Exhibit Q

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ANIMAL FEEDING OPERATIONS (CAFOS) ON
GROUNDWATER QUALITY

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Case Studies on the Impact of Concentrated Animal Feeding Operations (CAFOs) on Ground Water Quality

Office of Research and Development
National Risk Management Research Laboratory, Ada, Oklahoma 74820

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Notice

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Abbreviations

AFO	Animal Feeding Operation
BMP	Best Management Practices
CAFO	Concentrated Animal Feeding Operation
CTET	Chlortetracycline
CWAP	Clean Water Action Plan
DO	Dissolved Oxygen
DMA	Dimethylarsenate
ECTET	Epi-Chlortetracycline
EDC	Endocrine Disrupting Compound
EICTET	Epi-Iso-Chlortetracycline
EOTET	Epi-Oxytetracycline
EQBLK	Equipment Blank
ETET	Epi-Tetracycline
FD	Field Duplicate
FLDBLK	Field Blank
GC/MS/MS	Gas Chromatography Tandem Mass Spectrometry
ICP-MS	Inductively-Coupled Plasma Mass Spectrometry
ICP-OES	Inductively Coupled Plasma Optical Emission Spectrometry
ICTET	Iso-Chlortetracycline
IDS	Isotope Dilution Standard
IRMS	Isotope Ratio Mass Spectrometry
LC/MS	Liquid Chromatography Mass Spectrometry
LINC	Lincomycin
MCL	Maximum Contaminant Level
MDL	Method Detection Limit
MMA	Monomethylarsenate
MWL	Meteoric Water Line
MQO	Measurement Quality Objective
NMP	Nutrient Management Plan
NPDES	National Pollutant Discharge Elimination System
ORP	Oxidation-Reduction Potential
OTET	Oxytetracycline
PRB	Permeable Reactive Barrier
QA	Quality Assurance
QL	Quantitation Limit
RO	Reverse Osmosis
RRT	Relative Retention Time
SCLP	Sulfachloropyridazine
SDMX	Sulfadimethoxine
SDWA	Safe Drinking Water Act
SMOX	Sulfamethoxazole
SMZN	Sulfamethazine
SPE	Solid Phase Extraction
STHZ	Sulfathiazole
TC/EA	High Temperature Conversion Elemental Analyzer
TET	Tetracycline
TKN	Total Kjeldahl Nitrogen
TIC	Total Inorganic Carbon
TOC	Total Organic Carbon
TP	Total Phosphorus
TYL	Tylosin
VSMOV	Vienna Standard Mean Ocean Water

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Executive Summary

Concentrated Animal Feeding Operations (CAFOs) are increasing in size and generating considerably more waste requiring disposal over an increasingly limited area. As livestock production has become more spatially concentrated, the amount of available manure nutrients often exceeds the assimilative capacity of the land on the farms, resulting in both ground and surface water contamination. Ground water has long been recognized as an essential resource for drinking water and irrigation water, but it is also an important conduit for transfer of contaminants to surface waters. Potential ground water contaminants arising from CAFOs include dissolved solids, nutrients, metals and metalloids, pathogens, antibiotics, and natural and synthetic hormones. Ground water protection will only be as adequate as allowable under site-specific Nutrient Management Plans (NMPs), which serve as one of the few risk management tools for ground water protection. Few data exist which show whether proper NMPs are being developed or rigorously followed. Additional research on land application of CAFO wastewater on agricultural lands is also needed to assess whether risk management strategies used to prevent ground water contamination by nutrients (primarily nitrate) are equally protective with respect to other contaminants.

To address this issue, field studies were conducted to ascertain whether nitrate-impacted ground waters at commercial CAFO facilities were also contaminated with other stressors present in those CAFO wastes. Seven case studies are presented, including a swine finisher operation, a poultry layer operation (since closed), a swine nursery operation, a dairy operation, a combined swine operation (since closed), a beef feedlot operation, and a swine farrowing sow operation. With the exception of the swine farrowing sow operation, which essentially served as a relatively non-impacted site, each of these case study sites exhibited ground water contamination by nitrate and/or ammonium. For most sites, this resulted directly from the operation, either through leaking infrastructure piping, leaking lagoons, or land application of CAFO waste, as supported through the monitoring of stable nitrogen isotopes. The primary focus was on nutrients (nitrate, ammonium, orthophosphate) and natural estrogen hormones (estrone, 17α-estradiol, 17β-estradiol, and estriol), but other stressors that were analyzed at each site and are discussed included microbial indicators (total coliforms, fecal coliforms, fecal enterococci), metals and metalloids (arsenic, copper, nickel, selenium, and zinc), and antibiotics (selected macrolides, quinolines, sulfonamides, tetracyclines, and other antibiotics). These stressors were measured in CAFO lagoons as well as in ground waters from monitoring wells and other site wells. Extensive sampling and analyses were conducted during one to three sampling events per site, although two of these sites were monitored annually for approximately ten years, albeit for not all of the parameters listed above.

With the exception of the closed swine combined operation which had impacted ground water directly through a leaking lagoon, ground water contamination for most of these sites was limited to nitrate, and occasionally ammonium, only. This was somewhat surprising, since several contaminated ground water samples at each of the sites showed a moderate to strong animal waste signature based on stable nitrogen isotopes, and nitrate concentrations often exceeded 50 to100 mg/L NO_3- N in these samples. Fecal coliforms were only sporadically detected at high concentrations in a few wells at some of the sites, and in most cases there were greater numbers of fecal enterococci compared to fecal coliforms. There was little correlation between microbial indicator counts and nitrate concentrations. Similarly, concentrations of metals and metalloids were generally low in these ground water samples and showed little correlation with nitrate concentrations. Sulfonamides, tetracyclines, and other antibiotics were detected at high concentrations (mg/L range) in swine lagoons and, to a lesser extent, in dairy lagoons, but only lincomycin, sulfamethazine, and sulfamethoxazole were detected in any of the ground water samples. Even in these cases, concentrations were close to the method reporting limits (low to mid ng/L range). Of these three antibiotics, lincomycin was detected at the greatest frequency in ground water samples, being present in one or more ground water samples at three of the six sites with CAFO-impacted ground waters. Again, there was little correlation of the presence of antibiotics with either nitrate concentration or with animal waste signature as indicated by stable nitrogen isotopes. With the exception of the closed combined swine operation, estrogen hormones (primarily estrone) were only sporadically detected in ground water samples, and again concentrations were very low (0.5-2.7 ng/L).

The closed combined swine operation was the only site with significant ground water contaminants other than nitrate, and this was most likely due to direct and extensive leakage of the original lagoon swine waste into the shallow aquifer over several years. Unlike the other operations, ammonium concentrations were quite high in several of the ground water samples, with four wells generally ranging from 50-200 mg/L NH_4-N during annual monitoring from 2003 to 2011. These wells also had elevated levels of arsenic and estrogen hormones. Arsenic concentrations, as more accurately measured by ICP-MS in 2009, ranged from 84-126 µg/L in these ground water samples compared to much lower levels in most of the other wells. Estrogen hormones were also consistently detected in these ground water samples over this time period, with the levels of estrone being the highest (11,200 ng/L maximum), followed by estriol (824 ng/L maximum) and then 17β-estradiol (41 ng/L maximum). These estrogen concentrations have generally been decreasing over time, and the maximum concentrations in 2011 were 388 ng/L for estrone, < 0.3 ng/L for estriol, and < 2.1 ng/L for 17β-estradiol.

Collectively, these data show that ground water contamination by nitrate or ammonium can occur at very different types of CAFOs, whether through leaking lagoons, leaking pipes or infrastructure, land application of wastes in excess of agronomic needs, or other factors. However, we found little evidence of significant ground water contamination by other stressors at these sites, except in cases where CAFO wastes leaked directly from the lagoons into associated aquifers. Even in those cases, where ground water nitrate concentrations greatly exceeded the MCL and moderate to high levels of ammonium could also be detected, the other stressor concentrations were generally quite low. This suggests, but does not necessarily imply, that if CAFOs were properly managed so as to preclude ground water contamination by nitrate in excess of the MCL, then other stressors associated with CAFO wastes (metals, metalloids, antibiotics, and estrogen hormones) might also be attenuated to acceptable risk levels. Additional field studies are needed to test this hypothesis, preferably with more frequent sampling events to account for seasonal variations and long-term effects. It is important to note that this study did not evaluate true pathogens, other hormones (e.g., trenbolone), or other antibiotics (e.g., monensin), and additional research is also needed to ascertain whether these stressors would exhibit similar potential for contaminating ground water through leaking lagoons or land application of CAFO wastes. In addition, it should also be noted that this study does not address long-term effects from the buildup of salts and other compounds on ground water quality or soil productivity. Finally, these results should not obscure the fact that contamination of ground water by nitrate or ammonium is in itself a significant environmental problem and can lead to legacy impacts on receiving surface waters with direct hydrologic connection to contaminated ground waters. Much more work is needed to address the efficacy of current CAFO nutrient management strategies (i.e., BMPs) for ground water protection from contamination by nutrients as well as other stressors, and to ascertain whether additional guidance or regulatory controls are needed.

1.0 Introduction

In the United States, there are approximately one million farms with livestock, of which about 212,000 confine animals and are defined as animal feeding operations (AFOs) under current regulations (USEPA, 2012a). A facility is an AFO if animals are stabled or confined and fed or maintained for 45 days or more within any 12-month period, and crops, vegetation, forage growth, or post-harvest residues are not sustained in the normal growing season over any portion of the lot or facility. Approximately 20,000 of these are large enough to be classified as concentrated animal feeding operations (CAFOs) which, under previous definitions, were generally considered to be operations with more than 1,000 animal units (USEPA, 2012a). AFOs generate approximately 500 million tons of manure each year which must be properly treated and disposed or utilized to prevent contamination of soil, air, and water (USEPA, 2003). Animal manure is a valuable source of nutrients and has been used as fertilizer to enhance production of agricultural crops and forage. However, there have been substantial changes in the U.S. animal production industry over the past several decades. Although the total number of operations has decreased, overall production has increased. As a result, CAFOs are increasing in size and generating considerably more waste requiring disposal over an increasingly limited area. As livestock production has become more spatially concentrated, the amount of available manure nutrients often exceeds the assimilative capacity of the land on the farms, especially in high production areas, and studies have shown that this problem is becoming much more widespread (Kellogg et al., 2000). The problem is not just limited to nutrients, and the industrialization of livestock production in the U.S. over the past three decades has not been accompanied by either improvements in waste treatment technologies or the corresponding changes in environmental regulations necessary to protect public health (Thorne, 2007).

In addition, CAFO demographics are changing, and an increasing number of these operations are expanding to the central and western regions of the U.S., where land is often less expensive and located away from major population centers. This is especially true for dairy operations (USDA, 2002), but it has occurred for swine operations as well. For example, in 1997 Oklahoma became the 8th leading swine-producing state in the United States, up from 26th in 1992, with the production of swine increasing from 200,000 to 2,950,000 in a relatively short span of six years (Luckey and Becker, 1999). There are many reasons for these types of demographic shifts, but one of the most important may be the semiarid nature of the central and western regions, which results in limited rainfall and relative lack of number of rivers and larger surface water bodies compared to the eastern U.S. This means that CAFOs that rely on open lagoons for storage of liquid manure, as is often the case with swine operations, can have smaller lagoons in place because of enhanced evaporation rates and less rainfall. Also, the potential for surface water contamination by agricultural runoff is lessened in areas with more limited rainfall events and greater distances to rivers and streams, which makes it easier for these operations to dispose more waste and still achieve regulatory compliance. Finally, in some of these areas unaccustomed to experiencing growth in the animal industry, adequate state or local regulations may not yet be in place to sufficiently protect the environment, and the attraction of economic growth in poorer communities may outweigh the environmental concerns on a local level (Wing and Wolf, 2000; Donham et al., 2007).

1.1 Ground Water Vulnerability to CAFO Impacts

Ground water has long been recognized as an essential national resource, accounting for 40% and 36% of the nation's drinking water and irrigation water, respectively (Alley et al., 1999). However, it is seldom recognized that ground water is also an important conduit for transfer of contaminants to surface waters, and nationally, approximately 40% of the average annual streamflow in the U.S. is from ground water (Alley et al., 1999). In addition, much of the agricultural land in the Midwest and East Coast is underlain by tile drains which can also eventually discharge into wetlands, streams, rivers, and lakes (Jaynes and James, 2012). CAFO impacts on ground water quality can therefore be considered as an emerging risk issue for surface water quality. Unlike surface waters, ground waters are not exposed to light, are often low in dissolved oxygen, and generally contain less organic carbon, all of which can lead to greater persistence of organic and inorganic contaminants. A case in point is the High Plains aquifer, which underlies parts of eight states in the Midwest and is the most intensively used aquifer in the U.S., producing almost two times more water than any other U.S. aquifer (Luckey and Becker, 1999). Although this aquifer accounts for about 20% of all ground water used in the U.S., the natural rate of nitrate attenuation is slow in most parts and it is estimated that hundreds to thousands of years would be required to lower nitrate concentrations by just 1 mg/L NO_3-N (Gurdak et al., 2009).

Recent events regarding waste releases and algal blooms in the eastern U.S. have prompted a closer evaluation of the environmental impact of CAFOs on surface waters. Nationally, agriculture accounts for 59% of all sources of impairment for rivers, and CAFOs directly contribute 16% to total agricultural impact through surface water runoff (USEPA, 2001a). However, the impact of agriculture to ground water is relatively unknown, and there are no estimates for impairment due specifically to CAFOs. Contaminants can enter ground water from a variety of CAFO sources, including leaking lagoons, breaches in piping or barn infrastructure, and land application of liquid and solid wastes. There are guidelines for design and construction of barns, infrastructure piping, and lagoons that in theory would preclude leakage to ground water, but in practice these events do occur (Ham and DeSutter, 2000; Krapac et al., 2002). In fact, even when properly constructed, slow leakage from lagoons over time can release large amounts of contaminants such as ammonium (Ham and DeSutter, 1999). In the central and western regions of the U.S., sparse rainfall, relative lack of surface water streams, and the availability of readily-permeable soils promote land application of CAFO waste, exacerbating the potential for ground water contamination. Because depths to ground water can exceed hundreds of feet in these areas, ground water contamination may not be realized for years. Land application practices in theory are designed to allow nitrogen uptake by the crops and vegetation, and under appropriate conditions can be used in a sustainable manner (Bastian, 2005; O’Connor et al., 2005; Pierzynski and Gehl, 2005; Van Es et al., 2006; Hatfield, 2009). But there have been reports of ground water contamination by nitrate even when proper management strategies are being followed

(Karr et al., 2001; Decau et al., 2004; Showers et al., 2008), including a recent study in Arkansas, where high levels of nitrate were transported through the soil profile, despite adherence to an approved NMP which allowed dairy cows to loaf in the land application area (Moore and Brauer, 2009).

1.2 Regulatory Mechanisms and Needs

The Clean Water Act prohibits the discharge of pollutants from a point source to waters of the United States except as authorized by a National Pollutant Discharge Elimination System (NPDES) permit, and also requires EPA to establish national technology-based effluent limitations guidelines and standards for different categories of sources (USEPA, 2001a). In 1974 and 1976, EPA promulgated regulations that established these guidelines and permitting regulations for CAFOs. Since that time, these rules had not kept pace with the growth of the animal industry and had become inadequate for environmental protection. In 1998, President Clinton released the Clean Water Action Plan (CWAP), which described 111 specific actions to expand and strengthen existing efforts to protect water quality. As part of this effort, the CWAP called for the development of a USDA-EPA unified national strategy to minimize the water quality and public health impacts of CAFOs, and this strategy was published in 1999. In 2003, EPA addressed one of the strategic issues, implementing and improving the existing regulatory program, by finalizing revisions to NPDES permit regulation and effluent limitations guidelines and standards for CAFOs, 40 CFR Parts 122 and 412 (USEPA, 2003). This rule, also known as the CAFO Rule, replaced rules and regulations that had been governing CAFO operations for the previous 25 years. Under this rule, all Large CAFOs that had a potential to discharge or that actually discharged into waters of the U.S. were required to apply for an NPDES permit, develop and follow a site-specific Nutrient Management Plan (NMP), and submit an annual report. In addition, the rule eliminated the exemption that excuses CAFOs from applying for permits if they only discharged during large storms, eliminated the exemption for poultry operations with dry manure handling systems, and extended permitting requirements to immature swine and immature dairy cows. Since 2003, there have been challenges and revisions to the 2003

CAFO Rule, primarily concerning the provision of the potential to discharge and the disclosure and use of site-specific NMPs (USEPA, 2012b). Currently, for Large CAFOs, the 2012 Final CAFO Rule requires NPDES permits only for those operations that actually discharge into waters of the U.S., and requires that the terms of the NMPs must be publicly disclosed and included in the permit. Regarding land application, facilities are required to establish protocols to land apply manure, litter or process wastewater in accordance with site-specific nutrient management practices that ensure appropriate agricultural utilization of the nutrients in the manure, litter or process wastewater (USEPA, 2012c). Specifically, with respect to protocols for land application, the terms must include the fields, field-specific rates of application, and timing limitations for land applying manure as terms of the permit. Lastly, once the terms of the NMP becomes terms of the permit, the NMP and the permit terms are to be made available for public review and comment. Regarding unpermitted facilities, there is still some regulatory incentive to follow sound land application practices, in that for a precipitation-related discharge to qualify as an exemption under agricultural stormwater and not be subject to NPDES permit requirements, the wastewater must be applied in accordance with site-specific practices that ensure appropriate utilization of nutrients. Efforts are currently underway to address some of these nutrient management issues through a reworking of the CAFO Rule (USEPA, 2010a).

