ABSTRACT

MOORE, AMBER DAWN. Nitrogen availability of anaerobic swine lagoon sludge: Sludge source and temperature effects. (Under the direction of Robert L Mikkelsen.)

Increased numbers of swine producers in North Carolina will be removing sludge from their lagoons in the next few years, mainly due to an increase in lagoons exceeding sludge capacity. Information on availability of nitrogen (N) in the sludge is needed to make improved recommendations about its use as a nutrient source for crops. The objectives of this study were to investigate possible affects related to lagoon sludges from different companies and operation types and to evaluate effects of seasonal temperatures and various application dates on the availability of N in lagoon sludge. Two separate incubation studies and one greenhouse study were conducted to quantify the N availability of the sludge. Sludges were mixed with a Wagram soil (loamy, siliceous, thermic Arenic Kandiudult) and incubated for one year at fluctuating seasonal temperatures based on four application dates (Feb. 26, June 4, Sept. 3, and Dec. 3). A second incubation experiment was conducted using sludges from three different company and operation-types. Samples were analyzed monthly for nitrate and ammonium. These sludges were also applied as the primary N source for bermuda grass, which was grown in the greenhouse, harvested and analyzed for total N.

Operation effects were not detected in the incubation and greenhouse experiments. Company effects were detected in the incubation experiments yet considered to be negligible because differences were only significant ($p \leq 0.05$) at weeks

0, 2, 3, and 8. A quadratic plateau curve fit to N mineralization data for all sludge sources ($r^2 = 0.52$) demonstrated that most of the active organic N was mineralized after 8 weeks of incubation. Nitrogen availability for all sludges averaged 45 % after 8 weeks for the incubation study, but only 20 % for the 14-week greenhouse study. This may have been related to inconsistent moisture throughout the soil in pots.

In the incubators with fluctuating temperatures, NH_4 remained in the soil for 4 months in the simulated winter application and for only 1 month for the simulated fall and summer applications, illustrating a direct influence of temperature changes on nitrification. Sludge N availability was fit to a nonlinear regression model for a first order reaction as follows: $N_t = N_o (1 – e^{-kt}) + N_{os}$ where: N_t = total inorganic N concentration, over time (mg N/ kg); N_o = potentially available organic N (mg N/kg); k = first order rate constant ($month^{-1}$); t = time (month); and N_{os} = inorganic N concentration when time = 0. Rate constants (k) increased between simulated applications as follows: fall (0.07) < winter (0.075) < spring (0.22) < summer (0.36). Sludge applied during simulated winter temperatures released N at a relatively constant rate, as compared to simulated summer temperatures, which increased rapidly during the first 6 months, then stabilized to allow minimal increase of mineralized N for the remainder of the incubation.

Predicted N availability for all temperature treatments after one year of incubation averaged 74 % of the total N applied, supporting agronomic recommendations of 60 % first-year plant-available N for incorporated swine lagoon sludge (NCCES, 1997). Year-long coefficients are unable to provide N availability information for short time length for growing seasons. To account for this, N availability for each month after sludge application was estimated using the first order equations for each simulated application date.

NITROGEN AVAILABILITY OF ANAEROBIC SWINE LAGOON SLUDGE: SLUDGE SOURCE AND TEMPERATURE EFFECTS

by
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BIOGRAPHY

Amber was raised in Soddy-Daisy, Tennessee by her parents, Shirley and Roger, with her siblings, Eric and Kala. Growing up she enjoyed activities such as playing soccer, performing the clarinet, and spending summers riding horses at Girl Scout camp. After high school she moved to Alabama, where she completed a B.S. degree in Environmental Science at Auburn University. Wanting to combine her interests in working with children and protecting the environment, Amber moved to Trinity, Texas to work at the Outdoor Education Center, a state-funded outdoor education program for fifth grade children from Houston, TX. In Texas Amber decided that although she enjoyed teaching young children, she would be happier going back to college to strengthen her environmental science background. Two years later she moved to Raleigh, N.C. to pursue a M.S. in Soil Science at North Carolina State University under Rob Mikkelsen, where her research focused on estimating the availability of nitrogen from swine lagoon sludge to plants, mainly to reduce the potential for nitrate leaching into groundwater. After graduation, she will begin working with Rich McLaughlin toward a Ph.D. in the same department, focusing on the prediction of erosion losses from construction sites. Upon the completion of her Ph.D. degree, she plans to continue on in the field of environmental soil science in a university setting, focusing on research and teaching. When she finds a moment of free time, she enjoys running in her neighborhood, cooking new vegetarian dishes, baby-sitting for the Car and Renegar families, teasing her cats Oatmeal and Stirfry, and spending time with her friends and family.

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Chapter 1 - Introduction

BACKGROUND

The swine industry has made a significant economic, environmental, and social impact in North Carolina over recent years. The number of swine in the state almost doubled from 5.1 million to 9.6 million from 1992 to 1997, with 88% of farms containing at least one thousand swine (U.S. Census Bureau, 1999). High numbers of swine yield large amounts of waste. The most common method for managing swine waste in N.C. is treatment and temporary storage in anaerobic lagoons before applying effluent to crops (CAST, 1995).

Anaerobic lagoons are used because they reduce biological oxygen demand, are inexpensive to operate and maintain, and require a relatively small amount of land for treatment. Waste is generally moved to the lagoons by flushing the material from pits beneath the swine houses with recycled lagoon liquid (CAST, 1995). Anaerobic bacteria, originating from the intestinal tract of warm-blooded animals, help to decompose the waste. Acid-forming bacteria break down complex organic compounds, such as lipids and proteins, to simpler compounds such as monosaccharides and amino acids, which are further broken down to acetic acid and methylamines by a second group of acid-forming bacteria (Crites and Tchobanoglous, 1998).

Methanogenic bacteria convert these relatively simple organic compounds into methane, CO_2, and NH_3, which may be lost from the lagoon as gases (Crites and Tchobanoglous, 1998). Leftover bacteria and slowly decomposable organic materials settle and accumulate at the bottom of the lagoon as sludge. The liquid portion, or effluent, is irrigated onto nearby fields.

Sludge removal

Sludge from swine lagoons is removed less regularly than effluent because it is relatively small in volume and difficult to remove. Sludge removal has recently become a major interest for waste application in North Carolina because 1) active lagoons are aging and reaching their sludge storage capacity, and 2) new policies may lead to the permanent closure of all lagoons in the state.

Sludge can accumulate in anaerobic swine lagoons for a relatively long period of time, before exceeding the sludge accumulation zone, usually after 10 to 15 years of operation (Sheffield et al., 2000). Once the zone is exceeded, the sludge may be removed by one of three methods:

- Agitating the entire lagoon into a slurry to be pumped for irrigation onto adjacent fields,
- Dewatering the lagoon, and agitating and pumping the remaining sludge into tanker trucks for subsequent soil incorporation or injection with a spreader tank, or
- Dewatering the lagoon, dredging the sludge with a dragline, dewatering the sludge in a bermed area, and distributing on forage or cropland with a manure spreader (Sheffield et al., 2000)

Mike Easley, the current governor of N.C., proposed to improve water quality in the state by initiating his Clean Water Plan as one of his campaign promises, which included the elimination of all anaerobic swine lagoons within five years of its implementation (Dyer, 2000). Although future implementation of this plan is uncertain, future lagoon closures should still be considered a possibility. When a lagoon is closed, sludge depth must be reduced to a depth of one foot or less, at which point the farmer may 1) fill the lagoon with soil and grow crops, 2) breach the existing berm and grow crops, or 3) maintain the lagoon as a farm pond (Sheffield et al., 2000).

Due to this potential for large increases in sludge application, the fate of nutrients in sludge should be examined so that farmers can supply sludge in amounts that provide optimum yields without health or environmental risks.

Health concerns

Nitrate (NO_3) accumulation in wells can have significant impacts on health. Nitrate concentrations for drinking water must not exceed 10 mg NO_3-N/L to prevent health problems, such as methemoglobinemia in infants (EPA, 1986). Normal oxyhemoglobin is oxidized to methemoglobin once nitrite (NO_2) is adsorbed in the bloodstream. Methemoglobin cannot transport O_2, which causes suffocation-like symptoms that may lead to death (EPA, 1986). Stomach cancer may also be related to high NO_3 concentrations, although the supporting evidence is currently inconclusive (Stevenson and Cole, 1999).

Disease-causing pathogens are commonly a concern associated with the land-application of untreated waste, but less of a concern for treated wastes. Once pathogens and parasites are introduced to an environment outside the host, survival is difficult. The process of primary settling for municipal treatment has been shown to cause a 30 – 40 % reduction in pathogenic populations (Hoadly and Goyal, 1976). Foster and Engelbert (1973) estimated a 50 % reduction in *Salmonella* and *Mycobacterium* populations from raw wastewater to primary effluent in municipal treatment. However, disease outbreaks may still occur, especially if raw food is consumed from land receiving waste applications (Elliot and Ellis, 1977).

<u>Environmental concerns</u>

Elevated concentrations of NO_3 in stream and lake environments can trigger an environmentally devastating phenomenon called eutrophication (Pierzynski, 1994). Eutrophication begins when an increase in the nutrient concentrations (mainly nitrogen (N) and phosphorus (P)) in natural water environments stimulates the growth of algae and water plants. Bacteria use up available dissolved oxygen in the water to decompose the increased amounts of plant biomass, causing aquatic animal life to suffer. Increases in N and P in natural bodies of water are often delivered by surface runoff and groundwater discharge from nearby agricultural areas where excess nutrients have been applied. Concentrations ranging between 0.5 and 1.0 mg N/L in freshwater environments are commonly used as threshold concentrations for eutrophication, which is considerably lower than the previously stated acceptable concentrations based on health concerns (Pierzynski, 1994).

There is concern that applying animal waste to crops based on N content will allow P, copper (Cu), and zinc (Zn) to accumulate to high levels in soil because due to application in excess of crop requirements. This could increase P losses from surface runoff, and increase potential toxicities to Cu and Zn-sensitive plants. For example, if sludge was applied to a corn crop on a Norfolk soil based on the N requirement (154 kg N/ha), P would be applied in 13-fold excess and Cu and Zn would be applied at 3 times the required agronomic rate as recommended by NCDA & CS. The U.S. Natural

Resource Conservation Service (NRCS) have recently included P-based waste application recommendations in their policy for nutrient management (NRCS, 1999), which should help reduce P losses from land-applied waste materials in North Carolina once an effective P index rating system has been adopted.

Land applied wastes

Farmers who raise confined livestock commonly apply animal waste to surrounding crop fields as organic fertilizer. Land application of animal waste can be economically advantageous for the farmer, reducing financial costs related to both the purchase of chemical fertilizers and for methods of disposal such as off-site removal to a landfill or permanent storage.

Application rates of inorganic and organic nutrient sources are currently based on the N requirement of the growing crop. Nitrogen is generally considered the most limiting nutrient for plant growth, since it can be quickly leached out of the soil matrix, taken up by plants, transformed to gaseous forms through denitrification (N_2, N_2O, and NO) and volatilization (NH_3), or immobilized in insoluble organic compounds. Such losses may cause plants to become N deficient. To estimate how much waste should be applied, it is necessary to understand how much of the N in the waste will become available for plant use.

Nitrogen mineralization

Approximately 75 % of N in anaerobic swine lagoon sludge is organic N (Chapter 2). Organic N is unavailable to plants until it is converted to NH_4 through mineralization by heterotrophic bacteria and fungi (Haynes, 1986). The first step of this process is aminization, where proteins and peptides are converted to amines and amino acids (Stevenson and Cole, 1999). The amines and amino acids are converted to ammonia (NH_3) during ammonification, and reacts readily with water to form NH_4 (Stevenson and Cole, 1999). Ammonium in the soil environment is either adsorbed by plant roots or rapidly converted to NO_3 through nitrification (Schmidt, 1982).

Nitrification, similar to mineralization, is a biologically mediated two-step process, although it is dependent on a small group of autotrophic bacteria instead of a diverse group of heterotrophic bacteria (Stevenson and Cole, 1999). Ammonium is first oxidized to NO_2 by species such as *Nitrosomonas* bacteria using the following reaction:

$2\ NH_4 + 3\ O_2 \rightarrow 2\ NO_2 + 2\ H_2O + 4H$,

and may be further oxidized to NO_3 by species such as *Nitrobacter* bacteria as follows:

$2\ NO_2 + O_2 \rightarrow 2\ NO_3$

Nitrite rarely accumulates in agricultural soils, since the NO_2 is converted to NO_3 at a much faster rate than NH_4 is converted to NO_2.

Because N mineralization and nitrification are biological processes, they are heavily dependent on environmental factors. Optimum conditions include soil pH values between 5.0 and 7.8 (Barber, 1984), C:N ratios below 20:1, (Stevenson and Cole, 1999) moderate aeration and moisture, and temperatures between 25 and 35 ºC (Schmidt, 1982).

Before farmers can apply waste amendments to crops, they have to consider plant-available N (PAN), which is typically defined as the fraction of total N in inorganic forms such as NO_3 and NH_4, and readily decomposable organic N compounds. Plant-available N estimates for a specific waste material can vary greatly, depending on whether one month, six months, a year, or longer is used to define the period of release from organic N compounds. Plant availability coefficients, or percentages of plant-available N, are generally established one year after application (NCCES, 1997). Farmers use coefficients to apply waste to their crops at a rate intended to minimize NO_3 leaching while optimizing crop yields.

RESEARCH FOCUS

Current information for farmers regarding the amount of available N in anaerobic swine lagoon sludge is very limited. The North Carolina Department of Agriculture and Consumer Services (NCDA & CS) recommendations for farmers assume that 60% of total N in swine lagoon sludge incorporated in the soil will become available to plants during the first year following application (NCCES, 1997). This mineralization estimate does not consider differences in seasonal temperatures, varying application dates, available N over shorter periods of time, or sludges from various farming operations. These factors could influence N mineralization rates, allowing the sludge to be applied at rates different than required by the crop, and increasing the potential for NO_3 leaching and plant N deficiencies.

OVERALL OBJECTIVES

The objectives of this research were to:

1) evaluate effects of seasonal temperatures on the N mineralization of anaerobic swine lagoon sludge under simulated winter, spring, summer, and fall applications during long-term incubations,

2) compare N recovery from lagoon sludges from different operation types (sow, nursery, and finishing farms) and different companies by measuring plant N uptake of bermuda grass in the greenhouse and by measuring N release during aerobic incubations, and

3) provide research information to help develop recommendations for sludge application that would minimize NO_3 leaching and optimize N uptake by plants.

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Chapter 2 – Nitrogen Availability of Anaerobic Swine Lagoon: Sludge Source Effects

INTRODUCTION

When predicting N availability (percent of N either in plant-available forms such as NH_4 or NO_3, or in the mineralizable organic N fraction) of anaerobic swine lagoon sludge for crop production, it may be important to consider variability among companies and types of farming operations. These differences could affect the physical, chemical, and biological properties of sludge in anaerobic swine lagoons, which could also affect N mineralization rates.

