FLORIDA DEPARTMENT OF HEALTH

Florida Onsite Sewage Nitrogen Reduction Strategies Study

Task C.2

Literature Review of Nitrogen Reduction by Soils and Shallow Groundwater

Final Report

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In association with

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FINAL REPORT

Literature Review of Nitrogen Reduction by Soils and Shallow Groundwater

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Introduction

1.1 Project Background

As a result of the widespread impacts of nitrogen on groundwater and surface waters in Florida, the management of nitrogen sources, including onsite sewage treatment and disposal systems (OSTDS), is of paramount concern for the protection of the environment. As part of Task C of the Florida Onsite Sewage Nitrogen Reduction Strategies (FOSNRS) Study, a review of available research related to the fate and transport of nitrogen is being developed. The primary objectives of this review are to:

- Assess the current available information on nitrogen treatment in soils and the effects to the receiving groundwater;
- Develop a searchable database of available literature concerning nitrogen groundwater contamination and OSTDS;
- Assist in the conceptual understanding of the fate and transport processes that influence distribution of nitrogen in groundwater; and
- Guide future field evaluation efforts and provide additional information to the development of a modeling tool for simulation of nitrogen in groundwater (Task D).

The following presents a literature review to assess the current state-of-knowledge regarding the fate and transport of nitrogen and its movement and distribution in groundwater related to OSTDS. The review will identify existing studies and reports that examine the influence of OSTDS-derived nitrogen inputs, the transformative processes that impact nitrate distribution, and the key factors that result in a significant effect to groundwater quality from OSTDSs. As part of the literature review, a database of the references was developed in conjunction with this summary report. This database (see separate Excel file "Nitrogen Soil-GW Studies") includes a summary table of the relevant features and parameters of each modeling study. As a result of the large number of identified sources, some studies that were deemed as not valuable to this effort and are mentioned in this report, but are not described in detail and the reader is directed to the database for further information.

1.2 Nitrogen in Ground Water; Conceptual Considerations

Nitrogen is an important concern for water quality and nitrates represent perhaps the most common groundwater pollutant. Animals, crops, ecosystems, and human health can be adversely impacted by the presence of nitrogen in water supplies. Of these concerns, nitrate impacts to human health have been a primary consideration. The consumption of nitrates has been linked to various illnesses, including cyanosis in infants and some forms of cancer. As a result, in the United States, a maximum allowable nitrate concentration of 10 mg/L as N has been established as protective of human health (Canter, 1996). Other agencies around the world have also established such standards for nitrates in groundwater.

Also of concern are the environmental effects on groundwater and surface water that can result from nitrogen impacts. The degradation of groundwater quality can ultimately lead to the degradation of surface waters in watershed systems that have strong groundwater/surface water interactions. Nitrogen that enters surface water bodies via these interactions can lead to algal blooms and eutrophication. These processes lead to oxygen depletion in surface waters which can be harmful to natural aquatic life. In Florida, the protection of watersheds, in particular surface water bodies, has led to the legislation of protection of these areas (i.e., the Wekiva River Protection Act).

A survey of community service wells and private domestic wells performed by the U.S. Environmental Protection Agency (EPA) indicated that over half of these water supply wells contained detectable levels of nitrate (Canter, 1996). The sources of this contamination are various, and include agricultural and domestic fertilizer applications, natural sources, wastewater treatment applications, and the use of OSTDS. The last category is often of concern, as nearly 25% of the population in the U.S. and 30% of all new development utilize OSTDS (Lowe et al., 2007). In Florida, nearly a third of all house-holds are serviced by OSTDS and 92% of water supplies come from groundwater (Briggs et al., 2007; Lowe et al., 2007).

Due to the unique features of the geology and hydrogeology, the groundwater systems and ultimately ecological systems and human health may be adversely impacted by nitrogen contamination of groundwater. The geology in Florida is characterized by the presence of sinkholes and fractures that develop in the karst limestone prevalent in many areas (Briggs, Roeder et al., 2007). These features tend to act as preferential flowpaths that can contribute to widespread groundwater contamination and potentially can impact protected surface waters.

Nitrogen transport in the subsurface is a complex process, especially when considering the nitrogen inputs from OSTDS. Meeting the objectives of the FOSNRS project therefore requires the development of a conceptual understanding that includes the relevant fate and transport processes, parameters, and simulation approaches that will appropriately achieve the goals of the project. Figure 1-1 summarizes the conceptual understanding of the inputs of nitrogen and the transformative and advective processes that lead to nitrogen contamination of groundwater. The FOSNRS project should result in tools that will consist of the adequate level of complexity to represent these processes to accurately simulate the fate and transport of nitrogen species.

Proper OSTDS design, installation, operation, and management are essential to ensure protection of the water quality and the public served by that water source. Assuming soils and site conditions are judged suitable, a wide variety of OSTDS are designed and installed (U.S. EPA, 1997, 2002; Crites and Tchobanoglous, 1998; Siegrist, 2001). Conventional OSTDS rely on septic tanks for the primary digestion of raw wastewater followed by discharge of septic tank effluent (STE) to the subsurface soils for eventual recharge to underlying groundwater (Crites and Tchobanoglous, 1998; Metcalf and Eddy, 1991; U.S. EPA, 2002). However, increasing uses of alternative OSTDS rely on additional treatment of the STE prior to discharge to the environment in sensitive areas (e.g., aerobic filter) or in some designs may eliminate use of a septic tank altogether (e.g., membrane bioreactor).

Conventional septic tanks are anaerobic and have long solids retention times (e.g., years) that can enable digestion resulting in a reduction of sludge volume (40%), biochemical oxygen demand (60%), suspended solids (70%) and conversion of much of the organic nitrogen to ammonium (Reneau et al., 2001). Septic tanks are also important as they attenuate instantaneous peak flows from the dwelling unit or establishment. The effluent discharged from the septic tank (i.e., septic tank effluent or STE) then flows to subsequent treatment (e.g., aerobic treatment unit) or directly to the soil treatment unit where the processes of soil adsorption, filtration, and transformation (biological and chemical) occur.

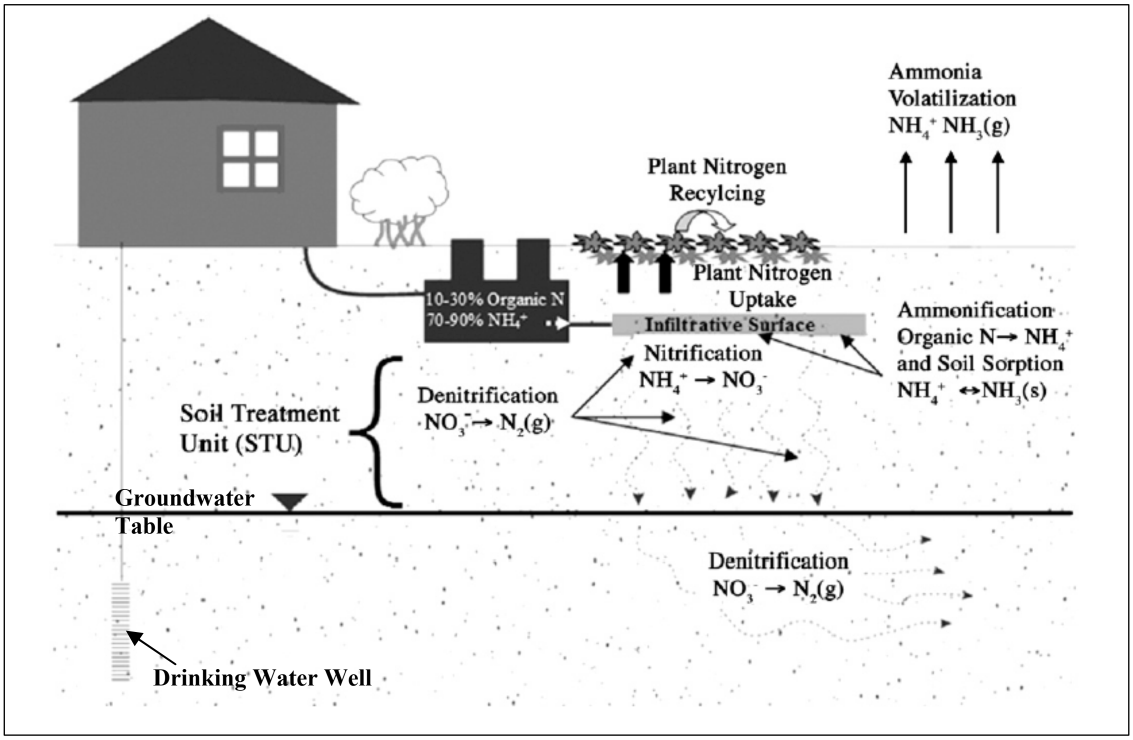

Figure 1-1: Nitrogen Processes Occurring in a Typical OSTDS (adapted from Heatwole and McCray, 2007)

Nitrogen waste products are a considerable component of septic tank effluent. Total nitrogen, composed primarily of organic nitrogen products and ammonium-nitrogen, is typically assumed to range between 20-190 mg-N/L in untreated waste water, and 26-125 mg-N/L in STE (Canter, 1996; Crites and Tchobanoglous, 1998; Lowe et al., 2009). Furthermore, in a recent study that evaluated the composition of raw wastewater and STE, the median total nitrogen concentration in STE specific to Florida was determined to be 65 mg-N/L (average = 61 mg-N/L) (Lowe et al., 2009). In terms of mass loading to the subsurface, the median loading rate was determined to be 10 g-N/capita/d (average = 13.3 g-N/capita/d) (Lowe et al., 2009). McCray et al. (2005) suggested that an average subdivision can generate up to 2880 kg/km^2 annually. While this value is significantly higher than estimates of naturally generated deposition (600-1,200 kg/km^2 annually), it is much lower than the loading that results from fertilizer application (10,000-20,000 kg/km^2 annually). Nonetheless, OSTDS should be considered a potential contributor to groundwater nitrogen concentrations.

The first stages of nitrogen transformation related to OSTDS occur in the septic tank. Organic nitrogen is mineralized to the inorganic form (ammonia) via the process of ammonification, followed by volatilization to ammonium ions.

$$NH_3(aq) + H_2O \rightarrow NH_4^+ + OH^- \qquad \text{(equation 1)}$$

Once the liquid portion of the wastewater enters the drainfield through the subsurface infiltration system, nitrogen species (specifically ammonium and nitrate) are further transformed in the soil by nitrification and denitrification. Nitrification is a two step process by which ammonium is converted first to nitrite than to nitrate via biological oxidation.

$$NH_4^+ + O_2 \rightarrow NO_2^- + O_2 \rightarrow NO_3^- \qquad \text{(equation 2)}$$

Although a two step process, it can be assumed to be a one step process since the conversion of nitrite to nitrate is relatively rapid. Nitrification is either described as a zero-order or first-order reaction or via Monod kinetics. This particular reaction is of importance, as it represents the transformation from the relatively immobile nitrogen form (ammonium) to the highly mobile form (nitrate). Most studies of OSTDS with suitable unsaturated soil have indicated that little ammonium reaches the underlying groundwater and that most impacts to groundwater from nitrogen are in the nitrate form. Nitrate behaves essentially as a conservative solute, with virtually no sorption or retardation processes affecting its movement in the aquifer. It is, however, subject to transformative processes.

