97	4	2.8	7.4	2	3.5	9.2	0	0.0	0.0
Oct-97	2	1.4	3.4	5	3.5	8.4	0	0.0	0.0
Nov-97	4	2.8	8.3	2	3.1	9.1	2	2.2	6.5
Dec-97	1	0.7	3.3	1	1.8	8.2	3	3.1	14.7
	2.8[a]	**19.9[b]**	**59.0[c]**	**2.3[a]**	**27.7[b]**	**82.9[c]**	**1.5[a]**	**28.2[b]**	**86.9[c]**

[a]average number of spray events per month, January through December 1997
[b]total centimeters applied January through December 1997
[c]total g N m^{-2} applied January through December 1997

Table 3.4. Number of spray events, centimeters of effluent applied, and g N m^{-2} applied, January 1997 through December 1997, Site B.

Month	Weekly			Biweekly			Monthly		
	Events per month	cm	g N m^{-2}	Events per month	cm	g N m^{-2}	Events per month	cm	g N m^{-2}
Jan-97	3	0.8	1.7	2	1.7	3.8	1	1.3	2.9
Feb-97	4	0.9	2.4	2	1.7	4.4	1	1.3	3.3
Mar-97	3	0.9	2.7	2	1.5	4.5	1	1.1	3.2
Apr-97	3	1.2	4.5	2	1.5	5.5	2	2.2	7.8
May-97	1	0.6	1.8	1	2.0	5.7	2	0.3	0.8
Jun-97	1	0.6	1.4	0	0.0	0.0	1	1.4	3.1
Jul-97	2	0.6	2.0	1	2.0	6.5	1	1.1	3.6
Aug-97	1	0.6	1.9	3	3.6	11.0	2	0.3	0.8
Sep-97	1	0.6	1.6	1	2.0	5.2	0	0.0	0.0
Oct-97	1	0.6	1.5	2	2.0	4.7	1	1.4	3.3
Nov-97	4	2.4	7.1	1	2.0	5.7	0	0.0	0.0
Dec-97	1	0.6	2.9	1	2.0	9.3	3	2.2	10.1
	2.1[a]	**10.6[b]**	**31.2[c]**	**1.5[a]**	**22.0[b]**	**66.4[c]**	**1.3[a]**	**12.5[b]**	**39.0[c]**

[a]average number of spray events per month, January through December 1997
[b]total centimeters applied January through December 1997
[c]total g N m^{-2} applied January through December 1997

Nitrogen Loading from Fertilization

Total-N concentrations and volumes of effluent applied (from facility spray logs) were used to calculate the total-N load received by each treatment plot at each site (Tables 3.3 and 3.4). An

assumption of the study was that each treatment plot would receive the same volume of effluent (and thus N load). However, the monthly effluent volume varied widely among treatments in some months. There were no months in which all three treatments at Sites A and B received the same N load. For example, during October 1997 at Site A, the weekly treatment received 1.4 cm, biweekly received 3.5 cm, and the monthly treatment received no effluent at all.

Total annual effluent volumes and nitrogen loads show that at Site A, the biweekly and monthly treatments received close to the same N load (83 and 87 g N m^{-2}, respectively), while the weekly plot received approximately 30% less (59 g N m^{-2}). At Site B, the weekly and monthly treatments received equivelant N loads (31 and 39 g N m^{-2}, respectively), while the biweekly treatment received twice that amount (66 g N m^{-2}).

Soil Physicochemical Properties

Ranges and annual means of soil physicochemical properties for homogenized composites in the 0 to 20 cm zone are shown in Table 3.5. The mean soil pH was somewhat lower at Site B than at Site A. At Site A, soil pH increased from summer to fall 1997 at each treatment plot (Figure 3.4). At Site B, a similar trend was observed for the weekly and monthly plots, although the magnitude of the increase was less.

Table 3.5. Range and (mean) of selected soil physicochemical characteristics for the study period December 1996 through December 1997. Data are pooled for all treatments within each study site.

Soil Characteristic	Site A	Site B
pH	6.3 – 7.5 (6.8)	5.5 – 7.1 (6.4)
WFPS (%)	2 – 55 (27)	4 – 74 (30)
Organic Matter (%)	1.1 – 2.0 (1.4)	1.2 – 5.0 (2.8)
Total-C (%)	0.52 – 2.02 (0.81)	0.66 – 2.79 (1.43)
Total-N (%)	0.03 – 0.09 (0.05)	0.04 – 0.15 (0.09)
NO_3-N ($\mu g\ g_{dw}^{-1}$)	0.1 – 19.4 (4.4)	1.2 – 18.1 (6.8)
NH_4-N ($\mu g\ g_{dw}^{-1}$)	0.3 – 11.3 (2.7)	0.6 – 11.0 (2.1)

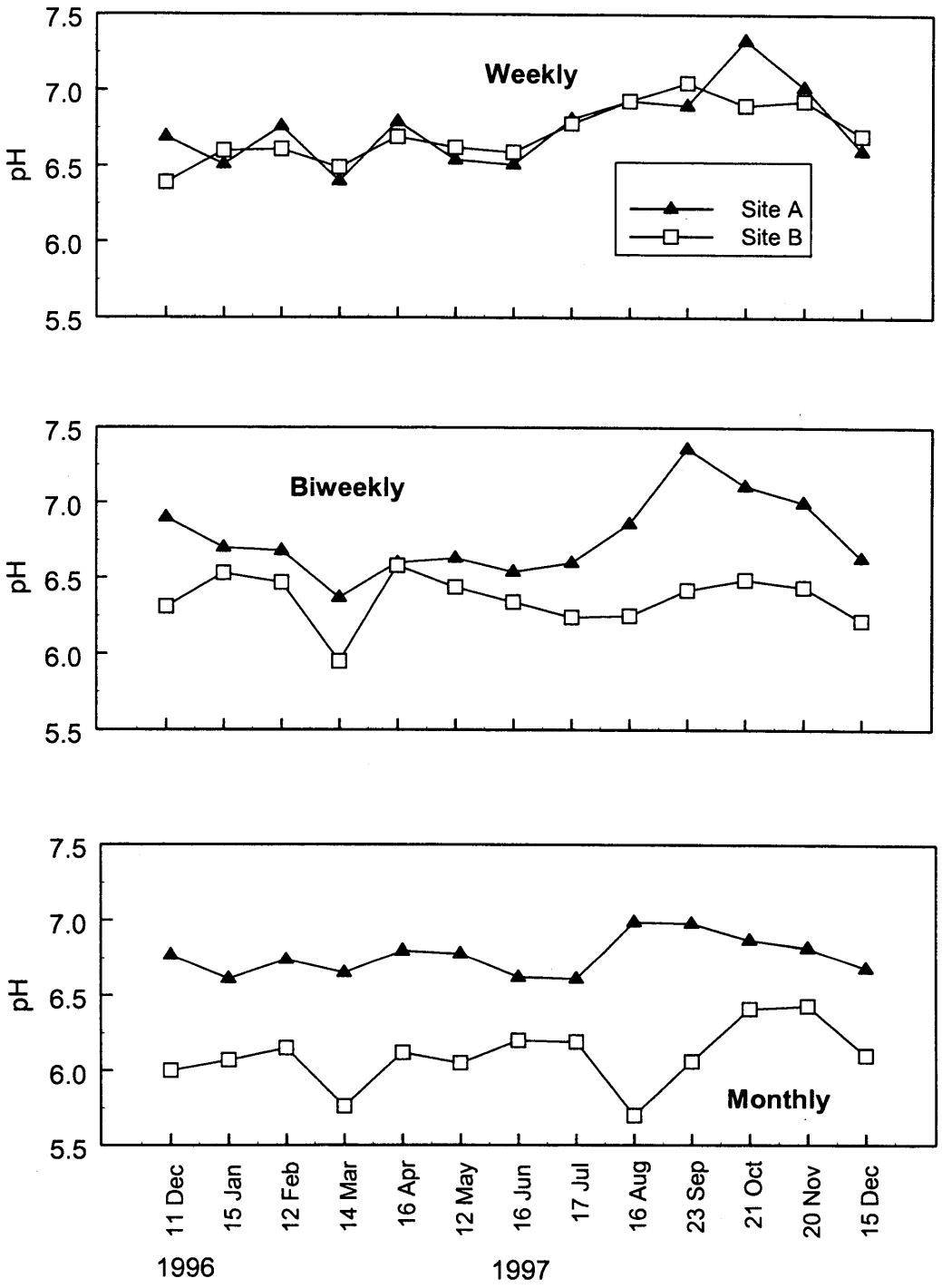
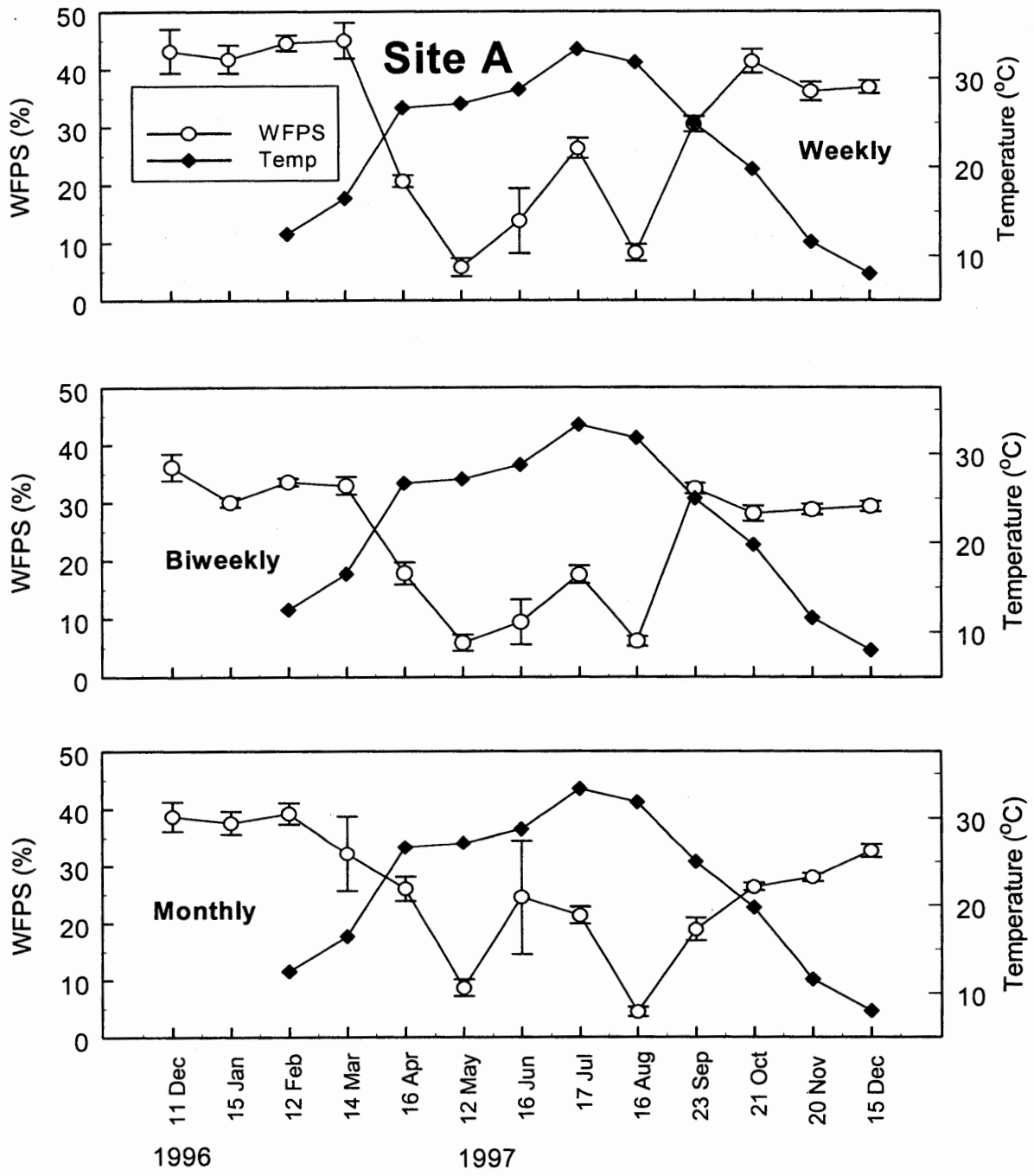


Figure 3.4. Soil pH in the 0 to 20 cm zone at Sites A and B, December 1996 through December 1997.

Sites A and B showed similar changes in %WFPS, with Site B showing somewhat higher values, notably in winter and spring 1997 (Figures 3.5 and 3.6). Percent WFPS decreased between 2 and 4-fold in summer 1997 at both sites, when soil temperature was approximately 30°C.

Figure 3.5. Soil temperature February through December 1997, and percent water-filled pore space (%WFPS) December 1996 through December 1997, Site A. Error bars



associated with %WFPS represent one standard error of the mean (n = 6).