Companies

There are several swine-producing companies in North Carolina. Each company uses specific practices designed to maximize pork production while minimizing financial costs and negative environmental impacts. These practices include proprietary selection of feed ingredients, methods of barn cleaning, types of detergents used, and lagoon management. Because the impact of companies on sludge composition has been assumed negligible compared to other possible factors, company differences in swine lagoon sludge have not been studied extensively.

Operations

In North Carolina, swine are typically divided among sow, nursery, and finishing farms. Sow farms contain female and newborn swine, which are raised to a weight of 10 kg (Ensminger and Parker, 1984). At this weight, the swine are considered to be in the prestarter stage, at which time they are transferred to a nursery, and raised to a weight of 25 kg (Ensminger and Parker, 1984). Finally, the swine are sent to a finishing farm, where they are raised until they reach market weight, ranging between 90 and 120 kg (Whittemore, 1998). Dividing swine by size among farming operations improves efficiency and helps to reduce production costs by phase feeding. Phase feeding allows optimum feed and nutrition to be provided for the swine at different stages of growth development, taking into account that swine require different levels of nutrients and energy during each stage of growth.

Relatively high concentrations of Cu and Zn are added to feed for nursery swine (Table 2-1), since these micronutrients are known to both stimulate growth and enhance disease resistance (NRC, 1998). Kornegay and Harper (1997) estimated that nursery and finishing swine digest approximately 10 to 25% of Cu and 10 to 45% of Zn in feed. Younger swine generally retain higher concentrations of minerals than older swine. Unabsorbed Cu and Zn are excreted in the manure and deposited mostly in the sludge layer of lagoons, excluding a small portion that remains in the effluent. This is a concern for subsequent sludge application to soil, since Cu and Zn in high concentrations can be harmful for plant growth.

Table 2 - 1. Typical metal concentrations present in swine feed during the three growth stages.

Phase	Diet	
	Cu	Zn
	mg/kg feed	
Sow	15	125
Nursery	240	2,000
Grower-Finisher	15	125

Adapted from Kornegay and Harper, 1998.

In North Carolina, the recognized critical toxic levels in soils that receive application of wastes products are 120 kg/ha for Cu and 240 kg/ha for Zn, based on a plow layer depth of 20 cm (NCDA & CS, 2001). These levels were established to prevent accumulations that could eventually result in toxicity to crops. Peanuts (*Arachis hypogaea*) are especially sensitive to Zn toxicities. Davis and Parker (1993) detected significant reduction of plant height and biomass weights in peanut plants that were cultivated in pots with Zn concentrations in the soil at 63 kg/ha. Toxic concentrations of Cu and Zn will generally inhibit root growth (Lexmond and Vorm, 1981; Godbold et al. 1983) and may stimulate chlorosis in the leaves (Sandman and Boger, 1983; Woolhouse, 1983), which is theorized to result from Fe deficiency (Woolhouse, 1983).

Since Cu and Zn could inhibit plant growth, there is concern that these metals could also inhibit microbial processes such as N mineralization. Researchers have found that soil additions of Cu and Zn can both stimulate (Quraishi and Cornfield, 1971; Premi and Cornfield, 1969; Premi, 1970) or inhibit (Liang and Tabatabai, 1978) N mineralization. Among these studies, stimulation of N mineralization was generally a result of low, growth-limiting concentrations of Cu and Zn initially present in the soil. Inhibition of N mineralization determined by Liang and Tabatabai was influenced by high, toxic concentrations of inorganic Cu and Zn in the soil (318 and 327 ppm, respectively). Other studies show that Cu and Zn in soils have little to no effect on N mineralization, especially over longer periods of time, leading to the theory that N mineralizing microbes adapt and become tolerant to the high concentrations of Cu and Zn (Minnich and McBride, 1986; Premi and Cornfield, 1969). Among all of these studies, differences included soil type, field or lab methods, whether Cu and Zn were added or already in the soil, how much was added, and varying Cu and Zn compounds, which helps explain inconsistency in results.

Measuring N availability

Several methods may be used to quantify N availability of land-applied waste materials, such as aerobic incubation and plant uptake experiments. In aerobic incubation studies, the organic amendment is added to the soil, incubated in a temperature-controlled environment, and periodically analyzed for the presence of NO_3 and NH_4. In plant uptake experiments, the amendment is applied to soil in a greenhouse setting, and the plants growing in the soil are analyzed for N. Aerobic incubations are more common than greenhouse experiments, because incubations are less inexpensive and require minimal work. However, greenhouse experiments provide environmental conditions that are more closely related to field conditions than incubator environments. They also allow for direct comparisons between organic and inorganic amendments, since chemical fertilizers can easily be incorporated into the treatment scheme. Many studies use both methods when estimating N availability and measuring how much N is contributed by the waste amendment for plant growth (King, 1981; King, 1984; Westerman et al., 1989; Duffera et al., 1999a: Duffera et al., 1999b).

Objective

The goals of this study were to characterize the chemical composition and plant availability of N from anaerobic swine lagoon sludge, and to evaluate potential differences related to varying companies and farming operations. Two methods, measuring N release through aerobic incubations and greenhouse bioassay of N availability, were used to estimate N availability over time.

METHODS AND MATERIALS

Soil

The top 23 cm of a Wagram sand (loamy, siliceous, thermic Arenic Kandiudult, 89% sand, 7% silt, and 4% clay) was collected from an unlimed forested area on the Central Crops Station near Clayton, North Carolina and passed through a 6-mm sieve. A Coastal Plain soil was used for this research since the majority of swine farms in North Carolina occur in the Coastal Plain area. The pH of the soil was raised from 3.6 to 6.5 by adding $CaCO_3$ fours days before the incubation at a rate of 2.0 g/kg, which was based on a preliminary two-week incubation using several different liming rates. The increase of soil pH after the addition of $CaCO_3$ was determined three weeks after the first day of incubation. The soil was amended with KH_2PO_4 at a rate of 0.20 g/kg to maintain environment that was not P or K limiting. The soil was stored in a shaded area under a polyethylene cover 2 months prior to the greenhouse study and for 6 months prior to the incubation study. This resulted in a lower soil pH for the incubation study (6.0) than for the greenhouse study (6.5). The greenhouse experiment was conducted from July 18th to October 26th in Raleigh, NC.

Sludge

Sludges were retrieved from anaerobic lagoons belonging to three swine companies, which will be referred to as Companies A, B, and C in this paper. Sow (S), nursery (N), and finishing (F) farm lagoons were sampled from each company. Lagoons were located in Duplin, Wayne, and Sampson counties in North Carolina. Samples were also taken from lagoons on farrow to finish operations from NC State University research stations in Rocky Mount (Rm) and Plymouth (Pl), NC. A total of 11 sludges ((3 companies X 3 operations) + 2 research station sludges) were collected.

Sludge samples were collected from 4 to 5 different locations throughout each lagoon. The sludge samples were retrieved by lowering an Ekman (3450 mL) grab sampler by rope. Once the metal, box-shaped sampler settled at the lowest possible depth, a weight attached to the rope was dropped to release the shutters, thereby closing and capturing the sample. The sampler was pulled to the surface, where excess liquid drained from the sampler for several minutes before releasing the sludge sample into 11 to 19 L plastic buckets, which were capped and refrigerated to reduce microbial activity.

Lagoon depth and sludge depth were measured at lagoons 1 through 6. Lagoon depth from the liquid surface was estimated by lowering a rope attached to a metal weight into the lagoon, marking the rope at the liquid-air interface, and recording the length of the rope and the weight. Sludge depth was estimated by lowering a flat, plastic disk with a 20 cm diameter and a 3 cm thickness into the lagoon until the disk could no longer be lowered, recording rope length and subtracting it from the measured lagoon depth. Lagoon characteristics are described in Table 2-2.

Measurements for pH were taken by inserting a pH electrode into 5 to 7 mL samples of undiluted sludge. The C:N ratio of oven-dried samples was determined through combustion using the Perkin Elmer 2400 CHN elemental analyzer. Samples were digested using the persulfate method for Total Kjeldahl N analysis (U.S. EPA, 1979a). Values for Total Kjeldahl N (TKN) and NH_4-N were determined within a few days of sludge collection using the ammonia salicylate method for automated analysis (U.S. EPA, 1979a). Total (TS) and volatile solid (VS) percentages were determined gravimetrically (U.S. EPA, 1979b). Total Cu, Zn, Mg, K, and P were determined following the nitric acid digestion using Inductively Coupled Plasma (Novazamsky et al., 1986).

Aerobic Incubation

Each of the eleven sludges were added to 100 g of soil (on a dry weight basis) to provide 200 mg N/kg soil. The soil and sludge were hand mixed in 0.033 mm thick Ziploc ® polyethylene sealable bags (16.5 cm X 14.9 cm) until the sludge appeared to be spread uniformly throughout the soil. Soil moisture was maintained at approximately 80% container capacity by adding water to the bags when the weight decreased by more than 5%. Container capacity was determined to be about 24 % using the method described by Cassel and Nielsen (1986).

All twelve treatments (including eleven sludge treatments and one unamended control) were replicated four times, divided randomly among blocks, and placed in temperature-controlled incubators. Block 1 and 2 bags were placed in incubator A, and Block 3 and 4 bags were placed in incubator B. Bags were removed from the incubators either immediately after mixing or 1, 2, 3, 4, 6, 8, and 12 weeks after mixing to evaluate production of inorganic N over time. A total of 384 samples were incubated (4 replications x 8 sampling periods x 12 treatments). Incubator temperatures were maintained at 25 ± 2 °C for the 12-week experiment.

At each sampling date, 10-g samples were weighed, shaken with 25 mL of 1M KCl for 30 minutes, and filtered through prewashed #2 Whatman filter paper. The extracts were frozen, until analysis of NO_3 (NO_3 and NO_2 concentrations combined) and NH_4 using Lachat QuikChem Methods 10-107-04-1-A and 10-17-06-2-A, respectively (Lachat, 1992).

Table 2 - 2. Selected anaerobic swine lagoon characteristics of sites used in incubation and greenhouse experiments.

Lagoon	Operation Type†	Company‡	Lagoon Vol.§	Sludge Vol.	Length of Operation
			- - - - m^3- - - -	- - - % - - -	- -years- -
1	S	A	85500	47	7
2	N	A	33200	43	7
3	F	A	15300	53	20
4	S	B	89100	61	7
5	N	B	1800	62	6
6	F	B	7900	23	5
7	S	C	17600	NA #	10
8	N	C	9600	NA	10
9	F	C	9300	NA	9
10	R	Rm	9000	NA	29
11	R	Pl	13300	NA	16

† S = Sow, N = Nursery, F = Finishing, and R = Research Station.
‡ Rm and Pl represent sludges from research stations in Rocky Mt. and Plymouth NC, respectively
§ Lagoon volume estimated based on length, width, and depth, with the assumption of 2 to 1 sideslopes for each lagoon.
# NA = Information is not available.

Plant Uptake

The previously collected samples of anaerobic swine lagoon sludge were added to soil at rates of 115 and 230 mg total N/kg soil. Ammonium nitrate (NH_4NO_3) was applied for comparison as a completely soluble N source at rates of 77, 153, and 230 mg total N/kg soil. A control treatment with no N added was also prepared. Two of the NH_4NO_3 rates were initially intended to match both sludge N rates, but this did not occur because the NH_4NO_3 treatments were applied to pots based on a soil weight of 2.0 kg as opposed to 1.5 kg. Pots containing 1.5 kg soil (on a dry weight basis) were seeded at a rate of 0.5 g seed/pot with hulled common bermuda grass seed (*Cynodon dactylon*). Pots were placed on two benches in a randomized block design using four replications. To ensure that all pots were receiving the same amount of moisture, pots were weighed monthly and water was added to maintain soil moisture content at 80% of field capacity. Grass blades were harvested by trimming the grass to a height of 2.5 cm at 3 to 4 week intervals for 14 weeks. Plants were watered 3 to 4 times per week. The remaining roots and stolons were removed from the soil at the end of the experiment. Both root and grass samples were dried for a minimum of 72 hours at 60°C, weighed, ground with a Wiley Mill to pass a 20 mesh screen, and analyzed for N through combustion as previously described. Approximately two-thirds of the remaining soil in the pots was air-dried and used for the measurement of pH using the 1:1 soil to water ratio.

Statistics

Company and operation effects on inorganic N concentrations in the sludge-amended (minus the inorganic N concentrations contributed by the unamended soil) were determined over the 3-month incubation period with SAS using PROC GLM (SAS, 1998). Company effects on inorganic N concentrations were determined at each sampling period by comparing means, which were determined to be significantly different at $p \leq 0.05$, as determined by the least square means test.

SAS PROC NLIN was used to fit a segmented regression model to a quadratic plateau, in order to illustrate sludge N mineralization during the 3-month incubation (SAS, 1998).

Company and operation effects on N concentrations and weights of bermuda grass over time were determined with SAS using REML analysis, while effects on total N accumulation was determined using PROC GLM (SAS, 1998).

RESULTS AND DISCUSSION

Sludge Characterization

Chemical and physical properties of all sludges are illustrated in Table 2 – 3. Differences among companies were determined by comparing means and ranges. General comparisons were based on the 11 lagoons that were sampled, and should not be considered entirely representative of sludge from all swine companies or farming operations using anaerobic lagoons.

Several differences were detected among sludges from the three operations (Table 2 – 4). Sludges from nursery and finishing farms had higher concentrations of Cu than sludges from sow farms. Nursery swine are fed a diet with elevated Cu and typically excrete 3 to 8 times as much Cu as sow and finishing swine (Kornegay and Harper, 1997), therefore sludges from nursery farms were expected to have higher Cu concentrations. Sludges from nursery farms contained 5 and 4 times as much Zn as sludges from sow and finishing farms, respectively. The diet for prestarter nursery swine generally contains 16 times as much Zn as swine in all other phases (Kornegay and Harper 1997), explaining why Zn concentrations in nursery farm sludges were so high. Differences among companies were relatively small (Table 2 – 5), indicating that differences between companies has little effect on chemical and physical differences of sludge.

The TKN concentrations observed in this study were 1.3 and 1.7 times higher than concentrations determined by Bicudo et al. (1999) and Barker et al. (1994) (Table 2 - 6). Differences were most likely related to sampling methods. Anderson et al. (2000) illustrated that total N in the sludge layer increased with depth. Samples taken in this

Table 2 - 3. Chemical composition of anaerobic swine lagoon sludge, sampled from eleven lagoons in North Carolina.