Denitrification is the transformation of nitrate to N_2 gas.

$$5(CH_2O) + 4NO_3 + 4H^+ \rightarrow 5CO_2 + 2N_2 + 7H_2O \qquad \text{(equation 3)}$$

Denitrification occurs in oxygen-free conditions, and is therefore seen in anoxic zones in the soil and groundwater. This reaction is typically described as first-order. However, nitrogen transformations are probably best modeled using Monod kinetics, which result in zero-order rate constants for concentrations typical of nitrate-impacted groundwater. The process, while studied extensively, is not well understood or well quantified.

Understanding denitrification in the saturated zone, while receiving much less focus in the literature, is nonetheless a potentially valuable topic. Korom (1982) provides a thorough review of denitrification in the saturated zone. Although not specific to OSTDS impacts, aquifer denitrification can naturally reduce nitrate concentrations, and can be potentially enhanced via the addition of *in situ* amendments such as sucrose or methanol. This review goes on to include data and estimated denitrification rates found in both laboratory and field studies. In order to assess the contribution denitrification makes to nitrate reductions, researchers will often use the ratio of non-reactive solute (typically chloride) to nitrate along the plume flowpath. Any relative reduction in nitrate can be attributed to denitrification, since a reduction due to mixing with ambient groundwater would not change the ratio. Depending on the aquifer conditions, previous studies concerning the reduction of nitrate concentrations specifically from OSTDS identify denitrification rates as relatively small, and that most reductions occur as a result of mixing with ambient groundwater (see Reneau et al., 1989). A small number of studies however indicate that denitrification may be the dominant process, perhaps characterizing aquifers with low groundwater flux (see Hantzche and Finnemore, 1992).

Section 2.0 Literature Review

The following presents a summary of available research related to the treatment of nitrogen in soils and the subsequent fate and transport of nitrogen in groundwater.

2.1 OSTDS Performance – Laboratory and Field Studies

A number of studies have looked at the performance of either experimental or conventional OSTDS in terms of the treatment of nitrogen wastes depends on the effluent quality dispersed into the environment and subsequent soil treatment, both of which have a significant influence on the resulting impacts to groundwater. These types of studies are valuable in that they can indicate which factors influence the transformative processes and how various loading rates, soil types, and geochemical parameters may lead to excessive nitrogen concentrations. Furthermore, these studies suggest ways of improving the performance of older or failing OSTDS. A large body of research has been dedicated to this topic and is important for assessing nitrogen in groundwater; however, a full discussion on this topic is beyond the scope of this review, and therefore only a few relevant studies are indicated below.

An in-depth review of the fate and transport of contaminants from on-site systems is provided by Reneau et al. (1989). This study considers multiple factors, including soil type, loading rates, effluent quality, and carbon content. In this review the author describes the important mechanisms related to OSTDS performance. Firstly, he describes the importance of conditions conducive to nitrification, namely coarse-textured soils in which aerobic conditions are dominant. This is even true in fine-grained clay soils as long as unsaturated conditions are present. Denitrification in soils utilized for OSTDS is expected to be minimal except in anaerobic microsites. However, soils that are influenced by fluctuating water tables in which saturated conditions can occur will see increases in denitrification rates. For groundwater, sites which are ideal for OSTDS are often the most vulnerable to nitrate impacts, since they are often well drained soils with limited capacity for denitrification. In this case, often the most important mechanism for nitrate reduction is dilution by ambient groundwater.

Cogger and Carlile (1984) looked at the performance of 15 conventional and alternative OSTDSs to determine their performance in soils with high water tables in North Carolina. The alternative methods included low-pressure pipe systems, soil replacement systems,

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and pressure-dosed mounds. At the study site, shallow groundwater wells were installed around the systems and monitored monthly for nitrogen species. The study found in general that nitrogen species concentrations were markedly influenced by seasonal variations in the water table, although some systems experienced continuous saturation. Those systems that were continually saturated had the poorest performance, as well as those with the heaviest effluent loadings. Additionally, transport of nitrogen products was facilitated by those systems located in areas with high gradients and continuous soil saturation. The low-pressure pipe systems, designed to distribute the effluent of the entire adsorption field and provide occasional dosing rest periods, performed the best in spite of any level of saturation from the water table. The mound system did not perform well, however the authors indicate the pumps feeding the system were not operating correctly and the dosing recommendations were being exceeded. The soil replacement systems showed no improved performance over the conventional systems.

Similarly, Costa (2002) conducted a series of experiments comparing the nitrogen removal capabilities of a conventional system, two proprietary nitrogen removal systems (the Waterloo Biofilter and the MicroFAST Model), and a recirculating sand filter (RSF) system. “Nitrogen losses” are described as reduction in nitrogen from the septic tank effluent to the groundwater. Measurements were conducted over an 18 month period. Results indicate that the conventional system removed 21-25%, the Waterloo 60%, the MicroFAST removed 55%, and the RSF removed 41%.

Cogger et al. (1988) examined the performance of an OSTDS on a coastal barrier island. The study considered loading rate and water table as the primarily influences on OSTDS performance. Two absorption fields were constructed and sampled biweekly for a period of 18 months. Three loading rates (one, four, and six cm/day) were applied in a random fashion. Loading rate was identified as significant. Additionally, periods with a high water table in the early part of the year resulted in anaerobic conditions which inhibited nitrification. Redox conditions were generally considered low. However, in drier conditions, aerobic conditions dominated and more nitrification resulted with corresponding increases in redox parameters. The authors concluded that although loading was a factor, the fluctuations in the water table were more influential in determining the rates of transformation.

Various loading rates were applied and the resulting leaching of nitrogen compounds in an OSTDS were measured (Uebler, 1984). Loading rates of 7.5, 11.3 and 15 L m^{-2} d^{-1} were tested. Additionally, soil amendments (cement and lime) were also part of the experiment. Transformation of ammonium to nitrate was enhanced by the soil amendments, particularly the cement amendment when water levels were higher. Interestingly, the nitrate concentrations were highest with the lowest loading rate, particularly during high water table conditions. This observation suggests that water table level influences the production of nitrate more than the loading rate.

Lowe and Seigrist (2008) describe a pilot-scale study to evaluate the effects of infiltrative surface architectures (ISA) and hydraulic loading rates (HLR) on soil treatment of septic tank effluent. A test site was established in Golden, Colorado with three different ISAs (open, stone, and synthetic) and with two different HLRs (four and eight cm/day). Monitoring was done over a two-year period to evaluate the infiltration capacity and purification performance of the different conditions. Results indicate improved infiltration using the higher HLR and using the open ISA. The higher HLR resulted in increased nitrogen mass removal (42%) compared to the lower HLR. No significant difference was reported for the different ISAs. The data suggests that improved purification can be achieved by applying higher HLRs to a portion of the soil treatment area rather than a low HLR over the entire area.

In another study, Lowe et al. (2007 and 2008) describes a large field-scale study examining the soil treatment performance receiving three different effluent qualities: septic tank effluent, textile filter unit effluent and membrane bioreactor effluent. The different units were operated over a period of 16 to 28 months, with water quality monitoring for different parameters including nitrogen. Results showed an improved performance for both the MBR and TFU over the conventional septic tank, with a 30% and 61% nitrogen removal rate for the TFU and MBR, respectively (compared to the conventional septic tank only). The systems including a textile filter or membrane bioreactor, generally performed better with respect to nitrogen removal and were less affected by hydraulic loading rate than the system comprised of only a septic tank and soil treatment.

2.2 Vadose Zone Processes and Impacts to Groundwater

Soil treatment of nitrogen from OSTDS in the vadose zone can also have a significant influence on the resulting nitrogen concentrations in the aquifer. The transformations and reactions of sorption, nitrification, and denitrification described earlier are present in this zone. Nitrogen that is present as ammonium is subject to adsorption to negatively charged soil particles, plant uptake, or microbial bioaccumulation. Nitrate, on the other hand, is mobile in the vadose zone but can be subject to denitrification. It is therefore important to quantify the vadose zone processes to assess nitrogen attenuation prior to entering the saturated zone.

Otis (2007) estimated the nitrogen loadings to groundwater from OWTS located within the Wekiva Study Area. Because most literature data lacked accurate wastewater flow data, mass loadings could not be determined from existing data and a range of percent removals were estimated based on the factors that affect biological denitrification (soil drainage, depth to water table, and organic soil matter content). While the study did not address nitrogen in groundwater, it did provide conservative estimates for nitrogen removal for soil types specific to the Wekiva Study Area. In another study conducted by the project team members, a summary of the available literature related to nitrogen attenuation in the soil treatment unit (STU) was done to identify the parameters that influenced transformations and reactions (McCray, et al., 2008). Data from available literature was collected and tabulated for nitrogen concentration vs. depth, vadose zone characteristics, and soil type. Additional data was collected considering wastewater type, hydraulic loading rates, and source type characterization. Data analysis was performed to indicate the correlation between nitrogen attenuation and the various parameters. Initial analysis indicated no significant relationship existed between expected nitrogen concentrations and depth, soil type, or HLR. A more in-depth analysis found that the data variability was most related to HLR, suggesting that this parameter may be more influential than soil type when considering nitrogen attenuation. However, the study also indicates that different soil types will have different hydraulic properties and this can influence nitrogen attenuation.

Ammonium that is not immobilized can be converted to nitrate via nitrification. This form of nitrogen, as mentioned before, is highly mobile and can impact aquifers under OSTDS. Within the vadose zone, the pathway of nitrate reduction is denitrification. In the vadose zone, denitrification is the dominant process affecting nitrate concentrations below the absorption field (Wilhelm, Schiff et al., 1998) and is therefore a key process in estimating the resultant nitrate loading to the aquifer. A body of research has been involved with understanding and quantifying denitrification in the vadose zone.

Ritter and Eastburn (1988) provide a summary of available literature related to denitrification and OSTDS. Based on their review of available literature, several factors which may influence nitrogen attenuation are:

- adequate supply of a carbon source;
- infiltrative surface biozones (the biozone has been shown to improve denitrification);
- OSTDS with high water tables (potentially insignificant dentrification due to lack of conditions conducive to nitrification);
- dosing (likely to improve denitrification); and
- recirculating sand filters (and other aerobic treatment units may improve denitrification).

Degen (1991) conducted a study that considered multiple factors that could potentially influence denitrification processes including effluent loading rates, effluent type, dosing rates, and temperature. This study included both experiments on soil cores in the laboratory and field sampling and measurements on sites in Virginia. The predominant soil types consisted mainly of silt loams collected in Blacksburg, Virginia. Soil cores collected for the laboratory experiments were subjected to a variety of effluent dosing rates and effluent types in order to quantify the response in a more controlled environment. The study attempted to quantify the denitrification via a number of methods, including nitrate/chloride ratios, soil chemical analyses, and microbial activity analyses. Field studies used similar analyses. Additionally, an attempt was made to model the expected denitrification in the field based on the lab results. The study made several key conclusions as follows:

- Carbon content was the limiting factor for denitrification.
- Applications every 48 hours (i.e., one dose every 48 hours) doubled the denitrification rates compared to applications every 24 hours.
- The model was not useful for predicting denitrification in the field, likely due to the more favorable anaerobic conditions present in the field study.

Tucholke (2006) provides an analysis for relating denitrification rates in the vadose zone with soil type. The study consisted primarily of identifying studies that measured denitrification rates and described the soil characteristics of the study site with the hypothesis that predictions of denitrification could be made based on soil type. While the data did not support the hypothesis, it did show that denitrification varied significantly with soil type. However, the study concluded that denitrification is a process dependent on many variables, such as organic carbon content, soil temperature, water content, and soil pH. This conclusion was verified by statistical analysis that demonstrated that data variability was dependent on the variability in the various parameters.