Despite these advances, it is still not clear whether the 2012 Final CAFO Rule will provide sufficient protection of ground water resources, since the emphasis is clearly on NPDES discharges, and therefore ground water protection will only be as adequate as allowable under site-specific NMPs. For example, CAFO operators are still permitted to apply manure to frozen, snow-covered, or saturated ground. In developing the 2003 CAFO Rule, EPA had originally proposed that all CAFOs be required to prevent discharges to the ground water beneath production areas, but received numerous comments opposing ground water protection and monitoring requirements. Arguments were based on the premise that EPA lacked the authority to directly regulate ground water contamination in this rule and that the cost to comply with the proposed requirements would threaten the viability of these operations. Under the Clean Water Act, EPA does have authority to control discharges to surface water via ground water when it has been established that ground water has a direct hydrologic connection with surface water. However, in the 2003 CAFO Rule, EPA rejected establishing national requirements on discharges to surface waters via ground waters with a direct hydrologic connection based on the scientific uncertainties and site-specific considerations required to regulate discharges. Direct ground water protection instead has been afforded through the Safe Drinking Water Act (SDWA) that was passed in 1974 to protect public health by regulating the nation's public drinking water supply (USEPA, 2004). The emphasis of the SDWA was on public water supplies, and it does not regulate private wells that serve fewer than 25 individuals. Originally, the SDWA focused primarily on treatment as the means of providing safe drinking water at the tap, but the 1996 amendments greatly enhanced the existing law by recognizing, among other things, source water protection. However, neither the SDWA nor the 2012 Final CAFO Rule requires monitoring of CAFOs for possible ground water contamination, and this is often left to the discretion of the individual states. In Oklahoma, for example, CAFOs are required to install monitoring wells upgradient and downgradient of waste storage facilities, but not at land application sites (ODAFF, 2011). In 2000, a review of all data from swine CAFOs in Oklahoma showed that 51% of all wells at swine CAFOs did not yield water; of those that did, 24% had nitrate levels exceeding 10 mg/L nitrate-nitrogen (NO_3-N), the maximum contaminant level (MCL) established under the National Primary Drinking Water Regulations of the SDWA (Becker et al., 2002). It is expected that this percentage would have increased substantially if monitoring wells were installed on or adjacent to land application areas of these facilities. Once ground water becomes contaminated, States and EPA may elect to pursue specific enforcement actions that may lead the way to prevent further contamination. For example, the SDWA has been used to require CAFO operators to provide drinking water to families whose wells have been contaminated by nitrate from CAFOs (USEPA, 2001b) and actions have also been taken using the Resource Conservation and Recovery Act (RCRA) to classify loss of effluent from swine lagoons and associated infrastructure as a discarded material and thus a solid waste (USEPA, 2001c). While effective, these actions require significant investments in time and resources for enforcement personnel, and prevention, rather than remediation and mitigation, is a much more preferable route towards sustainable agriculture.

Overall, this indicates that State regulations, as well as Federal regulations, for CAFOs may not be sufficiently protective of ground water, and there is a clear need to better assess the efficacy of existing Best Management Practices (BMPs) which form the basis of NMPs (Burkholder et al., 2007). It also remains to be seen whether a majority of CAFOs will develop adequate NMPs and whether these will be rigorously followed. Clearly, additional case studies are needed to provide both an assessment of existing BMPs as well as to provide the data required to support future rulemaking efforts.

1.3 CAFO Stressors Potentially Impacting Ground Water Quality

Potential ground water contaminants arising from CAFOs include dissolved solids, nutrients, pathogens, metals and metalloids, pharmaceutical chemicals, and natural and synthetic hormones. These stressors can enter ground water from a variety of CAFO sources, including disposal of dead animals, leaking lagoons, breaches in piping or barn infrastructure, and land application of liquid and solid wastes. Much of the ground water quality information centers on nutrients, and in fact nitrate may prove to be the primary ecological stressor. Reactive nitrogen (nitrate and ammonium) is considered to be a potent ecological stressor on a global scale, and recommendations for reducing it in the U.S. include increased controls of oxides of nitrogen, improved reactive nitrogen uptake by agricultural crops, large-scale creation and restoration of wetlands for nitrogen removal in agricultural landscapes, decreased loss of reactive nitrogen from agricultural lands and AFOs, and decreased discharge of reactive nitrogen from point sources and developed (urban) lands (USEPA, 2010b). CAFO lagoons contain high concentrations of ammonium nitrogen and organic nitrogen, both of which constitute important sources of nitrogen for crops when these wastes are land applied (Bradford et al., 2008). Ammonium nitrogen is available for direct uptake by plants, and organic nitrogen is readily broken down under a variety of conditions to produce additional ammonium nitrogen. However, although ammonium is strongly sorbed to the soil, it is also readily converted to nitrate under aerobic conditions through nitrification (Alexander, 1977). Because nitrate is readily mobile, it can be easily leached from soil and can contaminate ground water, and so it is essential that CAFO wastes be applied only at agronomic rates (Bradford et al, 2008). Although ground water bacteria can readily convert nitrate to nitrogen gas through denitrification, the process generally requires an easily assimilated organic carbon source and is not favored under aerobic conditions (Alexander, 1977). Nitrate is detected much more often in ground water than ammonium or phosphate (Nolan and Stoner, 2000), and nitrate-contaminated ground water can therefore serve as an important source for subsequent eutrophication of surface waters. Regarding nutrients, phosphate is also a major stressor and can lead to eutrophication of surface waters, but phosphate impacts to ground water from CAFO operations are expected to be low except for cases where over-application exceeds the assimilative capacity of the soil and crops (Bradford et al, 2008). Regardless, this has been documented in ground water impacted by agricultural operations, and transport of phosphate in ground water to surface water has been demonstrated as well (Domagalski and Johnson, 2011).

CAFOs can also provide the mechanisms for direct introduction of pathogens into subsurface systems through land application of liquid and solid wastes, and there have been numerous studies on the microbial ecology of pathogens in livestock wastes and their subsequent fate and transport (Guan and Holley, 2003; Hill, 2003; Gerba and Smith, 2005; Hutchinson et al., 2005). The main focus has been on the protozoa *Cryptosporidium parvum and Giardia* spp. (Atwill et al., 2006; Bradford et al., 2006; Jellison et al., 2007), the bacteria *Escherichia coli* O157:H7, *Salmonella enteri*ca, and *Campylobacter jejuni* (Cornick and Helgerson, 2004; Bae et al., 2005; You et al., 2006; Holley et al., 2008; Kunze et al., 2008; Sharma et al., 2008), and the enteric viruses (Meng et al; 1997; Fong and Lipp, 2005; Kasorndorkbua et al., 2005; Hundesa et al., 2006). Because of their smaller size, viruses can more easily be transported through the soil and vadose zone during land application than bacteria and protozoans, and can also remain infectious. Over half of the waterborne disease outbreaks in the U.S. between 1971 and 1994 were associated with ground water sources, and at least 10% of these were caused by viruses (Abbaszadegan et al., 2003). Because of the complexities and expense involved with analyzing water samples for all of these pathogens, microbial indicators (total coliforms, fecal coliforms, and fecal enterococci) are still widely used to provide an indication of microbial water quality, despite inherent limitations in underestimating levels of viruses and other pathogens (Noble et al., 2003; Skraber et al., 2004; Harwood et al., 2005; Haack et al., 2009).

Another group of stressors associated with CAFOs include trace elements, which are used extensively in the CAFO industry as growth promotants and for therapeutic purposes, and livestock manures are important sources for release of these elements back into the environment (Jackson et al., 2003; Bolan et al., 2004). Metals and metalloids of primary interest include copper, zinc, and arsenic, and to a lesser extent nickel and selenium. Copper and zinc are used in swine and poultry operations to increase weight gain (Rea et al., 1999). At higher concentrations, copper also inhibits microbial growth (Skrivan et al., 2006), and it has also been shown to improve odor characteristics of swine waste (Armstrong et al., 2000). Copper salts are also used as a footbath in milking yards to treat lameness in dairy cattle (Bolan et al., 2004). Arsenic, in the form of the organoarsenical antibiotic Roxarsone, has been used extensively in the poultry industry to improve weight gain, feed efficiency, and pigmentation, in addition to its use with ionophores to control coccidiosis (Chapman and Johnson, 2002). To a lesser extent, Roxarsone has also been used in the swine industry, and has been found in several swine lagoons (Makris et al., 2008). Roxarsone can be degraded under both aerobic and anaerobic conditions to release the more toxic inorganic arsenic species which can then be mobilized and contaminate ground water (Arai et al., 2003; Stolz et al., 2007; Church et al., 2011). Although the use of Roxarsone is declining in the U.S., this can lead to legacy contamination events from past practices, as illustrated in the Delmarva Peninsula where approximately 20-50 metric tons of Roxarsone had been applied yearly (Hileman, 2007).

Pharmaceutical chemicals used in CAFOs include antibiotics and synthetic hormones, and antibiotics in particular are of concern because of the large volumes that are used as livestock growth promotants and their potential to confer antibiotic resistance among commensal and pathogenic microorganisms. Although estimates vary, 40% to 87% of all antibiotics produced in the U.S. are used in animal feeds to promote growth (Gilchrist et al., 2007). Veterinary antibiotics vary widely in chemical properties and include many different sulfonamides, macrolides, fluoroquinolones, ß-lactams, tetracyclines, and aminoglycosides, and also include other chemicals which serve as anthelmintics, or dewormers (Halling-Sørensen et al., 1998; Lee et al., 2007). Hence, as might be expected, different compounds will vary widely with respect to sorption and degradation processes affecting fate and transport into ground water (Kemper, 2008; Khan et al., 2008). For example, compounds like tylosin are generally sorbed and rapidly degraded (Hu and Coats, 2007; Sassman et al., 2007), whereas the sulfonamides are generally more mobile and, in the case of sulfamethoxazole, more recalcitrant (Wang et al., 2006; Burkhardt and Stamm, 2007; Barber et al., 2009). Another complication is that antibiotic degradation products may be almost as potent as the parent compounds, and yet may be more mobile in soil (Boxall et al., 2003). In addition, there is some evidence that antibiotics like sulfamethazine and chlortetracycline can be taken up into plants (Kumar et al., 2005; Dolliver et al., 2007).

There have been relatively few studies on ground water contamination by antibiotics from CAFOs, in part because of some of the analytical challenges associated with the use of fairly sophisticated analytical methods and complex matrices such as CAFO lagoon wastes. However, studies are beginning to emerge that clearly demonstrate the presence of antibiotics in ground waters associated with beef, dairy, and swine CAFOs (Batt et al., 2006; Watanabe et al., 2010; Bartelt-Hunt et al., 2011). Perhaps of greater concern is the potential impact of the high use of antibiotics in CAFOs at subtherapeutic doses on the prevalence of antibiotic resistance (Pruden et al., 2006; Pruden, 2009a; McKinney et al., 2010). Numerous recent studies have demonstrated the distribution and prevalence of antibiotic resistance genes and pathogens at swine (Schroeder et al., 2002; Jindal et al., 2006; Bibbal et al., 2007; Kozak et al., 2009), dairy (Khachatryan et al., 2006; Sawant et al., 2007; Srinivasan et al., 2008), poultry (Harwood et al., 2001; Diarra et al., 2007; Smith et al., 2007; D’lima et al., 2007), and beef (Alexander et al., 2008) CAFOs. Not surprisingly, we are now starting to see evidence of antibiotic resistance genes and bacteria in impacted ground waters, especially at swine CAFOs (Chee-Sanford et al., 2001; Koike et al., 2007; Sapkota et al., 2007).

Other concerns involve the natural and synthetic steroid hormones associated with CAFOs that function as Endocrine Disrupting Chemicals (EDCs) and can interfere with the normal function of the endocrine system of humans and animals (Ying et al., 2002). The occurrence of EDCs in surface water is becoming of increasing concern worldwide, and has led to a growing awareness that animal, and perhaps human, health and function in ecosystems might become negatively impacted by continued release of EDCs into the environment (Ashby et al., 1997; Arcand-Hoy et al., 1998). As analytical techniques have become more robust and more sensitive, natural steroid hormones are now being detected at trace levels in streams (Kolpin et al., 2002) and in drinking water systems (Benotti et al., 2009). Natural steroid hormones include estrogens, androgens, and progestins, which are produced by metabolism and excreted by humans as well as animals (Arcand-Hoy et al., 1998). Although these compounds can be degraded biologically, they have been detected in sewage treatment effluents and receiving surface waters at ng/L levels (Ternes et al., 1999; Baronti et al., 2000; Joss et al., 2004; Shappell, 2006), and in some cases are transported into surface water from contaminated ground water (Standley et al., 2008). These concentrations are significant, because several of these steroid hormones are potent EDCs and can exert environmental effects at low ng/L levels. Hanselman et al. (2003) reported that natural estrogens possess estrogenic potency up to 10,000 to 100,000 times higher than exogenous EDCs (excluding the synthetic estrogen 17α-ethynylestradiol, used in birth control pills). Research has shown that male fish exposed to low ng/L levels of estrogens will exhibit estrogenic responses such as vitellogenin production (Routledge et al., 1998; Vajda et al., 2008; Shore, 2009a). Vitellogenein induction has also been observed in female turtles associated with farm ponds receiving steroid estrogens from direct contact with cattle as opposed to control ponds with no such contact (Irwin et al., 2001), and in fathead minnows exposed to runoff from fields amended with poultry litter under standard agricultural practices (Yonkos et al., 2010). Environmental effects can occur through short-term or intermittent exposure (Panter et al., 2000; Schultz et al., 2003; Martinović et al., 2008), or exposure to combinations of estrogens which individually are below their respective action levels (Silva et al., 2002a; Thorpe et al., 2003; Brian et al., 2007).

CAFOs constitute a source for release of steroid hormones into the environment, whether these are just produced naturally by the animals or used to promote weight gain, and high concentrations of natural estrogens are found in CAFO wastes (Arcand-Hoy et al., 1998; Hanselman et al., 2003; Hutchins et al., 2003; Lorenzen et al., 2004; Raman et al., 2004; Hutchins et al., 2007; Lee et al., 2007; Shore, 2009b; Bevacqua et al., 2011). The steroid hormones of particular interest that are endogenously produced and excreted by livestock include the natural estrogens estrone and 17β-estradiol. Of these, 17β-estradiol has the potential for exerting the greatest environmental impact, although estrone is almost as potent and in some cases can be converted back to 17α-estradiol or 17β-estradiol (Czajka and Londry, 2006). Estimates for the predicted-no-effect-concentration (PNEC) for the most active natural estrogens in surface water are 1 ng/L and 3-5 ng/L for 17β-estradiol and estrone, respectively (Young et al., 2004). It is estimated that estrogen loads from land application by livestock manure would account for greater than 90% of the total estrogen in the environment (Khanal et al., 2006), and emissions of estrogens from dairy and swine CAFOs exceed the mass flow of estrogens from municipal sewage treatment plants in the United States (Raman et al., 2004). In addition, other research has shown that estrogens can leach more readily from cow and chicken manure than from sludge from wastewater treatment plants (Suri et al., 2007). Research with swine manure has shown that steroid hormone levels can be reduced with additional treatment steps such as constructed wetlands (Shappell et al., 2007) or an upflow anaerobic sludge blanket with trickling filter (Furuichi et al., 2006), but by far most swine CAFOs in the United States store the liquid waste in lagoons and then land apply as needed. Several laboratory studies have focused on the sorption (Casey et al., 2003; Lee et al., 2003; Das et al., 2004; Mansell and Drewes, 2004; Casey et al., 2005; Zhou et al., 2007) and biodegradation (Colucci et al., 2001; Lorenzen et al., 2005; Jacobsen et al., 2005; Lorenzen et al., 2006; Khan et al., 2008) of steroid hormones in agricultural soils, and in general these studies show that steroid hormones are readily sorbed and degraded. However, there have been numerous studies showing that steroid hormones released from animal waste have been measured in both surface waters and ground waters associated with CAFOs (Nichols et al., 1997; Finlay-Moore et al., 2000; Peterson et al., 2000; Renner, 2002; Hanselman et al., 2003; Kolodziej et al., 2004; Kolodziej and Sedlak, 2007; Arnon et al., 2008; Zhao et al., 2010). One problem may be that laboratory studies represent very controlled environments that may or may not replicate all of the influencing factors found in the real environment. For example, Sangsupan et al. (2006) demonstrated significant transport of 17β-estradiol and testosterone in undisturbed soil columns which more appropriately replicated the macropores found in the real environment at most sites, and Kjær et al. (2007) similarly observed increased leaching of estrone and 17β-estradiol from manure-treated structured soils into tile drains. Another problem is that steroid hormones are excreted in either the free form or as sulfate or glucuronide conjugates, depending on the animal and the route of excretion. Because estrogen conjugates are more polar than the free forms, they are expected to be more mobile in the soil (Hanselman et al, 2003), and therefore have a greater potential for impacting ground water. CAFO lagoons have been found to contain estrogen conjugates as well as free estrogens (Hutchins et al, 2007). The conjugated forms of these steroid hormones are biologically inactive, but can be readily deconjugated to produce the active free steroid hormones (Arcand-Hoy et al., 1998; Ascenzo et al., 2003; Scherr et al., 2008).