Sludge I.D.	Operation †	Company ‡	TKN §	Cu	Zn	K	Mg	P	C:N	TS ¶	VS/TS ††	NH4-N	pH
			- - - - - - - - - - - - - - g/L					- - - - - - - - - - - - - -		%		%	
1	S	A	5.64	0.06	0.32	0.72	2.52	6.47	7.68	13.50	0.66	22.88	7.36
2	N	A	5.22	0.25	1.43	1.08	2.54	7.08	8.30	16.17	0.64	27.17	7.59
3	F	A	5.30	0.29	0.29	1.07	2.75	6.02	7.64	9.87	0.59	25.82	7.56
4	S	B	4.77	0.07	0.39	0.59	1.56	7.20	7.36	9.14	0.48	22.18	7.39
5	N	B	4.91	0.46	1.35	0.87	1.88	7.29	7.13	9.40	0.50	24.72	7.40
6	F	B	4.32	0.09	0.21	1.14	1.85	5.13	7.50	12.43	0.36	28.95	7.54
7	S	C	3.97	0.04	0.20	0.69	2.05	6.54	8.06	9.02	0.55	29.17	7.51
8	N	C	4.61	0.42	1.26	1.11	1.59	4.79	7.88	8.11	0.56	25.11	7.37
9	F	C	6.29	0.41	0.33	1.02	2.75	7.30	7.98	11.31	0.55	22.48	7.62
10	Rm	R	4.20	0.09	0.28	0.71	2.36	7.34	6.70	13.31	0.33	21.43	7.48
11	Pl	R	4.81	0.06	0.37	0.86	2.91	5.81	7.16	8.24	0.55	24.20	7.55
Average			4.89	0.19	0.54	0.88	2.19	6.54	7.57	10.71	0.52	24.63	7.47
Standard Deviation			0.62	0.16	0.46	0.20	0.48	0.95	0.43	2.46	0.10	2.53	0.10

† S = Sow, N = Nursery, F = Finishing, and R = Research Station.
‡ R and P represent sludges from research stations in Rocky Mt. and Plymouth NC, respectively.
§ TKN = Total kjeldahl nitrogen
¶ TS = Total solids
†† VS = Volatile solids

Table 2 - 4. Chemical composition of anaerobic swine lagoon sludge, as affected by the type of swine operation.

	Sow		*Nursery*		*Finishing*	
	Mean	Range	Mean	Range	Mean	Range
C:N	7.67	7.37 – 7.96	7.77	7.18 – 8.36	7.65	7.41 – 7.88
VS/TS	0.54	0.45 - 0.63	0.57	0.50 – 0.64	0.52	0.41 – 0.62
TS (%)	10.4	8.26 – 12.5	11.2	6.89 – 15.6	10.6	9.05 – 12.2
NH_4 - N (%) of TKN	24.4	21.2 – 27.6	25.7	24.4 – 27.0	25.0	22.0 – 28.0
pH	7.43	7.36 – 7.50	7.45	7.33 – 7.57	7.51	7.38 – 7.64
	- - - - - - - - - - - -	- - - - - - - - - - - -	- - - - - - - - g/L- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -
TKN	4.75	4.06 – 5.43	4.91	4.61 – 5.22	5.22	4.40 – 6.04
P	7.05	6.34 – 7.77	6.39	5.00 – 7.78	6.11	5.22 – 7.01
Cu	0.06	0.04 – 0.07	0.38	0.27 – 0.44	0.23	0.08 – 0.38
Zn	0.31	0.23 – 0.40	1.35	1.26 – 1.44	0.28	0.23 – 0.33
K	0.66	0.60 – 0.72	1.02	0.89 – 1.15	1.06	0.99 – 1.12
Mg	1.92	1.46 – 2.38	2.01	1.52 – 2.49	2.38	1.93 – 2.83

Table 2 - 5. Chemical composition of anaerobic swine lagoon sludge, as affected by swine company.

	Company A		*Company B*		*Company C*	
	Mean	Range	Mean	Range	Mea n	Range
C:N	7.87	7.50 – 8.24	7.41	7.23 – 7.58	7.97	6.16 – 8.06
VS/TS	0.63	0.60 - 0.66	0.48	0.40 – 0.55	0.55	0.55 – 0.56
TS (%)	13.2	10.0 – 16.3	9.93	8.49 – 11.37	9.48	7.83 – 11.13
NH_4 – N (%) of TKN	25.2	23.1 – 27.5	24.4	21.7 – 27.1	25.6	22.1 – 29.0
pH	7.50	7.38 – 7.63	7.42	7.34 – 7.51	7.50	7.37 – 7.63
	- - - - - - - - - - - -	- - - - - - - - - -	- - - - - - - - g/L- - - - - - - -	- - - - - - - -	- - - - - - - -	- - - - - - - -
TKN	5.38	5.16 – 5.61	4.72	4.45 – 4.98	4.96	3.76 – 6.16
P	6.52	5.99 – 7.06	6.72	5.58 – 7.87	6.21	4.92 – 7.50
Cu	0.20	0.08 – 0.33	0.16	0.00 – 0.33	0.29	0.07 – 0.51
Zn	0.68	0.03 – 1.33	0.52	0.05 – 0.99	0.60	0.02 – 1.18
K	0.96	0.75 – 1.16	0.84	0.61 – 1.08	0.94	0.72 – 1.16
Mg	2.60	2.48 – 2.73	1.80	1.55 – 2.05	2.13	1.55 – 2.72

Table 2 - 6. Chemical parameters of anaerobic swine lagoon sludge from various studies, within 95% confidence interval.

	Moore †	Bicudo †	Barker †	Sheffield ‡
# Sludges sampled	11	15	52 to 235	30
C:N	7.57 ± 0.23	NA	NA	NA
VS/TS	0.52 ± 0.05	0.44 ± 0.04	0.53	NA
TS (%)	10.7 ± 1.34	8.85 ± 1.48	10.0 ± 1.32	NA
NH_4-N (%) of TKN	24.6 ± 1.4	23.4 ± 3.8	24.3 ± 1.92	NA
pH	7.47 ± 0.05	7.77 ± 0.06	7.33 ± 0.06	6.53 ± 0.05
	- - - - - - - - - - -	- - - - - - - - - - - g/L	- - - - - - - - - - -	- - - - - - - - - - -
TKN	4.89 ± 0.34	3.85 ± 0.68	2.93 ± 0.21	2.97 ± 0.49
P	6.54 ± 0.52	3.75 ± 0.80	6.30	1.71 ± 0.33
Cu	0.19 ± 0.16	NA	0.04 ± 0.00	0.18 ± 0.05
Zn	0.54 ± 0.25	NA	0.10 ± 0.01	0.18 ± 0.03
K	0.88 ± 0.11	0.59 ± 0.08	0.78	0.18 ± 0.06
Mg	2.19 ± 0.26	1.13 ± 0.38	0.82 ± 0.09	0.53 ± 0.30

Adapted from Table 2-3 of this thesis, Bicudo et al.(1996), Barker et al. (1994), and Sheffield (2000).
† Sludges sampled from active lagoons.
‡ Sludges sampled from inactive lagoons.

study may have been extracted from a lower depth in the sludge layer than was the case for Bicudo et al. (1999) and Barker et al. (1994).

N availability (Aerobic Incubation)

Nitrogen concentrations in the control soil treatments were subtracted from the sludge amended soil N concentrations so that sludge N effects alone could be identified, assuming no interactions between soil and sludge N. Nitrogen concentrations in the soil alone are illustrated in Appendix Figure A2 - 1.

Farming Operation and Company Effects:

Nitrogen availability of the sludge was estimated by subtracting the N concentrations in the unamended soil control soil from the inorganic N concentrations in the sludge-amended soil. Analysis of the data showed that differences among farming operations had no significant effect on inorganic N accumulation over the 12-week experiment. Therefore the high concentrations of Cu and Zn present in the nursery sludges did not negatively affect N mineralization. Minnich and McBride (1986) found similar results in soils amended with Cu-rich sludges, theorizing that mechanisms such as intracellular immobilization of metals and extracellular precipitation of metal compounds may have enhanced microbial tolerance (Gadd and Griffiths, 1978).

Using PROC GLM, company x week interactions were significant at $p \leq 0.05$, therefore company effects at each sampling period were determined based on least squared differences. Significant company effects were detected at individual sampling periods (Table 2 – 7), however no one company was significantly higher or lower than the other two companies at all sampling periods. Therefore, company effects were considered to be negligible.

The TKN/Cu and TKN/Zn ratio of sludges were used to calculate the amount of Cu and Zn added to soil in the incubation study. Nursery sludge supplied the equivalent of 34 kg Cu/ha and 121 kg Zn/ha. Sludge from finishing farms supplied an equivalent of 19 kg Cu/ha and 23.5 kg Zn/ha. Assuming the unamended soil had low background concentrations of Cu and Zn, these concentrations are well below critical toxicity levels for crop plants (NCDA & CS, 2001). However, concentrations in the nursery sludges were higher than concentrations toxic to peanuts (63 kg Zn/ha) found by Davis and Parker (1993). Based on these findings, the application of nursery sludges to peanut crops would not be recommended.

Table 2 - 7. Inorganic N concentrations in sludge-amended soil (minus inorganic N concentrations in control soil), as affected by differences in companies.

Company	Week 0	Week 1	Week 2	Week 3	Week 4	Week 6	Week 8	Week 12
	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - mg N / kg	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -	- - - - - - - - - -
A	58.8a*	48.9a	87.6a	69.9ab	61.3a	88.5a	88.8ab	72.1a
B	46.8b	25.1ab	64.3b	64.5b	78.8a	89.7a	84.0b	82.8a
C	42.6b	20.6b	85.5a	75.5a	69.8a	100.7a	97.1a	86.4a

* Values in each column followed by the same letter are not significantly different as determined by the least square means test ($p \leq 0.05$).

Inorganic N:

The initial NH_4 fraction, averaged among all sludges, was about 25 % of the total N content (Table 2 - 3), and decreased to 20 % immediately after the initial mixing with soil. At the same time, nitrate concentrations contributed by sludge in the soil increased from 0 to 5 % after mixing, based on the fact that anaerobic sludges generally contain negligible amounts of NO_3. This was most likely a result of rapid nitrification of NH_4 to NO_3.

Almost all of the NH_4 initially present in the sludge was oxidized to NO_3 between week 0 and week 2 (Figs. 2-1, 2-2). It is likely that the NH_4 was converted to NO_3 through nitrification, since the soil NO_3 concentrations increased at the same rate that NH_4 concentrations decreased. Ammonium concentrations increased after 2 weeks of incubation for all treatments, peaking between week 6 and week 8 for sludges 1, 2, 4, 5, 8, 10, and 11 at 19 mg NH_4-N/kg, on average, and for sludges 3, 6, 7, and 9 at 10 mg NH_4-N/kg, on average (Figs. 2-1, 2-2). Ammonium accumulation was most likely related to the inhibition of nitrification. Factors such as low aeration in bags (bags were only opened once during the incubation period) and low moisture during the last month of incubation (about 5% moisture content) may have contributed to NH_4 accumulation in the bags.

Nitrate concentrations increased rapidly over the first two weeks of incubation, most likely due to nitrification of NH_4 in the applied sludges, and mineralization of the easily decomposable organic compounds that was subsequently nitrified. Nitrate accumulation appeared to have stabilized between 4 and 6 weeks for most sludges, after the readily decomposable organic compounds were mineralized.

Organic N:

Since company and operation effects were shown to be negligible, inorganic N concentrations of all sludges were combined in order to characterize the N availability of anaerobic swine lagoon sludge during the 3-month incubation period. Using segmented regression analysis, a best-fit curve in the form a quadratic plateau illustrates N release

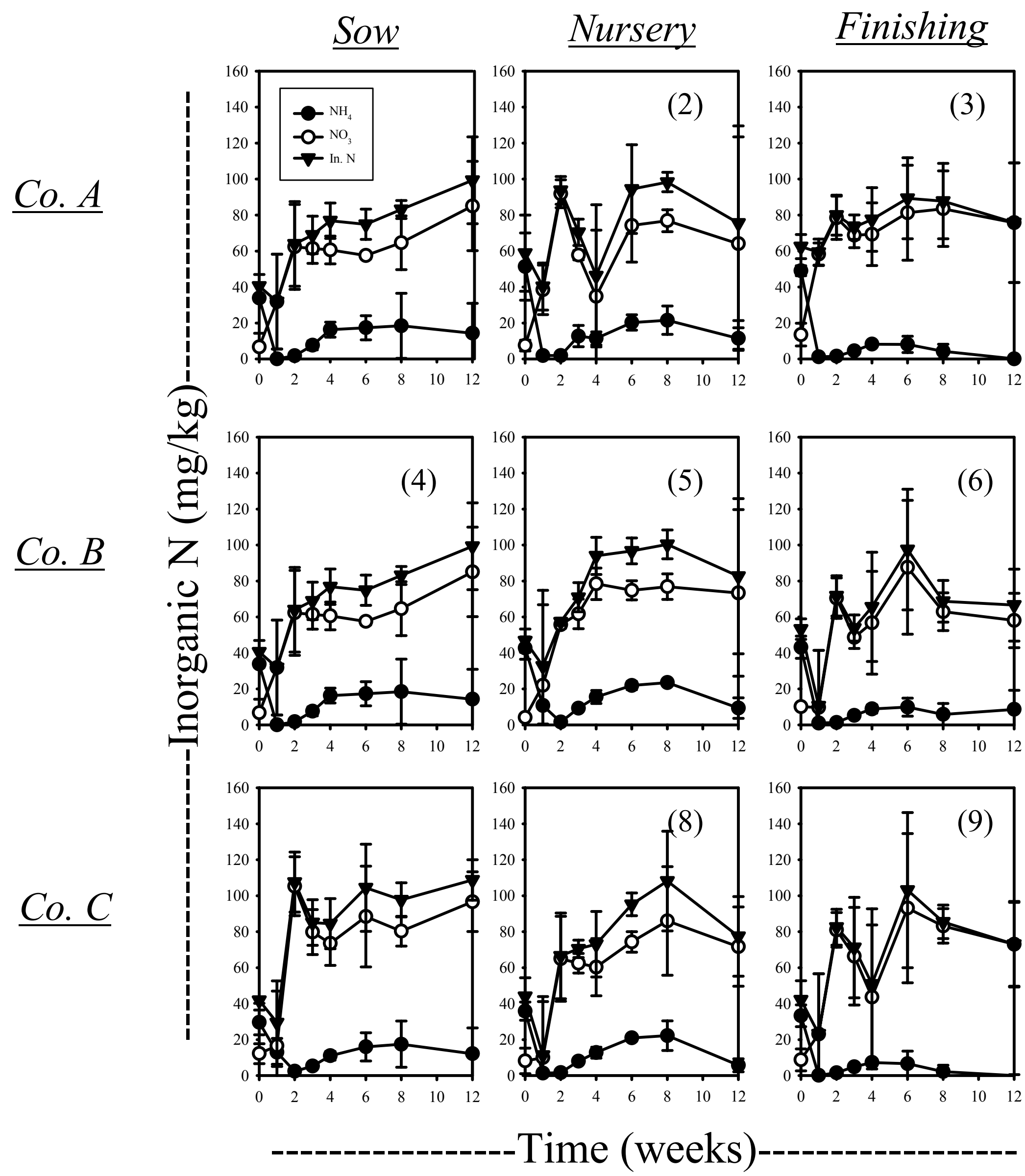
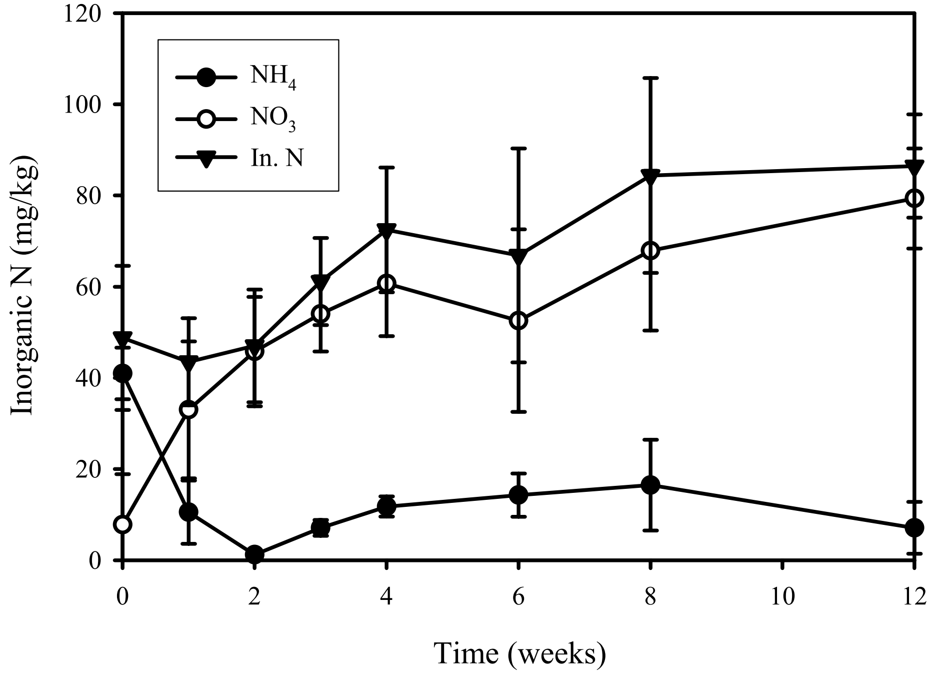


Figure 2 - 1. Inorganic N concentrations in soils to which nine anaerobic swine lagoon sludges (1-9) from three different companies (Co.) were added and incubated over a twelve week period. Nitrogen concentrations from the control soil treatment were subtracted from the sludge amended soil treatments. Error bars are based on standard deviations among 4 sampling units (one sludge treatment x four replications).