One of the major research concerns with quantifying denitrification is the wide variation in measured rates in different studies. This issue makes correlation of site characteristics and denitrification difficult. Tucholke et al. (2007) provides a review discussing the variability seen in the literature. This variation is attributed to variations in measurement method and wide variations observed spatially and temporally in the field. For example, rates determined in the laboratory as compared to the field varied widely, as did rates determined by isotope analysis as compared to other methods. Also, site heterogeneities in limiting factors such as water content and pH also impacted the rate determination.

Nitrogen in the vadose zone that results from OSTDS is subject to various transformations and reactions which are dependent on numerous factors within the soil and from the source. Attenuation of nitrogen is accomplished via sorption, plant uptake, bioaccumulation, or conversion of nitrate to nitrogen gas (denitrification). No single dominant process or parameter can be identified; rather, an interconnected complex of factors will ultimately influence the nitrogen attenuation. Due to the complexity of the issue, more research is required in the future to relate all of the processes and variables to observed changes in nitrogen concentration from the source to the groundwater.

2.3 Land Planning and OSTDS Density

While a large number of studies consider lot size or OSTDS density to be important factors, two studies were identified that examined these as primary characteristics for estimating potential groundwater impacts from OSTDS. Ultimately consideration of lot size or septic tank density will play a key role in land planning and developments considering OSTDS as the primary method of wastewater disposal.

A method of determining lot size and density related to land development in Pennsylvania was developed by Taylor that assumes the reduction of nitrate is primarily via groundwater dilution (Taylor, 2003). The author reiterates the discussion of whether or not denitrification is a significant process in groundwater, and ultimately concludes that land planning must consider dilution as the primary factor in nitrate reduction, since this approach is both conservative and simple. Also, the author indicates denitrification is a poorly understood process and should not be relied on for nitrate reductions.

Similarly, Yates concludes in her study of OSTDS distribution in various watersheds in the United States that the most important factor in limiting OSTDS impacts is restricting system density (Yates, 1985). The author looks at nitrate impacted areas in New Mexico, Colorado, New York, Massachusetts, Delaware, and North Carolina. The study cites other research in these areas that quantifies the number of septic tanks in a particular watershed and the level of nitrate impacts. However, little quantitative analysis is provided and no significant conclusions that specify lot size or density of septic tanks and how that relates to high nitrate concentrations in groundwater are given.

2.4 Groundwater Denitrification

An important component of the nitrogen cycle in the subsurface is denitrification in groundwater. This reaction, as described earlier, is essentially the conversion of nitrate to nitrogen gas. This transformative process is of particular importance when considering the reduction of impacts from OSTDS, since this is the primary pathway of nitrate reduction other than dilution by ambient groundwater. Most research on this topic has been focused on the nitrate impacts from agricultural activities or aquifer impacts that are not attributed to a specific source; therefore limited research directly related to OSTDS has been identified. However the body of research available is nonetheless valuable as the factors influencing denitrification are not exclusive to agricultural subsurface environments and can be applied when considering reductions related to OSTDS impacts. During this review, 47 references related to denitrification in groundwater were identified. The following summarizes natural denitrification in groundwater and provides an examination of the rates and processes described in deep and shallow aquifers and in riparian (streambed) environments. Additionally, the key factors identified as the most influential on the denitrification process are described.

In the literature review, studies by Hiscock et al. (1991), Korom (1992), Hill (1996), and Rivett et al. (2008) were reviews and discussions of the state-of-knowledge of the denitrification process in saturated subsurface environments. Hiscock et al. (1991) reviewed not only natural processes, but provided additional information on the progress of methods intended to enhance the denitrification process *in situ.* Korom (1992) provided a background of the denitrification process, a historical perspective on denitrification research, published rates of denitrification in the field and laboratory, and made recommendations for further research. The review done by Hill (1996) looked at the process of denitrification in the riparian zones of the stream/groundwater interface. Rivett et al. (2008) looked primarily at the biogeochemical factors and processes related to denitrification. This study was mainly focused on the limiting factors such as electron donor availability and attempts to identify the influence of chemical and physical factors. Since the organization and material presented in these four papers is generally similar, a summary of their findings is provided below to give a summary of the current understanding of the denitrification process.

Denitrification is generally accepted as the most significant pathway to nitrate attenuation in groundwater (Rivett et al., 2008; Korom, 1992). This process requires four key components for denitrification to occur (from Korom, 1992):

- N oxides (N_2O, NO_3^-, NO_2^- NO) as terminal electron acceptors;
- Bacteria with the appropriate metabolic capacity;
- Suitable electron donors; and
- Anaerobic conditions.

In general, the species of bacteria are fairly common in a variety of aquifer environments (Rivett et al., 2008). They can be found in shallow aquifers and at great depths. Bacterial species capable of denitrification have been found in clayey sands to 489 meters as well as deep limestone and granite aquifers. These bacteria either derive their energy through the oxidation of organic carbon or certain inorganic compounds. This distinction leads to the classification of the denitrification pathways available described below.

Depending on the electron donor used by the bacteria, denitrification can be classified as either heterotrophic or autotrophic. Heterotrophic denitrification utilizes organic carbon as an electron donor, whereas autotrophic denitrification utilizes reduced inorganic compounds such as sulfate or ferrous iron (Korom, 1992). Furthermore, autotrophic processes can follow heterotrophic processes in aquifers with both types of electron donors present when concentrations of nitrate become limited. Most researchers identified by Korom indicate the presence of either heterotrophic or autotrophic denitrification occurring but not both; however, most aquifers have both types of electron donors present. The review by Hiscock et al. (1991) also describes studies that identify the pathway of denitrification based on electron donors, but not in great detail.

Rivett et al. (2008) provides additional detail when considering the heterotrophic pathway to denitrification in terms of limiting factors. Their review indicates that in the case of the heterotrophic pathway, the rate of denitrification is most limited by the concentration of dissolved organic carbon in the pore water. Reseach indicates that the distinction between DOC and the total fraction of organic carbon (f_{oc}) or organic carbon present in soils is important based on bioavailability; soil organic carbon is generally not available to the microbial species that facilitate denitrification. Furthermore, studies are identified that correlate the rate of denitrification to the concentration of DOC in a given aquifer and since most aquifers have relatively low DOC concentrations, it is therefore critical to the occurrence of denitrification.

The review by Hill (1996) describing the denitrification process in riparian zones confirms the importance of organic carbon in the heterotrophic pathway. Organic carbon concentrations in these zones are typically high when compared to those found in aquifers and riparian zones often display complete reductions in nitrate. Also, Hiscock et al. (1991) describes methods, both above-ground and *in situ*, that attempt to enhance the denitrification process by the addition of organic carbon, usually in the form of methanol, ethanol, or sucrose. In most cases, an improved rate of denitrification was observed in the studies cited.

Temperature can also be an important factor. Hiscock et al. (1991) states that denitrification decreases markedly below 5°C, and generally, a doubling of denitrification occurs with each 10°C increase in temperature, other factors being equal. The temperature range for denitrification identified by Rivett et al. (2008) is from 2 - 50°C, however, their review indicates that the optimum temperature for denitrification is between 25 and 35°C. Thus, denitrification rates in Florida soils and groundwater should be higher than in cooler regions, as temperatures are typically near the lower end of this optimum range.

Rivett et al. (2008) indicate that the autotrophic process is less understood and Hiscock et al. (1996) describes autotrophic denitrification as “uncommon.” Both Rivett et al. (2008) and Korom (1992) cite studies that show that groundwater with high concentrations of ferrous iron typically do not contain nitrate. They also cite references that describe denitrification via reduced sulfure and pyrite, as does the Hiscock et al. (1991) review. As this process is not well understood, little detail describing how it proceeds and the limiting factors is provided, except to say that the desired endpoints may be less stable and the reaction can be pH dependent. Rivett et al. (2008) also indicates that using inorganic substrates from a remedial perspective may result in undesirable impacts to groundwater in terms of water hardness, corrosion to well materials and the formation of sulfate plumes.

Rivett et al. (2008) also looks at the influence of a number of biogeochemical factors that may influence denitrification. Nutrients and micronutrients such as phosphorus, boron, copper, and zinc are all important to the growth of denitrifying bacteria. In general, most aquifers have adequate concentrations of these nutrients, but they may be lacking in certain oligotrophic aquifers. Denitrifying bacteria prefer a pH range between 5.5 and 8, but the influence of pH is typically site-specific due to adaptive behaviors of the bacteria. Salinity, toxic substances, and small pore space that prevent the development of bacterial colonies are all identified as factors that may limit, but not prevent, denitrification. Based on the review, while these factors may influence the rate of denitrification but are not considered limiting factors as to whether denitrification occurs.

The review by Rivett et al. (2008) and Korom (1992) also describe an alternative pathway for nitrate in groundwater, dissimilatory nitrate reduction to ammonium (DNRA). In this case, it has been theorized in systems where nitrate supplies to the bacteria are limiting; however, this pathway is less common, mainly because the bacteria that facilitate this process cannot occupy as much substrate as those that facilitate denitrification. Though not common in the literature, some studies have identified DNRA process in aquifers. The main concern would be the cycling of ammonium back to nitrate in cases with a fluctuating water table that result in aerobic and anaerobic conditions.

Beyond the general review of research that these studies provide are research projects that collect field data from aquifers in an effort to characterize the nitrate reduction processes, if any exist. The descriptions of the studies represent a diverse collection of research in terms of site characteristics, aquifer type, and methods of determining whether or not denitrification is occurring. The value of the following material lies in this diversity of research as the material presented gives insight into how denitrification works in a variety of aquifer materials and which factors present in these materials may influence the reduction of nitrate.

Numerous studies considered the reduction of nitrate in shallow or unconfined aquifers, a relevant environment when considering the nitrate impacts that result from OSTDS. As stated earlier, a majority of the studies are field-scale studies that consider agricultural environments, but the processes and components that influence the denitrification reaction can be applied to nitrate plumes that result from OSTDS. The benefit is a developed understanding of the subsurface conditions that most influence the denitrification reaction and therefore source composition and orientation does not need to be considered. In fact, Anderson (1998) reviewed denitrification rates from some of the studies described here and identified the relevant factors for denitrification. Furthermore, the study then applied a statistically-generated regression to apply the rates to denitrificaiton related to an OSTDS site in Florida. Based on mass-balance calculations, the regression-derived rate indicated successfully that the nitrate reduction in this system was due to denitrification.

Over 20 studies were found during the review of the literature. Many of the studies are similar in their methodology and conclusions so a complete summary of each study is not warranted. Also, several studies looked to measure nitrate attenuation without consideration of the key parameters or pathway. Therefore, the following summarizes the important findings of the studies and highlights those that are the most relevant.

Many of the studies indicate heterotrophic denitrification as the dominant pathway in nitrate attenuation. This is likely due to the heterotrophic reaction being thermodynamically preferred. However, organic carbon concentration is often a limiting factor and in many cases natural levels of dissolved organic carbon are low. Bradley et al. (1992) show in their study of aquifer sediments collected in Tampa Bay, Florida that the levels of organic carbon limit the denitrification rate. They compared the natural *in situ* rates with those amended with organic carbon in the laboratory and found a significant difference in the results. Additionally, the heterotrophic pathway is strongly influenced by bioavailability of the organic carbon. In fact, many studies that indicate autotrophic reductions indicate that the carbon source present is not available to the bacteria in the aquifer. Siemens et al. (2003) describe an aquifer system that has relatively high concentrations of organic matter in the form of dissolved organic matter, but the matter is not spatially distributed to favor attenuation of nitrate and bioavailability is low.