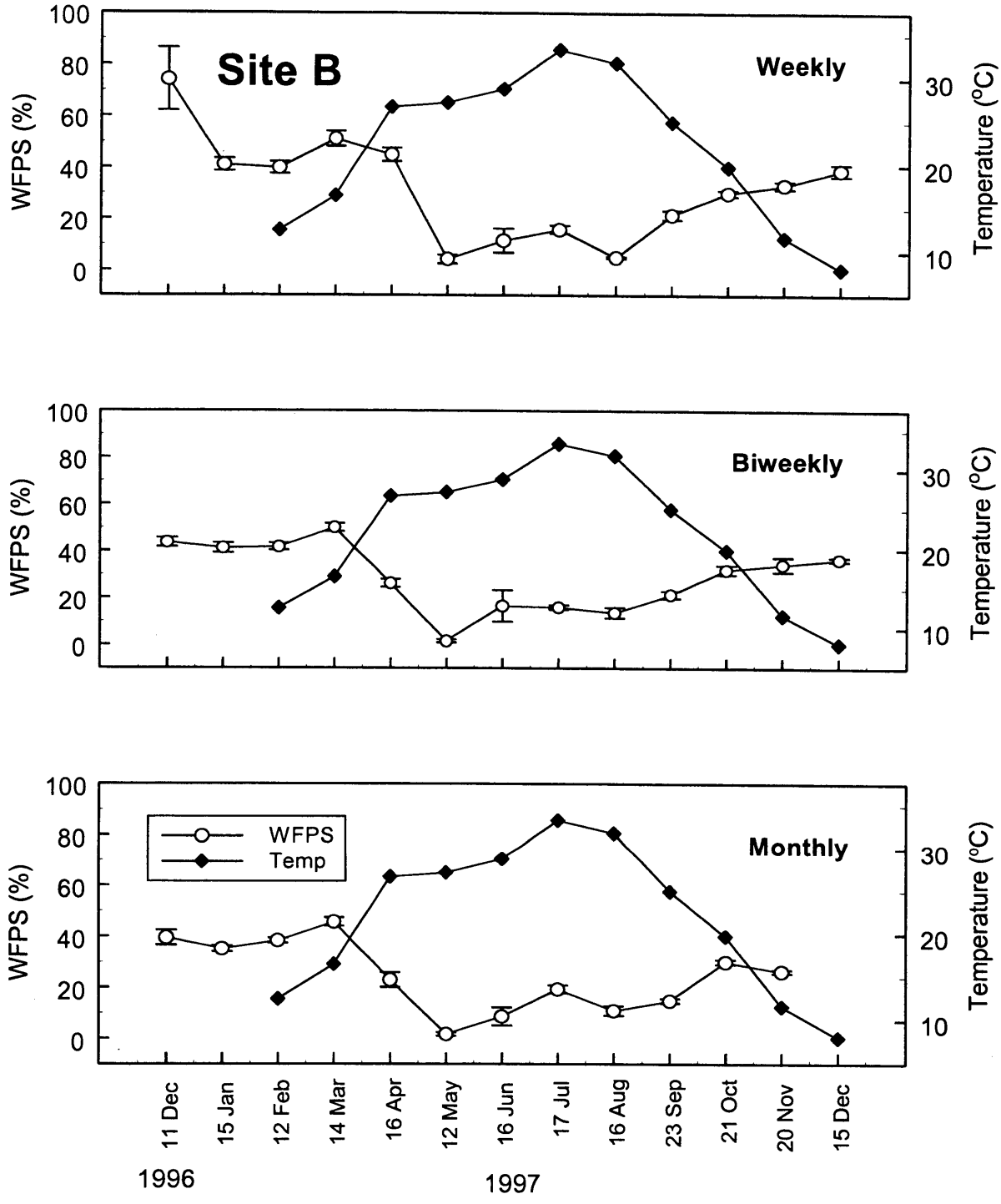


Figure 3.6. Soil temperature February through December 1997, and percent water-filled pore space (%WFPS) December 1996 through December 1997, Site B. Error bars associated with %WFPS represent one standard error of the mean (n = 6).

Soil organic content was generally 1.5 to 3 times higher at Site B than at Site A (Figure 3.7). At Site A, organic content was fairly consistent throughout the year at each treatment plot, varying over a factor of 1.5. At Site B, organic content showed more variation, over a factor of 2.9.

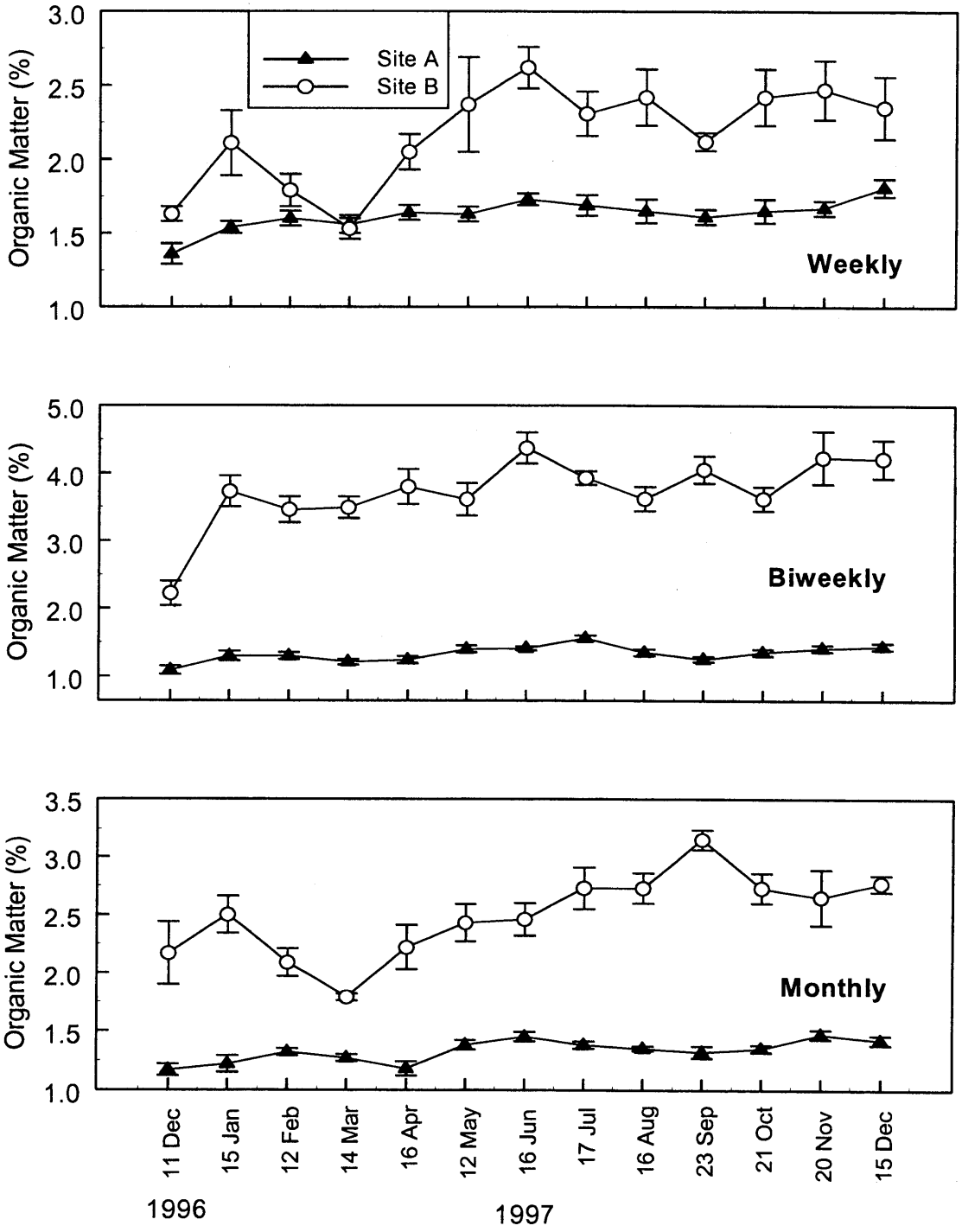


Figure 3.7. Soil organic content at Sites A and B, December 1996 through December 1997. Error bars represent one standard error of the mean (n = 6).

Total-C and total-N were generally two-fold higher at Site B than at Site A (Figures 3.8 and 3.9). At both sites, monthly fluctuations in total-C closely corresponded with monthly fluctuations in total-N. Both total-C and total-N increased over the course of 1997 at Site B. This trend was not apparent at Site A. Total-C, total-N and organic content were positively correlated for both sites (Tables 3.6 and 3.7).

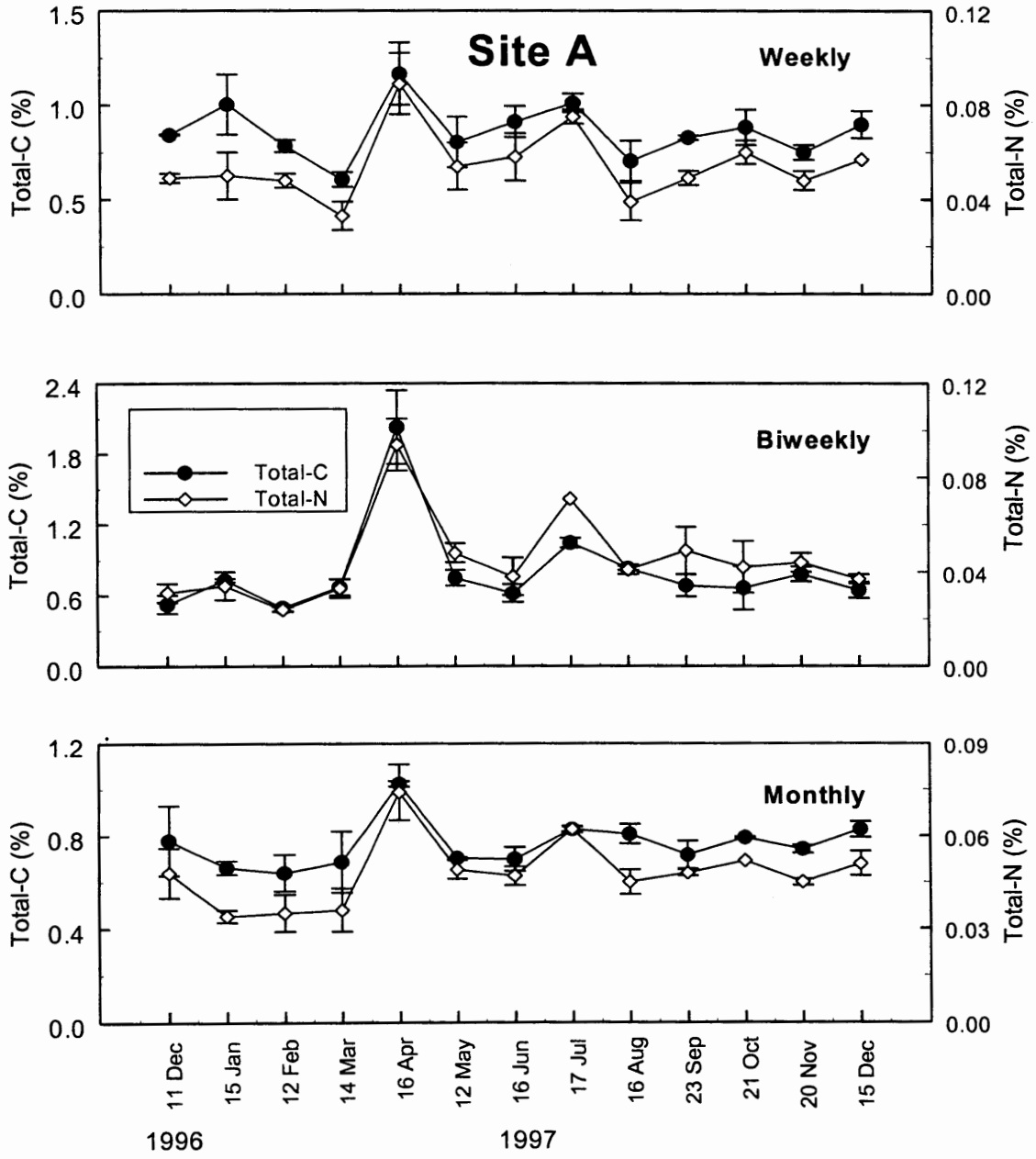


Figure 3.8. Soil total-C and total-N content at Site A, December 1996 through December 1997. Error bars represent one standard error of the mean (n = 2).