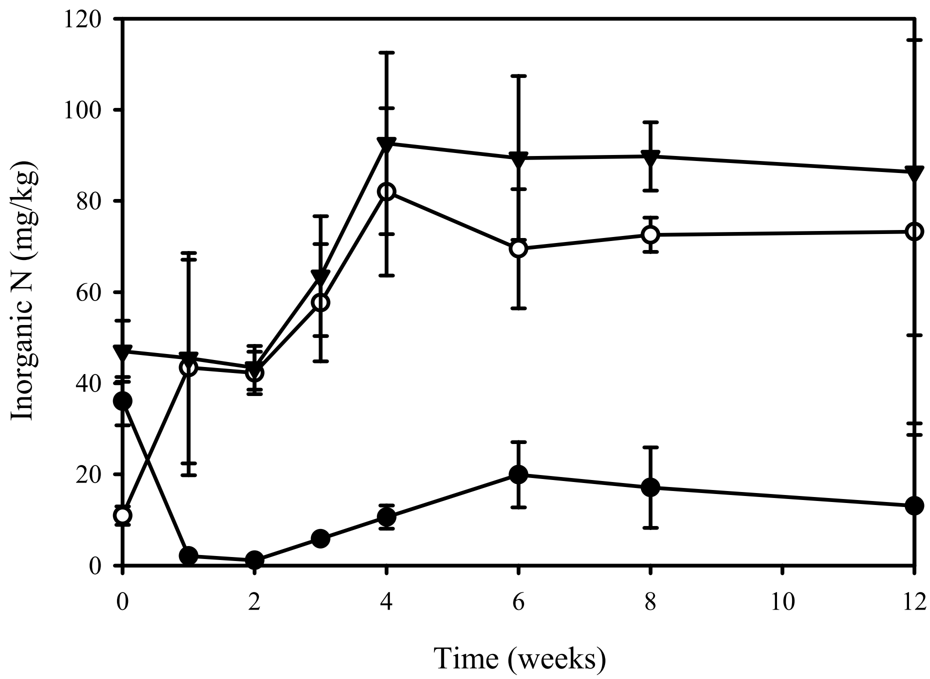


Figure 2 - 2. Inorganic N concentrations in soils to which two anaerobic swine lagoon sludges from research stations in Rocky Mt. (10) and Plymouth (11) were added and incubated over a twelve-week period. Nitrogen concentrations from the control soil treatment were subtracted from the sludge amended soil treatments. Error bars are based on standard deviations among 4 sampling units (one sludge treatment x four replications).

based on inorganic N means of each sludge at each sampling date (Fig. 2-3). From this analysis, it is estimated that most of the readily decomposable organic N compounds were mineralized after 8 weeks of incubation, where the curve begins to plateau. Immobilization may have caused the decrease in inorganic N concentrations at the twelfth week. Incubation experiments in N-rich environments often show a cycling pattern between increasing and decreasing concentrations of inorganic N (Westerman et al., 1988). A longer incubation experiment would be necessary to construct a curve that represented the mineralization of both active and stable organic N fractions.

Due to large variation and unusually low soil N concentrations measured at the 12th week sampling period, N availability estimates were compared at the 8th week sampling period instead. Nitrogen availability ranged from 34 to 54 %, averaging at about 45 % (Table 2 - 8). After 16 weeks of incubation, King (1984) found similar results, estimating 33% N availability for sludge from one anaerobic swine lagoon. Sludges 10 and 11 from this study were incubated in another study under relatively similar conditions and had 56 % available N after 8 weeks (Chapter 3), as compared to 44 % in the present study. This may have been a result of moderately higher temperatures ranging from 26 to 30°C in the other study. Also, increased initial NO_3 concentrations in the soil for the present study (47 ppm NO_3, as compared to 1.5 ppm NO_3) may have inhibited N mineralization. Soil was stored in a shaded area under a polyethylene 6 months prior to the present study, and 2 months prior to the other study. It is assumed that the longer storage period for the soil from the present study allowed more N

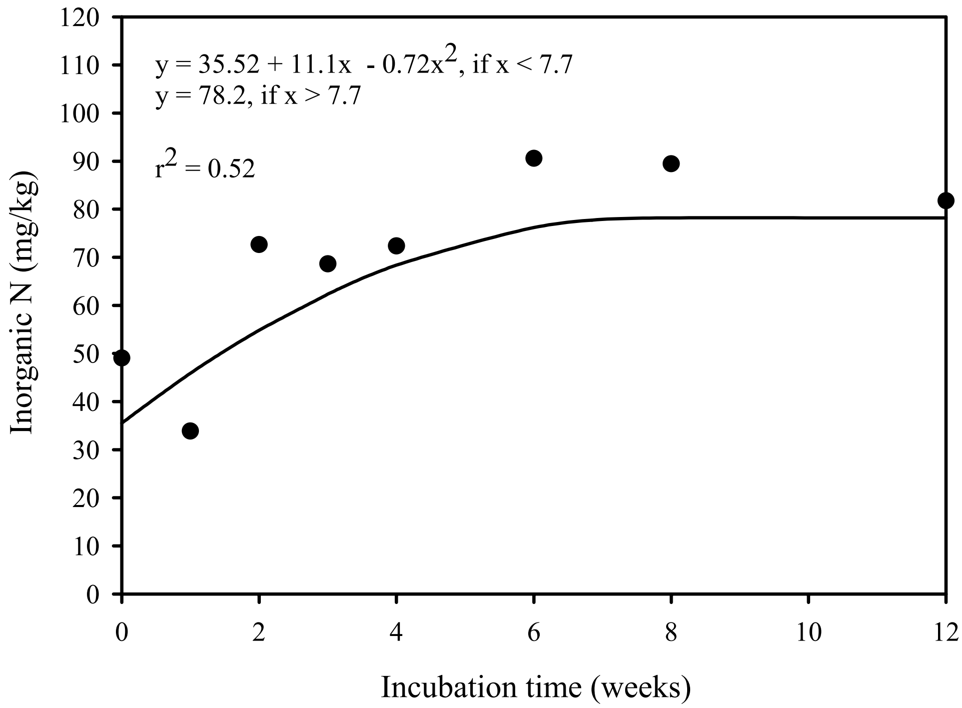


Figure 2 - 3. Composite N mineralization curve for sludges from 11 swine lagoons in N.C. Nitrogen concentrations from the control soil treatment were subtracted from the sludge amended soil treatments.

Table 2 - 8. Available and mineralized N from sludges after 8 weeks of incubation.

Sludge I.D.	Operation †	Company ‡	Organic N mineralized §	Available N ¶
			- - - - - - - - - %	- - - - - - - -
1	S	A	20.6	40.2
2	N	A	32.5	49.1
3	F	A	25.5	43.9
4	S	B	22.4	41.5
5	N	B	33.9	50.2
6	F	B	12.9	34.3
7	S	C	32.1	48.8
8	N	C	39.0	54.0
9	F	C	25.5	42.7
10	R	R	23.3	42.2
11	R	P	26.9	44.9
Average			26.7	44.7
Standard Deviation			7.3	5.5

† S = Sow, N = Nursery, F = Finishing, and R = Research Station.
‡ R and P represent sludges from research stations in Rocky Mt. and Plymouth NC, respectively.
§ % Organic N mineralized = ((Inorganic N in treated soil – inorganic N in control soil – inorganic N applied from sludge) / (Total N added from sludge – NH_4 added from sludge)) * 100
¶ % Available N = ((Inorganic N in treated soil – Inorganic N in control soil) / Total N added) *100

mineralization to occur. Also, because the tarp was beneath as well as on top of the stored soil, leaching of NO_3 was greatly reduced.

Nitrogen mineralized at the end of 8 weeks from the organic fraction ranged between 13 and 39 %, and averaged 27 %. This was comparable to King (1984), who found 21% mineralizable N from the organic fraction of sludge sampled from one anaerobic swine lagoon after 16 weeks.

Summary:

Aerobic incubation experiments were designed to measure N mineralization, under favorable moisture and temperature conditions in a 12-week incubation. Therefore N availability predictions for anaerobic swine lagoon sludge in this study should be considered as a high estimate of the amount of N available under field conditions.

<u>Nitrogen Availability (Greenhouse)</u>

Farming Operation and Company Effects:

Differences in sludges between companies had no significant effect on either N uptake or dry grass weights. Operational effects on sludge applied to the soil were not significant for N uptake, but significantly influenced cumulative shoot biomass accumulation. Total dry weights for grass fertilized with sludge from companies A and B were significantly higher from nursery farms than from finishing and sow farms. However, grass weights for company C were significantly higher on sow farms than nursery or finishing farms. Although these differences may be statistically significant, they do not show that one specific operation type consistently increases or decreases dry weights any more than another operation, indicating that operations in general do not influence grass growth fertilized with swine lagoon sludge.

Nitrogen Accumulation:

Nitrogen accumulation from either sludge or NH_4NO_3 was calculated by multiplying grass dry weights by N concentration of the plant tissue at each harvest. Sludge and NH_4NO_3 application rate effects on nitrogen and dry weight accumulation over time are illustrated in Figs. 2 – 4 and 2 – 6. Most of the plant growth occurred during the first 10 weeks, and essentially stopped after the 3rd harvest for sludge and NH_4NO_3 treatments, likely because N sources were exhausted. During aerobic incubation of sludge discussed previously, N mineralization rapidly decreased after approximately 8 weeks. This indicates that plant-available N would be released too slowly after 8 weeks to provide enough N for rapid plant growth.

Grass grown at both sludge application rates (115 and 230 mg N/kg) accumulated less N than all three NH_4NO_3 rates (77, 153, and 230 mg N/kg) (Fig. 2 – 4 and 2 – 5). Based on 25% inorganic N initially present in the sludges, a minimum of 29 and 55 mg N/kg would be plant-available at the low and high rates, respectively. That is less than the lowest rate of NH_4NO_3 applied, indicating that sludge N would initially supply less plant-available N than all three NH_4NO_3 rates used in this experiment, especially if N mineralization rates were low.

Nitrogen Recovery:

Plant recovery of sludge N is an estimate of N availability, and is defined as the percent of N recovered by the plant (both shoots and roots) from the applied treatment. Sludge applied at the low rate had higher N recovery than sludge applied at the higher

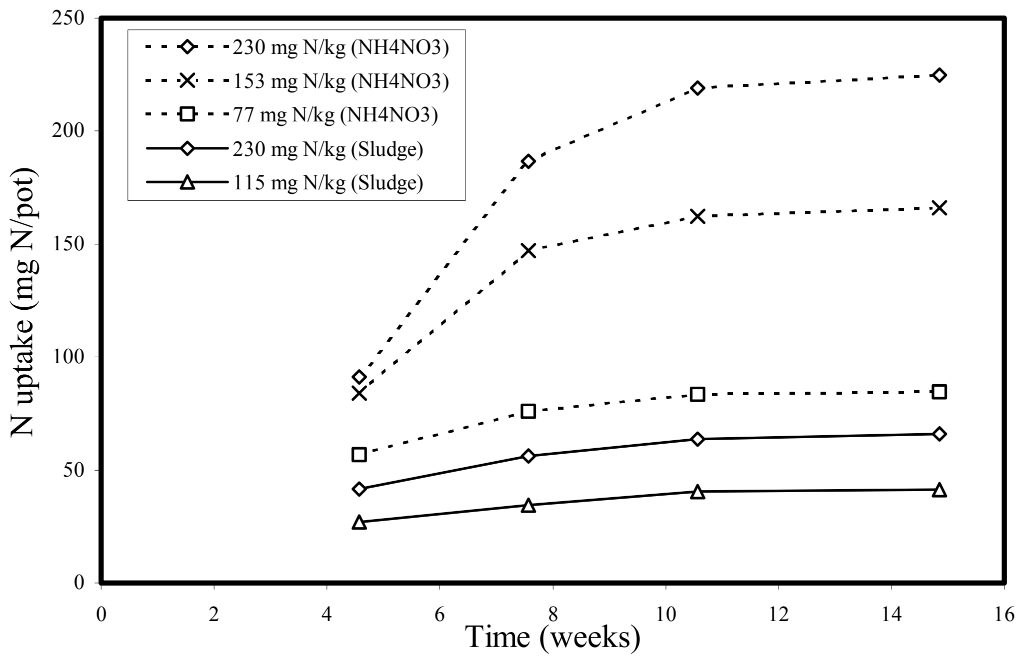


Figure 2 - 4. Source rate effects on N accumulation in bermuda grass shoots as a function of time. Nitrogen accumulated from the control soil treatments were subtracted from N accumulated from the sludge and NH_4NO_3 treatments.