Although numerous studies describe heterotrophic denitrification as the more dominant pathway, a surprisingly large number of studies identify autotrophic denitrification as the nitrate reduction pathway. These include the studies done by Robertson et al. (1996), Postma et al. (1991), Kolle et al. (1985), Pauwels et al. (1998), and Boettcher et al.

(1991). Robertson et al. (1996) look at nitrate attenuation in aquitard sediments in southern Ontario, Canada. The spatial relationship between reduced nitrate concentrations and a sulfur reservoir in the silty aquitard sediments suggest that sulfur is being used as the electron donor in this case. The authors conclude that similar silty or clayey materials, due to their relatively high concentrations of sulfur when compared to surrounding aquifer sands, have high potential for autotrophic denitrification. Boettcher et al. (1991) similarly conclude that sulfur is the dominant electron donor in the Fuhrberger aquifer in Germany due to mass balance calculations that correlate reductions in nitrate to increases in sulfate concentrations. The preference towards autotrophic denitrification is attributed to the lack of bioavailability of organic carbon in this aquifer.

Postma et al. (1991) look at denitrification in a sandy, unconfined aquifer in Denmark. Their findings indicate that pyrite is the dominant electron donor in spite of the fact that the aquifer has abundant amounts of organic carbon. While this observation seems contradictory, in this case the source of the organic carbon is brown coal fragments, and given the short residence time of the groundwater, cannot contribute enough to provide adequate bioavailability to the bacterial populations. Pauwels et al. (1998) describe autotrophic nitrate attenuation also due to pyrite oxidation in an aquifer in France characterized by silts, clays and sandstones with large amounts of pyrite. As with the previous study, the autotrophic reaction is favored; in this case, organic carbon concentrations are low and cannot contribute to nitrate reduction.

Some studies considered the denitrification processes that occur in deep or confined aquifers. These aquifers are typically characterized by sandstones, claystones, or limestones, and are typically fractured aquifer systems. Additionally, some systems in these studies consider the source of nitrate to be natural. Morris et al. (1988) examined deep coastal sediments in South Carolina to look for the occurrence of denitrification. Cores up to 185 meters in a silty and clay aquifer were sampled at discrete intervals, and samples were amended with 1 mM nitrate. Evidence of denitrification was seen at all the intervals, however the highest rates were observed in the near-surface sediments. The researchers suggest the rate is limited by the concentration of nitrate; however, they did not sample sediments for organic carbon, but they describe carbon as a limiting factor.

Two studies (Rivett et al., 2008; Wilson et al., 1990) looked for evidence of denitrification in limestone aquifers in England. The two studies result in contradictory evidence. Rivett et al. (2008), in taking a look at the hydrogeochemical conditions present in the major confined aquifers of England, have a pessimistic view of the potential for significant reductions in nitrate concentrations from denitrification. Although the conditions are anoxic, the bioavailability of organic carbon and the small pore space which can restrict bacterial colony growth are not favorable for denitrification. However, Wilson et al. (1990) in collecting aquifer data and comparing noble gas ratios, show evidence of significant denitrification in the Lincolnshire Limestone aquifer. As the objective of the study is to show evidence of denitrification, no data was collected concerning possible factors that could enhance or limit the nitrate reductions observed. The authors also do not indicate whether or not this observed reduction would have a significant impact on water quality. Both studies describe the need for further research in this aquifer types to determine denitrification potential in deep, confined aquifers in England.

Vogel et al. (1981) studied a deep confined sandstone aquifer in the Kalahari Desert. The nitrate in this groundwater system is from natural sources, but the study wanted to assess the rate of denitrification in such a system. Their findings, using noble gas ratios, indicate the denitrification rate is extremely slow and is essentially non-existent. This is attributed to the very low organic carbon content of the water.

Studies were found that examine denitrification in riparian zones (also known as buffer zones) and the results indicate that these regions have high potential for reduction of nitrate concentrations. In many cases, this can be attributed to the high organic carbon content of streambed sediments. However, heterotrophic reduction pathways are not the exclusive pathway in riparian zones, as the literature review revealed. Rather, a combination of biogeochemical factors in these zones seems to contribute to the ability to attenuate nitrate. Studies focused on this topic include Bohlke et al. (2002), Burt et al. (1999), Christensen et al. (1989), Devito et al. (2000), and Hill (1996). The study by Hill is a review of the current understanding of denitrification in these zones and is described earlier. Burt et al. (1999) studied the denitrification of riparian zones in England. Based on measurements of nitrogen gases that would be indicative of denitrification, the riparian zone showed very high potential for significant nitrate reductions, provided the water table adjacent to the buffer zone stayed adequately high to maintain anaerobic conditions. Also, the authors note that the silts and clays with the highest potential for reductions were often bypassed as groundwater flowed through gravel lenses above and below the lower permeability areas. Bohlke et al. (2002) conducted a similar study in a riparian zone on a stream in Minnesota. Nitrate concentrations were measured as well as other major groundwater chemistry parameters to determine the fate of agricultural nitrate contamination. Interestingly, the riparian zone in this case, which significantly reduced nitrate concentrations, was rich in pyrite and therefore the autotrophic pathway was favored. Organic carbon is present, but is in a form that is not biologically available. Christensen et al. (1989) conduct a study of denitrification in a riparian zone along three streams in Denmark. Significant reductions in nitrate were observed, however the authors do not attribute this to any factor such as organic carbon or pyrite minerals present in the sediments.

The researchers, as a result of the data collected in these studies, identify key factors in the process of denitrification which can be indicators of the occurrence of nitrate reduction. Although a precise correlation may not be identified, the presence or absence of certain groundwater components in the appropriate concentrations can, in some cases, lead to complete reduction of nitrate. Among the key findings related to denitrification in groundwater are the following:

- The primary limiting factor is most often related to the electron donor, whether it is organic carbon, ferrous iron, or sulfur compounds.

- The heterotrophic pathway, though thermodynamically favorable, is considerably dependent on the bioavailability of the organic carbon.

- It is important to consider both pathways of denitrification when assessing a particular aquifer as the conditions for both pathways may be present and may be active once a particular electron donor is depleted.

- Although there are optimal pH levels and temperatures for denitrification, the range is relatively wide when considering most aquifers and therefore they are generally not limiting. Soil and groundwater temperatures in Florida, however, are near optimum for denitrification. Therefore, denitrification rates should be higher than in cooler regions, other factors being equal.

- Certain limestone or sandstone environments are not favorable for the growth of bacterial colonies that can facilitate denitrification; however, given the typical aquifer environment common to OSTDS, this may not be relevant.

The factors influencing the denitrification reaction are various, as are the resultant rates of reduction. Studies that calculated or measured denitrification rates report values that vary by several orders of magnitude. This variability is indicative of not only the heterogeneity of the aquifers studied, but the importance of the identified biogeochemical factors observed in the research. Denitrification can occur in a variety of aquifers and utilize different pathways to achieve significant reductions of nitrate in groundwater. This results in a potential dichotomy; on one side, the process of denitrification may be fairly ubiquitous in most aquifer settings. On the other side, determining if it is actually occurring, at what rate, and what biogeochemical factors are driving the process may be difficult. Nonetheless, based on the literature review, a number of suggestions can guide sampling and assessment of natural denitrification in groundwater:

- Groundwater sampling directed at determining denitrification parameters can be focused on measurements of nitrate concentrations, denitrification end products, dissolved organic carbon, ferrous iron, pyrite minerals, and sulfur compounds.

- Groffman et al. (2006) describe several methods for the direct measurement of denitrification *in situ.* These methods should warrant further investigation and possibly lead to actual implementation in the field.

- Measurement of denitrification rates should be done in the context of other transport parameters. Smith et al. (2004) indicate that although the aquifer in their study had measurable rates of denitrification, these rates could not attenuate nitrate enough to overcome the transport rate and a large nitrate plume still resulted.

2.5 Groundwater Monitoring Studies and Reports

A relatively large number of studies and reports were found that considered nitrate distribution, plume delineation, and estimates of the source contribution of OSTDS. Generally these are characterized by various levels of groundwater sampling, and usually some effort to make conclusions as to the nature of the nitrogen impacts based on the results of the sampling. In some cases the studies or reports are quite simple, considering only nitrate concentrations. Others are highly detailed, considering not only nitrogen species concentrations, but a variety of hydraulic and geochemical parameters. Typically the more complex studies draw more conclusions as to the transport and transformative processes at work at the various sites. However, this level of complexity does not always correspond with superior results; in some cases, the simple study addresses the objectives and can make some significant conclusions related to nitrogen impacts.

A study in Helena, Montana, examined the change in groundwater nitrate distribution as correlated with the increase in population in the area (Drake and Bauder, 2005). The study indicates a potential relationship between the increase in observed nitrate concentrations and the increased use of OSTDS between 1971 and 2003. The study compiled data for aquifer nitrate concentrations from 10 publicly funded investigations in the defined time period. From this data, trend analysis with statistical significance methods was applied to identify any trend between the increasing population and nitrate concentration trends. Additionally, the data was plotted geographically for spatial trend analysis. The area surrounding Helena experienced a 17% increase in population and a 68% increase in septic tank use in the decade between 1990 and 2000. The statistical analysis confirmed a correlation between nitrate concentrations and increasing population. The geographical analysis also indicated a spatial trend, showing the highest increases occurred in rural areas. This was especially the case in areas overlying bedrock aquifers and areas with high density and unpermitted OSTDS.

A similar study summarized the overall impacts due to OSTDS in New Mexico that also considered nitrate distribution (McQuillan, 2004). In this study, data was compiled in a similar fashion to the study described above. The study compared the level of nitrate impacts of aquifers with largely oxic conditions to aquifers with anoxic conditions. Also, data results from geochemical isotopic fingerprinting are provided, to identify the source of nitrate contamination. Figure 2-1 shows the results using isotopic fingerprinting. This study indicated that areas with more significant nitrate concentrations occur in aquifers with oxic conditions, whereas aquifers with anoxic conditions have lower impacts due to conditions not being favorable for the transformation of ammonium to nitrate. The results of the study also indicated that isotopic fingerprinting can be a useful tool for identifying nitrate sources, which can be useful for targeting primary nitrate sources.