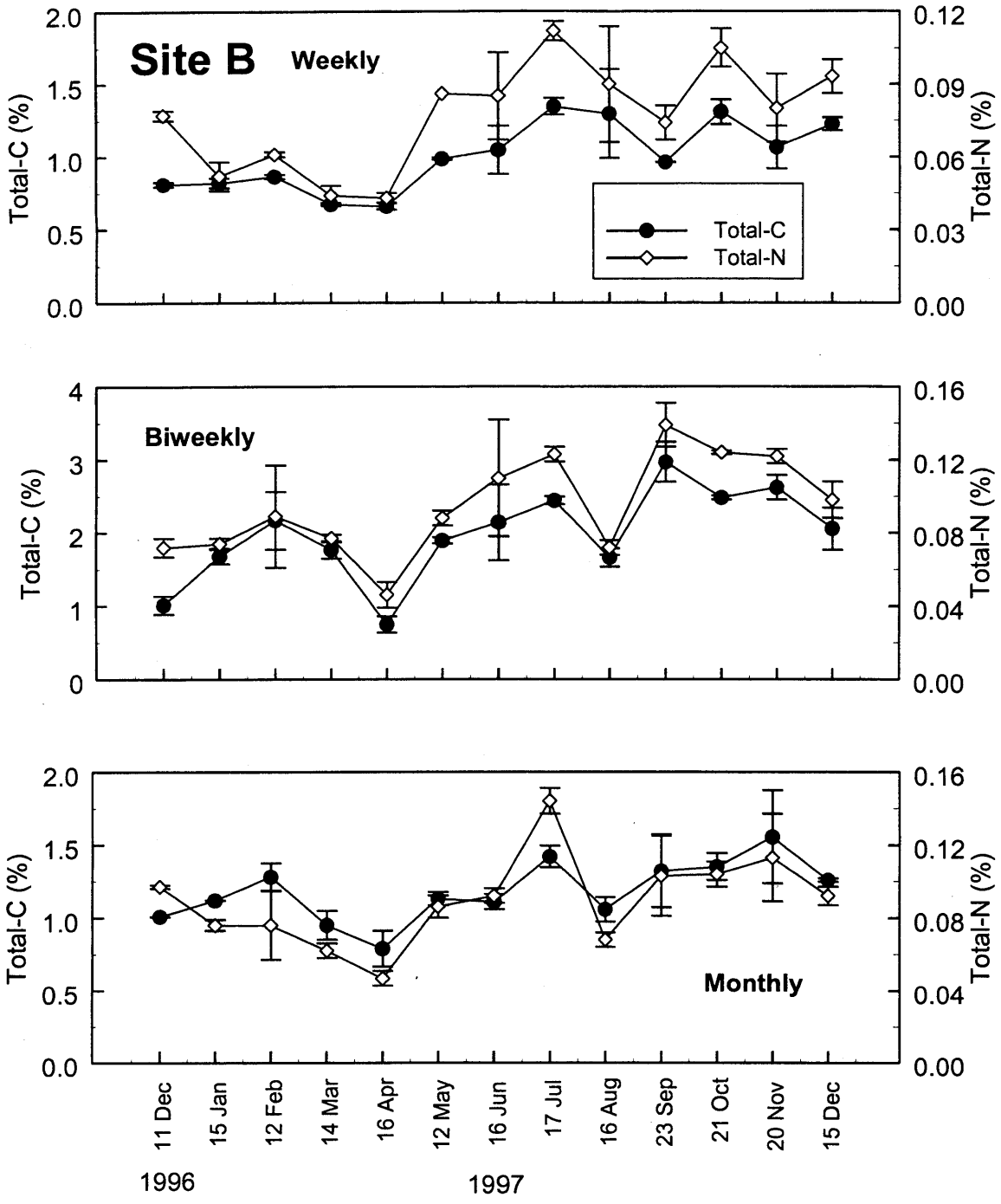


Figure 3.9. Soil total-C and total-N content at Site B, December 1996 through December 1997. Error bars represent one standard error of the mean (n = 2).

Table 3.6. Pearson product moment correlation coefficients for Site A. Only statistically significant correlations are given.

Site A	NA	DEA	WFPS	OM[a]	NO_3^--N	NH_4^+-N	pH	Total-N	Total-C	Temp[b]	Nitrif[c]	N min[d]
NA												
DEA												
%WFPS		-0.41										
OM												
NO_3^--N		0.63										
NH_4^+-N		0.35										
pH	0.45					0.63						
Total-N	-0.33				0.40							
Total-C								0.86				
Temp	-0.63		-0.75									
Nitrif	0.51					0.51	0.32					
N min								-0.35			0.73	

[a] OM = % organic matter
[b] Temp = soil temperature at 5 cm
[c] nitrif = net nitrification rate
[d] N min = net N-mineralization rate

Table 3.7. Pearson product moment correlation coefficients for Site B. Only statistically significant correlations are given.

Site B	NA	DEA	WFPS	OM[a]	NO_3^--N	NH_4^+-N	pH	Total-N	Total-C	Temp[b]	Nitrif[c]	N min[d]
NA												
DEA												
%WFPS		-0.65										
OM												
NO_3^--N	-0.37		-0.38	0.37								
NH_4^+-N		0.58		0.35								
pH	0.57											
Total-N		0.36	-0.32	0.46		0.45						
Total-C				0.83		0.37		0.71				
Temp			-0.79									
Nitrif			-0.40				-0.36					
N min			-0.34				-0.41				0.93	

[a] OM = % organic matter
[b] Temp = soil temperature at 5 cm
[c] nitrif = net nitrification rate
[d] N min = net N-mineralization rate

Results of t-tests performed on annual site means for the above soil physicochemical properties (Table 3.5) showed that total-N, total-C, %WFPS, and organic matter were significantly higher at Site B and that soil pH was significantly higher at Site A.

Data for soil inorganic-N concentrations were highly variable (Figures 3.10 and 3.11). Within each site, temporal changes were not consistent across treatments, which likely reflects differing times since fertilization relative to sampling date and differing masses of N applied for each treatment.

Results of t-tests showed the mean annual NO_3^--N concentration (Table 3.5) was significantly higher at Site B than at Site A, while the mean annual NH_4^+-N concentration was not significantly different between sites. Nitrate-N and NH_4^+-N were positively correlated for Site A but not for Site B (Tables 3.6 and 3.7).

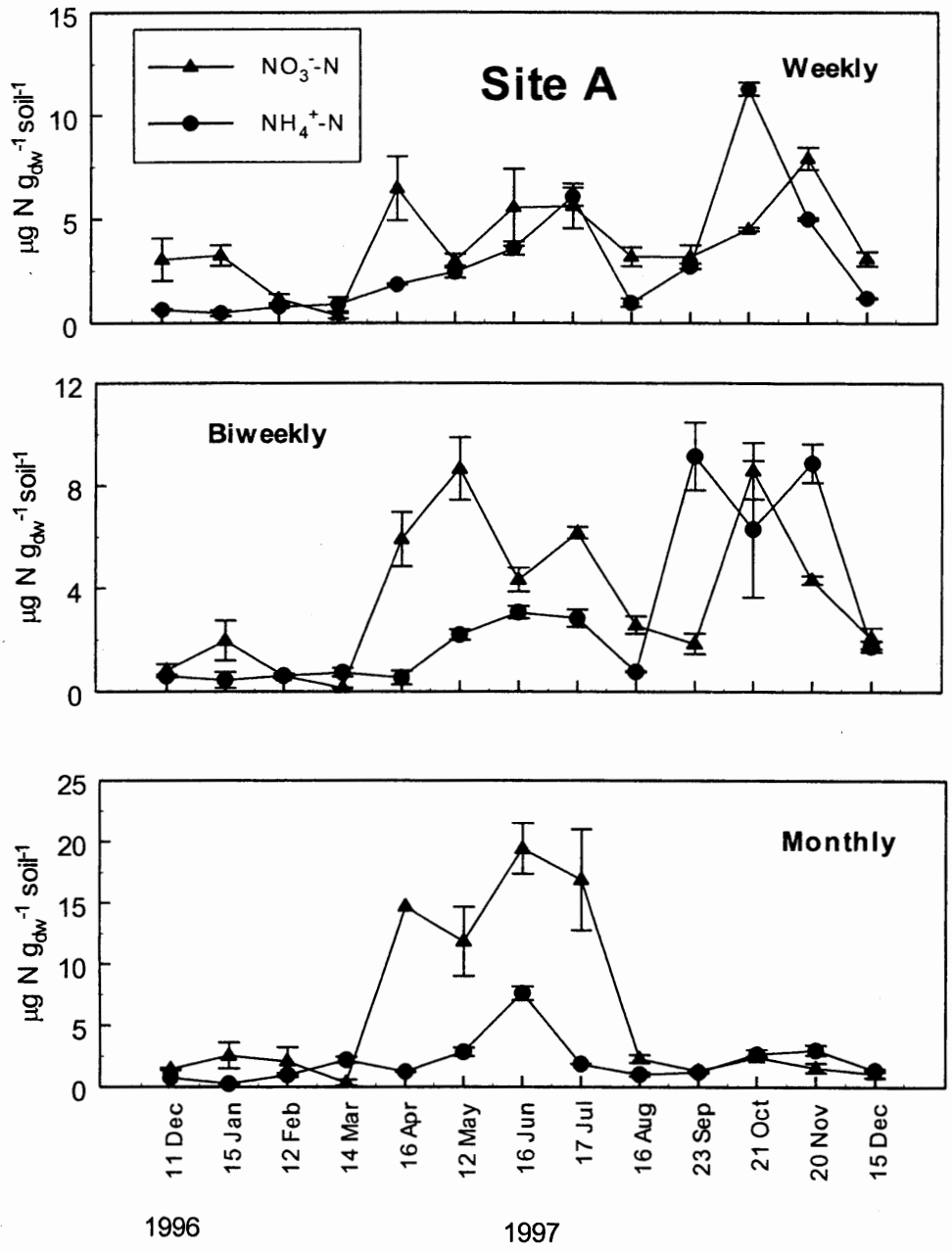


Figure 3.10. Soil NO_3^--N and NH_4^+-N concentrations at Site A, December 1996 through December 1997. Error bars represent one standard error of the mean (n = 2).

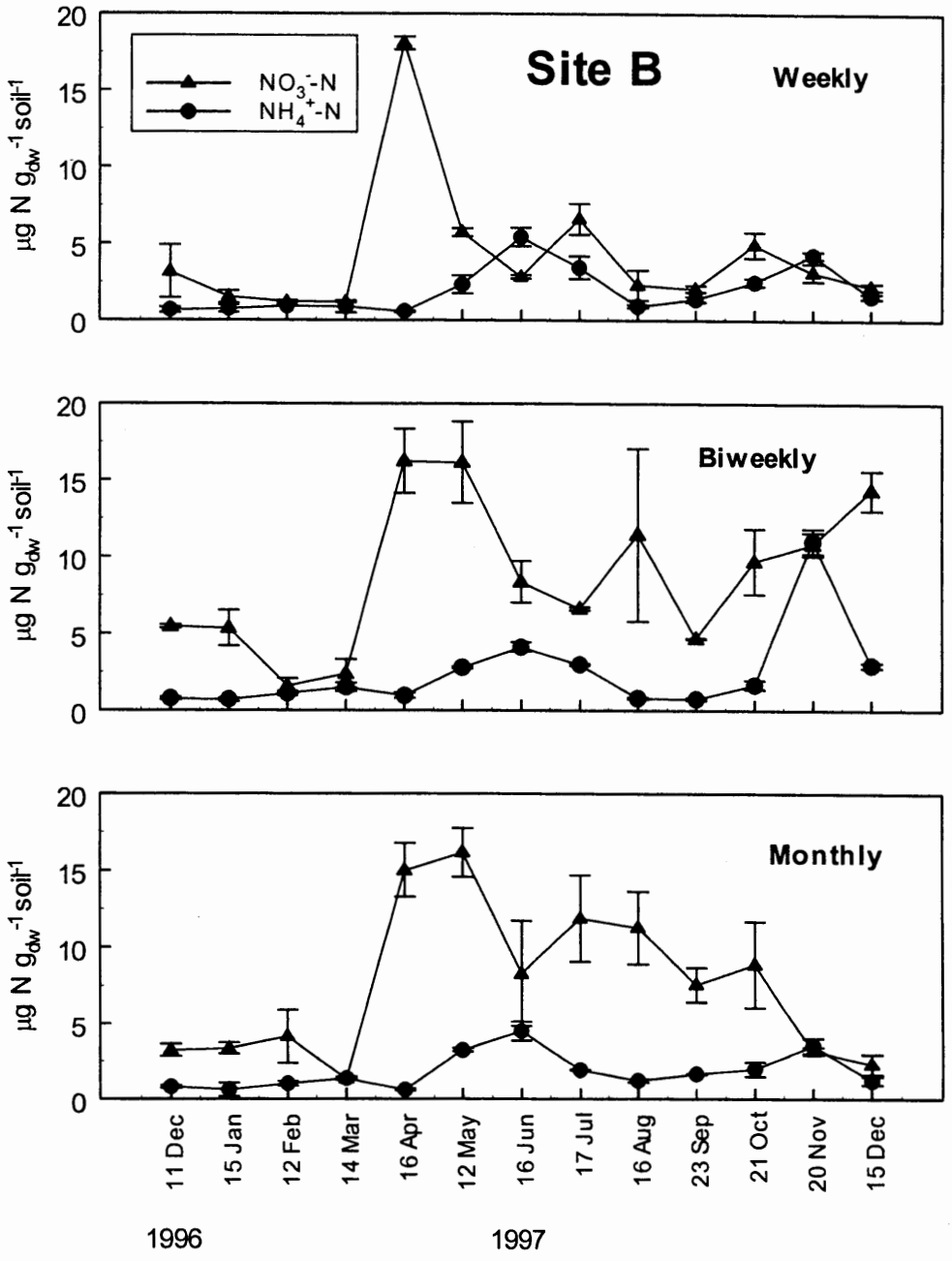


Figure 3.11. Soil NO_3^--N and NH_4^+-N concentrations at Site B, December 1996 through December 1997. Error bars represent one standard error of the mean (n = 2).