In summary, CAFOs have the potential for contaminating ground water with stressors other than just nutrients. Bradford et al. (2008) recently reviewed the theoretical impact of land application of CAFO wastewater on agricultural lands and recommended that additional research focus on whether risk management strategies used to prevent ground water contamination by nutrients (primarily nitrate) are equally protective with respect to other contaminants, including steroid hormones. We took a somewhat different approach to address this issue by posing the following question: if improper design or management practices at a CAFO results in ground water contamination by nitrate, is this ground water also contaminated by other stressors in that CAFO waste? These field studies were therefore conducted to ascertain whether nitrate-impacted ground waters at problematic CAFO sites were also contaminated with other stressors present in those CAFO wastes. The primary focus was on estrogen hormones, because of the importance of EDCs as ecological stressors and the lack of information on the contribution of CAFOs to estrogens in the environment. However, other stressors were also selected for study, based on expected prevalence and the availability of analytical resources, and the final analytical suite discussed in this report included nutrients (nitrate, ammonium, and orthophosphate), natural estrogen hormones (estrone, 17α-estradiol, 17β-estradiol, and estriol), microbial indicators (total coliforms, fecal coliforms, and fecal enterococci), metals and metalloids (arsenic, copper, nickel, selenium, and zinc), and antibiotics (selected macrolides, quinolines, sulfonamides, tetracyclines, and other antibiotics).

2.0 Materials and Methods

2.1 Field Site Selection Criteria

Seven separate CAFO field sites located in south central United States were selected for study, including swine, poultry, dairy, and beef operations. These were all commercial operations and were designated as Large CAFOs based on the number of animals at each facility. These sites were selected for study because each site, with one exception, had ground water contaminated with nitrate and/or ammonium, and previous investigations had either demonstrated or implicated site operations as the source of the ground water contamination. The exception was a relatively recent swine farrowing sow operation, which was included as a relatively non-impacted site to ascertain whether off-site wells which had very low nitrate levels and were downgradient of the site were also free of the other types of contaminants found in CAFO waste and would therefore provide suitable background conditions for evaluating any potential future impacts.

It was beyond the scope of this study to try to determine the relative contributions of the individual source terms to ground water contamination at each site because of limited site access and resource constraints. In addition, for most sites, ground water samples could only be obtained from the available facility sampling wells, which often were not distributed to the extent required for evaluating other source terms within the facilities.

In some cases ground water contamination most likely occurred through leakage of CAFO wastes from lagoons or associated barns and infrastructure, whereas in other cases excessive land application of CAFO waste effluents was the most likely source. Additional site information is provided for each site in the individual case studies.

2.2 Stable Isotope Evaluation Criteria

Stable isotopes have been used in previous ground water studies to provide information on the sources of ground water (Gat, 1971; Blasch and Bryson, 2007) as well as on the sources of nitrate in ground water (Sidle et al., 2000; Silva et al., 2002b; Jin et al., 2004; McMahon et al., 2008). Although CAFOs were already identified as the sources of nitrate in most of these case studies described in this report, we measured the stable isotopes of water, nitrate, and ammonium in CAFO lagoons and ground waters to evaluate whether the information could be used in these limited synoptic sampling events to facilitate the interpretation of the analytical data from these sites.

Stable isotope ratios of water ($\delta^{2}H-H_2O$, $\delta^{18}O-H_2O$) were used in a very simplified way to gather additional information about the sources of the ground water samples obtained from the site wells by comparing the distribution of these two isotopes to the meteoric water line (MWL) provided by Taylor (1974). The lighter isotopes of hydrogen and oxygen are lost more rapidly than the heavier isotopes during evaporation of water, but not proportionately so, and therefore surface water bodies tend to have isotopic ratios that deviate significantly from the MWL by becoming more depleted in $\delta^{2}H-H_2O$ relative to $\delta^{18}O-H_2O$. In this study, ground water samples whose isotope ratios fell significantly below the MWL compared to those which more closely aligned with the MWL were considered to be more influenced by recharge from an evaporative surface water body. Evaluation of stable water isotope data provides another generalization that can be made - if all of the wells at a site have similar screened intervals and the ground water samples have very similar water stable isotope values, this indicates a very similar ground water source and recharge history for the wells. Conversely, differences in water stable isotope values from ground water samples taken from wells with similar screened intervals could mean several things, including the possibilities that a well could be improperly sealed or that preferential flow paths may exist.

Stable nitrogen isotope ratios of nitrate (δ^{15}N-NO_3) and ammonium (δ^{15}N-NH_4) were also analyzed at these field sites. Because volatilization of ammonium from animal wastes results in an increased fraction of the heavier nitrogen isotope in the residual ammonium as well as in the nitrate produced from that ammonium, stable nitrogen isotopes of nitrate and ammonium have been used to indicate whether the source of ground water nitrate was from fertilizer or from animal waste (Becker et al., 2002; Karr et al., 2003; Widory et al., 2005). Although ranges vary (Bedard-Haughn et al., 2003; Mariappan et al., 2009), stable nitrogen isotope ratios (δ^{15}N) values in sewage and animal wastes typically exceed +8‰ to +10‰, and in general nitrate with a δ^{15}N value in excess of +10‰ indicates that sewage or animal waste is the source. One of the factors complicating this interpretation is that denitrification processes can cause the δ^{15}N of the residual nitrate to increase exponentially as nitrate concentrations decrease (Kendall et al., 2008), and this can lead to false assignments of animal waste signatures to δN^{15} values greater than +10‰. However, a consequence of this δN^{15} enrichment by denitrification is that nitrate concentrations will decrease as δ^{15}N values increase, and this can be used to indicate whether denitrification processes are occurring. These relationships were used in this study as follows: 1) a δ^{15}N value greater than +10‰ for either nitrate or ammonium was interpreted to be indicative of animal waste, and 2) any observed decreases in nitrate concentrations correlated with increases in δ^{15}N-NO_3 were interpreted as evidence that significant denitrification was occurring, and that δ^{15}N-NO_3 values greater than +10‰ might not be attributable to animal wastes.

The stable oxygen isotope ratio of nitrate (δ^{18}O-NO_3) was also analyzed at these field sites. Stable oxygen isotopes of nitrate have been used to help determine whether the source of nitrate derives from atmospheric precipitation, nitrate fertilizers or ammonium fertilizers, and also to evaluate the contribution of denitrification to enrichment of δ^{15}N-NO_3 (Silva et al., 2002; Deutsch et al., 2005; Kendall et al., 2008). Nitrate derived from atmospheric sources is more enriched in δ^{18}O-NO_3 than nitrate found in nitrate-based fertilizers or derived from nitrification of ammonium-based fertilizers, and this can help to determine the source of nitrate, especially where δ^{15}N-NO$_3$ values are low. An additional use of stable water oxygen isotope data derives from the fact that denitrification enriches δ^{18}O-NO$_3$ as well as δ^{15}N-NO$_3$, and therefore a positive correlation between δ^{18}O-NO$_3$ and δ^{15}N-NO$_3$ values would be expected under conditions of denitrification. These relationships were used in a very simplified manner as follows: 1) δ^{18}O-NO$_3$ and δ^{15}N-NO$_3$ values were compared graphically with published ranges adapted from Silva et al. (2002b) and Kendall et al. (2008) to attempt to discern the sources of nitrate, and 2) a positive correlation between δ^{18}O-NO$_3$ and δ^{15}N-NO$_3$ values was interpreted as evidence of denitrification, which could potentially lead to a false animal waste signature for δ^{15}N-NO$_3$ values in excess of +10‰.

Because there are many factors complicating the interpretation of stable isotope data (Mengis et al., 2001; Bedard-Haughn et al., 2003; Kendall et al., 2008), these criteria are not definitive and should be considered as supporting evidence only.

2.3 Field Site Sampling

CAFO lagoon samples were generally taken under vacuum using a stainless steel sample inlet suspended with a float and connected to a peristaltic pump. In some cases a stainless steel submersible pump (Grundfos Pumps Corporation, Clovis, CA) equipped with a float and suspended to a depth representative of effluent intake for land application was used. Lagoon water was pumped through polyethylene tubing at 500 mL/min through a sample filter bypass system into a flow-through cell for 15 min prior to electrode measurements and sampling. Ground water samples were generally taken using an identical, but separate, stainless steel submersible pump, filter by-pass system, and flow-through cell. In some cases where well casings were severely bent or pumping infrastructure partially blocked access, the peristaltic pump was used instead to sample ground water. Contrary to many common practices, wells were not purged for a specific number of well volumes prior to sampling. Rather, a low-flow sampling technique was used to ensure minimal disturbance of the water column and acquisition of representative ground water from the aquifer matrix (Puls and Powell, 1992). Ground water was pumped at a low flow rate (500 mL/min) and was monitored in-line for pH, oxidation-reduction potential (ORP), conductivity, temperature, and dissolved oxygen (DO) using the field meter electrodes (Orion Research, Inc., Beverly, MA) associated with the flow-through cell. Once conductivity and oxygen readings had stabilized, sample collection began. Sample collection generally began after about 15 min, but if these parameters had not stabilized additional time was allotted for purging the sampled well volume. When the sampled well volume was sufficiently purged, the ground water was diverted past the flow-through cell and samples were obtained and analyzed on-site for alkalinity, turbidity, and in some cases dissolved oxygen using field kits (CHEMetrics Inc., Calverton, VA). Certain unfiltered and filtered samples were routinely taken and transported back on ice for laboratory analysis, including 1) 60-mL unfiltered samples for analysis of nitrate (NO_3-N), nitrite (NO_2-N), orthophosphate (o-PO_4), chloride (Cl), sulfate (SO_4), total carbon (TC), total organic carbon (TOC), and total inorganic carbon (TIC); 2) 60-mL unfiltered samples, acidified with sulfuric acid to $pH < 2$, for analysis of combined nitrate and nitrite (NO_2/NO_3-N), ammonium (NH_4-N), total Kjeldahl nitrogen (TKN), total phosphorus (TP), and TOC; and 3) 30-mL filtered samples, filtered through 0.45μ syringe filters and acidified with nitric acid to $pH < 2$, for analysis of metals/cations. For certain lagoons with high suspended solids, additional unfiltered samples were taken, acidified with nitric acid to $pH < 2$, and then digested prior to analysis for metals/cations to better determine contaminant loading using whole effluents. Calibrated plastic bottles amended with sodium thiosulfate (IDEXX Laboratories, Inc., Westbrook, ME) were used to collect 100-mL unfiltered samples for microbial indicator counts. Unfiltered samples were also collected for dissolved methane (CH_4) and nitrous oxide (N_2O) analyses into each of two 60-mL serum bottles amended with sodium phosphate tribasic dodecahydrate and sealed with Teflon®-lined grey butyl rubber septa.

For large-volume filtered samples, the sample stream was diverted through a 0.45-μ high-capacity ground water sampling capsule (Geotech Environmental Equipment, Inc., Denver, CO) and 500 mL was collected and discarded prior to sample collection. Filtered samples for stable isotopes of water (δ^2H-H_2O, $\delta^{18}O$-H_2O) were collected into

30-mL plastic bottles and sealed with minimal headspace. Filtered samples for stable nitrogen isotopes of nitrate and ammonium ($\delta^{15}N-NO_3/NH_4$) were collected into 1,000-mL plastic bottles, and filtered samples for stable oxygen isotopes of nitrate ($\delta^{18}O-NO_3$) were collected into 500-mL plastic bottles. Filtered samples for estrogen analysis were collected into 2-L glass media bottles and preserved with formaldehyde (1% final volume). Filtered samples for antibiotics were collected into each of four separate 125-mL amber glass bottles. Filtered samples for arsenic speciation were collected into 30-mL amber plastic bottles and acidified to $pH < 2$ with hydrochloric acid. Some lagoon samples could not be filtered on-site and were instead collected into 2-L glass media bottles and transported back to the laboratory on ice for centrifugation and pressure filtration.

Extensive sampling and analyses generally occurred for one to three sampling events per site, although two of these sites were monitored annually for approximately ten years, albeit for not all analytical parameters. The complete data sets for all sampling events are provided in Appendix A. Equipment blanks (EQBLK) and field duplicates (FD) were collected for each sampling event, although not for all parameters each time. Equipment blanks were prepared by running laboratory reverse-osmosis (RO) water through the sampling pumps, filter bypass system, and flow-through cell during sampling events. The frequency of field duplicate collection was approximately 10%. Because a change in the estrogen hormone analytical method provided greater sensitivity in 2009, these measures were later expanded to include field blanks (FLDBLK) and multiple equipment blanks. Field blanks were prepared by pouring RO water directly into sample containers during sampling events.

2.4 General Laboratory Analyses

Samples which could not be field-filtered (particularly lagoons) required additional processing in the laboratory. Each lagoon sample was mixed and transferred to 500-mL centrifuge bottles and centrifuged at 9,000 RPM (13,700 RCF) for 1 h at 4°C. The supernatants were decanted directly into a 10-L stainless steel pressure tank and the sample was pressure filtered under 40 psi nitrogen through a stainless steel filter holder containing a 142-mm 0.45µ filter with a 124-mm 0.7µ glass fiber prefilter.

Nutrient and general parameter samples were analyzed by Standard Methods (American Public Health Association, 2005), whereas metals/cations were analyzed by inductively-coupled plasma-optical emission spectrometry (ICP-OES). Because high organic carbon can interfere with arsenic determination by ICP-OES, selected samples were also analyzed for arsenic by inductively-coupled plasma-mass emission spectrometry (ICP-MS), and some of these samples were also analyzed for arsenic speciation using ion chromatography coupled online to ICP-MS as described by Beak and Wilkin (2009). Arsenic speciation included arsenate (As (V)), arsenite (As (III)), monomethylarsenate (MMA), and dimethylarsenate (DMA). Dissolved gases were analyzed by exchanging part of the sample with helium and then measuring gas concentrations in the headspace with a gas chromatograph after headspace equilibrium was attained. Microbial indicator counts were performed with a Most Probable Number (MPN) method using the assay kits Colilert® for total and fecal coliforms and Enteroalert® for fecal enterococci (IDEXX Laboratories, Inc., Westbrook, ME). Stable isotopes of water were analyzed using a high temperature conversion elemental analyzer (TC/EA), a continuous flow unit, and an isotope ratio mass spectrometer (IRMS). The TC/EA reactor contains carbon chips that are used to react with water at high temperature to generate hydrogen and carbon monoxide gases. The isotopic composition is reported in permil (‰) relative to Vienna Standard Mean Ocean Water (VSMOV). The acceptable standard deviations of replicates and standards for this method are 1.0% and 0.2% for $\delta^{2}H\text{-}H_2O$ and $\delta^{18}O\text{-}H_2O$, respectively.

Outside laboratories were used for the analysis of the other stable isotopes and also for antibiotics. Following any required centrifugation and pressure filtration for lagoon samples, these stable isotope samples were immediately frozen and then shipped to the University of Nebraska's Water Sciences Laboratory (Lincoln, NE). Nitrogen isotopes of nitrate and ammonium ($\delta^{15}N\text{-}NO_3/NH_4$) were determined using alkaline distillation of ammonium, Devardas alloy reduction and separate distillation of nitrate as ammonium, and then oxidation of ammonium to nitrogen gas and dual inlet IRMS (Gormly and Spalding, 1979; Krietler, 1979). Oxygen isotope analysis of nitrate ($\delta^{18}O\text{-}NO_3$) was done separately by ion exchange separation of nitrate and conversion to silver nitrate prior to high temperature pyrolysis IRMS (Silva et al., 2000). Laboratory duplicates, reference standards, and blanks were run at a frequency of 5% of total sample throughput (Dan Snow, personal communication). The measurement quality objective (MQO) for the measured value of the stable isotope ratios in reference standards was set to within 0.5 permil or less of the nominal value in the calibration standards. Unfiltered samples for antibiotic analysis were shipped on ice to the U.S. Geological Survey's Organic Geochemistry Research Laboratory (Lawrence, KS). Samples were filtered and analyzed by solid phase extraction (SPE) and liquid chromatography mass spectrometry (LC/MS) as described by Meyer et al. (2007). Water blanks and standard samples were processed using the same method as the environmental samples. Blanks were analyzed after the last sample in the standard curve and after every check standard or matrix spiked sample. Two sample blanks were also interspersed among the environmental samples in each analytical run. A duplicate and matrix spiked sample was analyzed after every ten samples in each analytical run and a check standard was analyzed after every twenty samples in an analytical run. The particular assay used (LCAB analysis) measures selected macrolides, quinolines, sulfonamides, tetracyclines, and other antibiotics and pharmaceuticals (Mike Meyer, personal communication). The complete analyte list, along with their corresponding reporting limits, is shown in Table 1.