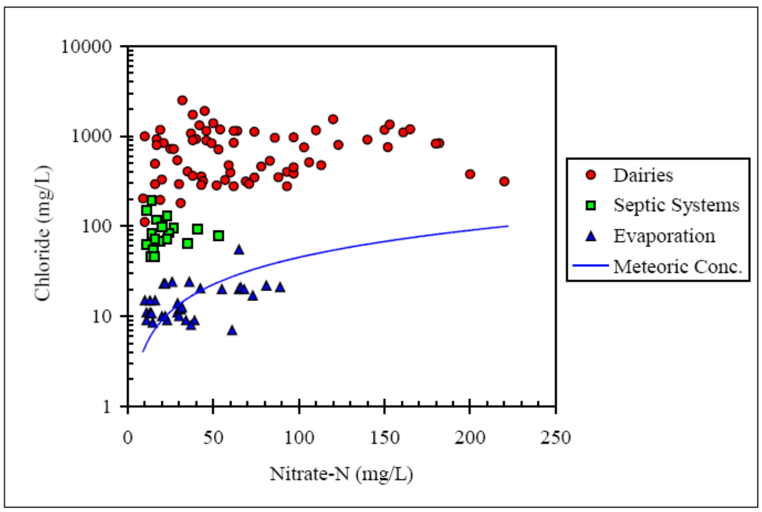

Figure 2-1: Nitrate Sources as Determined from Isotope Fingerprinting (McQuillan, 2004)

A study of the Darling Plateau region near Perth, West Australia also examined the nitrate contributions from OSTDS in a populated area served almost exclusively by individual OSTDS (Gerritse, Adeney et al., 1995). It was estimated in this study that nearly 80% of the nitrogen in the subsurface could be attributed to OSTDS source contributions. This study specifically looked at impacts of a neighboring surface water body approximately 70 meters downgradient. Monitoring of nitrogen species and bromide tracers showed significant decreases in inorganic nitrogen as the groundwater approached the creek. Interestingly, the surface water body had relatively high background concentrations of nitrate, but the study showed no significant contribution from this soil treatment unit.

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Lapointe et al. (1990) conducted a study to relate groundwater impacts to nearby marine surface waters via tidally-influenced groundwater recharge in the Florida Keys. The area in the study is characterized by typical tropical wet and dry seasons, with most of the precipitation falling between June and October. The subsurface is a highly porous and permeable limestone that allows for rapid lateral groundwater flow. For sampling, networks of monitoring wells were established on seven residences using OSTDS and one control site in a neighboring wildlife refuge. Wells were sampled monthly for approximately one year for nitrogen species and other biogeochemical factors. Groundwater flow was measured directly using an *in situ* flow meter. Surface water was also sampled. The results indicated that the contribution of nitrogen to the groundwater by OSTDS was significant in this area, in some cases as much as two orders of magnitude higher than when compared to control groundwater. Ammonium was the dominant species, the result of the largely unfavorable conditions for nitrification. Surface water showed a seasonal variation, with the highest concentrations occurring in the summer months. The study also concluded that seasonal variations in tides and groundwater levels result in significant contributions from OSTDS to surface waters in the Florida Keys.

A series of reports have been previously completed for assessment of OSTDS contributions to nitrate contamination of groundwater and surrounding surface waters in the Wekiva watershed in Florida. This includes reports prepared by: Anderson (2006); Briggs, Roeder et al. (2007); MACTEC (2007); Otis (2007); and Young (2007). The study was initiated to protect the Wekiva river system which had been assigned protection under the Wekiva River Protection Act. The watershed occupies roughly 304,000 acres and includes parts of Lake, Orange, and Seminole counties in central Florida. The project has been performed over a number of years and includes a series of tasks in order to assess the contribution to groundwater impacts from OSTDS and ultimately strategies to reduce these impacts. The tasks included:

- Field sampling for watershed characteristics, nitrogen concentrations, and OSTDS loading estimates.
- A literature review for refining estimates of OSTDS loading.
- Integrating these estimates with estimates of other source contributions.
- Development and discussion of alternatives for reducing OTWS contributions.

Three sites were selected for sampling that met the criteria and were deemed suitable for assessment of the desired data. After completion of the tasks, a number of conclusions were reached. For example, an average home with 2.6 people on average contributes 18 pounds of nitrogen to the groundwater annually with the main nitrogen contributor attributed to fertilizer use. This is slightly higher than reported by Lowe et al. (2008) of approximately 14 pounds of nitrogen annually (excluding outdoor residential nitrogen sources). The studies also concluded that OSTDS contribution to shallow groundwater contamination was similar in terms of intensity to atmospheric deposition, however, due to the areal distribution, nitrate impacts from OSTDS were approximately an order of magnitude higher and distinct plumes could be delineated. Furthermore, OSTDS tended to be in high-vulnerability areas and did not have effective nitrogen removal as compared to centralized wastewater methods. Figure 2-2 shows the estimated relative contributions for medium density residential land uses.

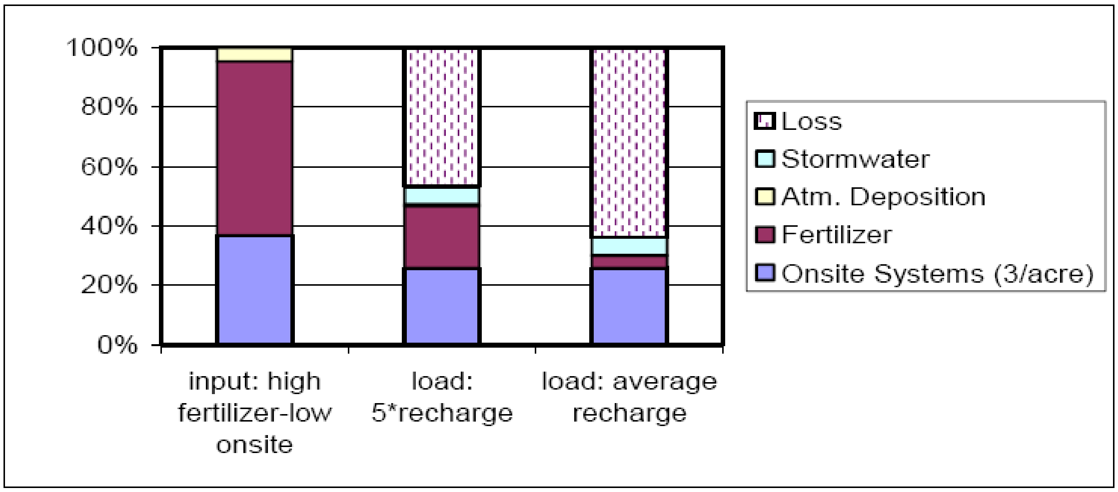

Figure 2-2: Source Contribution to Nitrate Impacts (Briggs, Roeder et al., 2007)

Other aspects of the study considered transport and transformation of nitrogen. Two factors were identified that influenced nitrogen entering the drainfield; the amount of nitrogen present in the effluent, and the level of pre-treatment prior to discharge. In the event pre-treatment was present then ammonia is converted to nitrate. However, nitrification will be limited in soils with high water tables. After discharge, if there is adequate organic carbon present, the nitrate can be denitrified to nitrogen gas.

Soils with moderate to poor drainage, fine loamy texture with clay, shallow water tables, and some organic matter have the highest potential for denitrification.

Ultimately the study found that contributions from OSTDS could be effectively minimized by reduced loading and improving OSTDS performance with pre-treatment methods and improvement of subsurface characteristics, especially considering high water table areas.

Andreadakis (1987) performed laboratory simulations of an alternative OSTDS in Greece to estimate the effectiveness of the system for nitrogen removal. The system consisted of a septic tank, gravel filter, two sand filters operated alternatively, and two soil absorption trenches operated alternatively. The study found the system could achieve approximately 70% nitrogen removal. The factors that influenced the effectiveness were the compaction characteristics of the filters and soil, loading rates, and variability in saturated/unsaturated conditions.

Reneau (1977) conducted a study of changes in inorganic nitrogen compound concentrations from a septic tank in a soil with a fluctuating water table in Virginia coastal plain area. Samples were collected and analyzed for nitrate, nitrite, and ammonium ions over a three year period. The relationship between nitrate and ammonium and distance is demonstrated by the ratio of these constituents to chloride (Cl^-). Assuming that chloride undergoes no significant transformations or adsorption, any variation in the ratio can be the result of either adsorption or transformation. In this case, the ratio of ammonium to chloride decreased with depth, indicating that at higher points anaerobic conditions dominated and nitrification could not take place. Following this trend, decreases in the nitrate to chloride ratio suggested that in some areas denitrification could take place due to the rising water table.

Arnade (1999) examined the relationship between nitrate well contamination and distance from OSTDS as related to seasonal variations in water level in Palm Bay, Florida. The study area experiences high precipitation during the summer months and results in high water tables in sandy soils that cause septic tank overflows and ultimately groundwater contamination. Results indicated that during the wet season, nitrate concentrations tended to be higher as distance increased as compared to the dry season, although the opposite was true closer to the OSTDS. The reasons provided for this observation were perhaps dilution, plant uptake, or enhanced transformation. This reasoning seems suspect, as if dilution is a factor in reduced concentrations, then concentrations should follow the same pattern throughout the flow path.

Walker et al. (1973a and b) describes two studies that look at nitrogen transformations of septic tank effluent in sands. The first study focused on transformations in sand while the second study examined transformations related to groundwater quality. Research was done at the field scale at five separate locations in Wisconsin. In all cases, effluent was ponded near the surface due to the formation of a “crust” (aka, the biozone) which was the result of biological processes. As a result, unsaturated flow rates were extremely low (8 cm/day). The biozone conditions were favorable for nitrification where groundwater was not present. Most of the sites showed complete nitrification was possible at six centimeters below the biozone. One site had a high water table and as a result nitrification did not occur unless seasonal variations resulted in a lowering of the water table. Denitrification was identified in an underlying clay layer at some of the sites, although this was not the case if the site had an underlying sandy layer. In the groundwater, the dominant process reducing the nitrate concentrations were dilution with ambient groundwater and not denitrification due to the nature of the well-aerated sandy soils and the low carbon content of the groundwater. The authors concluded that in order to minimize impacts from OSTDS in such aquifers, considerable land size is necessary in order to maximize the effects of dilution from clean water.

Harman et al. (1996) looked at the groundwater impacts resulting from an OSTDS at a school in Langton, Ontario, Canada. In this community, over 30% of the water supply wells exceeded the standard for nitrate. Multiple sources, primarily from OSTDS use and agricultural practices contributed to the high nitrate concentrations. The study aquifer in question was characterized by fine to medium sands and has a relatively high groundwater velocity (170 meters/year). The wastewater from the facility was largely from washrooms, as the site had no laundry facilities present. The effluent was primarily ammonium. At the site over 400 samples were collected at 45 multilevel monitoring points at various locations downgradient of the OSTDS. Samples were collected for all major ions, DOC, alkalinity, pH, and dissolved oxygen. The results found high nitrate concentrations were observed (20-120 mg/L) and extended over 100 meters downgradient owing to the high groundwater velocity. Vadose zone residence time was one to two weeks but did not appear to allow for complete conversion of ammonium to nitrate. However, geochemical analyses indicated reduced ammonium and organic carbon concentrations coinciding with increases in nitrate which suggest that nitrification was occurring. Denitrification was limited and isolated due to low levels of organic carbon and aerobic conditions. It appeared that most of the reduction of nitrate along the plume extent was likely due to natural dilution; denitrification was limited by low levels of organic carbon and aerobic conditions.

Robertson et al. (1991) studied the OSTDS impact to sand aquifer from two single-family homes in Ontario, Canada. The first site was a home in Cambridge, Ontario. The surficial aquifer was characterized as a coarse sand overlying a low permeability silt. The home was occupied by four people. The second site in Bracebridge, Ontario was situated on a fine sand aquifer with a household occupied by two people. Major ion geochemistry and typical septic tank nutrients were sampled. Bromide tracer tests were also performed. Both sites showed evidence of nitrification due to high concentrations of nitrate, and low concentrations of dissolved organic carbon and ammonium. High concentrations of nitrate were observed more than 130 meters downgradient from the sources which suggested little or no denitrification was occurring and that aquifer conditions were favorable for considerable nitrate migration. However, almost complete denitrification was observed in the carbon rich river sediments downgradient. In this aquifer, it was concluded that due to the low dispersive nature of this type of aquifer, current minimum distance to well regulations may not be protective. This was verified by natural-gradient bromide tracer tests.