Denitrifier Enzyme Activity

DEA was more variable at Site B than at Site A, ranging from 9 to 575 ng N_2O-N g_{dw}^{-1} h^{-1} at the former (Figure 3.12a) and from 5 to 133 ng N_2O-N g_{dw}^{-1} h^{-1} at the latter (Figure 3.12b). At both sites, DEA was significantly higher in the summer than in the winter or spring/fall (Table 3.8).

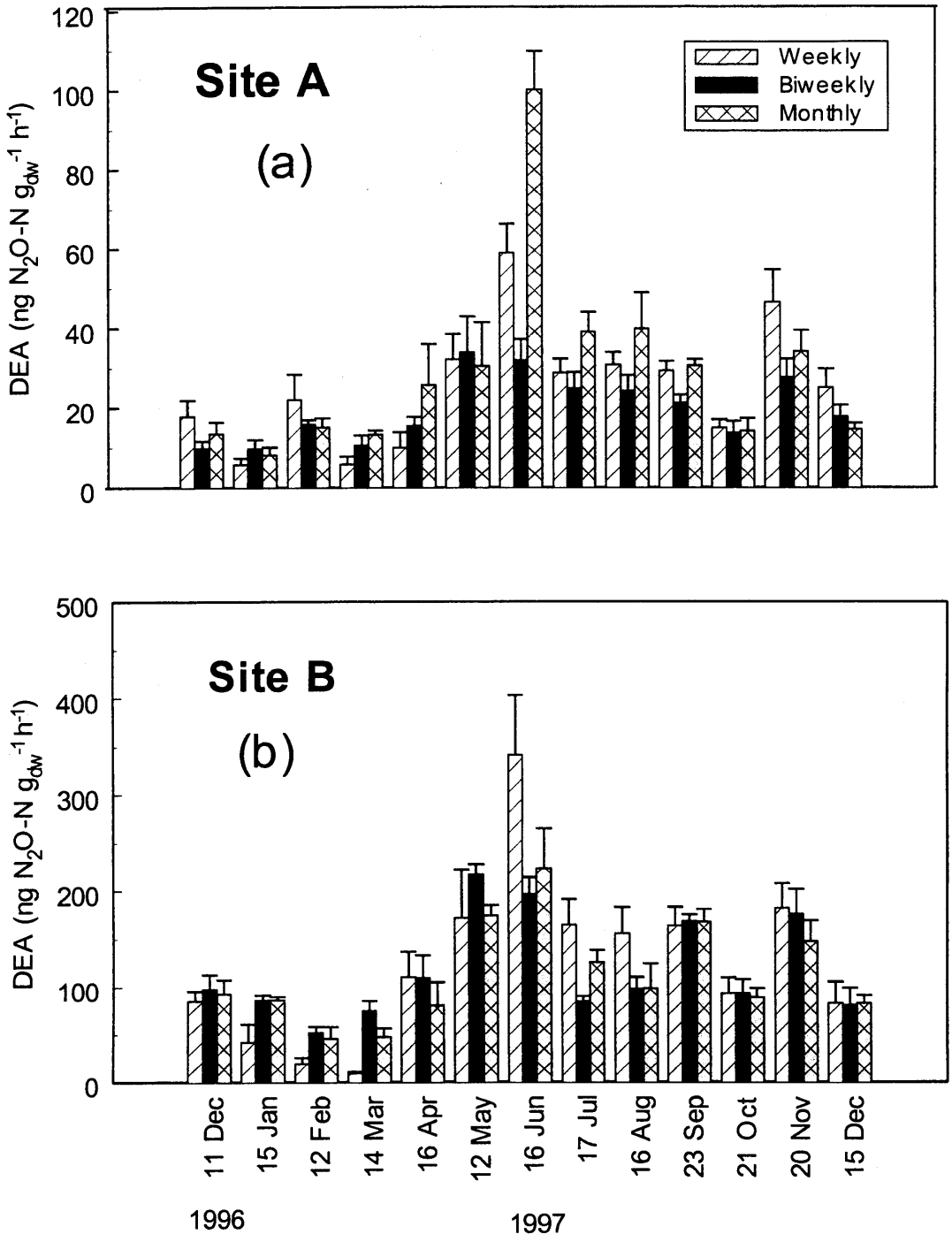


Figure 3.12. (a) Denitrifier enzyme activity (DEA) at Site A, December 1996 through December 1997. (b) DEA at Site B, December 1996 through December 1997. December 1996 data are pre-treatment and remaining data are post-treatment. The treatment is frequency of fertilization. Error bars represent one standard error of the mean (n = 6).

Table 3.8. Means and (standard error of the mean) for seasonal DEA, NA, net N-mineralization and net nitrification, Sites A and B.

Process	Site	Season		
		Winter	Spring/Fall	Summer
DEA ($ng\ N_2O\text{-}N\ g_{dw}^{-1}\ h^{-1}$)	A	20.1 (7.3)	18.0 (9.0)	38.4 (12.4)
NA ($ng\ NO_2^-\text{-}N\ g_{dw}^{-1}\ h^{-1}$)		28.4 (7.4)	11.0 (5.5)	18.4 (4.7)
Net N-mineralization ($\mu g\ NH_4^+\text{-}N\ g_{dw}^{-1}\ d^{-1}$)		0.27 (0.09)	0.24 (0.12)	0.27 (0.07)
Net Nitrification ($\mu g\ NO_3^-\text{-}N\ g_{dw}^{-1}\ d^{-1}$)		0.32 (0.09)	0.28 (0.14)	0.34 (0.09)
DEA ($ng\ N_2O\text{-}N\ g_{dw}^{-1}\ h^{-1}$)	B	20.1 (7.3)	18.0 (9.0)	38.4 (12.4)
NA ($ng\ NO_2^-\text{-}N\ g_{dw}^{-1}\ h^{-1}$)		37.9 (9.9)	13.9 (4.7)	24.7 (10.1)
Net N-mineralization ($\mu g\ NH_4^+\text{-}N\ g_{dw}^{-1}\ d^{-1}$)		0.26 (0.13)	0.36 (0.10)	0.51 (0.13)
Net Nitrification ($\mu g\ NO_3^-\text{-}N\ g_{dw}^{-1}\ d^{-1}$)		0.29 (0.08)	0.35 (0.11)	0.55 (0.13)

Monthwise comparisons of pooled DEA consistently revealed that DEA was significantly greater at Site B than at Site A (cf. Figures 3.12a and 3.12b). DEA at Site B was approximately 2 to 9 times higher each month, with the largest differences in site means (Sites B and A, respectively) occurring in December 1996 (92 vs. 14 ng $N_2O\ g_{dw}^{-1}\ h^{-1}$), January 1997 (72 vs. 8 ng $N_2O\ g_{dw}^{-1}\ h^{-1}$), May 1997 (227 vs. 32 ng $g_{dw}^{-1}\ h^{-1}$), and October 1997 (92 vs. 14 ng $N_2O\ g_{dw}^{-1}\ h^{-1}$).

Within-month comparison of treatments for each site (application frequency) usually showed no significant differences (20 of 26 comparisons). For Site A, the following differences among fertilization schemes were found: in March, monthly (13 ng $g_{dw}^{-1}\ h^{-1}$) was significantly greater than weekly (6 ng $N_2O\ g_{dw}^{-1}\ h^{-1}$); in June, monthly (100 ng $N_2O\ g_{dw}^{-1}\ h^{-1}$) was significantly

greater than weekly (59 ng N_2O g_{dw}^{-1} h^{-1}) and biweekly (32 ng N_2O g_{dw}^{-1} h^{-1}); and in September, weekly (29 ng N_2O g_{dw}^{-1} h^{-1}) and monthly (30 ng N_2O g_{dw}^{-1} h^{-1}) were significantly greater than biweekly (21 ng g_{dw}^{-1} h^{-1}). For Site B, the following differences were found: in February, biweekly (52 ng N_2O g_{dw}^{-1} h^{-1}) was significantly greater than weekly (19 ng N_2O g_{dw}^{-1} h^{-1}); in March, biweekly (74 ng N_2O g_{dw}^{-1} h^{-1}) and monthly (47 ng N_2O g_{dw}^{-1} h^{-1}) were significantly greater than weekly (9 ng N_2O g_{dw}^{-1} h^{-1}); and in July, weekly (165 ng N_2O g_{dw}^{-1} h^{-1}) was significantly greater than biweekly (84 ng N_2O g_{dw}^{-1} h^{-1}). In these 6 comparisons, DEA generally increased as fertilization frequency decreased.

Pearson product moment correlations for all pooled data showed that for Site A, DEA was positively correlated with NO_3^--N, and NH_4^+-N, and was inversely correlated with %WFPS (Table 3.6). For Site B, DEA was correlated with NH_4^+-N, total-N, and was inversely correlated with %WFPS (Table 3.7).

Plots of mean DEA per treatment (both sites) vs. total effluent load showed no relationship between these two variables (Figure 3.13). In addition, plots of monthly nitrogen load and monthly DEA for each treatment did not show a recognizable relationship between these two variables (Figures 3.14 and 3.15). The plot for Site A shows no relationship between seasonal effluent load and seasonal DEA.

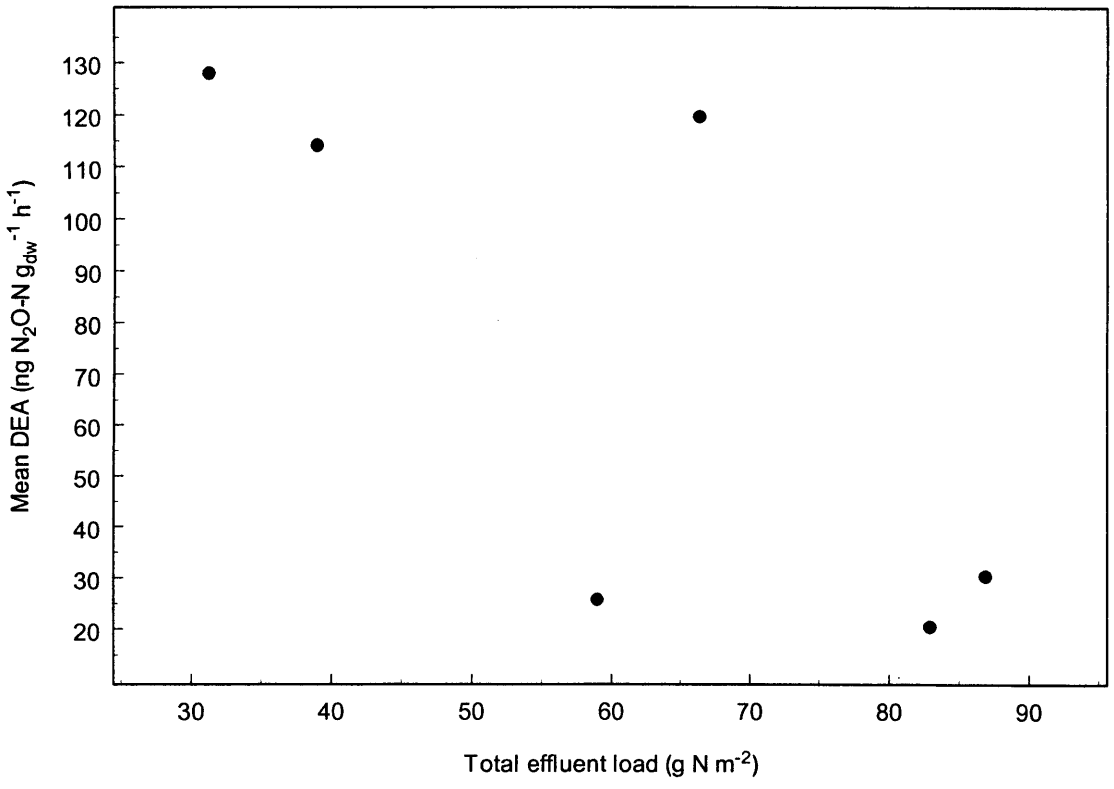


Figure 3.13. Mean DEA per treatment vs. total effluent load per treatment, Sites A and B.