2.5 GC/MS/MS Analysis for Estrogens

Estrogen hormones analyzed in this study include estrone, 17α-estradiol, 17β-estradiol, 17β-ethynylestradiol, and estriol. Estrogens were analyzed in ground water and lagoon samples by solid phase extraction (SPE), pentafluorobenzyl and trimethylsilyl derivatization, and gas chromatography tandem mass spectrometry (GC/MS/MS) using electron capture negative ionization. There were three iterations of this method used over the course of this ten-year study. From 2001 to 2008, samples were analyzed using a 60-m DB5-XLB capillary column and a Finnigan TSQ-7000 mass spectrometer with

Table 1: Antibiotics and Related Compounds Analyzed in this Study[a]

Compound Class	Compound	Compound Identifier[b]	Reporting Limit (ng/L)	CAS Number	General Animal and Human Usage
Pharmaceuticals	Carbamazapine	-	5	298-46-4	humans
	Ibuprofen	-	50	15687-27-1	humans
Macrolides and Degradation Products	Azithromycin	-	5	117772-70-0	humans
	Erythromycin	-	8	114-07-8	humans, poultry, swine
	Erythromycin-H2O	-	8	-	-
	Roxithromycin	-	5	80214-83-1	humans
	Tylosin	TYL	5	1401-69-0	cattle, chickens, swine
	Virginiamycin	-	5	21411-53-0	cattle, poultry, swine
Quinolines	Ciprofloxacin	-	5	85721-33-1	chickens, humans, swine
	Lomefloxacin	-	5	98079-51-7	humans
	Norfloxacin	-	5	70458-96-7	humans, poultry
	Ofloxacin	-	5	82419-36-1	humans, poultry
	Sarafloxacin	-	5	98105-99-8	fish, poultry
	Enrofloxacin	-	5	93106-60-6	cattle, cats, dogs,poultry, swine
Sulfonamides	Sulfachloropyridazine	SCLP	5	80-32-0	calves, dogs, swine
	Sulfadiazine	-	100	68-35-9	horses, humans
	Sulfadimethoxine	SDMX	5	122-11-2	fish, poultry
	Sulfamethazine	SMZN	5	57-68-1	cattle, swine
	Sulfamethoxazole	SMOX	5	723-46-6	human
	Sulfathiazole	STHZ	5	72-14-10	swine
Tetracyclines and Degradation Products	Chlortetracycline	CTET	10	57-62-5	cattle, ducks, poultry, sheep, swine
	Epi-chlortetracycline	ECTET	10	-	-
	Iso-chlortetracycline	ICTET	10	-	-
	Epi-iso-chlortetracycline	EICTET	10	-	-
	Doxycycline	-	10	564-25-0	dog, humans
	Oxytetracycline	OTET	10	79-57-2	bees, cattle, fish, lobsters, poultry, sheep, swine
	Epi-oxytetracycline	EOTET	10	-	-
	Tetracycline	TET	10	60-54-8	cattle, dogs, humans
	Epi-tetracycline	ETET	10	-	-
Other Antibiotics	Lincomycin	LINC	5	154-21-2	swine
	Trimethoprim	-	5	738-70-5	dogs, horses, humans
	Chloramphenicol	-	100	56-75-7	cats, dogs
	Ormetroprim	-	5	6981-18-6	fish, poultry

[a] modified from Meyer et al, 2007

[b] identifiers are provided only for those compounds that were reported in any of the samples in this study

methane (and then later carbon dioxide) as the chemical ionization reagent gas (Fine et al., 2003; Hutchins et al., 2007). In brief, ground water and lagoon samples were collected in the field and preserved with formaldehyde to a final concentration of 1% (w/v). Ground water samples were normally filtered in the field whereas lagoon samples were centrifuged and filtered through a 1.2 µm glass fiber filter pad in the laboratory.

Volumes used for extraction and analysis were typically 1,000 mL for ground water samples and 25 mL for lagoon samples. Deuterated analogs of the estrogens were used as isotope dilution standards (IDS) and were added to both ground water and lagoon samples before extraction. After the isotope dilution standard was added, the sample was drawn through an Oasis HLB SPE cartridge using vacuum. The cartridge was washed with polar solvents to remove highly polar compounds and then the adsorbed estrogens were eluted with more nonpolar organic solvents. The extract was taken to dryness and then reconstituted with acetone, after which phenolic functional groups of the estrogen residue were derivatized with pentafluorobenzyl bromide to make pentafluorobenzyl ethers. Following an additional evaporation and reconstitution step, any hydroxy groups present in the estrogens were then derivatized with N-trimethylsilylimidazole to make trimethylsilyl ethers. The derivatized extracts were then additionally spiked with derivatized pentafluorobenzyl 7-methylestrone (7-methylestrone-PFB) as a surrogate to allow monitoring of the performance of the mass spectrometer independent of the extraction and derivatization processes. The final extracts containing the derivatized estrogens, deuterated estrogen isotope dilution standards, and derivatized surrogate were injected onto a GC capillary column and the compounds were then separated and detected using GC/MS/MS. Multiple procedures were used to assess QA, including the use of blanks, laboratory replicates, field replicates, and matrix spikes.

This instrument was replaced in 2009 with a TSQ Quantum GC mass spectrometer (Thermo Fisher Scientific, Inc., Waltham, MA) and the method parameters were subsequently modified. Instead of the 60-m capillary column used earlier, two 15-m Zebron® ZB-XLB-HT capillary columns (Phenomenex, Torrance, CA) were used in series and a Deans switch (Agilent Technologies, Inc., Santa Clara, CA) was installed in the gas chromatograph to vent the solvent and derivatizing reagents eluting from the first capillary column away from the ion source. Quantitation of the derivatized estrogens was accomplished by selective reaction monitoring (SRM) mass spectrometry where one characteristic ion of the derivatized estrogen, the precursor ion, was filtered through the first quadrupole (Q1) of the mass spectrometer. Collision-induced dissociation of the precursor ion occurred in the collision cell where argon and collision offset energy caused the filtered ion to fragment. The most abundant fragment ion, the product ion, was then filtered by the third quadrupole (Q3). A unique set of SRM ions provided mass chromatographic peaks for each estrogen and isotope dilution analog. The peak areas were integrated and the peak area ratios of the target and labeled estrogen were interpolated into calibration curves allowing determination of estrogen concentration in the extract and initial sample. With 1,000 mL of ground water sample, 1.0 mL of extract volume, and the lowest level calibration standard of 1.0 pg/µL, the quantitation limit (QL) was 1.0 ng/L in ground water. For a 25-mL lagoon sample, the QL would correspond to 40 ng/L. Recoveries of the five estrogens spiked into 1,000 mL of lab purified water (n = 7) at 2.0 ng/L ranged from 98-101%. Recoveries of estrogens spiked into 100 mL of dairy lagoon water and 25 mL of swine and dairy lagoon water at 200, 2,500 and 8,000 ng/L ranged from 67-122%. The method detection limits (MDLs) of the five estrogens for a lab purified water sample volume of 1,000 mL ranged from 0.1-0.3 ng/L.

This method was used in 2009 to analyze estrogen hormones from several different field sites, which during that same time period were also sampled for antibiotics and stable isotopes and provide most of the data for this report. However, as more experience was gained using the new instrumentation, it was also recognized that low-level carryover and background noise were sometimes resulting in erroneous detections of some analytes, particularly estrone, at or near the QL (1.0 ng/L). Although this did not affect the confirmation or quantitation of analytes at higher concentrations (> 5.0 ng/L), it did bring into question whether analytes detected in the 1.0-5.0 ng/L range were truly present or represented artifacts. Considerable effort was done to minimize the carryover and background interferences in early 2010 before conducting additional field sampling. Background interferences of target estrogens in blanks were reduced by switching the surrogate from 7-methylestrone to 7-methylestradiol and by decreasing the concentration of isotope dilution standards that were added to standards, samples and blanks. Carryover of estrogens into the next sample during extraction was minimized by setting up separate sample queues for lagoon samples and using extraction apparatus dedicated to lagoons. The potential for estrogen carry-over and adsorption during SPE was also minimized by eliminating plastic funnels used to transfer sample to the SPE cartridges, replacing Teflon® sample transfer tubing with Siltek/Sulfinert® stainless steel megabore capillaries (Restek, Belefonte, PA), and replacing Teflon® solvent guide needles with stainless steel ones. Derivatization reactions were made more reproducible by using new sample processing stations (Reacti-Vap III®, Thermo Scientific Laboratories, Inc., Waltham, MA) that incorporated magnetic stirrers, centralized heating, and blow-down units to minimize sample handling. Teflon®-covered magnetic stirring bars were replaced with glass-covered magnetic stirring bars and reusable glass centrifuge tubes were replaced with disposable derivatization vials. In the previous GC autosampler method, a solvent plug was drawn up into the syringe barrel followed by the sample. This was modified so that instead sample would be drawn into the syringe and expelled multiple times to thoroughly rinse the syringe and minimize air bubbles. Then, following sample injection, both the syringe and injector port were flushed by injecting three 8-µL volumes of solvent into the injection port. Several additional improvements to the GC method were made to improve reproducibility and help prevent degradation of the capillary columns and ion source. These included the use of a Uniliner® injection port liner (Restek, Bellafonte, PA) that was press-tight sealed to the front of the capillary column, and pressurized pulse injection for more complete transfer of sample to the capillary column. High thermal stability capillary columns (Zebron® ZB-XLB-HT Inferno, (Phenomenex, Torrance, CA)) were used to replace the original capillary columns, and contamination of the mass spectrometer ion source was minimized by utilizing a cleaner chemical ionization gas, ammonium, instead of carbon dioxide.

Improvements were also made to better track instrument performance and to increase confidence in target estrogen identification and quantitation. Instrument stability during the analysis of a sample queue was followed by acquiring the spectrum of the ion source calibration gas and a mass chromatogram of an archived derivatized estrogen standard at the beginning and end of the sample queue. If these fell below stated MQOs at the beginning of the sample queue, the sample queue was stopped to preserve sample integrity while the underlying causes are addressed. The confidence in target estrogen identification and quantitation was improved by adding three or four additional MS/MS precursor/product ion pairs to the acquisition parameter list for each target estrogen and by establishing MDLs and calibration curves for each of the ion pairs. The sum of the ion pairs was normally used for quantitation, but this was often not possible in cases where matrix interferences masked the response of one or more of the ion pairs. This improvement allowed quantitation using those ion pairs unaffected by matrix peaks. In addition, evaluation of the relative intensities of the ion pairs provided additional confirmation of analyte identity and also allowed a calculation of index match between standards and samples. With these improvements, the MDLs of the five estrogens in 1,000-mL water samples ranged from 0.02-0.07 ng/L, and the QL was lowered to 0.25 ng/L. An example of the use of these methods is provided in Appendix B, which is an analytical report for one of the sites sampled in 2011. This report also shows data from field replicates, laboratory replicates, equipment blanks, laboratory blanks, derivatization blanks, extraction blanks, and matrix spikes, which were run with every sample set that was analyzed.

3.0 Summary Case Studies

Seven separate case studies are presented, including a swine finisher operation, a poultry layer operation (since closed), a swine nursery operation, a dairy operation, a combined swine operation (since closed), a beef feedlot operation, and a swine farrowing sow operation. With the exception of the swine farrowing sow operation, which served as a relatively non-impacted site, each of these case study sites exhibited ground water contamination by nitrate and/or ammonium. For most sites, this most likely resulted directly from the operation, either through leaking infrastructure piping, leaking lagoons, or land application of CAFO waste. Sampling events during 2001-2011 ranged from one-time (snapshot) to periodic, with some sites being sampled annually or even more frequently. Only the most relevant sampling events are described in these case studies, and only part of the data from these sampling events are presented in this report; the remainder of the data from these sampling events are provided in Appendix A.

3.1 CAFO Site #1 – Swine Finisher

Case Study Summary. This site was only sampled once, and in a way represents the most problematic of all of the sites in this report, having a complex hydrogeology that leads to problems in data interpretation. Ground water nitrate concentrations ranged up to 45 mg/L NO_3-N, and, although the source is not definitive, likely resulted from leaking lagoons and/or CAFO barn and piping infrastructure. Few additional stressors were detected in ground water at this site, other than the antibiotic lincomycin which was found at low levels in several wells. Estrogen hormones were not detected in ground water, with exception of one detection of estriol in one well replicate sample at 1.6 ng/L.

Site Description. CAFO Site #1 is a swine operation that began in April 1983 as a swine finisher, raising pigs from 40-60 lbs up to a market weight of 260-285 lbs. In May 1999, it switched to a swine nursery, raising pigs from about 15 lbs up to a weight of 40-60 lbs. In May 2004 it switched back to a swine finisher and was licensed for 16,320 hogs distributed among 20 barns. Waste from the barns flows into primary lagoons and then, where available, secondary lagoons. The lagoons are unlined, but are constructed in heavy clay. Effluent used for land application is pumped from the terminal lagoon for each set of barns to several spray heads installed in fields around the facility which are used for grazing or grass production and baling. The total acreage receiving effluent is approximately 300-500 acres, depending on the season. The topsoil is a thin layer of silty clay loam underlain by heavy clay and fractured shale. There were no monitoring wells associated with the land application areas, and so this site investigation was limited to the lagoons as well as the monitoring wells around each barn lagoon complex (Figure 1). Ground water, where found, is in shallow fractures located within the clay and shale matrix. It was not possible to determine regional ground water flow direction at this site, but a rough estimate was provided locally for each barn lagoon complex in 1999 by triangulation of water levels from each of three monitoring wells installed for each barn lagoon complex (Dan Parrish, personal communication), and these are shown in Figure 1. For this case study, sampling of the terminal lagoons (LAG1, LAG3, and LAG4) used for land application was conducted in late March 2009. Lagoon LAG2 was empty and could

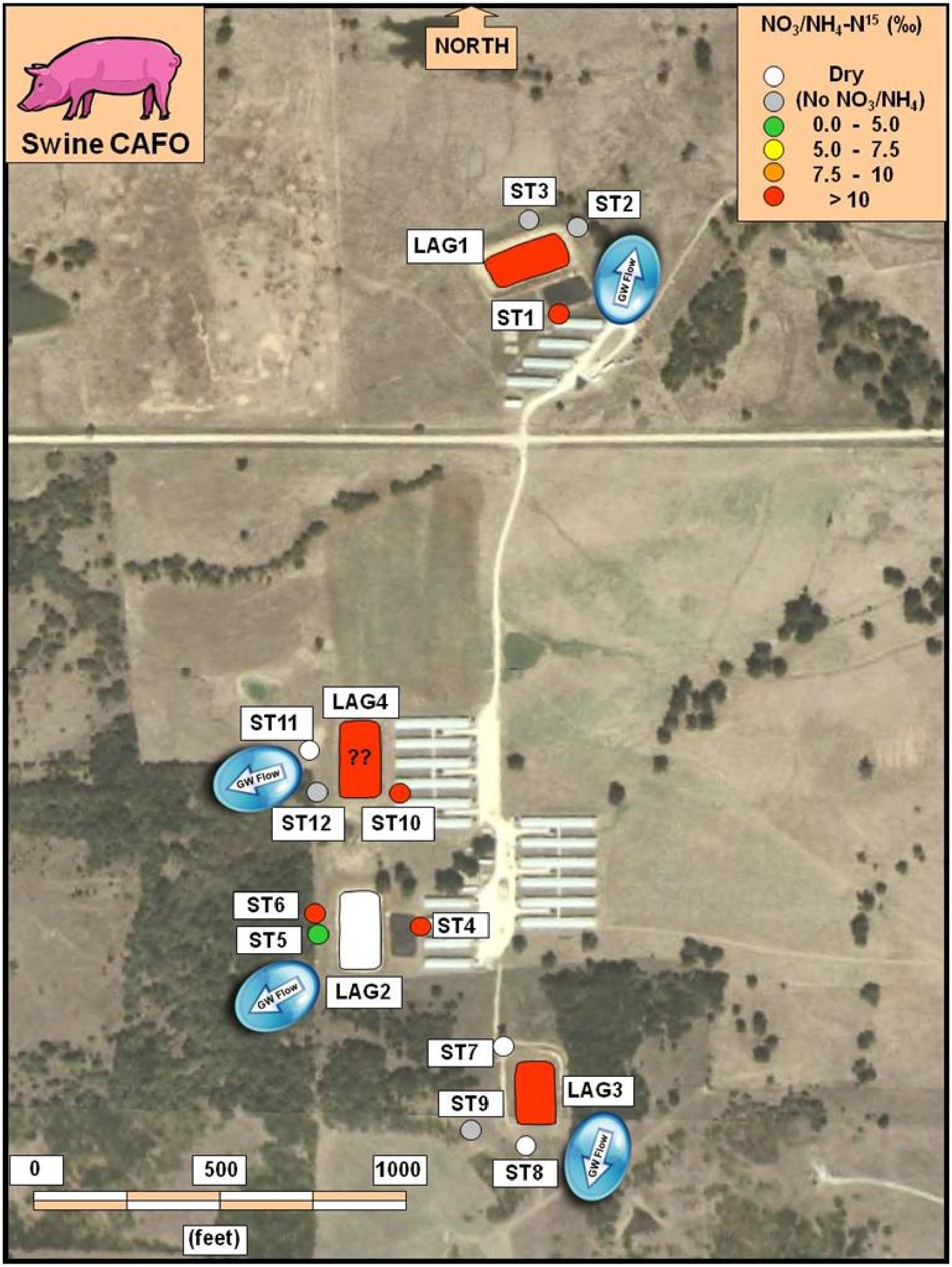


Figure 1. CAFO Site #1 schematic (swine finisher operation). Colors of lagoons and wells correspond to ranges of δ^{15}N of nitrate or ammonium as shown in legend at upper right based on 2009 data. Isotope sample was lost for lagoon LAG4.

not be sampled. The wells were sampled in early April 2009. Several of the monitoring wells were either dry or produced water so slowly that adequate volumes could not be obtained for all of the analyses (9 liters total required).