Another study conducted looked at water sampling from domestic supply wells in five unsewered subdivisions in Wisconsin (Tinker, 1991). The objective of the study was to identify the sources of nitrate impacts to drinking water. Water samples were collected on two separate occasions from supply wells in five subdivisions and tested for nitrate concentrations. Sources of nitrate impacts were assessed by the location of the OSTDS and the water supply well in relation to the groundwater flow direction and comparison of the results of three mass-balance models. The combination of methods resulted in a good correlation between the locations and the groundwater flow, as well as the results of the mass-balance modeling. The author concluded that elevated nitrate concentrations could be attributed to lot size (from the mass-balance modeling) and locations of water supply wells and OSTDS.

Reay (2004) examined the impacts from OSTDS to near shore areas along Chesapeake Bay. Due to the sandy characteristics of the aquifer and the shallow water table, significant nitrate impacts to near shore sediments were observed. Multiple characteristics were analyze at three separate sites in Virginia considered representative of the Virginia coastal plains. Among the characteristics were depth to water, aquifer thickness, soil characteristics, lot size, and persons per household. Groundwater was sampled for nitrogen species and phosphorus as was neighboring surface waters. The author noted the lot size and relatively high loading rates contributed to the observed concentrations. Furthermore, the sites showed potentially high nitrification rates are likely present due to the observed concentrations of nitrate versus ammonium, and that very little denitrification was occurring, which led to significant nitrate impacts to nearby surface waters.

A sampling study to quantify the nitrogen impacts from OSTDSs was performed for a community in Nevada (Rosen et al., 2006). This study combined field data and a mass-balance approach to assess the nitrogen impacts attributed to OSTDS. The area under study was a densely populated area north of Reno, Nevada. In this area, 2,070 septic tanks were in use. Annual precipitation was low (20-25 cm/year) and recharge water to the aquifer also came from irrigation ditches (54%) and septic tank effluent (17%). Four separate sites were sampled monthly for one year. No geochemical or hydraulic parameters were collected. The final results of the estimates indicated that 25-30 metric tons of nitrogen in the groundwater could be the result of OSTDS use, although the authors concede that considerable error is possible and that future studies considering more parameters will be needed.

2.6 OSTDS Plume Geochemistry

A number of researchers went beyond the approach of considering nitrate concentrations only and considered numerous factors of OSTDS-generated nitrate plumes to delineate the important parameters that may affect nitrate transport and transformation. In most cases, the study collected samples related to all major ions present in groundwater (K^+, Cl^-, NO_3^-, SO_4^{-2}, Ca^{+2}, Na^+, Mg^{+2}, PO_4^{-3}), field parameters such as pH, conductivity, alkalinity, dissolved oxygen, and other factors such as dissolved organic carbon. Additionally, complete characterization of the aquifer parameters were collected, such as those related to soil type and groundwater flow and velocity. These studies were often performed at the field scale, although some laboratory experiments were done as well. The value of these studies is the opportunity to understand how the aquifer responds to transformative processes in terms of changes to other constituents and physical characteristics, and provide a rationale for the extent of impacts observed.

Wilhelm et al. (1998) looked at changes in geochemistry for two operating OSTDS in a sandy aquifer in Ontario for evidence of nitrate transformation. The objective of the study was to confirm a conceptual model that indicated the transformative processes related to nitrogen would result in the creation of redox zones. Changes in geochemical parameters could be measured to confirm the presences of these zones. Sampling was performed along the wastewater flow path at two sites from 1987 to 1990. In the septic tanks themselves a primarily anaerobic environment existed, with low concentrations of nitrate and high concentrations of ammonium and carbon. Aerobic conditions dominated below the discharge pipes. The research indicated that nitrification zones could be identified in areas with decreases in pH and alkalinity, whereas zones of denitrification were characterized by increases in both parameters. Differences in the sediment composition led to different behaviors of nitrate in the groundwater. For example, the plume at the second site entered carbon-rich sediments near a river bed, ultimately leading to complete denitrification and an increase in alkalinity.

Another study looked primarily at changes in inorganic nitrogen compounds related to septic tank effluents, but also looked at subsequent changes in pH and Eh (redox potentials) in a groundwater system in Virginia (Reneau, 1979a). The objective of the study was to relate changes in concentrations as related to distance traveled, soil properties, and seasonal variation. At three different sites, rows of sampling wells were established at 1.5, 5, 10, and 13.5 meters downgradient and sampled semi-monthly for phosphate, nitrogen species, Eh, and pH. Sampling occurred over a two-year period. For nitrate, concentrations reached a maximum (average values ranging from 2.7 to 3.9 mg/L) at the five meter sampling points then decreased with distance. This was attributed to nitrification of ammonium and the subsequent denitrification of nitrate to a relatively large degree. This was accompanied by a drop in pH and a slight increase in Eh values.

A study conducted in Ontario, Canada examined multiple geochemical factors which can be related to OSTDS impacts (Ptacek, 1998). Temperature, pH, dissolved organic carbon redox conditions and nitrogen species concentrations were all sampled. The original OSTDS effluent contained 98 mg/L of nitrogen as ammonium. Nitrate concentrations were high in the shallow portions of the aquifer, along with diminishing concentrations of DOC downgradient. pH stayed near neutral which was attributed to the buffering capacity of the aquifer due to carbonate content. Nitrate concentrations were low, which may suggest low rates of denitrification.

Robertson and Blowes (1995) observed nitrate concentrations in an acidic OSTDS plume. The study site was again located in Ontario, Canada at a location using an OSTDS for wastewater at a seasonal-use cottage. Sampling was performed at 38 piezometers adjacent to and underneath the infiltration bed. Major ion geochemistry samples were collected. Subsurface soil characteristics were various, from clays to silts to sands. The water table was generally consistent (1.5 meters below the field tiles), but became much shallower during the off-season winter months. In this system, background pH was naturally low; however, more acidic conditions existed within the plume core. Ammonium levels dropped substantially suggesting nitrification was occurring. The authors suggest that changes in nitrate concentrations downgradient were due to denitrification that was facilitated by relatively high levels of dissolved organic carbon and anaerobic conditions. Furthermore, at greater depths in the groundwater, high levels of sulfate coinciding with drops in nitrate concentrations suggested an alternative pathway for consumption of nitrate via sulphur oxidation.

Section 3.0
Discussion and Analysis

The literature review revealed important conceptual information for the assessment of nitrogen impacts in groundwater due to OSTDS. One of the primary objectives of the review was to examine the current state-of-knowledge related to the primary influences on the fate and transport of nitrogen following the initial loading into the soil from the use of OSTDS. A cascade of processes and factors contribute to nitrogen contamination. These include loading rate, OSTDS density, soil characteristics, oxygen content, aquifer recharge, and water table elevation and fluctuation. Primary factors that can lead to significant nitrogen concentrations are found in both the septic tank and the vadose zone and an understanding of the processes within these is important rather than just considering processes in the aquifer.

The transformative processes of nitrification and denitrification require further study and quantification, especially when considering septic tank performance and processes within the vadose zone. Additionally, an understanding of the aquifer characteristics, such as groundwater velocity and flux estimates can greatly improve the quantification of dilution for reduction of nitrate. Nitrification can be inhibited by high water tables and overloading of OSTDS. Likewise, heterotrophic denitrification, is an important process in the reduction of nitrate in groundwater, requires anoxic conditions in the presence of adequate carbon sources.

An improved understanding and assessment of field conditions prior to septic tank design can improve performance and result in reduced impacts from OSTDS. A large number of reports have been generated that are essentially monitoring reports describing nitrogen levels in observation wells. In some cases, these reports considered factors beyond nitrogen concentrations and included multiple geochemical factors as well. These studies have immense value in the light of other studies, in which the influence of important factors for nitrogen contamination can be quantified in real field-scale studies. Specifically, these studies provide quantitative data concerning:

- Downgradient and cross gradient nitrogen concentrations in groundwater which provides plume delineation spatially and in some cases temporally;

- Site-specific subsurface characterization such as soil type and distribution;

- Groundwater measurements that provide data concerning groundwater flow paths, velocities, and fluxes which can strongly influence the extent of the impacts in terms of concentrations and distance from the OSTDS;

- Total nitrogen loading rates at the source, which when compared to downgradient nitrogen concentrations provide data concerning OSTDS performance, and nitrogen conversion rates; and

- In some cases, surface water sampling which may indicate the level of groundwater/surface water interaction and/or transformative processes present at the groundwater /surface water interface.

The conclusions reached using the data in these studies can then be applied for nitrogen impact estimates in future studies and how to appropriately monitor and sample a site that will utilize OSTDS. Furthermore, these studies can be examples for assisting in OSTDS design and installation to minimize nitrogen in groundwater. Lastly, data from these studies can be applied to the further study of the OSTDS and vadose zone processes affecting nitrogen transport and fate in groundwater and lead to better predictive methods for estimating nitrogen impacts.

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Section 4.0 Conclusions

The literature review revealed numerous factors that may influence nitrogen impacts to groundwater resulting from the use of OSTDS. Transport and fate processes that are present in the OSTDS, vadose zone, and saturated zone all will influence the extent of nitrogen impacts to groundwater. Furthermore, these factors, along with factors related to groundwater/surface water interactions will also determine if nearby surface water bodies are adversely affected. In doing site assessments, it is therefore important to develop sampling plans that can collect data for a majority of the factors described in the literature. Also, predictive efforts and efforts aimed at reduction of impacts should also consider the findings of the literature review. A brief summary of important points is as follows:

- Some studies identified lot size and location of water supply wells in relation to OSTDS as important factors in determining nitrate contamination to groundwater.
- OSTDS loading rate can significantly impact the performance of the soil and ultimately nitrogen concentrations in the aquifer.
- In certain cases, water table fluctuations may be a larger factor than loading rate of nitrogen on the overall OSTDS performance.
- Nitrogen reduction in the vadose zone is an important determining factor for nitrate concentrations in the groundwater. This is a complex process dependent on numerous factors that need to be studied in depth.
- Nitrification can be influenced by soil type and appropriate loading of an OSTDS. Sikora and Corey (1976) indicate that coarse-textured strongly-aggregated soils favor nitrification while finer textured soils lead to the development of anaerobic conditions and inhibit the process.
- Sandy soil aquifers are particularly susceptible to nitrate contamination, particularly in the case of low carbon content aquifers with relatively high groundwater velocities. In these cases, high concentrations and large areas of impact may be expected due to the lack of transformation and the distance nitrate can travel in a short time period.

- Denitrification occurs largely in anoxic soils and groundwaters with adequate carbon sources. In the soil column, denitrification may occur in systems with high or fluctuating water tables that allow the creation of anoxic conditions, providing the organic carbon content of the soil is adequate. In groundwater, dilution is often seen as the dominant mechanism for the reduction of nitrate, although some studies identify denitrification as the dominant factor. This is highly dependent on site-specific characteristics.

- Denitrification, while being a well-understood process is poorly quantified and not correlated with other site characteristics especially when considering the saturated zone. This should be a significant topic of further study.

- Some studies identified the relatively high denitrification capacity of river bed sediments, particularly if they contained high levels of organic carbon. This is especially relevant if the protection of adjacent surface water bodies is a key concern.