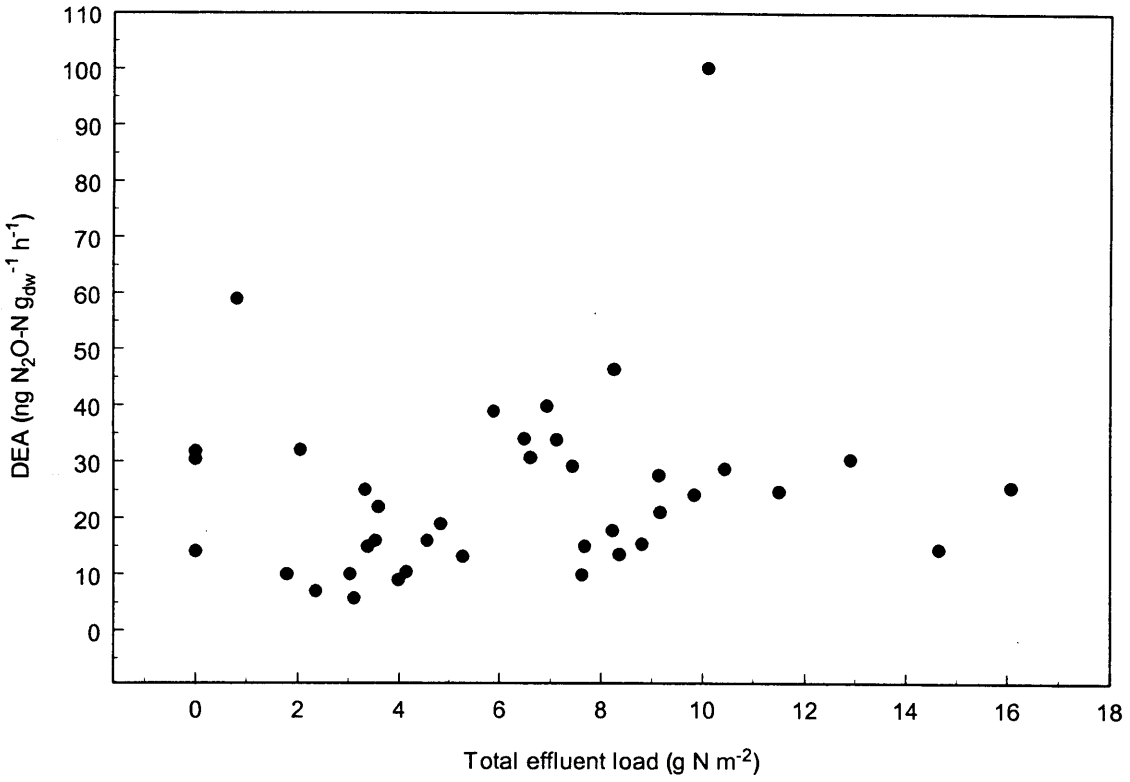


Figure 3.14. DEA (mean DEA per month per treatment) vs. monthly total effluent load, Site A.

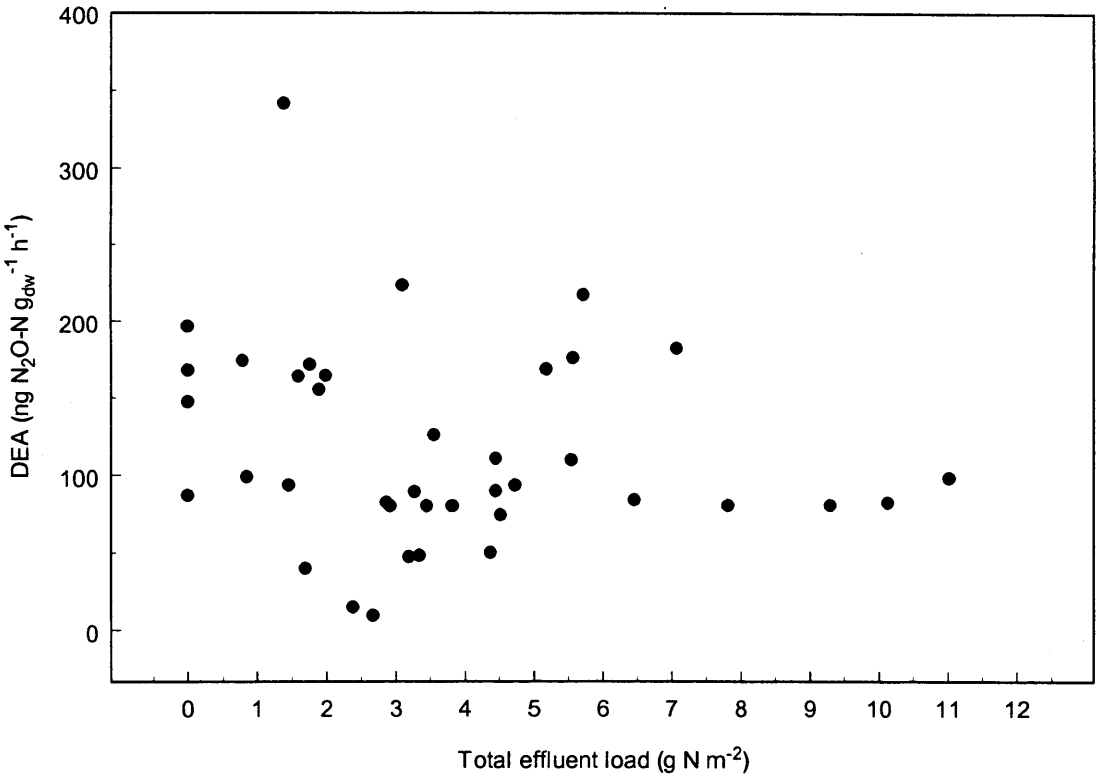


Figure 3.15. DEA (mean DEA per month per treatment) vs. monthly total effluent load, Site B.

Short-term Nitrifier Activity

NA was more variable at Site B than at Site A, ranging from 0.1 to 108 ng NO_2^--N g_{dw}^{-1} h^{-1} at the former (Figure 3.16b) and from 5 to 66 ng NO_2^--N g_{dw}^{-1} h^{-1} at the latter (Figure 3.16a). Results of NA experiments showed that for Site A as well as Site B, seasonal rates of NA were significantly different, and were ranked in the following order: winter > summer > spring/fall (Table 3.8).

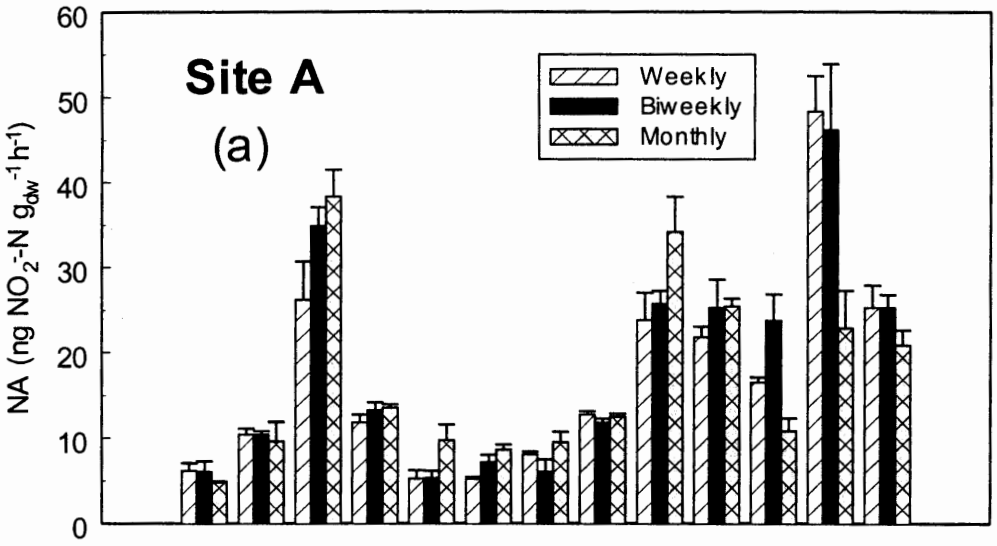
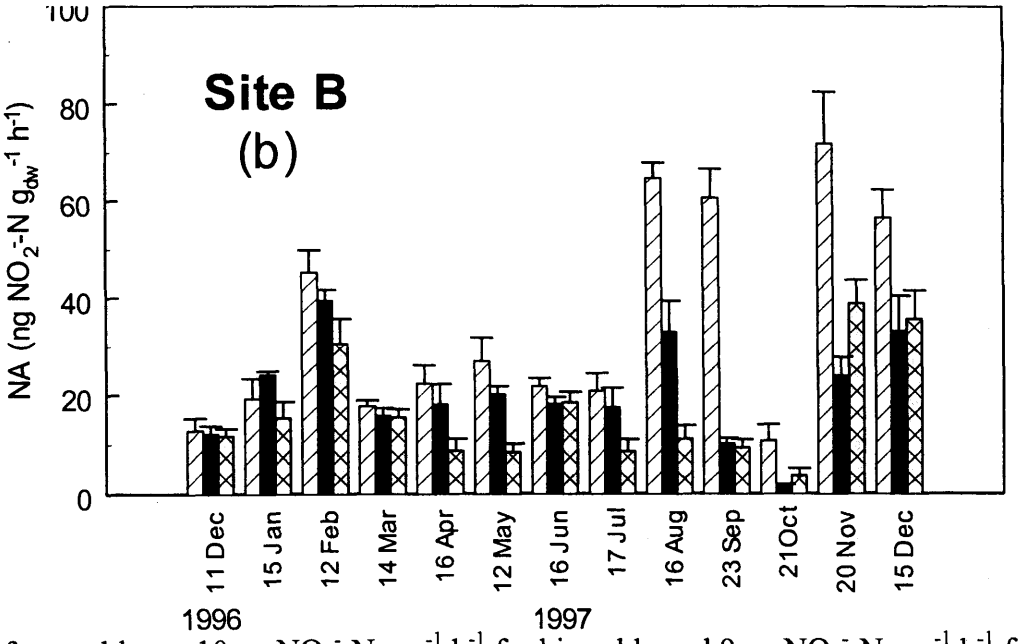


Figure 3.16. (a) Nitrifier enzyme activity (NA) at Site B, December 1996 through December 1997. (b) NA at Site B, December 1996 through December 1997. December 1996 data are pre-treatment and remaining data are post-treatment. The treatment

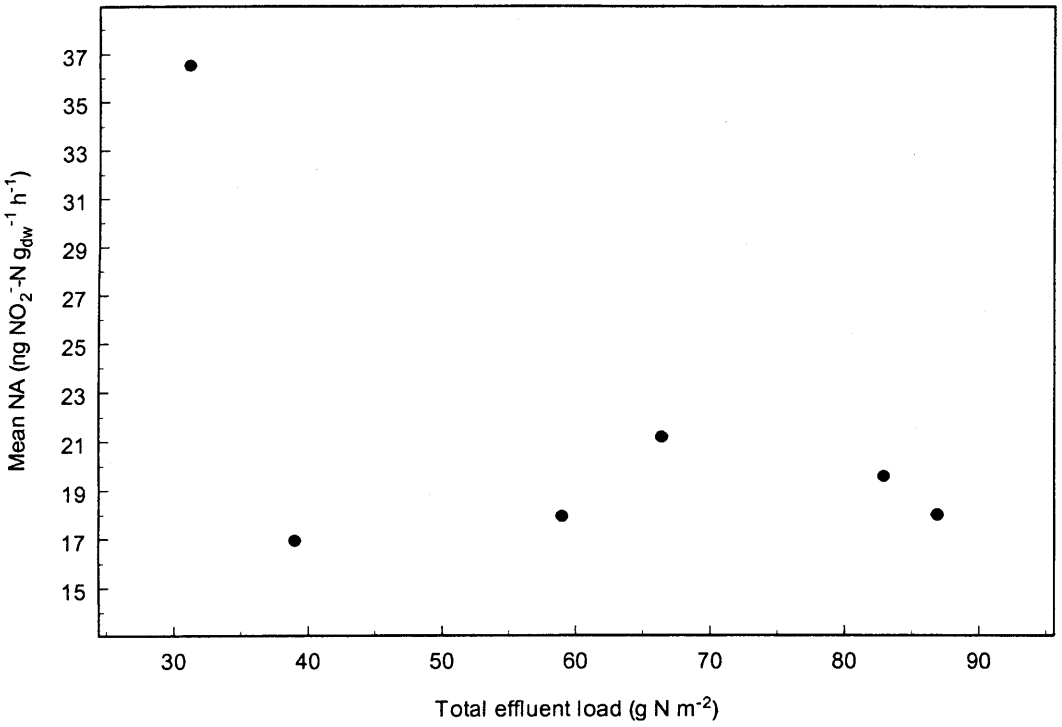
is frequency of fertilization. Error bars represent one standard error of the mean (n = 6).

Monthwise comparisons of pooled NA data showed that NA was significantly higher at Site B than at Site A, except for February and July through September 1997, when sitewise differences were not significant (cf. Figures. 3.16a and 3.16b). In comparisons in which significant differences were detected, NA was approximately 1.3 to 2.6 times higher at Site B, with the highest differences in April, May and June 1997 (16 vs. 7 ng NO_2^--N g_{dw}^{-1} h^{-1}, 18 vs. 7 ng NO_2^--g_{dw}^{-1} h^{-1}, and 20 vs. 8 ng NO_2^--N g_{dw}^{-1} h^{-1}, respectively). In October 1997, NA at Site A was significantly greater than Site B (17 vs. 5 ng NO_2^--N g_{dw}^{-1} h^{-1}). However, this result is suspect due to prolonged sample storage before analysis that month.