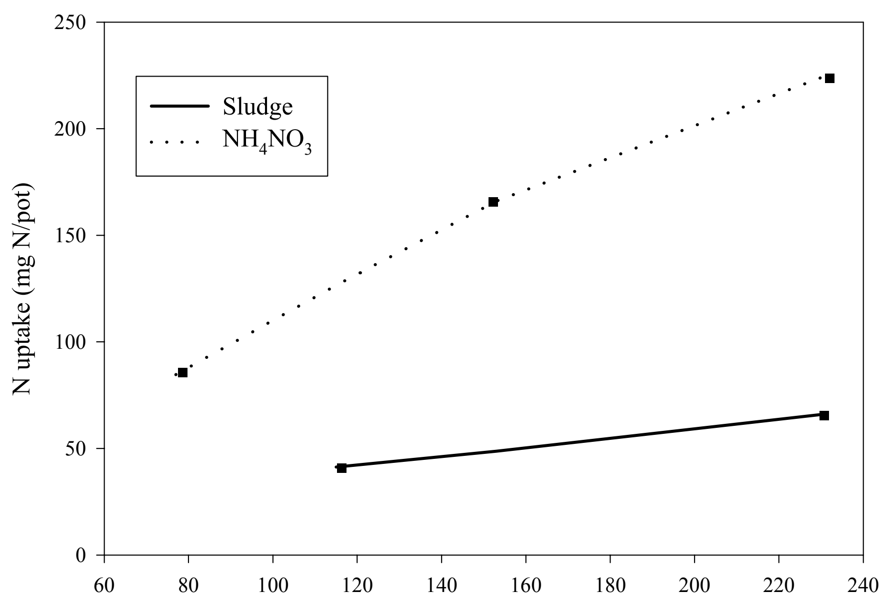


Figure 2 - 5. Source rate effects on N accumulation in bermuda grass shoots and roots based on amount of N applied, summed over all harvests. Nitrogen accumulated from the control soil treatments were subtracted from N accumulated in the sludge and NH_4NO_3 treatments.

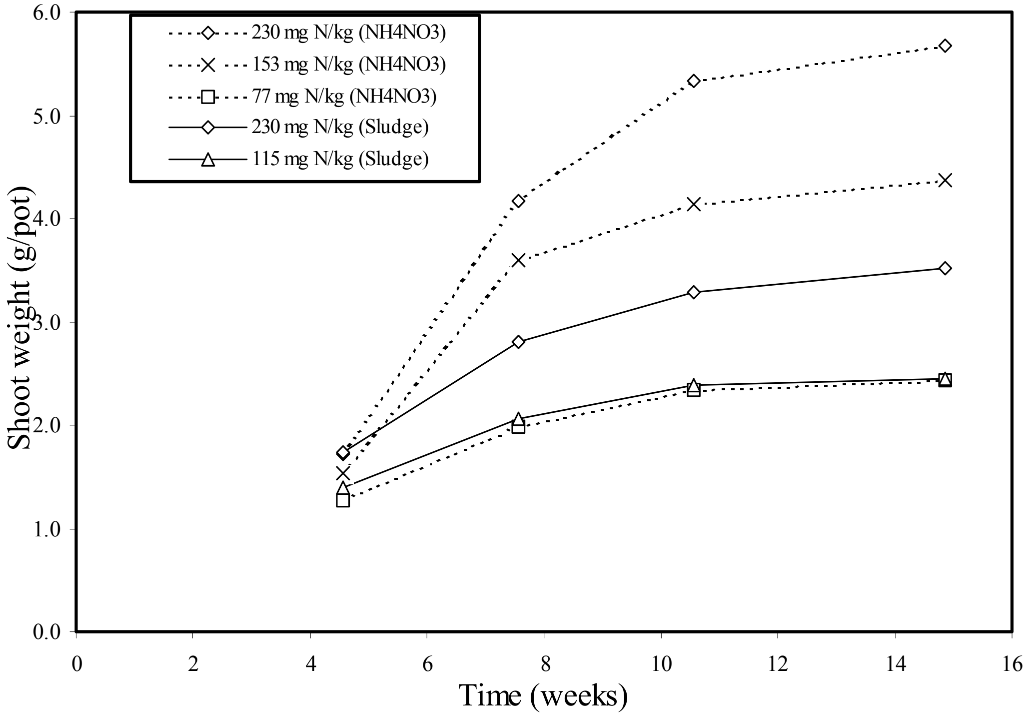


Figure 2 - 6. Source rate effects on dry weights of bermuda grass shoots as a function of time. Nitrogen concentrations from the control soil treatments were subtracted from the sludge and NH_4NO_3 treatments.

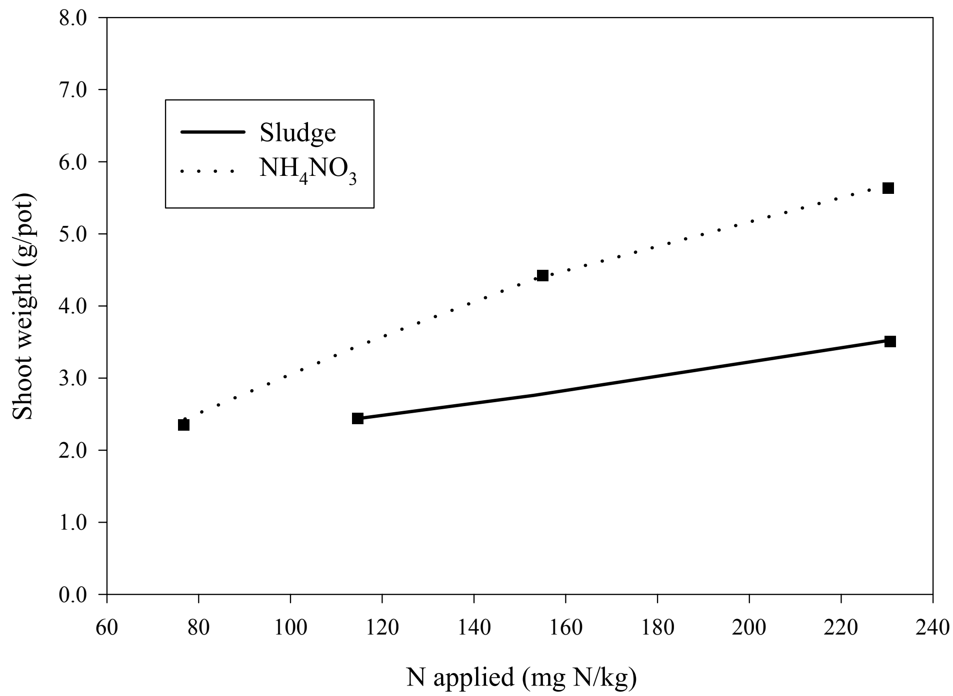


Figure 2 - 7. Source rate effects on dry weights of bermuda grass shoots based on amount of N applied, summed over all harvests. Nitrogen concentrations from the control soil treatments were subtracted from the sludge and NH_4NO_3 treatments.

rate (22.4 % compared to 17.7 %, respectively). The rate effect on N recovery was significant ($p = 0.001$), yet was considered to be relatively small and insignificant for land application purposes. King (1981) found that N recovery of sludge by fescue grass decreased from 22% to 18% as application rate increased. The differences King found, however, were insignificant ($p>0.05$), supporting that rate may have an effect on N recovery, but not a great enough effect to be considered when applied at agronomic rates.

Overall plant N recovery among all 11 sludges ranged from 17 to 28, averaging 20 %, which is significantly less than the predicted amount of N available during the aerobic incubation experiment (45 %). Temperatures above 35 ºC are often associated with a dramatic reduction of microbial activity (Killham, 1994). Daily high temperatures in the greenhouse were over 40 ºC for 11 of the 14 weeks of the experiment, with the highest daily temperature reaching 53 ºC. However, some thermophilic bacteria can mineralize organic N at temperatures of 60 ºC and higher (Panganibani, 1925). Therefore, it is unlikely that mineralization was affected by high temperatures. It is more likely that warm temperatures decreased soil moisture, which decreased N mineralization in the soil.

Nitrogen Efficiency:

Nitrogen efficiency was determined by dividing cumulative N accumulation of grass fertilized with sludge by N accumulation of grass fertilized with NH_4NO_3 at the same N rate. Efficiency for the lowest sludge rate (115 mg N/kg) was estimated by approximating N uptake of NH_4NO_3, with the assumption that N uptake from NH_4NO_3 increases linearly as rate increases (Fig. 2 - 5). Sludge applied at the low rate had higher N efficiency (29 %) than sludge applied at the high rate (25 %). As with N recovery, the rate effect was statistically significant ($p = 0.028$), yet insignificant for practical purposes.

Overall efficiency of the 11 sludges averaged 27 %, ranging from 22 to 37 % (Table 2 – 9). Because sludge contains about 25 % plant-available N (as NH_4), and NH_4NO_3 contains 100 % plant-available N, it is expected that sludge N would be at least 25 % as efficient as NH_4NO_3, which would indicate that no N mineralization occurred in the soil. However, since only 76 % of NH_4NO_3 was recovered by the plant, potentially only 76 % plant-available N from the sludge would be from the NH_4 fraction, with the remaining 24 % mineralized from organic N.

Table 2 - 9. Plant recovery of nitrogen, and efficiency of plant N uptake from sludge, as compared to NH_4NO_3. Values were averaged over high and low rates.

Sludge I.D.	Operation †	Company ‡	Recovery §	Efficiency ¶
			- - - - - - - - - - %	- - - - - - - - - -
1	S	A	17.5	23.4
2	N	A	27.8	36.8
3	F	A	17.7	23.4
4	S	B	16.8	22.2
5	N	B	25.1	33.5
6	F	B	22.7	30.4
7	S	C	19.7	26.3
8	N	C	17.3	23.2
9	F	C	17.6	23.5
10	R	R	20.7	27.7
11	R	P	17.3	23.0
Average			20.0	26.7
Standard Deviation			7.4	9.6

† S = Sow, N = Nursery, F = Finishing, and R = Research Station.

‡ R and P represent sludges from research stations in Rocky Mt. and Plymouth NC, respectively

§ % N Recovery = ((Grass N in sludge treated soil – grass N in control soil) / (Total N applied))* 100

¶ % N Efficiency = ((Grass N in sludge treated soil – grass N in control soil) / (Grass N in NH_4NO_3 treated soil – grass N in control soil)) * 100

CONCLUSION

Company and operation effects on the N mineralization of anaerobic swine lagoon sludge were determined to be negligible by both the incubation and greenhouse experiments. Therefore high concentrations of Zn detected in nursery sludges did not negatively affect N availability. Based on these results, it would not be necessary for agencies, such as NCDA & CS, to incorporate company or operation effects when recommending how much sludge to apply based on N concentrations

Predicted N availability of anaerobic swine lagoon sludge after two months of aerobic incubation, averaged over all 11 sludges, was approximately 45 %. Because aerobic incubation experiments are used to determine the highest possible rate of N mineralization, such a number should be considered a high estimate of the amount of N available under field conditions. Overall plant N recovery among all 11 sludges averaged 20 %. Greenhouse plant uptake estimates are often lower than aerobic incubation estimates of N availability, as a result of inconsistent moisture throughout the soil in pots, as compared to incubated bags, where moisture remains constant throughout the experiment. Nitrogen availability estimates from these studies, which are based on 3 to 4 month long growing seasons, are considerably lower than the recommendation of 60 % from NCDA & CS, which is based on N availability one year after application. Farmers often use first-year availability coefficients to estimate available N for a specific growing season, and therefore are at risk for N deficiencies, illustrating the necessity for regulatory agencies to convert first-year coefficients to 3 to 4 month coefficients.

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Chapter 3 – Effects of Seasonal Temperature Fluctuations and Application Date on N Availability of Anaerobic Swine Lagoon Sludge

INTRODUCTION

Nitrogen mineralization and nitrification, which are biologically mediated processes where organic N is converted to NH_4 and NH_4 is oxidized to NO_3. The most limiting environmental factors influencing N mineralization and nitrification are temperature, moisture, pH, the presence of organic N and NH_4, and the presence of O_2 (Stevenson and Cole, 1999). Most of these factors are relatively easy to manage; drought problems may be relieved by irrigation, acid soils can be limed, O_2-deprived soils are often drained, and lack of organic N and NH_4 can be amended through fertilization with organic and inorganic materials. Soil temperature, however, is almost impossible to control. Therefore, it is important to understand the relationship between N mineralization and temperature in order to predict how much N will be available to plants when organic amendments such as sludge are applied to the soil.

It is generally accepted that temperature has a significant effect on N mineralization and nitrification (Sabey et al., 1956; Ellert and Bettany, 1992; Killham, 1994; Sierra, 1996). In addition, N mineralization can occur at temperatures as low as 0°C, while nitrification rarely occurs below 7 °C (Killham, 1994). Because only a few genera of bacteria are capable of nitrifying NH_4, this process is more sensitive to temperature than mineralization, which is executed by a large, diverse population of microorganisms (Killham, 1994).

The rate at which N mineralization increases as a function of temperature is generally understood. Mathematical models, such as Arrhenius and Q_{10} temperature functions, are commonly used to predict the effects of temperature on N mineralization rates. Both of these functions relate rates of biological processes (such as N mineralization) to temperature changes between 5 and 35 ºC, based on 10 ºC increments (Ellert and Bettany, 1992). The Arrhenius function shows a decrease in differences in the rates of biological processes as temperatures increase, while the Q_{10} function expresses a constant value for any 10 ºC difference between 5 and 35 ºC (Ellert and Bettany, 1992). Therefore, the curve created by the Arrhenius function eventually reaches an asymptote, while the curve created by the Q_{10} function increases continuously. Many researchers have evaluated both of these models by incubating soil at various constant temperatures (Campbell et al., 1981; Sierra, 1996; Vigil and Kissel, 1995; Addiscott, 1983). These types of studies are helpful, although they do not incorporate changes in temperature that occur in the field. Some researchers are beginning to test the models against diurnal fluctuating temperatures (Das et al., 1995), which are more representative of field conditions.

Several lab incubation studies have reviewed the effect of fluctuating temperature on N mineralization. Soils subjected to different sequences of temperatures changed at four days increments between 5 and 35 °C showed no difference in inorganic N accumulation at the end of each sequence, showing that fluctuations in temperature did not effect N mineralization (Stanford et al., 1975). Campbell et al. (1971) found similar results when comparing diurnally fluctuating temperatures between 3 and 14°C to a constant mean temperature at 8.5 °C. However, the fluctuating temperatures almost completely inhibited nitrification, and reduced bacterial counts by half. The authors attributed the lack of difference in net N mineralization to the resilience of the ammonifiers in the soil.

The effect of temperature on N mineralization has also been studied using field incubations of soil in buried polyethylene bags (Westerman and Crothers, 1980; Foster, 1989). This technique incorporates natural patterns of temperature fluctuation that are more realistic than controlled changes found in lab incubations. Disadvantages with the buried bag method include unexpected climatic changes (severe flooding, unusually high or low temperatures), deterioration of polyethylene films, and difficulty maintaining constant moisture in the bags.

Nitrogen mineralization studies in the laboratory typically extend for three to four months, the time required for readily available organic N pools such as amino acids and amino sugars to be mineralized (Stevenson and Cole, 1999). The disadvantage of these types of studies is that they do not account for N released by labile, or stable, organic pools, which release N at a relatively slow but constant rate. Bundy and Meisenger (1994) suggest longer incubation periods (up to 30 weeks) to quantify both the active and stable fractions. Although shorter incubation studies are often preferred due to fewer expenses and time constraints, long-term incubations provide information that would be useful for more accurately estimating N mineralizing capabilities of a soil, along with the ability of organic amendments to be mineralized.