The literature review suggests reductions in groundwater nitrogen impacts associated with OSTDS are achievable with a few steps. Nitrate is highly mobile in groundwater and the only significant methods of natural attenuation is denitrification, a process that the review indicates is not always present in natural aquifers (however, it should be noted that saturated zone denitrification can be enhanced with amendments as a potential treatment process, see Korom (1992)). Therefore, reduction of nitrate contamination may be most efficiently approached in the design and installation processes when considering OSTDS as a treatment alternative. Appropriate land planning and density of OSTDS in new developments is a first step. OSTDS should be placed within protective distance of downgradient groundwater and surface water resources. Additionally, recognizing the importance of dilution for nitrate concentration reductions, appropriate lot size should be in the design to allow adequate dilution from recharge water. Within the design of OSTDS, appropriate loading rates and an understanding of OSTDS effluent can achieve lower levels of nitrogen entering the subsurface. Lastly, the review indicates the performance value of appropriate treatment units can improve effluent quality by reducing nitrogen prior to infiltration.

Additional optimization can be achieved by a thorough understanding of site characteristics and how these may influence OSTDS performance and ultimately nitrogen concentrations in groundwater. Numerous studies were identified that have data related to existing systems and their performance within the framework of the characteristics of the site. Certain water table conditions, soil types, and other subsurface characteristics such as pH or temperature can have an effect on the treatment ability of OSTDS by varying oxygen content and redox conditions. If detrimental conditions are seen at a site being considered for OSTDS, other methods of wastewater treatment may be appropriate. This can also be true for areas identified as “high-risk,” such as areas adjacent to a protected water body. Alternatively, it may be possible to amend the site conditions or use an effluent pre-treatment method to improve OSTDS performance. Future work may be needed to examine the data in such studies and make attempts to correlate hydraulic and reactive parameters to observed nitrogen impacts.

Section 5.0
References

Aley, W.C.; M. Mark Mechling, G.S. Pastrana, and E.B. Fuller (2007). Multiple Nitrogen Loading Assessments from Onsite Wastewater Treatment and Disposal Systems within the Wekiva River Basin; Wekiva Study Area. Prepared for State of Florida, Department of Health, Tallahassee, FL, by Ellis & Associates, Inc., Jacksonville, FL.

Anderson, D.L. (2006). A Review of Nitrogen Loading and Treatment Performance Recommendations for Onsite Wastewater Treatment Systems (OWTS) in the Wekiva Study Area. Report submitted to the Florida Department of Health, Technical Review and Advisory Panel.

Anderson, D.L. (1998). Natural Denitrification in Groundwater Impacted by Onsite Wastewater Treatment Systems. Proceedings of the 8th National Symposium on Individual and Small Community Sewage Systems. American Society of Agr. Engineers., St. Joseph, MI.

Andreadakis, A.D. (1987). Organic Matter and Nitrogen Removal by an On-site Sewage Treatment and Disposal System. *Water Research,* **21**: 559-565.

Aravena, R. and W.D. Robertson (1998). Use of Multiple Isotope Tracers to Evaluate Denitrification in Ground Water: Study of Nitrate from a Large-flux Septic System Plume. *Ground Water,* **36**(6): 975-982.

Arnade, L.J. (1999). Seasonal Correlation of Well Contamination and Septic Tank Distance. *Ground Water*, **37**(6): 920-923.

Bates, H.K. and R.F. Spalding (1998). Aquifer Denitrification as Interpreted from in situ Microcosm Experiments. *Jour. Environ. Qual.,* **27**(1): 174-182.

Beller, H.R.; V. Madrid, G.B. Hudson, W.W. McNab, and T. Carlsen (2004). Biogeochemistry and Natural Attenuation of Nitrate in Groundwater at an Explosives Test Facility. *Applied Geochem.,* **19**(9): 1483-1494.

Bengtsson, G. and H. Annadotter (1989). Nitrate Reduction in a Groundwater Microcosm Determined by 15N Gas Chromatography-Mass Spectrometry. *Appl. And Environ. Microbiology,* **55**(11): 2861-2870.

Bohlke, J.K.; R. Wanty, M. Tuttle, G. Delin, and M. Landon (2002). Denitrification in the Recharge Area and Discharge Area of a Transient Agricultural Nitrate Plume in a Glacial Outwash Sand Aquifer, Minnesota. *Water Resources Research,* **38**(7): 10.11-10.26.

Bottcher, J.; O. Strebel, S. Voerkelius, and H.L. Schmidt (1990). Using Isotope Fractionation of Nitrate Nitrogen and Nitrate Oxygen for Evaluation of Microbial Denitrification in a Sandy Aquifer. *Jour. Of Hydrology,* **114**: 413-424.

Bradley, P.M.; M. Fernandez Jr., and F.H. Chapelle (1992). Carbon Limitation of Denitrification Rates in an Anaerobic Groundwater. *Environ. Sci. and Tech.,* **26**: 2377-2381.

Briggs, G.R.; E. Roeder, E. Ursin (2007). Nitrogen Impact of Onsite Sewage Treatment and Disposal Systems in the Wekiva Study Area. Florida Department of Health Report.

Burt, T.P.; L.S. Matchett, K.W.T. Goulding, C.P. Webster, and N.E. Haycock (1999). Denitrification in Riparian Buffer Zones: The Role of Floodplain Hydrology. *Hydrol. Processes,* **13**(10): 1451-1463.

Canter, L.W. (1996). Nitrates in Groundwater.

Christensen, P.B.; L.P. Nielsen, N.P. Revsbech, and J. Sorensen (1989). Microzonation of Denitrification Activity in Stream Sediments as Studied with a Combined Oxygen and Nitrous Oxide Microsensor. *Appl. And Environ. Microbiology,* **55**(5): 1234-1241.

Cogger, C.G. and B.L. Carlile (1984). Field Performance of Conventional and Alternative Systems in Wet Soil. *Jour. Environ. Quality* **13**: 137-142.

Cogger, C.G.; L.M. Hajjar, C.L. Moe, M.D. Sobsey (1988). Septic System Performances on a Coastal Barrier Island. *Jour. Environ. Quality* **17**(3): 401-407.

Costa, J.E.; G. Heufelder, S. Foss, N.P. Milham, and B. Howes (2002). Nitrogen Removal Efficiencies of Three Alternative Septic System Technologies and a Conventional Septic System. *Environment Cape Code* **5**(1): 15-24.

Degen, M.J.; C. Hagedorn, and D.C. Martens (1991). Denitrification in Onsite Wastewater Treatment and Disposal Systems. *Virginia Water Resources Research Center Bulletin #171*. V. W.R.R. Center.

o:\44237-001R009\Wpdocs\Report\Final

Devito, K.J.; D. Fitzgerald, A.R. Hill, and R. Aravena (2000). Nitrate Dynamics in Relation to Lithology and Hydrologic Flow Path in a River Riparian Zone. *Jour. Environ. Qual.,* **29**(4): 1075-1084.

Drake, V.M. and J.W. Bauder (2005). Ground Water Nitrate-Nitrogen Trends in Relation to Urban Development, Helena, Montana, 1971-2003. *Ground Water Monit. & Remed.* **25**(2): 118-130.

Fontes, J.-C.; J.N. Andrews, W.M. Edmonds, A. Guerre, and Y. Travi (1991). Paleorecharge by the Niger River (Mali) Deduced from Groundwater Geochemistry. *Water Resources Research,* **27**(2): 199-214.

Foster, S.S.D.; D.P. Kelly, and R. James. (1985). The Evidence for Zones of Biodenitrification in British Aquifers. In *Planetary Ecology,* Brierely, C.L. *(ed.)* pp. 356-369.

Francis, A.J.; J.M. Slater, and C.J. Dodge (1989). Denitrification in Deep Subsurface Sediments. *Geomicrobiology Journal,* **7**(1): 103-116.

Gerritse, R.G.; J.A. Adeney, and J. Hosking (1995). Nitrogen Losses from a Domestic Septic Tank System on the Darling Plateau in Western Australia. *Water Research* **29**(9): 2055-2058.

Groffman, P.M.; M.A. Altabet, J.K. Bohlke, K. Butterbach-Bahl, M.B. David, M.K. Firestone, M. K., et al. (2006). Methods for Measuring Denitrification: Diverse Approaches to a Difficult Problem. *Ecol. Appl.,* **16**(6): 2091-2122.

Hantzche, N.N. and E.J. Finnemore (1992). Predicting Ground-Water Nitrate-Nitrogen Impacts. *Ground Water* **30**(4): 490-499.

Harman, J.; W.D. Robertson, J.A. Cherry, L. Zanini (1996). Impacts on a Sand Aquifer from an Old Septic System: Nitrate and Phosphate. *Ground Water* **34**(6): 1105-1114.

Heatwole, K.K. and J.E. McCray (2007). Modeling Potential Vadose-zone Transport of Nitrogen from Onsite Wastewater Systems at the Development Scale. *Jour. Of Contam. Hydrology* **91**: 184-201.

Hill, A.R. (1996). Nitrate Removal in Stream Riparian Zones. *Jour. Environ. Qual.,* **25**(44): 743-755.

Hiscock, K.M.; J.W. Lloyd, and D.N. Lerner (1991). Review of Natural and Artificial Denitrification of Groundwater. *Water Research,* **25**(9): 1099-1111.

Kelly, W.R. (1997). Heterogeneities in Ground-water Geochemistry in a Sand Aquifer Beneath an Irrigated Field. *Jour. Of Hydrology,* **198**(1): 154-176.

Kolle, W.; O. Strebel, and J. Bottcher (1985). Formation of Sulfate by Microbial Denitrification in a Reducing Aquifer. *Water Supply,* **3**(1): 35-40.

Korom, S. (1992). Natural Denitrification in the Saturated Zone: A Review. *Water Resources Research* **28**(6): 1657-1668.

Korom, S.F.; A.J. Schlag, W.M. Schuh, and A.K. Schlag (2005). In-situ Mesocosms: Denitrification in the Elk Valley Aquifer. *Ground Water,* **25**(1): 79-89.

Lapointe, B.E.; J.D. O'Connell, G.S. Garrett (1990). Nutrient Couplings Between On-site Sewage Disposal Systems, Groundwater, and Nearshore Surface Waters of the Florida Keys. *Biogeochemistry* **10**: 289-307.

Lind, A.-M. (1983). Nitrate Reduction in Subsoil. In *Denitrification in the Nitrogen Cycle,* Golterman, H.L. (ed.) pp. 145-156.

Lowe, K.S.; M.B. Tucholke, J.M.B. Tomaras, K. Conn, C. Hoppe, J.E. Drewes, J.E. McCray, J. Munukata-Marr (2009). Influent Constituent Characteristics of the Modern Waste Stream from Single Sources: Final Report. WERF Report #04-DEC-01. 206 pp.

Lowe, K.S.; S.M. Van Cuyk, R.L. Siegrist (2007). Soil Treatment Unit Performance as Affected by Hydraulic Loading Rate and Applied Effluent Quality. *11th Individual and Small Community Sewage Systems Conference Proceedings*, Warwick, RI. 10 pp.

Lowe, K.S.; S.M. Van Cuyk, R.L. Siegrist, and J.E. Drewes (2008). Field Evaluation of the Performance of Engineered On-Site Wastewater Treatment Units. *Journal of Hydrologic Engineering* **13**(8): 735-743.

Lowe, K.S. and R.L. Siegrist (2008). Controlled Field Experiment for Performance Evaluation of Septic Tank Effluent Treatment during Soil Infiltration. *Journal of Environmental Engineering* **134**(2): 93-101.