Within-month comparison of treatments for each site (application frequency) showed no significant differences for 16 of 26 comparisons. When differences were detected for Site A (3 of 13 comparisons (May, October, November 1997), no consistent rank order of treatments was observed. In May, monthly (9 ng NO_2^--N g_{dw}^{-1} h^{-1}) was greater than weekly (6 ng NO_2^--N g_{dw}^{-1} h^{-1}). In October, biweekly (24 ng NO_2^--N g_{dw}^{-1} h^{-1}) was greater than monthly (11 ng NO_2^--N g_{dw}^{-1} h^{-1}). In November, weekly (48 ng NO_2^--N g_{dw}^{-1} h^{-1}) and biweekly (46 ng NO_2^--N g_{dw}^{-1} h^{-1}) were greater than monthly (23 ng NO_2^--N g_{dw}^{-1} h^{-1}). When differences were detected for Site B (7 of 13 comparisons (April, May, August through December 1997)), NA in weekly treatments was greater than NA in biweekly and monthly treatments. Differences were especially apparent in August 1997 (65 ng NO_2^--N g_{dw}^{-1} h^{-1} for the weekly treatment, vs. 33 ng NO_2^--N g_{dw}^{-1} h^{-1} for the biweekly treatment and 16 ng NO_2^--N g_{dw}^{-1} h^{-1} for the monthly treatment), September 1997 (61 ng NO_2^--N



g_{dw}^{-1} h^{-1} for weekly vs. 10 ng NO_2^--N g_{dw}^{-1} h^{-1} for biweekly and 9 ng NO_2^--N g_{dw}^{-1} h^{-1} for monthly), November 1997 (72 ng NO_2^--N g_{dw}^{-1} h^{-1} for weekly vs. 39 ng NO_2^-- g_{dw}^{-1} h^{-1} for biweekly and 24 ng NO_2^--N g_{dw}^{-1} h^{-1} for monthly), and December 1997 (57 ng NO_2^--N g_{dw}^{-1} h^{-1} for weekly vs. 33 ng NO_2^--N g_{dw}^{-1} h^{-1} for biweekly and 36 ng NO_2^--N g_{dw}^{-1} h^{-1} for monthly).

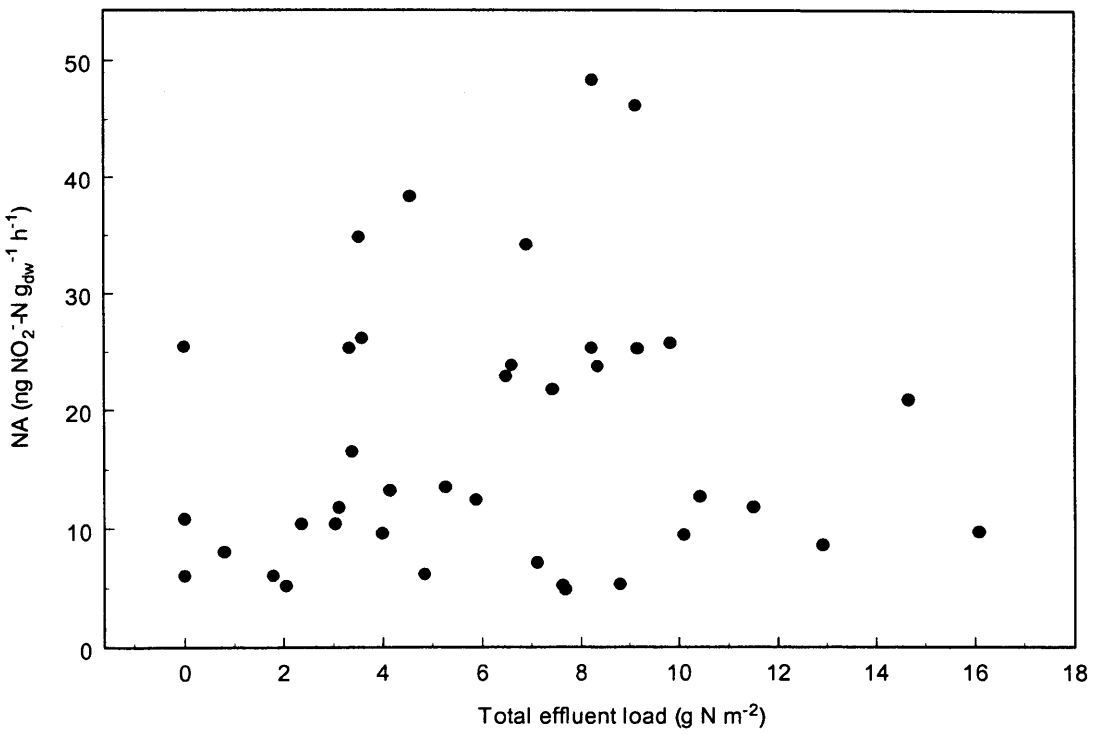


Pearson product moment correlations for all pooled data showed that for Site A, NA was positively correlated with pH and net nitrification, and was inversely correlated with total-N and soil temperature (Table 3.6). For Site B, NA was positively correlated with pH and inversely correlated with soil NO_3^--N (Table 3.7).

Plots of mean NA per treatment vs. total-N load show no correlation between these two variables (Figure 3.17). Plots of monthly nitrogen load and monthly DEA for each treatment did not show a recognizable relationship (Figures. 3.18 and 3.19).

Figure 3.17. Mean NA per treatment vs. total effluent load per treatment, Sites A and B.

Figure 3.18. NA (mean NA per month per treatment) vs. monthly effluent load, Site A.



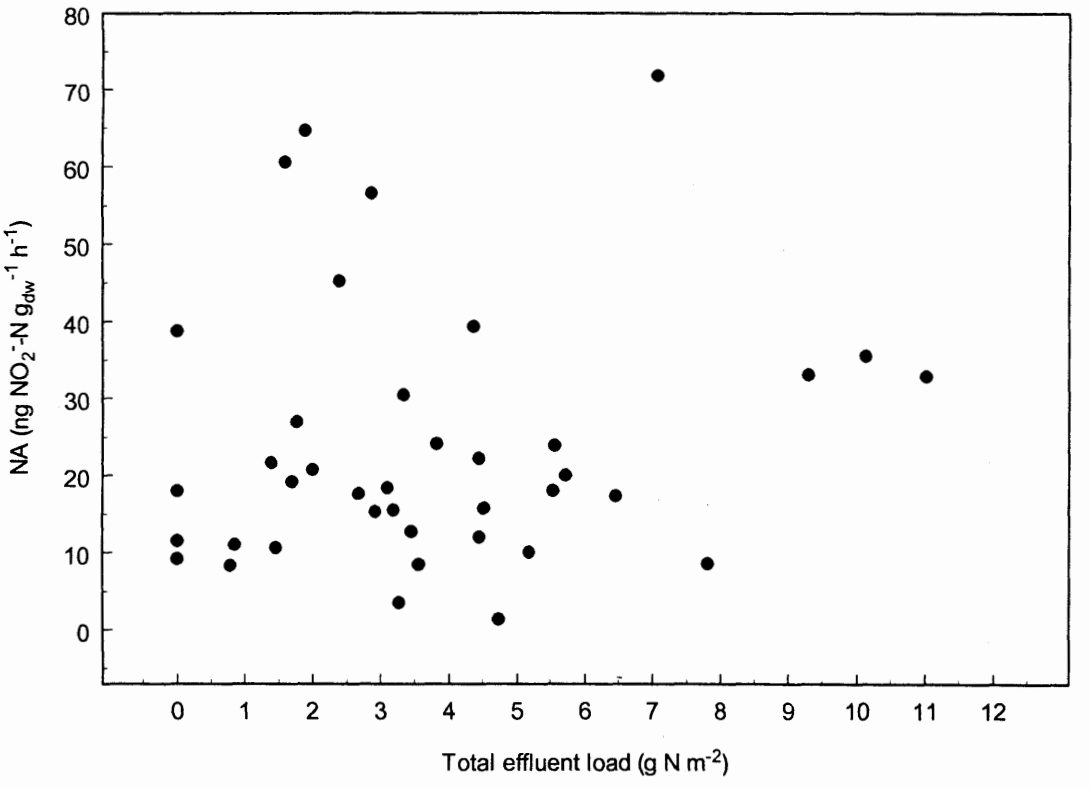


Figure 3.19. NA (mean NA per month per treatment) vs. monthly effluent load, Site B.

Net Nitrification Rates

Rates of net nitrification were low, ranging from -0.08 to 0.69 μg NO_3^--N g_{dw}^{-1} d^{-1} for Site A, and from 0.06 to 1.05 μg NO_3^--N g_{dw}^{-1} d^{-1} for Site B (Figures. 3.20a and 3.20b). In general, net nitrification rates were approximately 25% lower for Site A, a trend that was most apparent in the summer months. When pooled data for all treatments were grouped into winter, spring/fall and summer months for Site B, the net nitrification rate in summer (0.55 μg NO_3^--N g_{dw}^{-1} d^{-1}) was significantly (almost two-fold) higher than in winter (0.29 μg NO_3^--N g_{dw}^{-1} d^{-1}). However, no statistically significant seasonal differences in net nitrification rates were noted for Site A, where seasonal rates of net nitrification consistently averaged about 0.32 μg NO_3^--N g_{dw}^{-1} d^{-1} (Table 3.8).

Within-month net nitrification rates for Site B were generally higher for the biweekly and monthly treatments than for the weekly treatment (Figure 3.20b). The highest rates of net nitrification for this site were observed April through July 1997, when net nitrification for the monthly treatment was roughly 2 to 6 times higher than the weekly or biweekly treatments. Net nitrification for all other months demonstrated higher rates for the biweekly treatments. No pattern of treatment effect was apparent for Site A (Figure 3.20a).

The net nitrification rate for Site A was significantly correlated with NA, soil NH_4^+-N, net N-mineralization, and pH (Table 3.6). For Site B, net nitrification was significantly correlated with net N-mineralization, and inversely correlated with %WFPS and pH (Table 3.7).

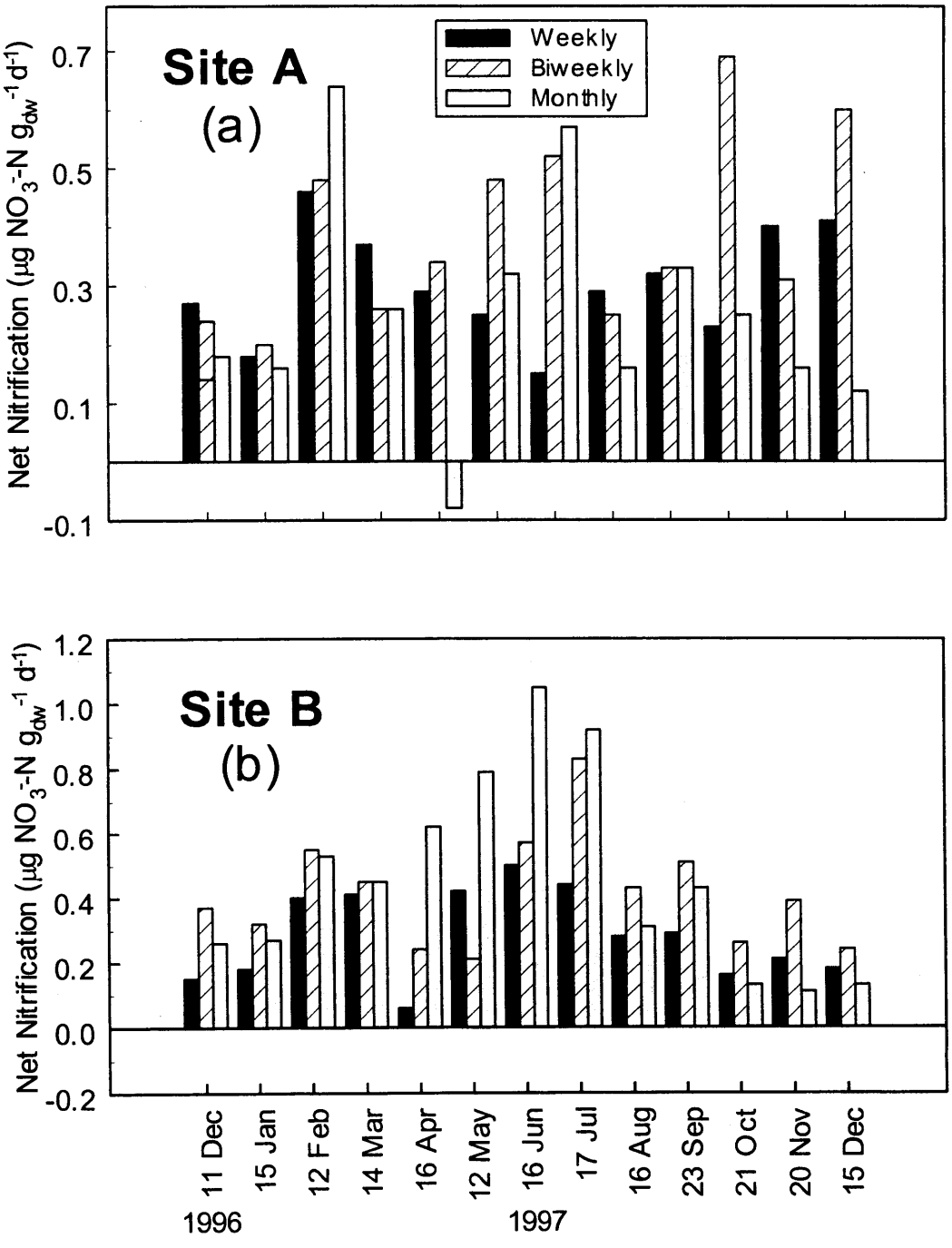


Figure 3.20. (a) Net nitrification at Site A, December 1996 through December 1997. (b) Net nitrification at Site B, December 1996 through December 1997. December 1996 data are pre-treatment and remaining data are post-treatment. The treatment is frequency of fertilization.