<u>General Chemistry and Stable Isotope Interpretation.</u> Figure 1 shows the orientation of the wells with respect to the lagoons, and illustrates which lagoons and wells had either a nitrate or ammonium stable nitrogen isotope δ^{15}N value that exceeded +10‰ and that therefore represented an animal waste signature as defined in this study. Table 2 shows the well water levels, well screened intervals, and general chemistry parameters for each of the wells and lagoons sampled, and Table 3 shows corresponding values for reactive nitrogen (nitrate, nitrite, nitrous oxide, and ammonium) as well as the stable isotope information for nitrate, ammonium and water. As expected, lagoons were high in ammonium (950-3,190 mg/L NH_4-N) with δ^{15}N values exceeding +10‰ (Table 3). Well ST5 had a nitrate concentration (12.8 mg/L NO_3-N) that exceeded the MCL for nitrate, but did not have a discernible animal waste signature based on δ^{15}N (+4.1‰). In contrast, wells ST1, ST4, ST6, and ST10 had nitrate concentrations ranging from 23 to 45 mg/L NO_3-N (Table 3), and these all showed animal waste signatures (δ^{15}N-NO_3 > +10‰). Three of these four wells were located between the barns and the associated lagoons, and

Table 2. CAFO Site #1 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[a]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Swine Secondary Lagoon	LAG1	03/31/09	NA	NA	0.5	1.88	2,580	< 7.0	108	1,530	137	4,020	3,070
Swine Primary Lagoon	LAG3	03/31/09	NA	NA	NA[b]	15.4	1,390	< 5.0	553	4,730	1,000	13,000	4,950
Swine Primary Lagoon	LAG4	03/31/09	NA	NA	0.8	15.8	1,800	< 7.0	918	5,390	1,320	13,400	7,860
Monitoring Well	ST1	04/07/09	13.04	3-15	NA	< 0.01[c]	14.2	25.0	0.086	0.17	0.064	0.85	106
Monitoring Well	ST2	04/07/09	16.35	4-14	NA	< 0.01	400	1,640	0.079	0.49	0.099	1.35	135
Monitoring Well	ST3	04/07/09	9.31	4-14	NA	< 0.01	407	36.5	0.025	0.62	0.056	3.75	134
Monitoring Well	ST4	04/07/09	17.38	7-17	NA	< 0.01	163	22.9	0.049	0.72	0.066	3.36	148
Monitoring Well	ST5	04/06/09	12.62	5-15	NA	< 0.01	70.6	19.3	0.176	1.10	0.443	7.62	71.7
Monitoring Well	ST6	04/07/09	3.92	4-14	NA	< 0.01	336	17.9	0.026	1.05	0.433	4.56	140
Monitoring Well	ST9	04/06/09	19.20	9-19	0.8	< 0.01	3,730	3,180	0.030	1.47	0.818	5.96	100
Monitoring Well	ST10	04/06/09	13.86	5-15	NA	< 0.01	186	18.4	0.018	0.33	0.063	1.06	98.8
Monitoring Well	ST12	04/06/09	16.62	9-19	NA	< 0.01	109	26.2	0.036	0.14	0.060	0.44	90.5

[a] Feet from top of casing

[b] DO probe failure at these locations

[c] Values reported as "<" are below detection limits

the directions of ground water flow at these three locations implies that the source of ground water contamination may be from the barns or piping out to the lagoons rather than from the lagoons themselves. However, there were several anomalies at this particular field site complicating this interpretation. First, because chloride is a conservative tracer and is found at high levels in these lagoons (Table 2), it would be reasonable to expect a positive correlation between ground water nitrate and chloride concentrations if CAFO waste was the source of the nitrate. Although there is a weak positive correlation ($r^2 = 0.2939$) between nitrate and chloride concentrations for these wells, there is actually a weak negative correlation ($r^2 = -0.1497$) when all of the wells are considered. In fact, well ST9 had the lowest nitrate concentration (< 0.01 mg/L NO_3-N) and the highest chloride concentration (3,730 mg/L Cl). This well also showed some evidence of poor integrity, based on the observation that ground water was difficult to filter due to suspended grey colloidal material which probably originated from bentonite used to seal the well casing. Regardless, even excluding this well, there was still no correlation between ground water nitrate and chloride levels. An argument could be made that, for some wells, nitrate concentrations were reduced through microbial denitrification, but neither the general chemistry nor the stable isotope data provide any evidence to support this. Nitrite and nitrous oxide are produced as intermediates of microbial denitrification, and levels of both of these were very low in all of the wells at

Table 3. CAFO Site #1 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N	NO_3-N	NO_2-N	N_2O-N	δ^{2}H-H_2O	δ^{18}O-H_2O	δ^{15}N-NO_3	δ^{18}O-NO_3	δ^{15}N-NH_4
			(mg/L)	(mg/L)	(mg/L)	(mg/L)	(‰)	(‰)	(‰)	(‰)	(‰)
Swine Secondary Lagoon	LAG1	03/31/09	951	1.44	4.98	< 0.01	+12.1	+3.0	NA	-11.8	+37.6
Swine Primary Lagoon	LAG3	03/31/09	3,190	10.1	12.9	< 0.01	-4.6	-0.7	NA	+2.4	+10.6
Swine Primary Lagoon	LAG4	03/31/09	2,980	12.8	15.6	< 0.01	+0.5	-0.3	NA	NA	NA
Monitoring Well	ST1	04/07/09	< 0.02[a]	23.4	< 0.01	0.01	-28.8	-4.4	+11.0	-1.1	NA
Monitoring Well	ST2	04/07/09	< 0.02	0.16	< 0.01	< 0.01	-29.5	-4.3	NA	NA	NA
Monitoring Well	ST3	04/07/09	< 0.02	0.07	< 0.01	< 0.01	-11.3	-1.0	NA	NA	NA
Monitoring Well	ST4	04/07/09	0.03	42.5	0.01	0.02	-22.9	-3.8	+28.0	+14.3	NA
Monitoring Well	ST5	04/06/09	0.02	12.9	0.12	< 0.01	-25.8	-4.6	+4.1	-9.9	NA
Monitoring Well	ST6	04/07/09	< 0.02	37.1	0.01	0.03	-13.0	-1.8	+36.0	-5.8	NA
Monitoring Well	ST9	04/06/09	0.06	< 0.01	< 0.01	< 0.01	-25.1	-3.9	NA	NA	NA
Monitoring Well	ST10	04/06/09	< 0.02	44.6	< 0.01	< 0.01	-20.9	-3.4	+23.6	-24.6	NA
Monitoring Well	ST12	04/06/09	< 0.02	0.46	< 0.01	< 0.01	-26.2	-3.8	NA	NA	NA

[a] Values reported as "<" are below detection limits

this site (Table 3). Stable isotope relationships can also be used to look for evidence for denitrification (Figure 2). For example, Figure 2a shows the relationship between δ^{15}N-NO_3 values and nitrate concentrations for the wells in which nitrate was detected. If each well had the same source of nitrate and if denitrification was occurring, there should be a negative correlation between δ^{15}N-NO_3 values and nitrate concentrations. In this case, there is instead a positive correlation ($r^2 = 0.7173$) between these two parameters (Figure 2a). Furthermore, if each well had the same source of nitrate and if denitrification was occurring, there should be a positive correlation between δ^{15}N-NO_3 and δ^{18}O-NO_3 values. Instead, there is actually a weak negative correlation ($r^2 = -0.0720$) between these two parameters (Figure 2b). These data do not support the possibility that denitrification is significantly enriching the δ^{15}N signature, and therefore it is likely that the observed enriched δ^{15}N values are correctly indicating that the nitrate found in these wells is derived from animal waste, with swine CAFO waste being the most probable source. Figure 2b also provides some general ranges expected for different sources of nitrate, and most of the ground water samples from these wells indicate an animal source. Well ST10 falls well below these expected ranges due to depletion of δ^{18}O in the nitrate, and the reason for this is not clear. As noted previously, there are many factors complicating the interpretation of stable isotope data (Mengis et al., 2001; Bedard-Haughn et al., 2003; Kendall et al., 2008).

In contrast to stable isotopes of nitrate, stable isotopes of water were used to try to provide some additional information about the recharge history of the ground water samples collected from these wells. Figure 2c shows the relationship between the meteoric water line (MWL) and the stable isotopes of water in these wells and lagoons. Values further below the MWL are more indicative of water showing evaporative losses. Thus LAG1, a secondary lagoon which should exhibit more evaporative losses than a primary lagoon, is more enriched in δ^{18}O-H_2O and lies further below the MWL than the two primary lagoons LAG3 and LAG4 (Figure 2c). Interestingly, ground water from well ST3 also shows an evaporative signature, and it might seem reasonable to assume that it is due in part to leakage from the adjacent lagoon LAG1 (Figure 1). However, well ST3 shows no sign of impact from CAFO wastes, and there is no other adjacent

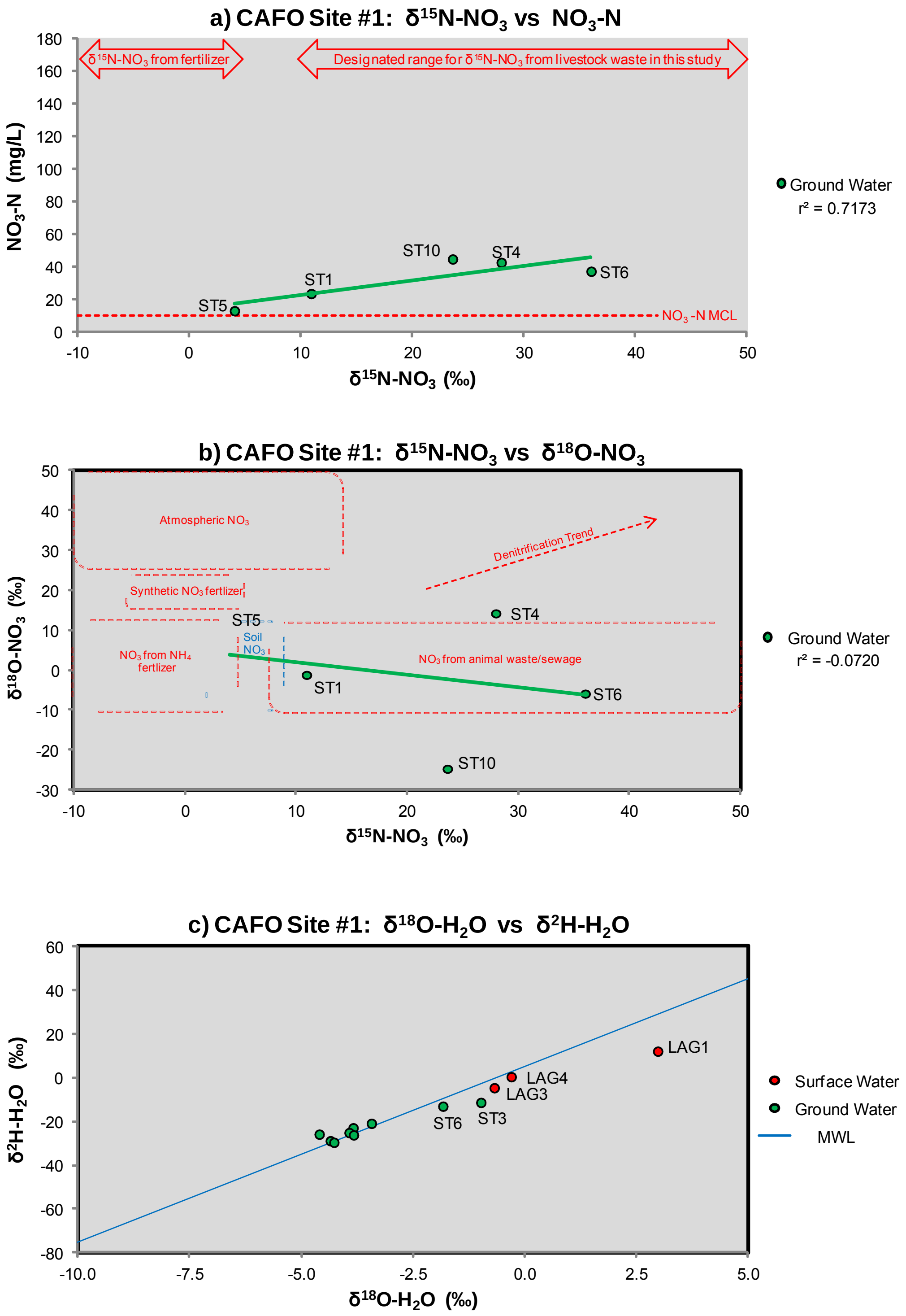


Figure 2. CAFO Site #1 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for NO_3-N. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

surface water body, and so the reason for this evaporative signature is not clear. Also note that ground waters from wells ST3 and ST6 have greater $\delta^{18}O\text{-}H_2O$ values compared to those of the other wells (Figure 2c). This indicates that these two wells are in some way different with respect to their history of recharge, since the values of $\delta^{18}O\text{-}H_2O$ in rainfall can vary widely depending on the season and other climatic factors (Harvey and Welker, 2000; Liu et al., 2010). Because these are all very shallow wells screened very near to the surface (Table 2), it might also indicate preferential flow through faults or down well casings in these two particular wells. This can easily occur during dry years through soft faulting in the heavy clay, which might help explain why well ST6, located only about 30 ft from ST5, has a water table that is over eight ft higher even though both wells are screened across the same intervals (Table 2). Regardless of the exact reason, the water stable isotope data show that these two wells are somehow different than the others with respect to recharge history, and more sampling events at different times of the year would be needed to determine whether these differences are consistent and whether transient contaminant events may have been missed.

<u>Evaluation of Additional Stressor Impact</u>. A few other stressors were identified in nitrate-impacted ground water at CAFO Site #1, but in most cases detections were sporadic and/or concentrations were low. Ground water orthophosphate concentrations ranged from 0.018-0.176 mg/L $PO_4\text{-}P$ and were not correlated with nitrate concentrations ($r^2 = -0.0270$). Microbial indicator numbers were quite low in almost all of the wells, except for well ST5 where very high numbers were recorded for total coliforms, fecal coliforms, and fecal enterococci (Table 4). The reason for this is not clear, since the ground water for this well was relatively low in chloride (70.6 mg/L Cl), had only moderate levels of nitrate (12.9 mg/L $NO_3\text{-}N$), and did not exhibit an animal waste signature, with a $\delta^{15}N\text{-}NO_3$ value of only +4.1‰ (Tables 2-3). It is possible that the total coliform counts could have been biased high, since the IDEXX method used in this study is a most-probable-number method and has been shown to overestimate total coliform numbers by several fold in surface waters compared to standard membrane filtration methods (Griffith et al., 2006). Regardless, well ST5 also had very high numbers of fecal enterococci (Table 4), and fecal enterococci counts have been shown to be comparable

Table 4. CAFO Site #1 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococci (cells per 100 mL)	As by ICP-MS (µg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Swine Secondary Lagoon	LAG1	03/31/09	43,800	50,900	8,300	14.1	0.036	0.210	0.357	0.067	3.77
Swine Primary Lagoon	LAG3	03/31/09	228,000	1,660,000	480,000	9.4	< 0.006[a]	0.358	0.247	0.162	2.89
Swine Primary Lagoon	LAG4	03/31/09	520,000	1,110,000	143,000	10.1	< 0.006	0.388	0.292	0.161	3.04
Monitoring Well	ST1	04/07/09	435	0	5	0.8	< 0.006	< 0.004	< 0.003	0.048	< 0.040
Monitoring Well	ST2	04/07/09	1	0	1	4.6	< 0.006	< 0.004	0.006	0.117	< 0.040
Monitoring Well	ST3	04/07/09	0	0	4	1.3	< 0.006	< 0.004	< 0.003	0.098	< 0.040
Monitoring Well	ST4	04/07/09	6	0	3	1.0	< 0.006	0.005	0.004	0.073	< 0.040
Monitoring Well	ST5	04/06/09	> 2,420	27	> 2,420	1.0	< 0.006	0.007	0.004	0.018	< 0.040
Monitoring Well	ST6	04/07/09	2	0	0	1.1	< 0.006	< 0.004	0.009	0.121	< 0.040
Monitoring Well	ST9	04/06/09	0	0	3	3.8	< 0.006	0.005	0.009	0.078	< 0.040
Monitoring Well	ST10	04/06/09	1	0	1	0.9	< 0.006	< 0.004	< 0.003	0.047	< 0.040

[a] Values reported as "<" are below detection limits

across those methods (Griffith et al., 2006). Well ST5 also had fairly high levels of fecal coliforms, and these counts tend to be actually somewhat lower than those obtained with standard membrane filtration methods (Francy and Darner, 2000). It is therefore unlikely that the MPN method used in this study significantly underestimated the numbers of fecal coliforms and fecal enterococci in the ground water sample from this well, and so the source remains unknown. Concentrations of metals and metalloids were also low in these ground water samples, with the possible exception of selenium, which ranged from 47-121 µg/L Se (Table 4). This may represent natural sources of selenium in the soil, since ground water selenium levels did not correlate with nitrate levels ($r^2 = -0.0007$). Arsenic was not detected in ground water using ICP-OES (< 6 µg/L As), and a more rigorous analysis by ICP-MS confirmed that arsenic levels were quite low (0.8-4.6 µg/L As). What little arsenic was found in the lagoons was generally present as As (III) and, to a lesser extent, DMA (Appendix A). Veterinary antibiotics were found in the lagoons at high levels and included lincomycin along with several tetracyclines, but only lincomycin was detected in the ground water samples (Table 5). Ground water lincomycin concentrations were quite low (< 5-55 ng/L) and represented a 10^5-10^6 decrease compared to lagoon concentrations. Although these low concentrations were often close to the reporting limits, lincomycin was detected in three of the five nitrate-impacted wells and probably represents a low-level contamination event originating from the swine waste. Note, however, that lincomycin was also detected (47 ng/L) in Well ST3, which