Along with temperature changes, initial soil temperature should also be considered when estimating N mineralization. The initial temperature will dictate how much of the active organic fraction is released from the organic amendment shortly after application. Anaerobic swine lagoon sludge is applied to fields during all months of the year, since sludge removal can be an ongoing process on many farms in North Carolina. Therefore, it is important to understand how application times along with temperature fluctuations affect the release of N from sludge over time.

Objective

The goal of this study was to evaluate effects of simulated fluctuating seasonal temperatures and various application dates on N availability of anaerobic swine lagoon sludge applied to soil. Information produced by this study may be used by agronomic laboratories to recommend monthly N availability constants depending on the season of application, which will allow farmers to optimize crop yields while minimizing negative environmental impacts related to NO_3 leaching.

METHODS AND MATERIALS

Soil

The top 23 cm of a Wagram sand (loamy, siliceous, thermic Arenic Kandiudult; 89% sand, 7% silt, and 4% clay) was collected and passed through a 6-mm sieve from an unlimed forested area near Clayton, NC. A Coastal Plain soil was used for this research since the majority of swine farms in North Carolina occur in the Coastal Plain area. The pH of the soil was raised from 3.6 to 5.1 by adding $CaCO_3$ two days before the incubation at a rate of 1.3 g/kg. This rate was based on a preliminary two-week incubation using several different liming rates. The increase of soil pH after the addition of $CaCO_3$ was determined one month after the first day of incubation. The soil was amended with KH_2PO_4 at a rate of 0.17 g/kg to maintain environment that was not P or K limiting.

Sludge

Sludges were retrieved from anaerobic swine lagoons at Upper Coastal Plain Research Station in Rocky Mount (Rm) and the Tidewater Research Station in Plymouth (Pl), NC. Sludge samples were collected from 4 to 5 different locations throughout each lagoon. The sludge samples were retrieved lowering an Ekman (3450 mL) grab sampler by rope. Once the metal, box-shaped sampler settled at the lowest possible depth, a weight attached to the rope was dropped to release the shutters, thereby closing and capturing the sample. The sampler was pulled to the surface, where excess liquid drained from the sampler for several minutes before releasing the sludge sample into 11 to 19-L plastic buckets, which were capped and refrigerated for 3 months at 11 ^{O}C to reduce microbial activity.

Selected chemical characteristics of the sludges are shown in Table 3-1. Measurements for pH were taken by inserting a pH electrode into 5 to 7 mL samples of undiluted sludge. The C:N ratio of oven-dried samples was determined through combustion using the Perkin Elmer 2400 CHN elemental analyzer. Samples were digested using the persulfate method for Total Kjeldahl N analysis (U.S. EPA, 1979a). Values for Total Kjeldahl N and NH_4-N were determined within a few days of sludge collection using the ammonia salicylate method for automated analysis (U.S. EPA, 1979a). Total and volatile solid percentages were determined gravimetrically (U.S. EPA, 1979b). Total Cu, Zn, Mg, K, and P were determined following the nitric acid digestion using Inductively Coupled Plasma (Novazamsky et al., 1986).

Table 3 - 1. Selected chemical and physical characteristics of anaerobic swine lagoon sludge used in the incubation study.

	Rm	Pl
C:N	6.70	7.16
VS/TS	0.33	0.55
TS (%)	13.3	8.24
NH_4 (% of TKN)	21.4	24.2
pH	7.43	7.55
	- - - - - - - g/L- - - - - - -	
TKN	4.20	4.81
P	7.34	5.81
Cu	0.09	0.06
Zn	0.28	0.37
K	0.71	0.86
Mg	2.36	2.91

Aerobic Incubation

Sludge was added to 100 g of soil (on a dry weight basis) to provide 200 mg N/kg soil. The soil and sludge were hand mixed in 0.025-mm thick Ziploc ® polyethylene sealable bags (16.5 cm X 14.9 cm) until the sludge appeared to be spread uniformly throughout the soil. Gordon (1988) reported that polyethylene films ranging in thickness between 0.015 and 0.032 were suitable for preventing water loss, while allowing for sufficient CO_2 exchange during aerobic incubations. Control bags (soil only) were prepared to account for N mineralization contributed by pre-existing organic N pools in the soil. Each treatment (2 sludge treatments and 1 control treatment) was replicated 48 times per chamber, so that four replications of each treatment could be removed during each of the 12 sampling periods during the year-long incubation.

Bags were placed in incubation chambers at the North Carolina State University Phytotron (Downs and Thomas, 1991). Binder clips attached the left upper corner of each bag to two shelving units, each unit consisting of 18 metal wires, allowing for four bags to hang per wire. Bags were placed randomly in each of the four separate chambers, with each chamber representing the beginning of winter, spring, summer, and fall, using the following simulated application dates: December 3, February 26, June 4, and September 3. Temperatures in each chamber were constant each day but changed weekly, based on soil temperatures recorded between 15 and 20 cm depth at the Lower Coastal Plain Tobacco Research Station in Kinston, NC, averaged over a three-year period (Fig. 3 – 1, Tables A3 - 1, A3 - 2, A3 - 3, and A3 - 4).

The moisture was maintained at approximately 80% field capacity (19% moisture) throughout the incubation period. Field capacity was determined using the method described by Cassel and Nielsen (1986). Each bag was weighed biweekly to determine moisture loss, and water was added to bags that had decreased in weight by 5% or more. Bags were aerated at least monthly by opening and closing the bags.

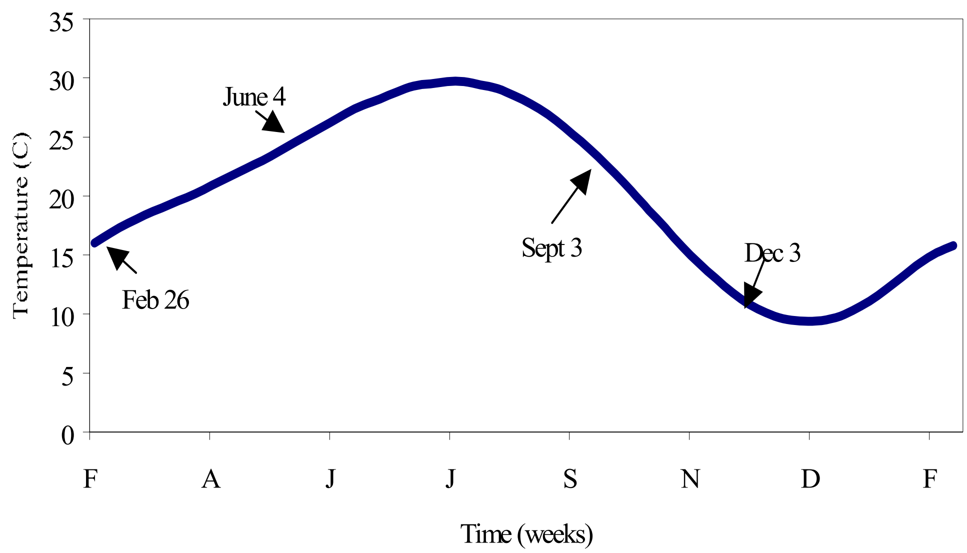

Figure 3 - 1. Mean daily temperature regime used in the incubation study, based on soil temperatures at a depth of 15 – 20 cm in Kinston, N.C.

Bags were removed monthly from each incubator (four replication) and returned to the laboratory for analysis, with the first samples taken on day that the sludge was added to the soil. A 10-g sample was weighed and extracted with 25 mL of 1M KCl for 30 minutes, then filtered through prewashed #2 Whatman filter paper. The extracts were frozen until analysis of NO_3^- and NH_4^+ using Lachat QuikChem Methods 10-107-04-1-A and 10-17-06-2-A, respectively (Lachat, 1992). Soil pH was measured during the first three months of incubation by inserting a pH meter electrode into a 1:1 mixture of soil and deionized water.

Nitrogen availability of both sludges among each chamber, over time, was compared with a one-way ANOVA using SAS PROC GLM, using NO_3, NH_4, and inorganic N concentrations minus controls as dependent variables, and chamber (temperature treatment), period (four-month increments over the year-long incubation) and sludge (both sludges) as independent variables (SAS, 1998).

RESULTS AND DISCUSSION

Nitrate and NH_4 concentrations in the control treatments were subtracted from the sludge treatments in order evaluate the mineralization of N contributed by the sludge. Nitrogen concentrations in the soil alone are illustrated in Appendix Figure A3 - 1.

Differences in total inorganic N and NO_3 concentrations between the two sludges for each temperature treatment over the whole year were insignificant at the $p<0.05$ level, based on temperature treatment and sludge interactions. Ammonium concentration differences between the two sludges for each temperature treatment were significant over the whole year ($p=0.0010$), mainly existing among summer and winter application treatments. Because the temperature treatment and sludge interactions were only significant for NH_4 concentrations based on two of the four temperature treatments, the two sludges were combined to show temperature effects.

Inorganic N

The nitrification rate of NH_4 applied initially in the sludge varied among the four temperature treatments. Ammonium initially contributed by the sludge was depleted after the first month of incubation for the simulated summer and fall applications, as a result of rapid nitrification (Fig. 3 – 2). When the two sludges were incubated at 25 ºC in a related experiment, NH_4 initially in the sludge was completely nitrified after two weeks; therefore NH_4 may have been nitrified in considering less than one month for both summer and fall treatments in this experiment.

Temperatures decreased from 27 to 23 ºC during the first month for the fall application chamber, and increased from 26 to 28 ºC for the summer application

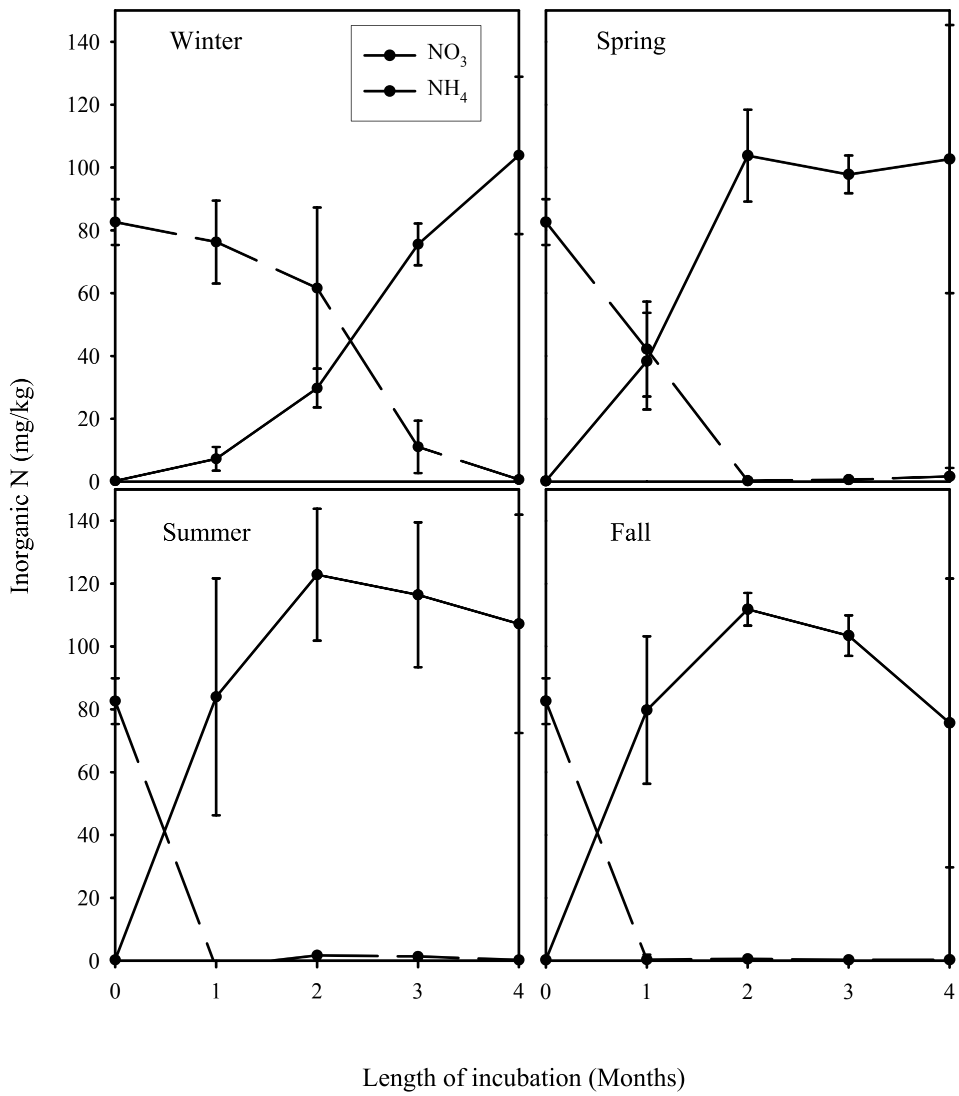

Figure 3 - 2. Concentrations of NH_4^+ and NO_3^- in the sludge amended soils minus concentrations of NH_4^+ and NO_3 in the control soil, during the first four months of incubation for each simulated application season. Error bars are based on standard deviations among 8 sampling units (two sludge treatments x four replications).

chamber. Optimum temperature for nitrification is about 30 ºC (Stevenson and Cole, 1999), explaining the rapid nitrification of NH_4. Nitrate concentrations after one month were similar to initial NH_4 concentrations, indicating that most of the NO_3 originated from the NH_4 fraction in the sludge, as opposed to significant contribution to the organic N fraction. After the first month, both N mineralization and nitrification processes contributed to NO_3 production, because of the general net increase of inorganic N.

Ammonium in the simulated winter application treatment was depleted after 4 months for (Fig. 3 – 2). In this treatment, temperatures ranged from 9 to 12 ºC during the first two months of incubation. Nitrification is generally inhibited below 7 ºC (Killham, 1994), therefore nitrification proceeds at a relatively slow rate at temperatures at or slightly above 7 ºC. Ammonium concentrations rapidly decreased between the second and third months of incubation, when temperatures in the chamber reached a maximum of 16 ºC. Ammonium initially contributed by the sludge was depleted after 2 months of incubation for the simulated spring application treatment (Fig. 3 – 2), with temperatures increasing from 16 to 21 ºC. Because nitrification and mineralization occur at slower rates during cooler seasons, NH_4 from applied sludge can remain unchanged in the soil, which reduces the potential for NO_3 leaching until the temperatures increase and nitrification increases.