MACTEC (2007). Phase I Report Wekiva River Basin Nitrate Sourcing Study. Florida Department of Environmental Protection Report.

McCray, J.E.; K.S. Lowe, M. Geza, J. Drewes, S. Roberts, A. Wunsch, D. Radcliffe, J. Amadore, J. Atoyan, T. Boving, D. Kalen, and G. Loomis (2008). Development of Quantitative Tools to Determine the Expected Performance of Unit Processes in Wastewater Soil Treatment Units: Literature Review. WERF Report # DEC1R06. 182 pp.

McCray, J.E.; S.L. Kirkland, R.L. Siegrist, G.D. Thyne (2005). Model Parameters for Simulating Fate and Transport of On-Site Wastewater Nutrients. *Ground Water* **43**(4): 628-639.

McGuire, J.T.; D.T. Long, M.J. Klug, S.K. Haack, and D.W. Hyndman (2002): Evaluating Behavior of Oxygen, Nitrate, and Sulfate during Recharge and Quantifying Reduction Rates in a Contaminated Aquifer. *Environ. Sci. and Tech.,* **36**(12): 2693-2700.

McQuillan, D. (2004). Ground-water Quality Impacts from On-Site Septic Systems. National Onsite Wastewater Recycling Association (NOWRA), 13th Annual Conference, Albuquerque, N.M.

Morris, J.T.; G.J. Whiting, and F.H. Chappelle (1988). Potential Denitrification Rates in Deep Sediments from the Southeastern Coastal Plain. *Environ. Sci. and Tech.,* **22**: 832-836.

Oehler, F.P.B., and P. Durand (2007). Variations of Denitrification in a Farming Catchment Area. *Agriculture, Ecosystems, and Environment,* **120**(2-4): 313-324.

Otero, N.; C. Torrento, A. Soler, A. Mencio, and J. Mas-Pla (2009). Monitoring Groundwater Nitrate Attenuation in a Regional System Coupling Hydrogeology with Multi-Isotopic Methods: The Case of Plana de Vic (Osana, Spain). *Agriculture, Ecosystems, and Environment,* **133**(1-3): 103-113.

Otis, R.J. (2007). Estimates of Nitrogen Loadings to Groundwater from Onsite Wastewater Systems in the Wekiva Study Area: Task 2 Report. Florida Department of Health Report.

Pauwels, H.; W. Kloppmann, J.-C. Foucher, A. Martelet, and V. Fritsche (1998). Field Tracer Test for Denitrification in a Pyrite-bearing Schist Aquifer. *Applied Geochem.,* **13**(6): 767-778.

Postma, D.; C. Boesen, H. Kristiansen, and F. Larsen (1991). Nitrate Reduction in a Unconfined Sandy Aquifer: Water Chemistry, Reduction Processes, and Geochemical Modeling. *Water Resources Research,* **27**(8): 2027-2045.

Ptacek, C.J. (1998). Geochemistry of a Septic-System Plume in a Coastal Barrier Bar, Point Pelee, Ontario, Canada. *Jour. Contam. Hydro.* **33**: 293-312.

Reay, W.G. (2004). Septic Tank Impacts on Ground Water and Nearshore Sediment Nutrient Flux. *Ground Water* **42**(7): 1079-1089.

Reneau, R.B.J. (1977). Changes in Inorganic Nitrogenous Compounds from Septic Tank Effluent in a Soil with a Fluctuating Water Table. *Jour. Environ. Quality* **6**: 173-178.

Reneau, R.B.J. (1979a). Changes in Concentrations of Selected Chemical Pollutants in Wet, Tile-drained Soil Systems as Influenced by Disposal of Septic Tank Effluents. *Jour. Environ. Quality* **8**: 189-196.

Reneau, R.B.J.; C. Hagedorn, M.J. Degen (1989). Fate and Transport of Biological And Inorganic Contaminants from On-site Disposal of Domestic Wastewater. *Jour. Environ. Quality* **18**(2): 135-144.

Ritter, W.F. and R.P. Eastburn (1988). A Review of Denitrification in On-site Wastewater Treatment Systems. *Environ. Pollution* **51**: 49-61.

Rivett, M. O.; J.W.N. Smith, S.R. Buss, and P. Morgan (2007). Nitrate Occurrence and Attenuation in the Major Aquifers of England and Wales. *Quarterly Jour. of Engin. Geol. and Hydrogeol.,* **40**(4), 335-352.

Rivett, M.O.; S.R. Buss, P. Morgan, J.W.N. Smith, C.D. Bemment (2008) Nitrate Attenuation in Groundwater: A Review of Biogeochemical Controlling Processes. *Water Research*, **42**(2008): 4215-4232.

Robertson, W.D. and D.W. Blowes (1995). Major Ion and Trace Metal Geochemistry of an Acidic Septic-System Plume in Silt. *Ground Water* **33**(2): 275-283.

Robertson, W.D.; J.A. Cherry, E.A. Sudicky (1991). Ground-Water Contamination from Two Small Septic Systems on Sand Aquifers. *Ground Water* **29**(1): 82-92.

Robertson, W D.; B.M. Russell, and J.A. Cherry (1996). Attenuation of Nitrate in Aquitard Sediments of Southern Ontario. *Jour. Of Hydrology,* **180**: 267-281.

Rosen, M.R.; C. Kropf, K.A. Thomas (2006). Quantification of the Contribution of Nitrogen from Septic Tanks to Groundwater in Spanish Springs Valley, Nevada. USGS Investigations Report # 2006-5206. United States Geological Society.

Siemens, J.; M. Haas, and M. Kaupenjohann (2003). Dissolved Organic Matter Induced Denitrification in Subsoils and Aquifers? *Geoderma,* **113**(3-4): 253-271.

Sikora, L.J. and R.B. Corey (1976). Fate of Nitrogen and Phosphorus in Soils Under Septic Tank Waste Disposal Fields. *Transactions of ASAE* **19**: 866-870.

Sikora, L.J. and D.R. Keeney (1976). Denitrification of Nitrified Septic Tank Effluent. *Jour. Water Poll. Control Fed.,* **48**, 2018-2025.

Singleton, M. J.; Esser, B. K., Moran, J. E., Hudson, G. B., McNab, W. W., & Harter, T. (2007). Saturated Zone Denitrification: Potential for Natural Attenuation of Nitrate Contamination in Shallow Groundwater under Dairy Operations. *Environ. Sci. and Tech., 41(3)*, 759-765.

Slater, J.M., and D.G. Capone (1987). Denitrification in Aquifer Soil and Nearshore Marine Sediments Influenced by Groundwater Nitrate. *Appl. And Environ. Microbiology,* **53**(6): 1292-1297.

Smith, R.L.; J.K. Bohlke, S.P. Garabedian, K.M. Revesz, and T. Yoshinari (2004). Assessing Denitrification in Groundwater Using Natural Gradient Tracer Tests with 15N: In situ Measurement of Sequential Multistep Reaction. *Water Resources Research,* ***40***, 17 pp.

Smith, R.L. and J.H. Duff (1988). Denitrification in a Sand and Gravel Aquifer. *Appl. and Environ. Microbiology,* **54**(5): 1071-1078.

Smith, R.L.; S.P. Garabedian, and M.H. Brooks (1996). Comparison of Denitrification Activity Measurement in Groundwater Using Cores and Natural-gradient Tracer Tests. *Environ. Sci. and Tech.,* **30**(2): 3448-3456.

Smith, R.L.; B.L. Howes, and J.H. Duff (1991). Denitrification in Nitrate-contaminated Groundwater; Occurrence in Steep Vertical Geochemical Gradients. *Geochimica et Cosmochimica,* **55**, 1815-1825.

Starr, J.L. and R.W. Gillham (1993). Denitrification and Organic Carbon Availability in Two Aquifers. *Ground Water,* **31**(6): 934-947.

Taylor, J.R. (2003). Evaluating Groundwater Nitrates from On-Lot Septic Systems, A Guidance Model for Land Planning in Pennsylvania.

Tesoriero, A.J.; H. Liebscher, and S.E. Cox (2000). Mechanism and Rate of Denitrification in an Agricultural Watershed: Electron and Mass Balance along Groundwater Flow Paths. *Water Resources Research,* **36**(6): 1545-1559.

Tinker, J.R.J. (1991). An Analysis of Nitrate-Nitrogen in Ground Water Beneath Unsewered Subdivisions. *Ground Water Monitoring Review* **Winter**: 141-150.

Trudell, M.R.; R.W. Gillham, and J.A. Cherry (1986). An In-Situ Study of the Occurrence and Rate of Denitrification in a Shallow Unconfined Sand Aquifer. *Jour. Of Hydrology,* **83**, 251-268.

Tucholke, M.B. (2006). Statistical Assessment of Relationships between Denitrification and Easily Measured Soil Properties: A Simple Predictive Tool for Watershed Modeling. M.S. Thesis, Colorado School of Mines. 187 pp.

Tucholke, M.B.; J.E. McCray, G.D. Thyne, and R.M. Waskom (2007). Variability in Denitrification Rates: Literature Review and Analysis. NOWRA 16th Annual Technical Education and Exposition Conference, Baltimore, MD. 18 pp.

Uebler, R.L. (1984). Effect of Loading Rate and Soil Amendments on Inorganic Nitrogen and Phosphorus Leached from a Wastewater Soil Absorption System. *Jour. Environ. Quality* **13**: 475-479.

van Beek, C.G.E.M. and J. van Puffelen (1987). Changes in the Chemical Composition of Drinking Water after Well Infiltration in an Unconsolidated Sandy Aquifer. *Water Resources Research,* **23**(1): 69-76.

Vogel, J.C.; A.S. Talma, and T.H.E. Heaton (1981). Gaseous Nitrogen as Evidence for Denitrification in Groundwater. *Journal of Hydrology,* ***50***, 191-200.

Walker, W.G.; J. Bouma, D.R. Keeney, F.R. Magdoff (1973). Nitrogen Transformations During Subsurface Disposal of Septic Tank Effluent in Sands: I. Soil Transformations. Jour. Environ. Quality **2**(4): 475-480.

Walker, W.G.; J. Bouma, D.R. Keeney, F.R. Magdoff (1973). Nitrogen Transformations During Subsurface Disposal of Septic Tank Effluent in Sands: II. Ground Water Quality. *Jour. Environ. Quality* **2**(4): 521-525.

Well, R.; J. Augustin, K. Meyer, and D.D. Myrold (2003). Comparison of Field and Laboratory Measurement of Denitrification and N_2O Production in the Saturated Zone of Hydromorphic Soils. *Soil Biology and Biochemistry,* ***35***, 783-799.

Wilhelm, S.R.; S.L. Schiff, W.D. Robertson (1998). Biochemical Evolution of Domestic Waste Water in Septic Systems: 2. Application of a Conceptual Model in Sandy Aquifers. *Ground Water* **34**: 853-864.

Wilson, G.B.; J.N. Andrews, and A.H. Bath (1990). Dissolved Gas Evidence for Denitrification in the Lincolnshire Limestone Groundwaters, Eastern England. *Jour. Of Hydrology,* **113**(1-4), 51-60.

Yates, M.V. (1985). Septic Tank Density and Ground-Water Contamination. *Ground Water* **23**(5).

Young, L.J. (2007). Final Report, Task 3: Assess Contributions of Onsite Wastewater Treatment Systems Relative to Other Sources; Wekiva Onsite Nitrogen Contribution Study June 4, 2007. Florida Department of Health Report.