Table 5. CAFO Site #1 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date	CTET[b] (ng/L)	ICTET (ng/L)	EICTET (ng/L)	OTET (ng/L)	TET (ng/L)	EOTET (ng/L)	LINC (ng/L)
Swine Secondary Lagoon	LAG1	03/31/09	9,770	2,660,000	2,110,000	67,000	29,000	3,340	2,010,000
Swine Secondary Lagoon	LAG1(FD)[a]	03/31/09	16,000	7,420,000	4,660,000	109,000	15,000	1,820	2,980,000
Swine Primary Lagoon	LAG3	03/31/09	10	7,800,000	9,400,000	110,000	46,000	1,800	7,300,000
Swine Primary Lagoon	LAG4	03/31/09	30,000	6,300,000	5,990,000	64,000	14,000	1,490	2,180,000
Monitoring Well	ST1	04/07/09	< 10[c]	< 10	< 10	< 10	< 10	< 10	5
Monitoring Well	ST3	04/07/09	< 10	< 10	< 10	< 10	< 10	< 10	47
Monitoring Well	ST4	04/07/09	< 10	< 10	< 10	< 10	< 10	< 10	16
Monitoring Well	ST4(FD)	04/07/09	< 10	< 10	< 10	< 10	< 10	< 10	18
Monitoring Well	ST6	04/07/09	< 10	< 10	< 10	< 10	< 10	< 10	55
Monitoring Well	ST9	04/06/09	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	ST10	04/06/09	< 10	< 10	< 10	< 10	< 10	< 10	< 5

[a] FD is field duplicate

[b] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table were not detected in any of these samples

[c] Values reported as "<" are below reporting limits

shows no nitrate contamination (Table 3), and the reason for this is unclear. As discussed earlier, the water stable isotope data show this well to have a stronger evaporative signature than the other wells, indicating a very different recharge history, and it is possible that there could have been a contamination event which was missed in this sampling period. In a separate study, lincomycin was also the only antibiotic detected in ground water samples impacted by a swine CAFO in North Carolina (Harden, 2009). Estrogen hormones were also found in these swine lagoons, but at concentrations much lower than those found for antibiotics (Table 6). The estrogen levels in the swine finisher lagoons are very similar to what had been observed for a separate swine finisher lagoon, although here concentrations of estriol were about seven times lower (Hutchins et al., 2007). There was severe matrix interference with the analysis of 17α-ethynylestradiol in these lagoon samples, and these results are flagged with the data label “NC” (not confirmed). This does not mean that this compound was detected. A peak eluted in the mass chromatogram at the correct retention time as this compound, but the relative intensities of the MS/MS ion pairs of the sample did not match that of the standards, and it cannot be determined whether or not this compound was present. An estimate of the concentration of the unconfirmed peak is included in the data label, and if 17α-ethynylestradiol is hidden within this unconfirmed peak, its concentration will be less than this estimate. Because 17α-ethynylestradiol is a synthetic hormone used exclusively

Table 6. CAFO Site #1 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17α-Ethynylestradiol (ng/L)	Estriol (ng/L)
Swine Secondary Lagoon	LAG1	03/31/09	1,660	84.9	142	NC (< 235)[c]	230
Swine Secondary Lagoon	LAG1(FD)[a]	03/31/09	2,290	128	149	NC (< 185)	242
Swine Primary Lagoon	LAG3	03/31/09	1,060	117	517	NC (< 110)	116
Swine Primary Lagoon	LAG4	03/31/09	973	85.7	231	NC (< 189)	299
Monitoring Well	ST1	04/07/09	< 1.0[b]	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	ST3	04/07/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	ST4	04/07/09	< 1.0	< 1.0	< 1.0	< 1.0	1.6
Monitoring Well	ST4(FD)	04/07/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	ST6	04/07/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	ST10	04/06/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0

[a] FD is field duplicate
[b] Values reported as "<" are below quantitation limits
[c] NC is not confirmed due to interference; if analyte is present, it is below estimate shown in parantheses

for human contraception, it is highly unlikely that it would be found in these CAFO lagoons. Estrogen hormones were not detected in any of the ground water samples at this site, with the exception of estriol which was detected at 1.6 ng/L in only one of the field duplicates for well ST4 (Table 6). This is near the quantitation limit for this compound (1.0 ng/L), and it was not detected in the other field duplicate. Collectively, these data provide few indications of ground water contamination at this site by stressors other than nitrate, with the exception of very low levels of the antibiotic lincomycin.

3.2 CAFO Site #2 – Poultry Layer (Closed)

Case Study Summary. Even though this site also exhibits a complex hydrogeology, here there is evidence for direct impact to ground water from unlined leaking lagoons. Impacted ground waters were contaminated by ammonium as well as nitrate, and ranged up to 32 mg/L NH_4-N and up to129 mg/L NO_3-N. Few additional stressors were detected in ground water at this site, and these generally occurred only at very low levels and were not consistent with separate sampling events. The estrogen hormone estrone was detected at a very low level (1.1 ng/L) in one ground water sample in 2009, but not in the duplicate ground water sample taken from that same well (< 1.0 ng/L). Estrone was also detected in another well (2.7 ng/L) in 2011. The antibiotic lincomycin was also detected (34 ng/L) in one of these wells along with estrone. This operation had been closed and the lagoons had been decommissioned prior to the installation and sampling of the

Figure 3. CAFO Site #2 schematic (poultry layer operation, since closed). Colors of decommissioned lagoons and wells correspond to ranges of $\delta^{15}N$ of nitrate or ammonium as shown in legend at upper right, based on 2009 data.

monitoring wells, and the frequency of detection of these other stressors in ground water might have been higher had these lagoons still been in use.

Site Description. CAFO Site #2 was a wet (i.e., lagoon) poultry layer operation that began in 1971 with about 365,000 chickens distributed among six barns (Figure 3). Waste from the barns flows into primary lagoons LAG1 and LAG2, with overflows being diverted to secondary lagoon LAG3 as needed. Effluent used for land application was pumped from lagoon LAG2 to a center pivot located south of the lagoons onto a bermudagrass pasture, and at times a reel gun was used to distribute effluent on pastureland north of the lagoons. Ground water, where found, is in shallow unconsolidated zones and fractures located within the granite bedrock. One barn was still operational in early 2006 when lagoon samples were first obtained for steroid hormone analysis (Hutchins et al., 2007), but the site was later decommissioned and the lagoon contents were cleaned out by late 2007. The lagoons now primarily serve as catchment basins for rainwater and runoff. Because no liner was observed during decommissioning of the lagoons and there was no certification of a lack of hydraulic connection between the lagoons and the underlying ground water, four monitoring wells were constructed around the lagoons in 2008 to assess ground water impacts. No monitoring wells were available to assess ground water impacts from previous land application practices at this site.

General Chemistry and Stable Isotope Interpretation. We sampled one of the operational lagoons in 2006, all three decommissioned lagoons and the recently-constructed monitoring wells in 2009, and the monitoring wells again in 2011 (Table 7-8). Site operations showed a direct impact on ground water as evidenced by relatively high ammonium concentrations in downgradient wells GR2 and GR3 during both sample events (Table 8), and this most likely occurred from previous leakage of CAFO poultry waste from the lagoons. Samples taken in 2006 from three locations in the operational lagoon LAG2 showed high ammonium levels (281-302 mg/L NH_4-N) with a strong animal waste signature (+27.8‰ to +28.6‰ δ^{15}N-NH_4), and the ammonium in ground water samples taken from these two wells in 2009 also showed a strong animal waste

Table 7. CAFO Site #2 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[c]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Poultry Primary Lagoon	LAG2-1[b]	03/01/06	NA	NA	0.0	4.03	542	< 0.6	9.60	409	22.9	372	1,175
Poultry Primary Lagoon	LAG2-2	03/01/06	NA	NA	0.0	1.92	554	< 0.6	9.31	405	23.3	390	1,084
Poultry Primary Lagoon	LAG2-3	03/01/06	NA	NA	0.0	1.84	554	< 0.6	9.68	406	23.6	361	1,029
Poultry Primary "Lagoon" [a]	LAG1	02/25/09	NA	NA	12.4	0.00	76.2	250	6.30	8.02	7.56	31.0	106
Poultry Primary "Lagoon"	LAG2	02/25/09	NA	NA	12.0	0.01	107	194	2.30	13.4	3.95	53.1	132
Poultry Secondary "Lagoon"	LAG3	02/25/09	NA	NA	17.8	0.00	174	41.3	0.121	9.51	1.69	42.4	151
Monitoring Well	GR1	02/25/09	11.70	8-18	2.1	0.00	140	54.4	0.041	1.17	0.032	7.99	228
Monitoring Well	GR2	02/25/09	8.09	8-18	0.6	0.00	90.9	18.8	0.030	31.5	0.036	9.53	144
Monitoring Well	GR3	02/25/09	6.75	3-15	0.7	< 0.01[d]	140	287	0.026	10.3	0.198	34.2	54.7
Monitoring Well	GR4	02/25/09	6.85	3-15	2.9	< 0.01	337	88.2	0.031	2.36	0.611	22.9	259
Monitoring Well	GR1	02/22/11	8.54	8-18	1.8	NA	34.8	34.1	0.029	0.47	0.121	1.27	108
Monitoring Well	GR2	02/22/11	6.16	8-18	1.3	NA	131	69.0	0.021	33.5	0.059	13.3	156
Monitoring Well	GR3	02/22/11	5.37	3-15	1.0	NA	58.0	30.8	0.019	22.0	0.158	39.8	101
Monitoring Well	GR4	02/22/11	6.75	3-15	0.7	NA	185	124	0.019	0.44	0.045	5.80	126

[a] Lagoons were emptied in 2007; matrix is primarily runoff and rainfall

[b] One of three sampling locations in this lagoon

[c] Feet from top of casing

[d] Values reported as "<" are below detection limits

signature (+25.7‰ to +26.6‰ δ^{15}N-NH_4). Because ammonium is tightly bound to surface soils, it is unlikely that the concentrations of ammonium observed in these ground water samples would be derived from surface application of poultry waste unless the application rates were excessive. Nitrate was also detected in ground water samples taken from these two impacted downgradient wells, but with variable results between the two sampling events (Table 8). Ground water nitrate concentrations at well GR2 increased from 10.4 mg/L NO_3-N in 2009 to 23.1 mg/L NO_3-N in 2011, whereas the corresponding concentrations in well GR3 dropped from 129 to 0.60 mg/L NO_3-N. There were also significant changes in ground water chloride concentrations between 2009 and 2011 for three of the four monitoring wells (Table 7), and there was no correlation (r^2 = - 0.0040) between ground water nitrate and chloride concentrations. Nitrate was not only detected in the ground water samples from the two impacted downgradient wells, but also in those from the upgradient well GR1 (Table 8), and isotope analyses conducted in 2009 showed that ground water samples taken from all three of these wells exhibited an animal waste signature (+20.8‰ to +40.7‰ δ^{15}N-NO_3).

Table 8. CAFO Site #2 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	N_2O-N (mg/L)	δ^2H-H_2O (‰)	$\delta^{18}O$-H_2O (‰)	$\delta^{15}N$-NO_3 (‰)	$\delta^{18}O$-NO_3 (‰)	$\delta^{15}N$-NH_4 (‰)
Poultry Primary Lagoon	LAG2-1[b]	03/01/06	302	< 0.01	0.52	0.04	+4.2	+1.8	NA	NA	+27.8
Poultry Primary Lagoon	LAG2-2	03/01/06	284	< 0.01	0.53	0.08	+4.1	+1.6	NA	NA	+28.4
Poultry Primary Lagoon	LAG2-3	03/01/06	281	< 0.01	0.51	0.12	+4.2	+1.5	NA	NA	+28.6
Poultry Primary "Lagoon" [a]	LAG1	02/25/09	0.24	5.99	0.10	< 0.01	+10.2	+2.1	+43.3	+13.0	NA
Poultry Primary "Lagoon"	LAG2	02/25/09	0.17	0.26	0.12	< 0.01	+13.4	+3.1	NA	NA	NA
Poultry Secondary "Lagoon"	LAG3	02/25/09	0.11	0.17	0.08	< 0.01	+12.2	+2.7	NA	NA	NA
Monitoring Well	GR1	02/25/09	< 0.02[c]	16.4	0.07	0.14	-19.1	-4.4	+22.4	+9.1	NA
Monitoring Well	GR2	02/25/09	30.2	10.4	0.02	0.10	-16.7	-3.7	+40.7	+12.5	+26.6
Monitoring Well	GR3	02/25/09	6.56	129	0.07	1.61	-0.9	-0.2	+20.8	+5.2	+25.7
Monitoring Well	GR4	02/25/09	< 0.02	0.25	< 0.01	< 0.01	-8.3	-1.6	NA	NA	NA
Monitoring Well	GR1	02/22/11	< 0.02	17.1	< 0.01	NA	NA	NA	NA	NA	NA
Monitoring Well	GR2	02/22/11	31.9	23.1	0.01	NA	NA	NA	NA	NA	NA
Monitoring Well	GR3	02/22/11	17.9	0.60	0.02	NA	NA	NA	NA	NA	NA
Monitoring Well	GR4	02/22/11	< 0.02	0.15	< 0.01	NA	NA	NA	NA	NA	NA

[a] Lagoons were emptied in 2007; matrix is primarily runoff and rainfall
[b] One of three sampling locations in this lagoon
[c] Values reported as "<" are below detection limits

One explanation for this might be that when the lagoons were decommissioned in 2007, the solids were spread in the area where this upgradient well was later constructed.

In one respect, there is no need to evaluate whether denitrification processes are enriching $\delta^{15}N$-NO_3 values and leading to a false animal waste signature, because high $\delta^{15}N$-NH_4 values were also observed, and this signature would be unaffected by denitrification. However, it is of interest to determine whether denitrification processes are active and therefore might facilitate nitrate removal from the contaminated ground water. The ground water environment reflected in the impacted wells GR2 and GR3 is low in DO and high in TOC, conducive for denitrification, and in fact there is evidence of this, based on the very high nitrous oxide level observed in the 2009 ground water sample from well GR3 (Table 8). Stable isotope relationships also provide some evidence for this (Figure 4). There is a moderate negative correlation ($r^2 = -0.3612$) between $\delta^{15}N$-NO_3 values and nitrate concentrations (Figure 4a), and a strong positive correlation ($r^2 = 0.7811$) between $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values (Figure 4b), both of which could indicate that denitrification is occurring, but the data are too few to attach strong significance to these

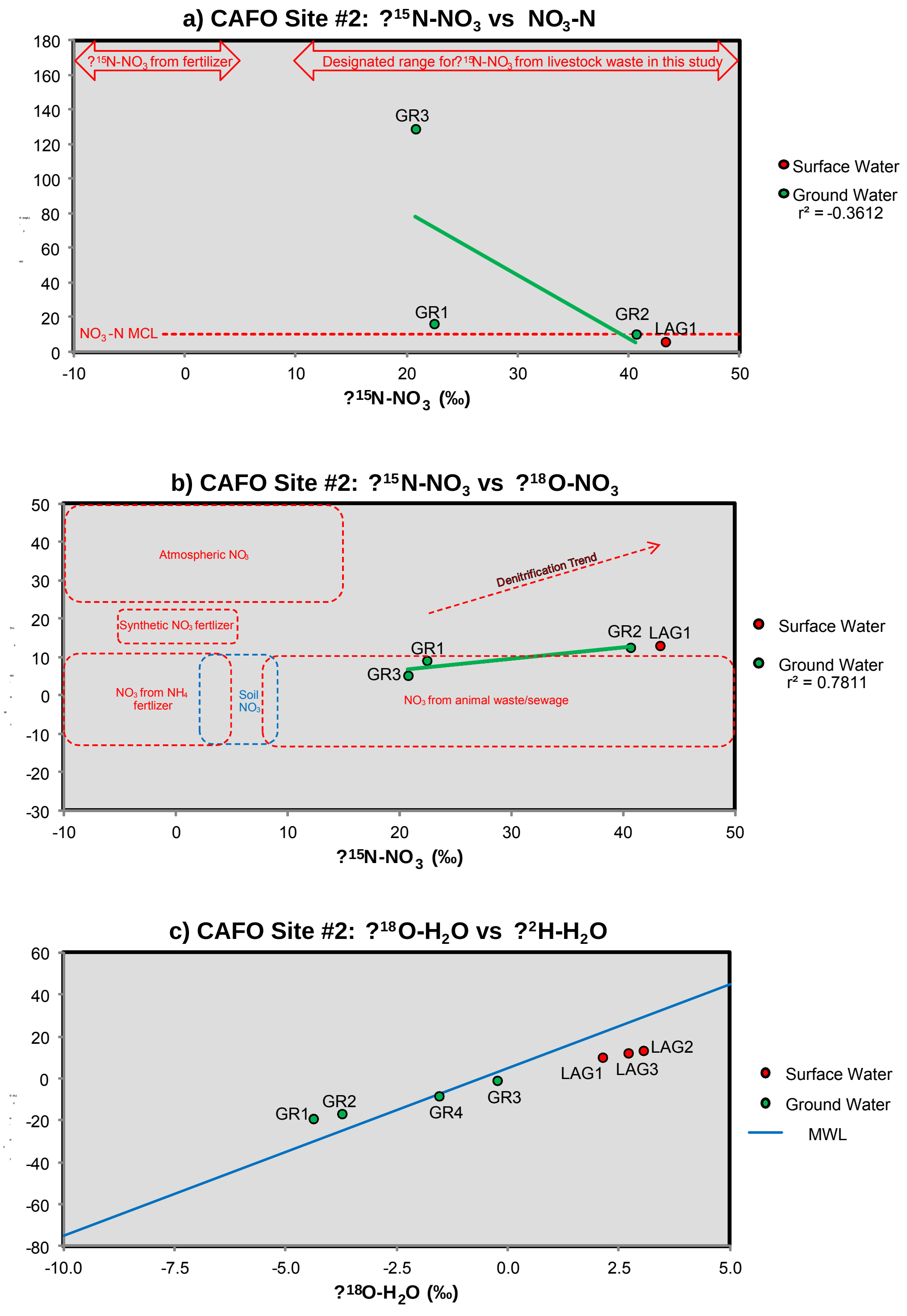


Figure 4. CAFO Site #2 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for NO_3-N. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

particular observations. Collectively, these data show that denitrification may be occurring in the contaminated ground water, but it is not known whether this will significantly reduce nitrate concentrations over time. Organic carbon or other electron donors may not be available at the levels needed to continually support denitrification as the sorbed ammonium is nitrified to nitrate.