Soil pH

The addition of sludge to the soil initially increased soil pH from 5.1 to 6.6. The pH increase was caused by the alkalinity of sludge, which has an average pH of 7.5. The pH decreased to an average of 4.8 one month after application in simulated summer and

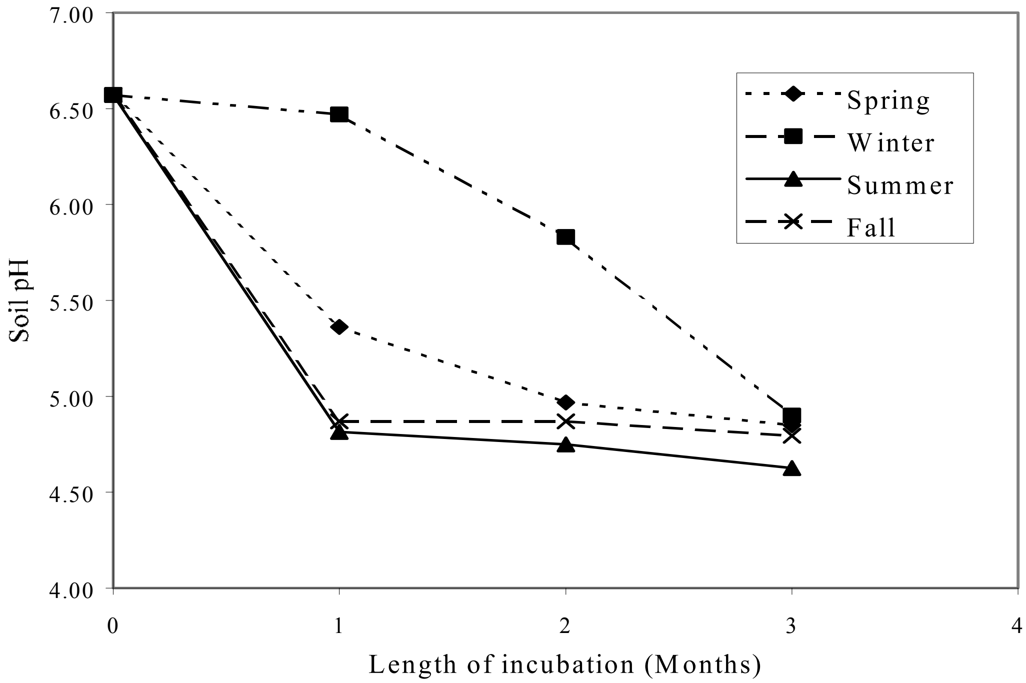

Figure 3 - 3. Soil pH of sludge amended soil during the first 3 months of incubation.

fall application chambers, after two months in spring application chamber, and after three months in the winter application chamber (Fig. 3 - 3). The pH decrease was a direct result of nitrification, an acid-forming process as follows:

$$2\ O_2 + NH_4 \rightarrow NO_3 + H_2O + 2\ H$$

Nitrogen may be removed from the soil as a gas, depending on soil pH. Ammonia volatilization occurs at a pH of 7.0 or higher (Stevenson and Cole, 1999). Therefore NH_4 losses as NH_3 gas are unlikely from these treatments. Nitrogen losses related to denitrification would also be unlikely, since soil in incubated under aerobic conditions, and because denitrifying bacteria are sensitive to acidic conditions (Stevenson and Cole, 1999).

Organic N

Sludge N availability was fit the nonlinear regression model using SAS PROC NLIN for a first-order reaction (adapted from Chescheir et al., 1986), as follows:

$$N_t = N_o\ (1 - e^{-kt}) + N_{os}$$

where:

> N_t = Total inorganic N concentration minus control concentrations, over time (mg N/ kg);
> N_o = Potentially available organic N (mg N/kg);
> k = First order rate constant ($month^{-1}$);
> t = Time (month);
> N_{os} = Inorganic N concentration minus control concentrations when time = 0.

Values for N_t, t, and N_{os} were measured, while values for N_o and k were fitted into the model with SAS PROC NLIN (SAS, 1998). Equations and first-order curves for each simulated application season are illustrated in Fig. 3 –4.

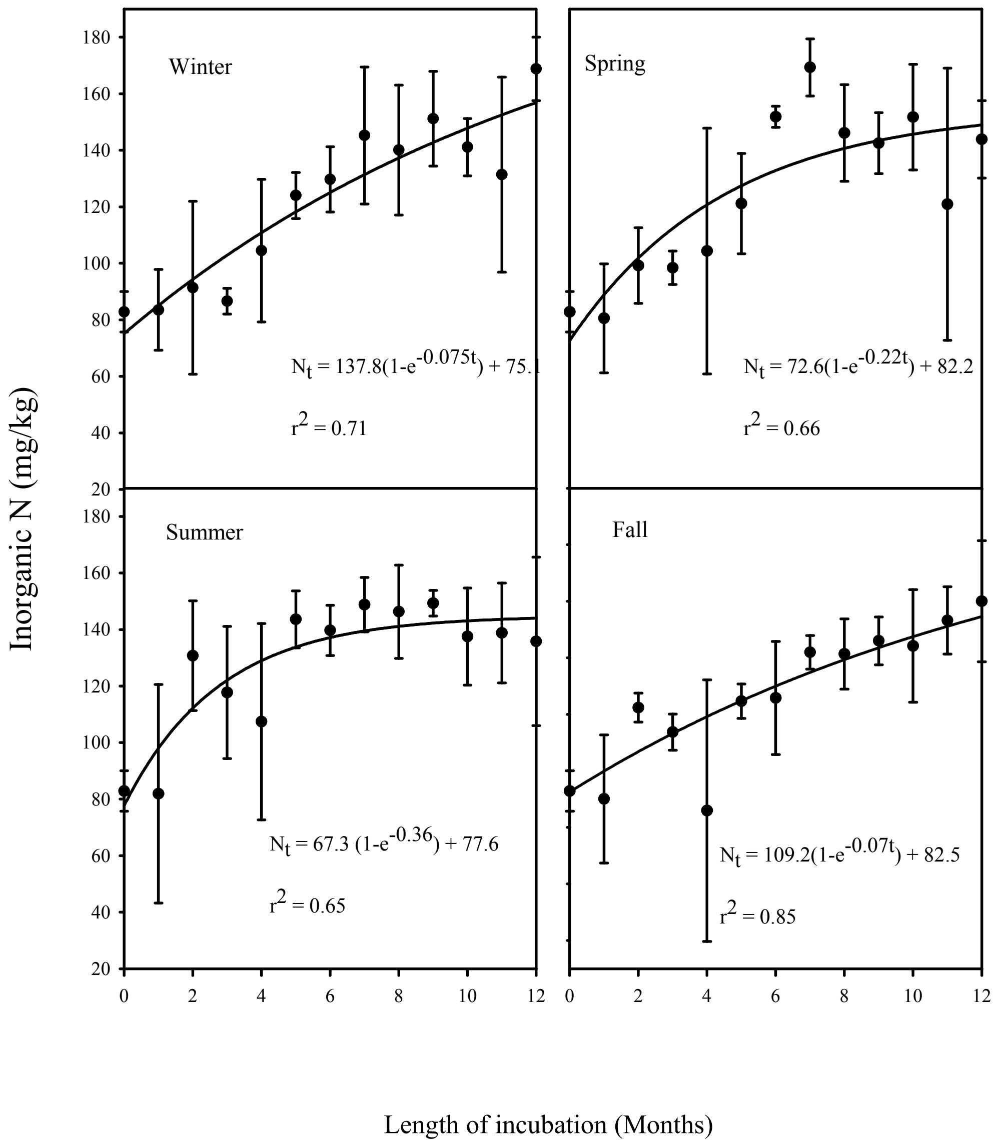

Figure 3 - 4. Total inorganic N availability of sludge during 12-month incubation applied in four simulated seasons. The line is based on first order reaction. Error bars are based on standard deviations among 8 sampling units (two sludge treatments x four replications).

Rate constants (k) increased between simulated applications as follows: fall (0.07) < winter (0.075) < spring (0.22) < summer (0.36). Campbell et al. (1984) also illustrated that rate constants increase as temperatures increase. In this study, sludge applied during simulated fall and winter temperatures mineralized N at a relatively constant rate over time. This is in contrast to the simulated summer temperatures, where N release increased rapidly during the first six months, then stabilized to allow no net significant increase of N during the last six months (Fig. 3 – 5). Fall and summer rate constants might be expected to be similar, since the initial temperatures were similar. However, actual inorganic N concentrations do not appear to increase after five months for the fall treatment (Fig. 3 – 4).

To obtain numbers for the fall treatment that were supported by the first order model, the month 4 inorganic N concentrations for the Rm sludge treatment were removed from the data set prior to statistical analysis. The sudden and unexplained decrease of inorganic N during month four for the Rm sludge may either be a result of immobilization, stimulated by a temperature decrease from an average of 13 ºC during the third month to an average of 9 ºC during the fourth month, or a result of experimental error, since inorganic N concentrations in the Pl treatment did not decrease sharply during the fourth month.

Nitrogen availability for each month after sludge application was estimated using the first order equations for each simulated application date (Table 3 – 2). Such estimates would be useful for North Carolina swine farmers who are applying the sludge to their land. For example, if sludge was applied to cropland in March, about 41 % of the total N would be available immediately after application. Three months after application,

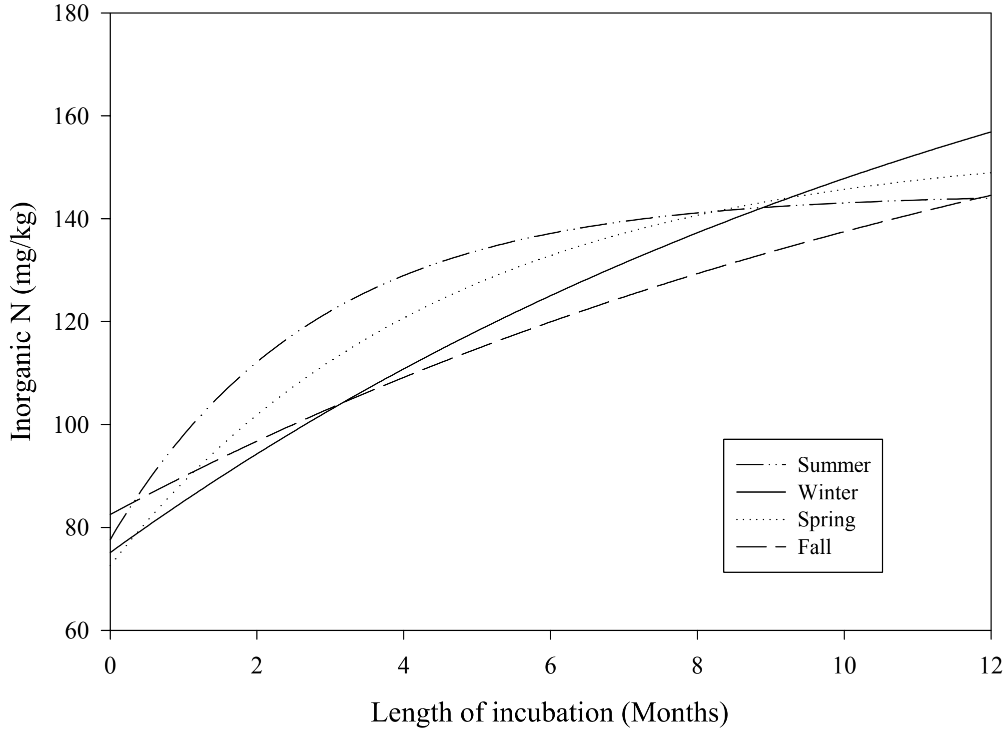

Figure 3 - 5. Predicted N availability of sludge during 12-month incubation based on four simulated seasons. The lines are based on first order reaction kinetics.

Table 3 - 2. Predicted N availability from anaerobic swine lagoon sludge incorporated in a sandy soil near 80 % field capacity.

Time after application	Month of initial application			
	December	March	June	September
Months	Available N (% of added N) †			
0	38	41	39	41
1	42	48	49	45
2	47	54	56	48
3	51	59	61	52
4	54	62	64	55
5	58	65	67	60
6	61	68	68	62
7	64	70	69	62
8	67	71	70	65
9	70	72	71	67
10	72	73	71	69
11	74	74	72	71
12	77	75	72	73

% Available N = ((Inorganic N in treated soil – Inorganic N in control soil) / Total N added) *100 approximately 59 % of the sludge N would be available, meaning that an additional 18 % of the sludge N was mineralized after initial application. Knowing potential N availability on a monthly basis, as opposed to a yearly basis, will allow farmers to improve yields while minimizing NO_3 leaching.

A quick method for predicting N mineralization based on temperature is the application of degree-days. Honeycutt et al. (1988) applied the concept of degree-days when comparing N mineralization of soil at several different constant temperatures. Because the degree-days approach can incorporate both time and temperature into one unit, N mineralization rates at different temperatures follow the same pattern. In preliminary tests, degree-days were not applicable in our study, due to the fact that temperatures for each incubation chamber were variable, as opposed to Honeycutt's incubators, which were maintained at constant temperatures.

Predicted N availability after one year of incubation averaged 74 % of the total N applied, ranging from 72 % for the simulated summer application treatment, to 77 % for the simulated winter treatment (Table 3 – 2). These estimates are higher than the current recommendation of 60 % plant-available N for incorporated anaerobic swine lagoon sludge during the first year of application (NCDA & CS, 2000). However, 60 % should still be considered a realistic recommendation, since sludge-amended soil to the field can be exposed to greater environmental changes such as changes temperature and moisture, which usually slow N mineralization rates. Nitrogen mineralization of the organic fraction averaged 42 % at the final sampling date (Table 3 – 3). Only 27 % of organic N was mineralized after 3 months of incubation in the sludge source experiment, illustrating the slow release of N from the stable organic fraction over time in the long-term experiment.

Table 3 - 3. Predicted available and mineralized N from sludges after 12 months of incubation.

	Available N †	Organic N mineralized ‡
	- - - - - - - - - - - - - % - - - - - - - - - - - - -	
Winter	77	46
Spring	75	43
Summer	72	39
Fall	73	40
Average	74	42
Standard Deviation	2.2	2.9

† % Available N = ((Inorganic N in treated soil – Inorganic N in control soil) / Total N added) *100

‡ % Organic N mineralized = ((Inorganic N in treated soil – inorganic N in control soil – inorganic N applied from sludge) / (Total N added from sludge – NH_4 added from sludge)) * 100

CONCLUSION

Oxidation of NH_4 to NO_3 was four times slower for the winter simulated application date as compared to the summer application date during the first four months of incubation. This is due to nitrification sensitivity to cool temperatures. Soil pH increased from 5.1 to 6.6 as a result of sludge addition. However, the ph subsequently dropped to 4.8 in all treatments as a result of acidification properties related to nitrification. Sludge applied during simulated winter temperatures initially mineralized N at a relatively constant rate, as compared to simulated summer temperatures, where N mineralized rapidly during the first six months, then stabilized with no net increase of N during the remaining six months. Nitrogen availability for each month after sludge application was estimated using the first order equations for each simulated application date. This estimation of N availability will be useful for North Carolina swine farmers who are applying the sludge to their land. Nitrogen availability after one year of incubation averaged about 74 %, which is higher than the current NCDA & CS recommendation of 60 % plant-available N for incorporated anaerobic swine lagoon sludge applied under natural field conditions. This higher estimate of N availability may reflect the optimal conditions maintained during incubation.

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Chapter 4. Conclusions

Plant recovery of N from sludge and mineralization of sludge N in soil incubated at controlled temperatures and moisture indicated that type of swine operation nor differences in management by different companies significantly impacted availability of N from anaerobic swine lagoons. The sludge source incubation experiment provided higher estimates for plant availability in 8 weeks (44 %) than the crop N recovery in the greenhouse experiment in 15 weeks (20 %), possibly a result of inconsistent moisture throughout the soil in pots.