Net N-mineralization Rates

Rates of net N-mineralization were low and ranged from -0.07 and 0.61 μg NH_4^+- g_{dw}^{-1} d^{-1} for Site A (Figure 3.21a), and from -0.02 and 1.01 μg NH_4^+-N g_{dw}^{-1} d^{-1} for Site B (Figure 3.21b). In general, net N-mineralization rates were approximately 25% lower for Site A, with the trend especially pronounced in the summer months. When pooled data for all treatments were grouped into winter, spring/fall and summer months for Site B, net N-mineralization in summer (0.51 μg NH_4^+-N g_{dw}^{-1} d^{-1}) was significantly (two-fold) higher than in winter (0.26 μg NH_4^+-N g_{dw}^{-1} d^{-1}). However, no statistically significant differences were noted for Site A, where seasonal rates consistently averaged about 0.25 μg NH_4^+-N g_{dw}^{-1} d^{-1} (Table 3.8).

Within-month rates for net N-mineralization for Site B generally increased as the application frequency decreased (Figure 3.21b). Months in which this trend was especially obvious were February, and April through July 1997, when net N-mineralization rates were 2 to 7 times higher in the monthly treatment than in the weekly treatment, and were approximately 2 times higher in the monthly treatment than in the biweekly treatment. Treatment effects on net N-mineralization for Site A were less obvious (Figure 3.21a). The only apparent trend was that from April to November 1997, net N-mineralization in the biweekly treatment was consistently higher than in the monthly or weekly treatments. For Site A, no pattern of treatment effect on monthly and weekly study plots was evident (Figure 3.21a).

Pearson product moment correlation showed that net N-mineralization was positively correlated with net nitrification, and inversely correlated with total-N for Site A (Table 3.6). Net N-mineralization was positively correlated with net nitrification, and was inversely correlated with pH and %WFPS for Site B (Table 3.7).

No relationship between net N-mineralization and effluent nitrogen load is apparent at either site in the plots comparing these two variables (Figures. 3.22 and 3.23).

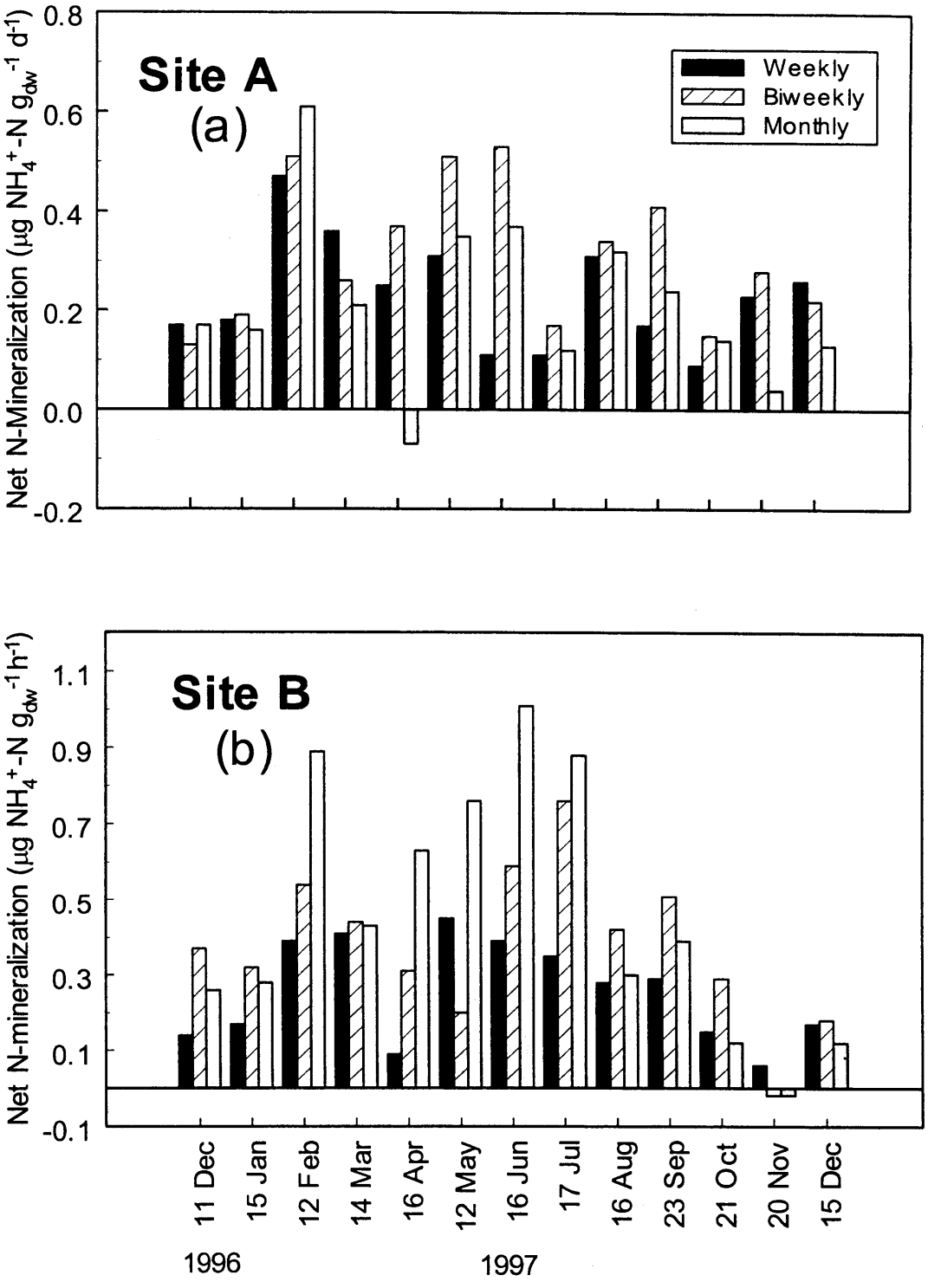


Figure 3.21. (a) Net N-mineralization at Site A, December 1996 through December 1997. (b) Net N-mineralization at Site B, December 1996 through December 1997. December 1996 data are pre-treatment and remaining data are post-treatment. The treatment is frequency of fertilization.

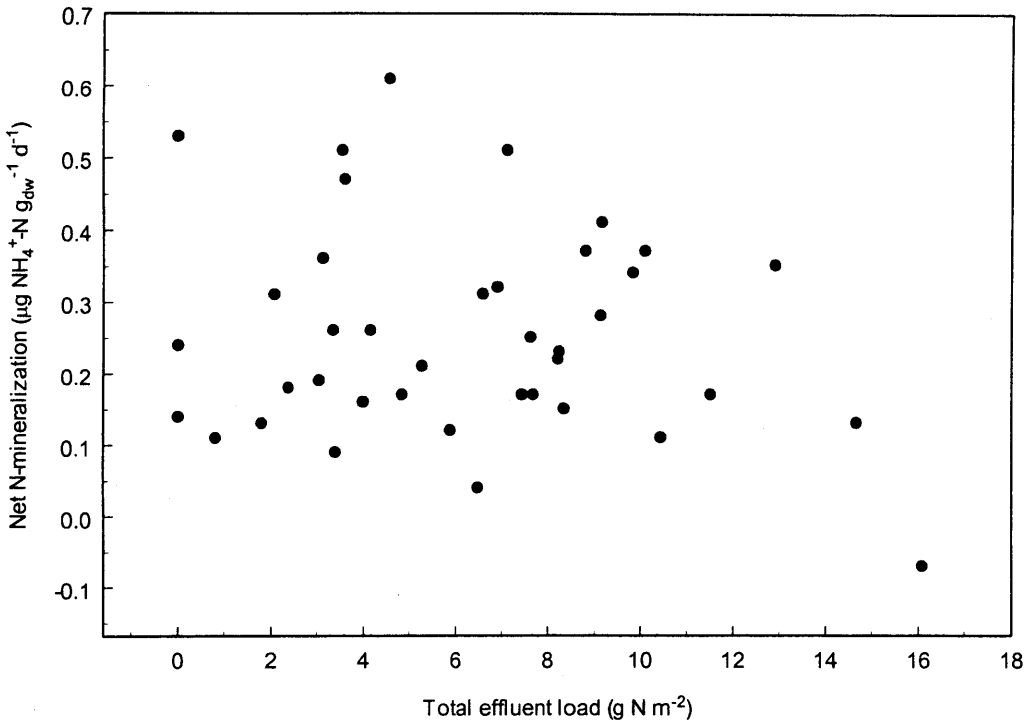


Figure 3.22. Mean monthly net N-mineralization vs. monthly effluent load, Site A.

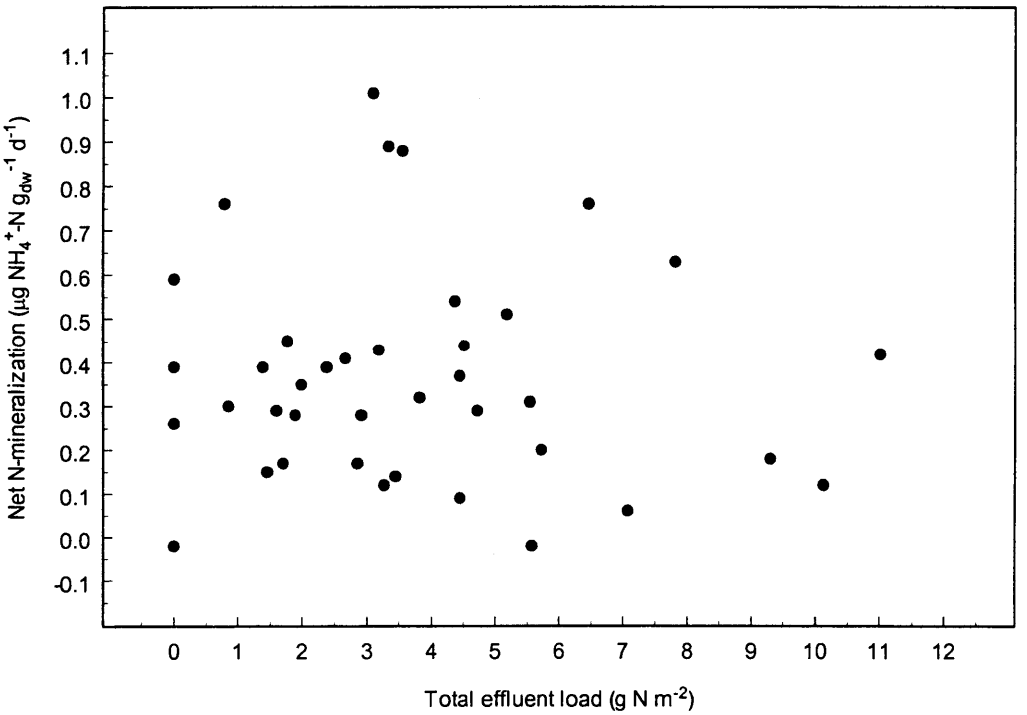


Figure 3.23. Mean monthly Net N-mineralization vs. monthly effluent load, Site B.

4. DISCUSSION

Lagoon Waste

The total-N and NH_4^+-N contents of swine lagoon samples measured in this study (Table 3.2) compare favorably to values from other studies (Safley et al. 1992; Harvey et al. 1996; Westerman et al. 1996; Liu et al. 1997) which show values ranging from 130 to 400 mg L^{-1} total-N, and from 110 to 340 mg L^{-1} NH_4^+-N. In this study, NH_4^+-N content of lagoon waste was generally 80% or higher (Table 3.2). Thus, the N in applied lagoon effluent was largely in a form favorable for immediate plant assimilation and microbial transformation (immobilization, nitrification and denitrification). N-mineralization does not figure significantly in the cycling of effluent N following application to soils, since its organic-N content is generally less than 20%.

Denitrifier Enzyme Activity

The observed range of values for DEA in this study, approximately 0 to 700 ng N_2O-N g_{dw}^{-1} h^{-1}, corresponds to rates of DEA observed in other studies on agricultural soils and grasslands (Martin et al. 1988; Ambus 1993; Bergstrom and Beauchamp 1993; Sotomayor and Rice 1996; Loro et al. 1997; Maag et al. 1997; Mulvaney et al. 1997), which ranged from 0 to 2000 ng N_2O-N g_{dw}^{-1} h^{-1}.

Results of the DEA experiments suggest that application frequency of lagoonal swine effluent has no statistically significant effect on potential rates of denitrification, at least after one year of treatment. The significant differences that were detected for Sites A and B showed no consistent rank order of effect. These differences appear to be random with respect to fertilization frequency and effluent load. DEA was also not affected by timing of sample collection with respect to spray events, as the time course experiment indicated (Table 3.1).