Similar to Site #1, the water stable isotope data at this site show that the surface water lagoons have an evaporative signature and again there is a wide range of $\delta^{18}O$-H_2O values (Figure 4c). These are very shallow wells constructed in a fractured matrix, which can lead to very different rates of recharge. Evidence of this can be seen in the changes in the water table as measured in the individual wells (Table 7). Wells that have similar histories of recharge would be expected to show similar changes in water table elevations over time, whereas in these wells the differences in water table elevations from 2009 to 2009 range from 0.1 ft in well GR4 to 3.2 ft in well GR1. These data highlight the probability that the presence of specific contaminants can be very transitory in these wells once the original sources (ie, CAFO wastes in the lagoons) have been removed.

Evaluation of Additional Stressor Impact. Surprisingly, even though direct leakage from the lagoon caused ground water contamination by ammonium as well as nitrate, there was little consistent evidence of additional stressors in the impacted wells. Ground water orthophosphate levels were very low (0.019-0.041 mg/L PO_4-P) and were not correlated with nitrate levels ($r^2 = 0.0000$). Fecal coliforms and fecal enterococci were not detected in any of the 2009 ground water samples, although total coliforms were found in moderate numbers in well GR3 (Table 9), which also had the highest nitrate concentration at that time (Table 8). Metals and metalloid concentrations were relatively low in the operational primary lagoon LAG2 sampled in 2006, and ground water concentrations were generally low in samples taken in 2009 and 2011. Arsenic concentrations, as measured by ICP-OES, appeared to be moderately high (up to 169 µg/L As) in some of the wells sampled in 2009, but this may be due to background interferences, since analysis by ICP-MS showed arsenic concentrations to be less than 5 µg/L in these same ground water samples (Table 9). Because this was a poultry layer

Table 9. CAFO Site #2 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococci (cells per 100 mL)	As by ICP-MS (μg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Poultry Primary Lagoon	LAG2-1[b]	03/01/06	> 2,420	> 2,420	53,400	NA	0.029	0.021	0.090	0.020	0.100
Poultry Primary Lagoon	LAG2-2	03/01/06	> 2,420	> 2,420	101,000	NA	0.025	0.026	0.089	0.017	0.091
Poultry Primary Lagoon	LAG2-3	03/01/06	> 2,420	> 2,420	101,000	NA	0.026	0.016	0.089	0.021	0.089
Poultry Primary "Lagoon" [a]	LAG1	02/25/09	1,483	70	234	9.4	0.023	0.011	0.011	0.008	< 0.040
Poultry Primary "Lagoon"	LAG2	02/25/09	2,420	1	291	6.0	0.015	0.006	0.012	< 0.005	< 0.040
Poultry Secondary "Lagoon"	LAG3	02/25/09	921	5	387	2.7	0.026	0.005	0.014	0.008	< 0.040
Monitoring Well	GR1	02/25/09	0	0	0	1.6	0.169	< 0.004[c]	0.010	0.113	< 0.040
Monitoring Well	GR2	02/25/09	2	0	0	1.3	0.075	0.007	0.005	0.051	< 0.040
Monitoring Well	GR3	02/25/09	461	0	0	2.2	0.029	0.005	0.022	0.026	< 0.040
Monitoring Well	GR4	02/25/09	1	0	0	1.7	0.055	0.014	0.017	0.043	< 0.040
Monitoring Well	GR1	02/22/11	NA	NA	NA	NA	0.024	< 0.003	0.002	0.035	< 0.040
Monitoring Well	GR2	02/22/11	NA	NA	NA	NA	0.055	0.008	0.005	0.077	< 0.040
Monitoring Well	GR3	02/22/11	NA	NA	NA	NA	0.030	< 0.003	0.008	0.040	< 0.040
Monitoring Well	GR4	02/22/11	NA	NA	NA	NA	0.025	0.007	0.007	0.037	< 0.040

[a] Lagoons were emptied in 2007; matrix is primarily runoff and rainfall

[b] One of three sampling locations in this lagoon

[c] Values reported as "<" are below detection limits

rather than a poultry broiler operation, it is not likely that the arsenical antibiotic Roxarsone® was ever used, and arsenic concentrations in lagoons would therefore be expected to be low. Ground water selenium concentrations were moderate (26-113 µg/L) and could be derived from natural sources in the soil, since selenium concentrations were actually higher in ground water than in the original lagoon (Table 9). The only veterinary antibiotics found in the operating lagoon LAG2 in 2006 were isochlortetracycline and epi-isochlortetracycline, and these were only detected in one or two of the three locations sampled in this lagoon and at levels close to the reporting limit (Table 10). Regardless, these determinations seem plausible based on the fact that these compounds are the principal metabolites of chlortetracycline in hen's eggs (Kennedy et al., 1998). Lincomycin was detected at low concentrations (30-38 ng/L) in both of the field duplicates for well GR2 in 2009, even though it was not detected in lagoon LAG2 during 2006. Estrogen hormones were detected in the operational lagoon LAG2 in 2006, with good reproducibility among the three locations sampled (Table 11). Again estrone was the predominant estrogen found, followed by estriol and then 17α-estradiol; 17β-estradiol was at or below detection limits for these lagoon samples. Estrone was the only estrogen

Table 10. CAFO Site #2 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date	ICTET[d] (ng/L)	EICTET (ng/L)	LINC (ng/L)
Poultry Primary Lagoon	LAG2-1[b]	03/01/06	45	18	< 5
Poultry Primary Lagoon	LAG2-2	03/01/06	< 10[e]	< 10	< 5
Poultry Primary Lagoon	LAG2-3	03/01/06	< 10	15	< 5
Poultry Primary "Lagoon" [a]	LAG1	02/25/09	< 10	< 10	< 5
Poultry Primary "Lagoon"	LAG1(FD)[c]	02/25/09	< 10	< 10	< 5
Poultry Primary "Lagoon"	LAG2	02/25/09	< 10	< 10	< 5
Poultry Secondary "Lagoon"	LAG3	02/25/09	< 10	< 10	< 5
Monitoring Well	GR1	02/25/09	< 10	< 10	< 5
Monitoring Well	GR2	02/25/09	< 10	< 10	38
Monitoring Well	GR2(FD)	02/25/09	< 10	< 10	30
Monitoring Well	GR3	02/25/09	< 10	< 10	< 5
Monitoring Well	GR4	02/25/09	< 10	< 10	< 5

[a] Lagoons were emptied in 2007; matrix is primarily runoff and rainfall

[b] One of three sampling locations in this lagoon

[c] FD is field duplicate

[d] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table detected in any of these samples

[e] Values reported as "<" are below reporting limits

detected (1.1 ng/L) in the 2009 ground water samples, but only for well GR2, and this occurred just above the quantitation limit (1.0 ng/L) and was not confirmed with the field duplicate (Table 11). With improvements in the analytical method, these wells were again sampled in 2011, and estrone was again detected in duplicate samples from GR2, although at levels (0.21-0.23 ng/L) below the quantitation limit (Appendix B). This time, however, estrone was also detected (2.7 ng/L) in well GR3 (Table 11). Although these detections are strongly supported by tighter quality controls and transition ion profiles (Appendix B), these concentrations are still quite low and are less than the predicted-no-effect-concentration of 3-5 ng/L for estrone (Young et al., 2004). These data show that leakage of CAFO wastes from the unlined lagoons resulted in ground water contamination by both nitrate and ammonium, but only in a few instances could the other

Table 11. CAFO Site #2 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17α-Ethynylestradiol (ng/L)	Estriol (ng/L)
Poultry Primary Lagoon	LAG2-1[b]	03/01/06	1,470	137	< 60.0	< 60.0	191
Poultry Primary Lagoon	LAG2-2	03/01/06	1,610	127	< 60.0	< 60.0	193
Poultry Primary Lagoon	LAG2-3	03/01/06	1,580	118	< 60.0	< 60.0	184
Poultry Primary "Lagoon" [a]	LAG1	02/25/09	< 1.0[d]	< 1.0	< 1.0	< 1.0	< 1.0
Poultry Primary "Lagoon"	LAG1(FD)[c]	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Poultry Primary "Lagoon"	LAG2	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Poultry Secondary "Lagoon"	LAG3	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	GR1	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	GR2	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	GR2(FD)	02/25/09	1.1	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	GR3	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	GR4	02/25/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	GR1	02/22/11	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	GR2	02/22/11	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	GR2(FD)	02/22/11	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	GR3	02/22/11	2.7	< 0.3	< 0.3	NC (<0.5)[e]	< 0.3
Monitoring Well	GR4	02/22/11	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

[a] Lagoons were emptied in 2007; matrix is primarily runoff and rainfall
[b] One of three sampling locations in this lagoon
[c] FD is field duplicate
[d] Values reported as "<" are below quantitation limits
[e] NC is not confirmed due to interference; if analyte is present, it is below estimate shown in parantheses

stressors monitored in this study be detected. However, monitoring wells were only installed after the original lagoons had been decommissioned, and it is likely that additional detections would have occurred had the original lagoons still been operating and receiving CAFO waste.

3.3 CAFO Site 3 – Swine Nursery

Case Study Summary. Most of our research has focused on this site, and a full site investigation will be published separately. Ground water contamination by nitrate has been conclusively linked to land application of liquid swine waste at this site, and EPA enforcement actions have brought about changes in land management practices which have caused ground water nitrate levels in the closest monitoring wells to slowly drop from a high of about 120 mg/L NO_3-N in 2006 to about 30 mg/L NO_3-N in 2011 (unpublished data). Based on multiple sampling events, there were essentially no additional stressors detected in ground water at this site, including antibiotics and estrogen hormones. The only exception was estrone, which was detected in two well samples at very low levels (1.6-2.4 ng/L) during only one of three sampling events in which ground water samples were analyzed for estrogens.

Site Description. CAFO Site 3 is a swine nursery operation that was started in June 1995 and houses 21,000 feeder pigs distributed among four barn/lagoon complexes (Figure 5). Each lagoon is constructed with a synthetic liner and receives swine waste directly from its associated barns without any additional treatment. The lagoon effluents are disposed by land application through a center pivot over approximately 80 acres of grasses, and sometimes other crops, used for grazing and/or feed production. The soil matrix consists of thin dunes of fine to loamy sand overlying an unconfined alluvial aquifer with the base of the aquifer terminating in red bedrock 60-70 ft below ground surface. Intermittent clay lenses are found within the vadose zone, and the depth to ground water ranges from 20-30 ft. A wildlife management unit is located just one mile downgradient of the land application area, and there were concerns that ground water could become contaminated and ultimately discharge into a series of wetlands within this unit.

Unlike the situation with the other case studies, we were granted routine access to private lands surrounding this facility and were therefore able to conduct a thorough subsurface site characterization. Based on the information obtained, we constructed several monitoring wells both upgradient and downgradient of the land application area screened at different levels within the aquifer. Generally, two wells were constructed at each location, with the shallow well (designated with an “A” suffix) screened across or just beneath the water table and the deeper well (designated with a “B” suffix) screened just above the bedrock base of the aquifer (Figure 5). Wells were situated in transects roughly parallel to the flow of ground water so that changes in ground water quality could be monitored as ground water moved south underneath the land application area and continued on towards the wetlands. Quarterly monitoring of these wells began in 2000 and was conducted for two years, and then annual sampling has been conducted since that time. Limited site access was also allowed for lagoon sampling in 2002 and 2007. Because early data showed that over-application had resulted in ground water

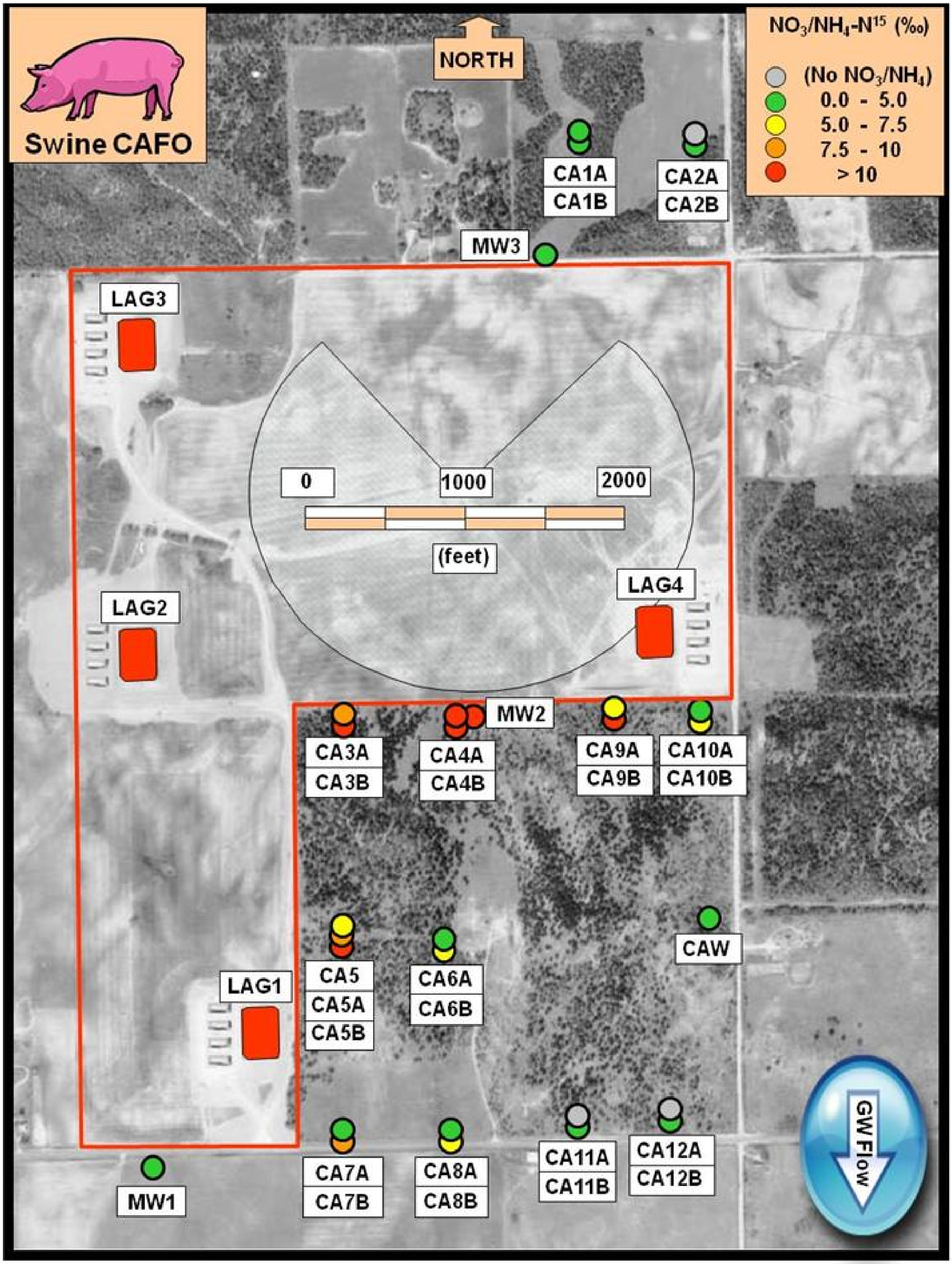


Figure 5. CAFO Site #3 schematic (swine nursery operation). Colors of lagoons (2007) and wells (2009) correspond to ranges of δ^{15}N of nitrate or ammonium as shown in legend at upper right.

contamination by nitrate in wells immediately downgradient of the land application area, several corrective actions were taken in 2002 leading to a substantial decrease in the amount of manure nitrogen applied to this field. Since then, there has been a low decline in ground water nitrate levels immediately downgradient of the land application area (unpublished data). This decline began in 2006 and continues to present day.