Different temperatures associated with simulated application dates significantly affected nitrification during the first four months of incubation, and also affected N mineralization first order rate constants. More N was mineralized from the organic fraction in the year-long seasonal incubations (42 %) than in the three-month sludge source experiment (27 %), illustrating the slow release of N from the stable organic fraction over time. First-year N availability coefficients for incorporated swine lagoon sludge recommended by NCDA & CS (60 %) were smaller than N availability predicted from the four temperature treatments (74 %).

Appendix

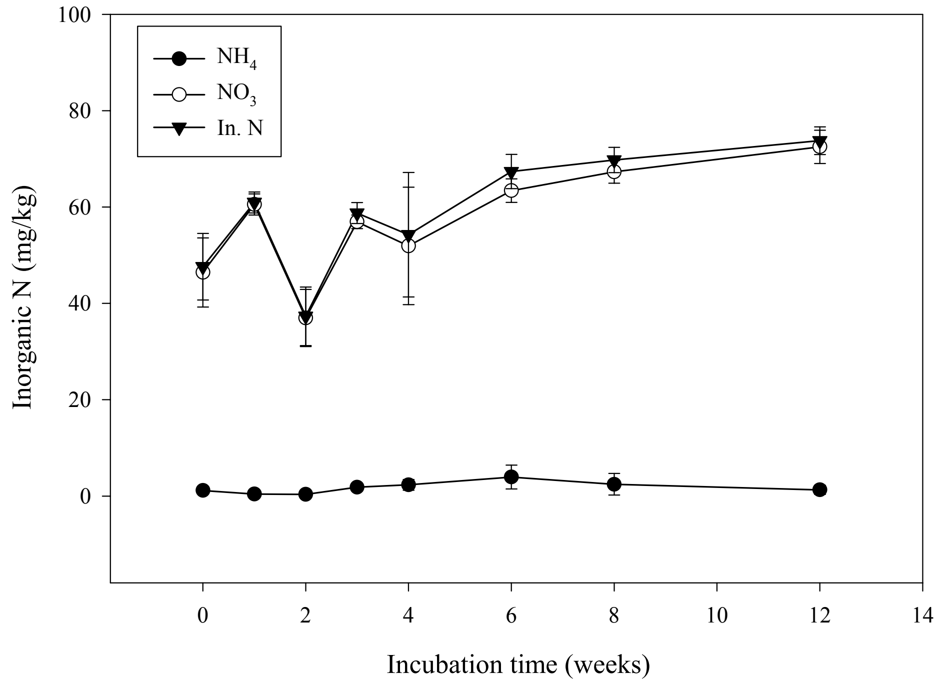

Figure A2- 1. Inorganic N concentrations in the control treatment (no N source added) from the sludge source incubation study over a twelve-week period. Error bars are based on standard deviations among 4 sampling units (one control treatment x four replications).

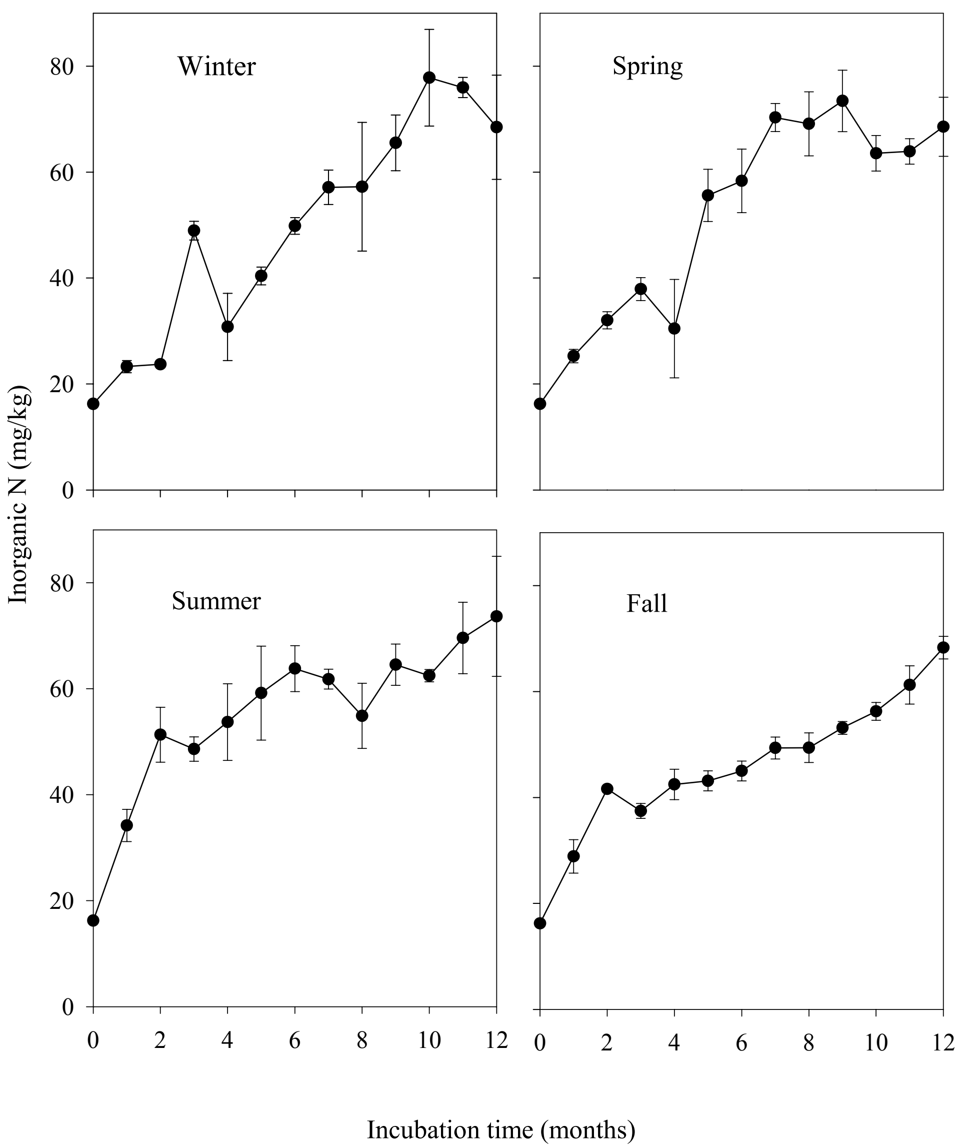

Figure A3 - 1. Total inorganic N availability in the control treatments (no N source added) from the seasonal temperature incubation study. Error bars are based on standard deviations among 4 sampling units (one control treatment x four replications).

Table A3 - 1. Temperature regime for the simulated fall application treatment.

Represented week	Time (weeks)	Incubation temperature (ºC)
Sept 3 - 9 †	1	26.9
Sept 10 - 16	2	25.8
Sept 17 - 23	3	24.6
Sept 24 - 30	4	23.3
Oct 1 - 7 †	5	21.9
Oct 8 - 14	6	20.4
Oct 15 - 21	7	18.8
Oct 22 - 28	8	17.3
Oct 29 - Nov 4 †	9	15.7
Nov 5 - 11	10	14.3
Nov 12 - 18	11	13.0
Nov 19 - 25	12	11.8
Nov 26 - Dec 2 †	13	10.8
Dec 3 - 9	14	10.1
Dec 10 - 16	15	9.6
Dec 17 - 23	16	9.4
Dec 24 - 31 †	17	9.4
Jan 1 - 7	18	9.7
Jan 8 - 14	19	10.3
Jan 15 - 21	20	11.1
Jan 22 - 28 †	21	12.1
Jan 29 - Feb 4	22	13.2
Feb 5 - 11	23	14.3
Feb 12 - 18	24	15.2
Feb 19 - 25 †	25	15.8
Feb 26 - Mar. 4	26	16.0
Mar 5 - 11	27	16.9

Table A3 – 1, cont.

Mar 12 - 18	28	17.7
Mar 19 - 25 †	29	18.4
Mar 26 - Apr 1	30	19.0
Apr 2 – 8	31	19.6
Apr 9 - 15	32	20.2
Apr 16 - 22 †	33	20.9
Apr 23 - 29	34	21.6
Apr 30 - May 6	35	22.3
May 7 - 13	36	23.0
May 14 – 20 †	37	23.8
May 21 - 27	38	24.6
May 28 - June 3	39	25.4
June 4 - 10	40	26.2
June 11 - 17 †	41	27.0
June 18 - 24	42	27.7
June 25 - July 1	43	28.2
July 2 - 8	44	28.8
July 9 - 15 †	45	29.3
July 16 - 22	46	29.5
July 23 - 29	47	29.7
July 30 - Aug 5	48	29.7
Aug 6 - 12 †	49	29.4

† Represents sampling period.

Table A3 - 2. Temperature regime for the simulated winter application treatment.

Represented Week	Time (weeks)	Incubation temperature (ºC)
Dec 3 - 9 †	1	10.1
Dec 10 - 16	2	9.6
Dec 17 - 23	3	9.4
Dec 24 - 31	4	9.4
Jan 1 - 7 †	5	9.7
Jan 8 - 14	6	10.3
Jan 15 - 21	7	11.1
Jan 22 - 28	8	12.1
Jan 29 - Feb 4 †	9	13.2
Feb 5 - 11	10	14.3
Feb 12 - 18	11	15.2
Feb 19 - 25	12	15.8
Feb 26 - Mar. 4 †	13	16.0
Mar 5 - 11	14	16.9
Mar 12 - 18	15	17.7
Mar 19 - 25	16	18.4
Mar 26 - Apr 1 †	17	19.0
Apr 2 - 8	18	19.6
Apr 9 - 15	19	20.2
Apr 16 - 22	20	20.9
Apr 23 - 29 †	21	21.6
Apr 30 - May 6	22	22.3
May 7 - 13	23	23.0
May 14 - 20	24	23.8
May 21 - 27 †	25	24.6
May 28 - June 3	26	25.4
June 4 - 10	27	26.2

Table A3 – 2, cont.

June 11 - 17	28	27.0
June 18 - 24 †	29	27.7
June 25 - July 1	30	28.2
July 2 - 8	31	28.8
July 9 - 15	32	29.3
July 16 - 22†	33	29.5
July 23 - 29	34	29.7
July 30 - Aug 5	35	29.7
Aug 6 - 12	36	29.4
Aug 13 - 19 †	37	29.1
Aug 20 - 26	38	28.5
Aug 27 - Sept 2	39	27.8
Sept 3 - 9	40	26.9
Sept 10 - 16 †	41	25.8
Sept 17 - 23	42	24.6
Sept 24 - 30	43	23.3
Oct 1 - 7	44	21.9
Oct 8 - 14 †	45	20.4
Oct 15 - 21	46	18.8
Oct 22 - 28	47	17.3
Oct 29 - Nov 4	48	15.7
Nov 5 - 11 †	49	14.3

† Represents sampling period.

Table A3 - 3. Temperature regime for the simulated spring application treatment.

Represented Week	Time (weeks)	Incubation temperature (ºC)
Feb. 26 - Mar 4 †	1	16.0
Mar 5 - 11	2	16.9
Mar 12 - 18	3	17.7
Mar 19 - 25	4	18.4
Mar 26 - Apr 1 †	5	19.0
Apr 2 - 8	6	19.6
Apr 9 - 15	7	20.2
Apr 16 - 22	8	20.9
Apr 23 – 29 †	9	21.6
Apr 30 - May 6	10	22.3
May 7 - 13	11	23.0
May 14 - 20	12	23.8
May 21 – 27 †	13	24.6
May 28 - June 3	14	25.4
June 4 - 10	15	26.2
June 11 - 17	16	27.0
June 18 – 24 †	17	27.7
June 25 - July 1	18	28.2
July 2 - 8	19	28.8
July 9 - 15	20	29.3
July 16 – 22 †	21	29.5
July 23 - 29	22	29.7
July 30 - Aug 5	23	29.7
Aug 5 - 12	24	29.4
Aug 13 – 19 †	25	29.1
Aug 20 - 26	26	28.5
Aug 27 - Sept 2	27	27.8

Table A3 – 3, cont.

Sept 3 - 9	28	26.9
Sept 10 - 16 †	29	25.8
Sept 17 - 23	30	24.6
Sept 24 - 30	31	23.3
Oct 1 - 7	32	21.9
Oct 8 - 14 †	33	20.4
Oct 15 - 21	34	18.8
Oct 22 - 28	35	17.3
Oct 29 - Nov 4	36	15.7
Nov 5 - 11 †	37	14.3
Nov 12 - 18	38	13.0
Nov 19 - 25	39	11.8
Nov 26 - Dec 2	40	10.8
Dec 3 - Dec 9 †	41	10.1
Dec 10 - 16	42	9.6
Dec 17 - 23	43	9.4
Dec 24 - 30	44	9.4
Jan 1 - 7 †	45	9.7
Jan 8 - 14	46	10.3
Jan 15 - 21	47	11.1
Jan 22 - 28	48	12.1
Jan 29 - Feb 4 †	49	13.2

† Represents sampling period.

Table A3 - 4. Temperature regime for the simulated summer application treatment.

Represented Week	Time (weeks)	Incubation temperature (ºC)
June 4 - 10 †	1	26.2
June 11 - 17	2	27.0
June 18 - 24	3	27.7
June 25 - July 1	4	28.2
July 2 - 8 †	5	28.8
July 9 - 15	6	29.3
July 16 - 22	7	29.5
July 23 - 29	8	29.7
July 30 - Aug 5 †	9	29.7
Aug 6 - 12	10	29.4
Aug 13 - 19	11	29.1
Aug 20 - 26	12	28.5
Aug 27 - Sept 2 †	13	27.8
Sept 3 - 9	14	26.9
Sept 10 - 16	15	25.8
Sept 17 - 23	16	24.6
Sept 24 - 30 †	17	23.3
Oct 1 - 7	18	21.9
Oct 8 - 14	19	20.4
Oct 15 - 21	20	18.8
Oct 22 - 28 †	21	17.3
Oct 29 - Nov 4	22	15.7
Nov 5 - 11	23	14.3
Nov 12 - 18	24	13.0
Nov 19 - 25 †	25	11.8
Nov 26 - Dec 2	26	10.8
Dec 3 - 9	27	10.1

Table A3 – 4, cont.

Dec 10 - 16	28	9.6
Dec 17 - 23 †	29	9.4
Dec 24 - 31	30	9.4
Jan 1 - 7	31	9.7
Jan 8 - 14	32	10.3
Jan 15 - 21 †	33	11.1
Jan 22 - 28	34	12.1
Jan 29 - Feb 4	35	13.2
Feb 5 - 11	36	14.3
Feb 12 - 18 †	37	15.2
Feb 19 - 25	38	15.8
Feb 26 - Mar. 4	39	16.0
Mar 5 - 11	40	16.9
Mar 12 - 18 †	41	17.7
Mar 19 - 25	42	18.4
Mar 26 - Apr 1	43	19.0
Apr 2 - 8	44	19.6
Apr 9 - 15 †	45	20.2
Apr 16 - 22	46	20.9
Apr 23 - 29	47	21.6
Apr 30 - May 6	48	22.3
May 7 - 13 †	49	23.0

† Represents sampling period.