The lack of correlation between monthly DEA and monthly N load at sites A and B (Figures 3.14 and 3.15), suggests that substrate supply had no clear influence on DEA in this study. These results differ from previous studies (Wheatley et al. 1991; Petersen et al. 1991; Dendooven and Anderson 1995; Murray et al. 1995; Chantigny et al. 1996; Dendooven et al. 1996; Weier et al. 1996; Paul and Zebarth 1997; Paul et al. 1997), which indicate that manipulations of substrate and C supply to denitrifiers produce significant effects on DEA within one year of treatment initiation. Hence, the lack of effect of fertilization frequency on DEA may be because soil microbial populations are capable of fully exploiting the N in lagoonal swine waste regardless of spray regime, or because the fertilization schedule was not enforced strictly enough to produce differences.

The main objective of this study was to vary fertilization frequency while delivering similar nutrient loads to the study plots. However, in reality, both fertilization frequency and nutrient load were inconsistent from month to month. Therefore, in the correlation analysis, the effects of fertilization frequency and nutrient load are mixed and perhaps non-linear.

The denitrifying enzyme content of a soil reflects the history of the environmental variables affecting DEA at that site (Groffman and Tiedje 1989; Tiedje et al. 1989). Rates of DEA occurred on a scale 3 to 4 times higher at Site B than at Site A (Figures 3.12a and 3.12b), suggesting that the longer history of fertilization, higher soil organic matter and nutrient contents had a significant effect on DEA. Nitrate-N and NH_4^+-N were significantly correlated to DEA at Site A (Table 3.6), and total-N and NH_4^+-N were significantly correlated to DEA at Site B (Table 3.7). Previous studies on agricultural soils have also shown significant correlations between DEA and soil concentrations of inorganic-N (van Kessel et al. 1993) or total-N and total-C (Staley et al. 1990). Moreover, others (Liang and McKenzie 1997, Loro et al. 1997) have reported significant correlation between *in situ* denitrification rates and soil inorganic-N. Collectively, this evidence suggests that potential denitrification is likely to increase as soil nitrogen and organic content increase.

Denitrification has been shown to be C-limited in many agricultural soils (Seech and Beauchamp 1988; Aulakh et al. 1991; Weier et al. 1993; Sotomayor and Rice 1996; Mulvaney et al. 1997; Palma et al. 1997), and DEA has been correlated with mineralizable and soluble organic-C (Bijay-Singh et al. 1988; Drury et al. 1991; Ambus 1993; Chantigny et al. 1996; Maag et al. 1997). In addition, Liang et al. (1996) reported that after the addition of water-soluble organic-C extracted from dairy manure to sandy soil, the mineralizable-C content of the soil was 7.5 to 8 times greater than the amount of C added, indicating that most of the C mineralized comes primarily from the indigenous soil organic-C pool. Therefore the organic content of the soil is an important factor in determining energy supply for denitrifiers. Zak et al. (1990) found that microbial biomass C was highly correlated with water-soluble organic-C. Although water-soluble C was not determined in this study, it is reasonable to assume that the higher organic matter and total-C content at Site B provided a larger labile-C resource, supporting a greater denitrifier population, hence the enhanced DEA observed at that site.

Soil moisture has been shown to be an important factor determining *in situ* denitrification rates (Pennock et al. 1992; van Kessel et al. 1993; Bergstrom and Beauchamp 1993), and has been strongly correlated with DEA (Rice and Smith 1982; Parsons et al. 1991; Ambus 1993). In saturated soil, microbial activity is stimulated by availability of labile-C and nitrogenous substrate, increasing the oxygen demand and creating anaerobic microzones. Therefore, we would have expected DEA to be highest in winter months, when %WFPS was highest at both sites, ranging from 30 to 80% (Figures 3.5 and 3.6). However, DEA was significantly higher in summer months (Figure 3.12a and 3.12b), when soil temperature ranged from 25 to 35°C and %WFPS was generally lower, ranging between 0 and 20% (Figures 3.5 and 3.6). This points to persistence of denitrifying enzymes in dry aerobic soil. In a laboratory study, Giambiagi et al. (1993) dried soil samples at room temperature for 13 weeks. At the end of the study, DEA was 4 times greater than at the beginning of the study. Giambiagi et al. (1993) found that soil aggregate size decreased with the length of drought. Higher denitrification rates have been shown to occur in small soil aggregates (Seech and Beauchamp 1988). Extractable-C content has also been shown to increase upon soil dessication (Birch 1958; Davidson et al. 1987; Sparling and West 1989). Soil aggregate size and extractable-C content were not determined in this study; however, it is possible that the combined effects of decreased aggregate size and higher extractable-C

content of dry soils may have been at least partially responsible for increased DEA in summer months.

Another explanation for increased DEA in summer is that, in general, larger volumes of effluent are sprayed per month during the summer season. This was the case at several of our study plots (Tables 3.3 and 3.4). Even though the soil at both sites was drier in summer, DEA could have increased in response to increased availability of soil nitrogen.

Desiccation tolerance of denitrifying enzymes has been demonstrated by Smith and Parsons (1985), Groffman and Tiedje (1988), Martin et al. (1988) and Peterjohn (1991). The mechanism for desiccation tolerance of denitrifying enzymes is unknown. At my study site, high DEA would have allowed rapid response of denitrifiers to episodic fertilization and rainfall events. This would have been especially advantageous in summer, when warm soil temperatures were favorable for denitrification.

Nitrifier Activity

The observed range of NA in this study, approximately 0 to100 ng NO_2^--N g_{dw}^{-1} h^{-1}, falls within the range of NA found in previous studies on arable soils and grass pastures (Berg and Roswall 1985; Hopkins et al. 1988; Bramley and White 1989; Berg and Roswall 1989), which ranged from about 0 to 500 NO_2^--N g_{dw}^{-1} h^{-1}.

Frequency of fertilization did not have a clear effect on NA. At Site A in May 1997, the monthly treatment was sprayed 3 times, receiving 13 g N m^{-2}, while the weekly treatment was sprayed only once, receiving 2 g N m^{-2} (Table 3.3). Nitrifier activity was significantly greater at the monthly treatment plot during that month, which could indicate a significant effect of fertilization frequency and effluent load on NA. The other significantly greater rates of NA detected at Site A (the biweekly treatment in October, and both weekly and biweekly treatments in November) also correspond with higher fertilization frequency and higher effluent loads at those plots. However, such a trend was not apparent for Site B. For example, in August 1997, NA was significantly greater at the weekly plot, even though the biweekly plot was fertilized twice more and received roughly 10 times the effluent load (Table 3.4). In September 1997, NA was significantly greater at the weekly plot, even though the biweekly plot received roughly 4 times the effluent load, and the monthly plot received no effluent at all. Overall, the small number of significant differences found at Site A, and the inconsistencies in the effect (positive or negative) of fertilization frequency or effluent load on NA during the months when significant differences were found at Site B, suggest that no trends of fertilization frequency on NA were developing after one year of study.

Previous studies (Wheatley et al. 1991; Chantigny et al. 1996) indicate that manipulations of substrate supply to nitrifiers produce significant effects on NA within one year of treatment initiation. Hence, the duration of the study was likely sufficient to elicit an effect on NA. The lack of response may be because the soil microbial populations are capable of fully exploiting the N in lagoonal swine waste regardless of spray regime. Alternatively, it may be because the fertilization schedule was not strictly enough enforced to produce differences.

There are several possible explanations for why NA was significantly greater in winter than in summer. First, NA has been shown to increase with increasing soil moisture (Gilmour 1984; Staley et al. 1990), and %WFPS ranged from 2 to 10 times higher in the winter than in the summer (Figures 3.5 and 3.6). Percent WFPS was as low as 4 to 5% at Sites A and B in August 1997. Nitrifier population size and respiration are subject to significant declines during dry summers (Kandeler and Bohm 1996; Jha et al. 1996), as nitrifying bacteria are particularly sensitive to water stress (Domsch 1985; Killham 1990; Alef 1991).

Secondly, NA may have been affected by root development and growth of the Bermuda grass. Nitrification can decline because of microbial N-immobilization and root uptake of N (Breland and Bakken 1991; Verhagen et al. 1994), and because of O_2 depletion following the stimulation of heterotrophic respiration by root-released C (Klemedtsson et al. 1987; Crescenzi et al. 1988). Laboratory studies have shown the inhibitory effects of grass and other plant root washings on nitrification (Neal 1969; Moore and Waid 1971). It is possible that in this study, exudates from growing roots in summer inhibited nitrifier activity at both sites.

Third, NA may have responded to changes in pH. The highest values of pH and NA occurred toward the end of 1997 (Figures. 3.4, 3.16a, and 3.16b). The positive correlation of pH and NA found in this study has also been reported previously (Gilmour 1984; Staley et al. 1990). Nitrifier activity may be correlated with pH because at higher pH, more total ammoniacal-N (NH_4^+ and NH_3) is present as NH_3 in the soil, resulting in more substrate for nitrification (Prosser 1989).

A general pattern of greater NA at Site B was observed, except during the summer. Low summer NA at both sites could be explained by soil desiccation, as previously described. Several explanations can account for higher NA at Site B during the rest of the year. First, Site B generally had higher %WFPS than Site A (Figures 3.5 and 3.6), particularly in winter. Nitrification has been observed to be greater in fields with high (but not saturated) soil water content (Hatch et al. 1998). Second, Site B had a higher total-N content (Table 3.5). Nitrifier populations are greater where total-N (organic-N, NH_4^+-N, and NO_3^--N) is higher (Adrakani et al. 1974), and NA has been significantly correlated with total-N (Staley et al. 1990). Finally, addition of fertilizer-N to soils have been shown to increase populations of *Nitrosomonas* and *Nitrobacter* (Adrakani et al. 1974; Berg and Rosswall 1985; Martikainen 1985; Donaldson and Henderson 1990). Site B had a longer history of fertilization (ten years) than Site A (one year).

Net Nitrification Rates

The rate-limiting step for nitrification in most soils is conversion of NH_4^+ to NO_2^- (Tate 1995), hence the positive correlation of net nitrification rates with soil NH_4^+-N concentration (Site A) and net N-mineralization rates (Sites A and B) (Tables 3.6 and 3.7). In general, the data for net nitrification rates do not correlate well with NA. Unlike NA, net nitrification was not highest in winter and lowest in spring/fall at either site. For Site A, no seasonal differences were observed, although net nitrification rates and NA were significantly correlated (Figure 3.20a, Table 3.6). For Site B, net nitrification rates were highest in summer (Figure 3.20b). This could be because

soil NH_4^+-N reached a peak in June 1997 at all three Site B plots (Figure 3.11), although there was another NH_4^+-N peak at the biweekly plot in November 1997. Higher soil temperature in summer was likely a very important factor influencing *in situ* nitrification, since nitrification rates increase with temperature (Tate 1995). Since *in situ* net nitrification rates were higher in summer for Site B, NA was expected to be higher in summer as well. However, this was not the case. One explanation for this discrepancy could be that the moisture content of the field-incubated soil cores was kept higher and more constant by the polyethylene incubation bags. Thus net nitrification rates (and perhaps NA) would have been greater in the field-incubated cores, where warm and more moist conditions would have been favorable for decomposition of organic-N (Tate 1995). Nitrifier activity was not determined for field incubated soil cores in this study, therefore this hypothesis would need to be tested in a separate study.

Higher overall net nitrification rates in the biweekly treatment at Site B could be explained by the fact that the biweekly plot received approximately twice the N load as the weekly and monthly plots (Table 3.4). However, higher net nitrification rates at the monthly plot in April through July 1997 do not correspond with higher N load at that plot.

Net N-mineralization Rates

High correlation of net nitrification rates and net N-mineralization rates for both Sites A and B (Tables 3.6 and 3.7) was expected because these processes are closely linked (Hatch et al. 1998). Application of animal waste has been shown to increase soluble organic-N and C in soil (Reddy et al. 1980), as well as N-mineralization rates (Jha et al. 1996). Since generally 80% or more of the total-N in applied lagoon effluent was in the form of NH_4^+, the effluent itself was probably not a significant source of organic-N substrate for N-mineralization. Higher net N-mineralization rates at Site B were likely due to its longer fertilization history, and the subsequent larger amounts of crop residues and higher levels of root exudates in the soil.

Summer net N-mineralization rates at Site B could have been enhanced by soil drying and wetting cycles (Figures 3.21a and 3.21b). The desiccation of soil in summer probably caused partial senescence of the soil microbial population (Vinten and Smith 1993). Net N-mineralization may have increased due to the decomposition of the dead microbes by the living bacteria. While the buried bag technique stabilizes the moisture regime of the soil core during its incubation period, it is possible that drying and wetting cycles (and the effects outlined above) affected the population of N-mineralizing bacteria before the soil was bagged. For example, if dry soil was wetted by rainfall and then bagged the next day, the moisture of the core could have supported decomposition of organic matter for the entire incubation period. Warm summer temperatures would have also enhanced rates of net N-mineralization (MacDuff and White 1985), further promoting organic matter breakdown and contact between N-mineralizing bacteria and substrate (Vinten and Smith 1993; Giambiagi et al. 1993).

Analysis of within-month treatment effects for Site B (February and April-June 1997) do not indicate an observable pattern, since the prescribed fertilization schedule was largely violated during those months (Table 3.4). The same is true for Site A (Table 3.3). The manner in which net N-mineralization was measured at each treatment plot (average of 5 soil composites divided by the number of days) provided only one rate per plot, which prevented statistical analysis of data.

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