General Chemistry and Stable Isotope Interpretation. For brevity, these discussions focus on the 2007 data for the lagoons and the 2009 data for the wells (Tables 12-13), since this is generally when additional samples were analyzed for stable isotopes and the other stressors. Unlike the situation with the two previous case studies, interpretations were

Table 12. CAFO Site #3 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[a]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Swine Primary Lagoon	LAG1	4/18/2007	NA	NA	0.2	26.3	440	< 0.1	44.6	820	55.6	678	874
Swine Primary Lagoon	LAG2	4/18/2007	NA	NA	0.2	7.20	516	< 0.1	50.4	751	55.8	852	1,445
Swine Primary Lagoon	LAG3	4/18/2007	NA	NA	0.2	8.35	437	< 0.1	43.3	949	59.7	819	1,130
Swine Primary Lagoon	LAG4	4/18/2007	NA	NA	0.2	17.2	509	< 0.1	55.9	971	65.1	865	969
Monitoring Well	MW1	06/09/09	8.61	10-35	6.4	< 0.01[b]	11.2	31.0	0.262	0.19	0.177	0.56	35.6
Monitoring Well	MW2	06/08/09	29.66	30-60	6.5	< 0.01	87.0	58.8	0.063	0.31	0.048	1.34	18.6
Monitoring Well	MW3	06/08/09	18.32	50-80	7.2	< 0.01	37.5	66.5	0.128	< 0.02	0.118	0.50	35.8
Monitoring Well	CA1A	06/08/09	24.20	21-31	5.8	0.05	10.1	26.0	0.036	0.05	0.133	0.54	38.1
Monitoring Well	CA1B	06/08/09	24.40	36-46	7.5	< 0.01	25.7	68.2	0.136	< 0.02	0.144	0.31	32.0
Monitoring Well	CA2A	06/08/09	22.51	21-31	4.7	< 0.01	1.1	12.9	0.044	0.07	0.053	0.53	87.9
Monitoring Well	CA2B	06/08/09	22.41	35-45	7.0	< 0.01	48.6	56.1	0.167	0.20	0.241	0.57	37.6
Monitoring Well	CA3A	06/08/09	28.49	30-40	6.7	< 0.01	66.1	56.5	0.047	0.34	0.080	0.99	22.9
Monitoring Well	CA3B	06/08/09	32.49	49-59	5.6	< 0.01	76.0	48.8	0.071	0.16	0.101	0.92	137
Monitoring Well	CA4A	06/08/09	26.79	30-40	6.1	< 0.01	108	71.8	0.028	0.48	0.071	1.74	15.9
Monitoring Well	CA4B	06/08/09	30.30	50-60	5.6	< 0.01	87.2	52.5	0.043	0.36	0.085	1.08	21.2
Monitoring Well	CA5	06/08/09	25.27	25-35	4.5	< 0.01	12.5	42.6	0.132	0.15	0.219	1.51	58.7
Monitoring Well	CA5A	06/08/09	25.23	35-45	6.3	< 0.01	45.9	22.7	0.198	0.14	0.102	0.39	26.7
Monitoring Well	CA5B	06/08/09	25.27	50-60	5.6	< 0.01	71.6	35.3	0.111	0.21	0.113	0.57	27.8
Monitoring Well	CA6A	06/08/09	20.69	20-30	3.8	< 0.01	2.2	30.9	0.081	0.43	0.094	3.64	39.8
Monitoring Well	CA6B	06/08/09	20.72	40-50	6.6	< 0.01	41.6	45.8	0.141	0.20	0.110	0.54	30.3
Monitoring Well	CA7A	06/09/09	11.41	14-24	1.6	< 0.01	4.8	28.9	0.140	0.21	0.133	1.63	56.9
Monitoring Well	CA7B	06/09/09	11.15	34-44	4.9	< 0.01	43.5	74.7	0.197	0.21	0.146	0.57	33.3
Monitoring Well	CA8A	06/09/09	10.24	15-25	5.7	< 0.01	1.5	8.5	0.095	< 0.02	0.086	0.29	22.1
Monitoring Well	CA8B	06/09/09	10.12	34-44	7.0	< 0.01	33.5	44.8	0.090	0.03	0.100	0.41	30.0
Monitoring Well	CA9A	06/08/09	15.22	20-30	6.2	< 0.01	19.5	74.8	0.074	0.12	0.090	0.43	39.4
Monitoring Well	CA9B	06/08/09	22.54	49-59	7.1	< 0.01	58.8	48.9	0.065	0.18	0.088	0.71	29.2
Monitoring Well	CA10A	06/08/09	16.12	20-30	4.7	< 0.01	4.1	385	0.046	0.08	0.044	0.59	40.5
Monitoring Well	CA10B	06/08/09	25.25	48-58	6.7	< 0.01	22.3	42.3	0.133	0.10	0.096	0.47	30.9
Monitoring Well	CA11A	06/09/09	16.96	20-30	1.0	< 0.01	133	409	0.085	0.52	0.167	4.97	112
Monitoring Well	CA11B	06/09/09	17.24	35-45	7.1	< 0.01	41.1	55.8	0.144	0.05	0.080	0.38	30.1
Monitoring Well	CA12A	06/09/09	13.22	15-25	1.0	< 0.01	517	1,050	0.083	0.87	0.085	8.94	101
Monitoring Well	CA12B	06/09/09	13.36	35-45	6.2	< 0.01	18.2	68.6	0.092	0.04	0.092	0.41	33.2
Pond Water Well	CAW	06/09/09	NA	NA	6.7	< 0.01	20.5	246	0.146	0.11	0.152	0.44	28.9

[a] Feet from top of casing

[b] Values reported as "<" are below detection limits

pretty straightforward with this site. The four swine nursery primary lagoons all had high ammonium levels (664-879 mg/L NH_4-N) with animal waste signatures ranging from +13.4‰ to +17.6‰ δ^{15}N-NH_4 (Table 13). Land application resulted in increases in ground water concentrations of chloride (Table 12) and nitrate (Table 13) in both the shallow and deeper wells immediately downgradient of the land application area compared to upgradient wells. Ground water samples from several of these wells showed animal waste signatures ($\delta^{15}N > +10$‰) similar to those of the applied ammonium (Figure 5). There was no correlation ($r^2 = 0.0002$) between ground water nitrate and chloride levels across this site, but this was only because of very high chloride levels in ground water samples from the shallow wells CA11A and CA12A (Table 12). The

Table 13. CAFO Site #3 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	N_2O-N (mg/L)	δ^2H-H_2O (‰)	$\delta^{18}O$-H_2O (‰)	$\delta^{15}N$-NO_3 (‰)	$\delta^{18}O$-NO_3 (‰)	$\delta^{15}N$-NH_4 (‰)
Swine Primary Lagoon	LAG1	4/18/2007	698	0.41	0.89	< 0.01	-12.1	-0.9	NA	NA	+14.3
Swine Primary Lagoon	LAG2	4/18/2007	664	0.37	0.72	< 0.01	-11.0	-0.3	NA	NA	+14.2
Swine Primary Lagoon	LAG3	4/18/2007	879	0.55	1.02	< 0.01	-15.8	-1.5	NA	NA	+17.6
Swine Primary Lagoon	LAG4	4/18/2007	806	0.06	0.97	< 0.01	-12.4	-1.1	NA	NA	+13.4
Monitoring Well	MW1	06/09/09	< 0.02[a]	9.67	0.02	< 0.01	-43.2	-6.5	+3.5	-8.5	NA
Monitoring Well	MW2	06/08/09	< 0.02	47.4	0.02	< 0.01	-41.3	-6.2	+11.6	+11.7	NA
Monitoring Well	MW3	06/08/09	< 0.02	6.70	0.02	< 0.01	-40.9	-6.2	+2.8	+10.0	NA
Monitoring Well	CA1A	06/08/09	< 0.02	1.63	0.02	0.01	-44.5	-6.7	+2.2	+2.6	NA
Monitoring Well	CA1B	06/08/09	< 0.02	6.23	0.02	< 0.01	-38.7	-5.9	+3.7	+1.8	NA
Monitoring Well	CA2A	06/08/09	< 0.01	0.13	0.02	< 0.01	-46.6	-7.1	NA	NA	NA
Monitoring Well	CA2B	06/08/09	< 0.02	5.94	0.04	< 0.01	-40.6	-6.2	+2.8	+7.5	NA
Monitoring Well	CA3A	06/08/09	< 0.02	41.5	0.02	0.01	-40.3	-6.1	+9.7	+11.2	NA
Monitoring Well	CA3B	06/08/09	< 0.02	46.8	0.02	0.01	-40.7	-5.9	+10.5	+12.9	NA
Monitoring Well	CA4A	06/08/09	< 0.02	52.7	0.02	< 0.01	-40.3	-5.6	+12.5	+11.7	NA
Monitoring Well	CA4B	06/08/09	< 0.02	50.3	0.02	0.01	-42.8	-5.6	+11.8	+13.2	NA
Monitoring Well	CA5	06/08/09	< 0.02	9.52	0.03	0.05	-42.2	-6.2	+6.4	+12.5	NA
Monitoring Well	CA5A	06/08/09	< 0.02	38.7	0.02	0.01	-40.6	-6.1	+9.5	+19.1	NA
Monitoring Well	CA5B	06/08/09	< 0.02	51.9	0.02	0.01	-38.0	-5.9	+10.2	+18.2	NA
Monitoring Well	CA6A	06/08/09	< 0.02	11.2	0.03	0.01	-43.0	-6.5	+3.5	+13.6	NA
Monitoring Well	CA6B	06/08/09	< 0.02	27.8	0.04	< 0.01	-42.1	-6.3	+7.0	+14.3	NA
Monitoring Well	CA7A	06/09/09	< 0.02	4.83	0.02	< 0.01	-44.5	-6.7	+4.8	+18.4	NA
Monitoring Well	CA7B	06/09/09	< 0.02	33.6	0.03	0.01	-39.6	-5.8	+9.1	+18.8	NA
Monitoring Well	CA8A	06/09/09	< 0.02	5.11	0.02	< 0.01	-36.5	-5.5	+0.4	+16.0	NA
Monitoring Well	CA8B	06/09/09	< 0.02	20.4	0.02	< 0.01	-41.8	-6.1	+7.2	-3.0	NA
Monitoring Well	CA9A	06/08/09	< 0.02	30.4	0.02	0.02	-39.1	-5.9	+37.8	+7.8	NA
Monitoring Well	CA9B	06/08/09	< 0.02	30.1	0.02	< 0.01	-41.7	-6.0	+13.3	+13.6	NA
Monitoring Well	CA10A	06/08/09	< 0.02	4.85	0.02	0.02	-40.8	-5.8	+4.9	+0.0	NA
Monitoring Well	CA10B	06/08/09	< 0.02	9.73	0.02	< 0.01	-40.1	-5.6	+5.3	+0.5	NA
Monitoring Well	CA11A	06/09/09	< 0.02	0.15	0.02	< 0.01	-43.7	-6.1	NA	NA	NA
Monitoring Well	CA11B	06/09/09	< 0.02	27.4	0.02	< 0.01	-40.4	-5.7	+1.7	+5.9	NA
Monitoring Well	CA12A	06/09/09	< 0.02	0.17	0.02	< 0.01	-42.0	-6.0	NA	NA	NA
Monitoring Well	CA12B	06/09/09	< 0.02	11.2	0.02	< 0.01	-40.1	-5.6	+2.7	+6.9	NA
Pond Water Well	CAW	06/09/09	< 0.02	9.47	0.02	0.01	-40.1	-5.7	+4.0	+0.0	NA

[a] Values reported as "<" are below detection limits

reason for this is unknown, and the ground water samples from these two wells also showed very high levels of sulfate compared to those from all of the other wells. The swine CAFO lagoon waste is very low in sulfate, and it is unlikely that this is the cause of the high chloride levels found in the ground water samples from these two wells. If the results from these two wells are excluded, there is a good correlation ($r^2 = 0.7878$) between ground water nitrate and chloride levels across this site. This nitrate plume has been slowly migrating south and is currently impacting the next well transect (CA5, CA6). There are definitive animal waste signatures as indicated by increased $\delta^{15}N$ values in the deeper wells downgradient of the land application area, and these values do not appear to be caused by denitrifying activity. Ground water DO levels are moderate to high whereas TOC levels are generally low, which is not conducive to denitrification (Table 12), and concentrations of both nitrite and nitrous oxide were very low across the site (Table 13). Denitrification is also not indicated by stable isotope relationships (Figure 6). In fact, there was actually a strong positive correlation ($r^2 = 0.7381$) between ground water nitrate concentrations and $\delta^{15}N$ values (Figure 6a), contrary to what would be expected based on denitrification. There was some observed enrichment in $\delta^{18}O\text{-}NO_3$ with increasing $\delta^{15}N\text{-}NO_3$ values which could indicate denitrification (Figure 6b), but this correlation was weak ($r^2 = 0.1700$). In general, then, nitrate concentrations in this ground water are expected to persist. Ground water chemistry was not influenced by selective rapid recharge events at this site, in part due to the relatively uniform sand matrix and the increased isolation of well screens from surface effects, and therefore ground water samples from these wells were very uniform with respect to water stable isotope ratios (Figure 6c).

Evaluation of Additional Stressor Impact. Additional CAFO swine waste stressors were generally not detected in any of the ground water samples at this site, even with multiple sampling events. Ground water orthophosphate levels were somewhat high and ranged from 0.044-0.262 mg/L PO_4-P (Table 12), but these levels did not correlate with either nitrate ($r^2 = -0.0288$) or chloride ($r^2 = -0.0269$) levels. The highest orthophosphate concentrations were generally found in the deeper ground water samples, and this was observed upgradient as well as downgradient of the land application area (Table 12).

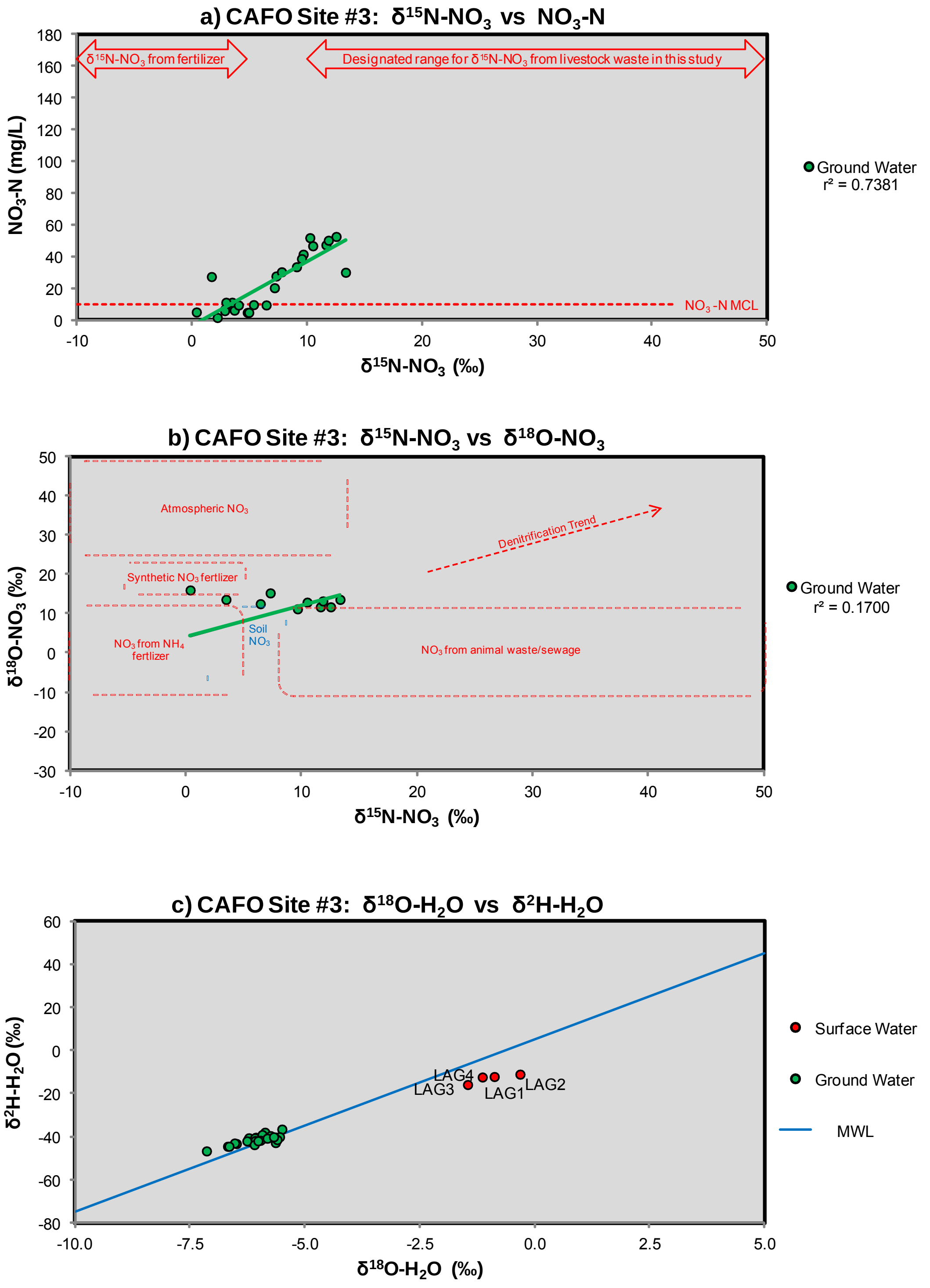


Figure 6. CAFO Site #3 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for NO_3-N. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

Microbial indicators were not monitored in 2009, but data from the 2011 sample event showed that numbers were very low, with the exception of the ground water sample taken from well CA4B, which had a moderately low count of 26 cells/100 mL for fecal enterococci (Table 14). Metals and metalloid concentrations were also very low in ground water at this site. Arsenic concentrations, as measured by ICP-MS, were low in the original swine lagoons (< 10 μg/L), and correspondingly arsenic concentrations were below 5 μg/L in all ground water samples (Table 14). What little arsenic was found in the lagoons was generally present as As (V) and, to a lesser extent, DMA (Appendix A). All other monitored metals and metalloids were at or near the detection limits, except for

Table 14. CAFO Site #3 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococci (cells per 100 mL)	As by ICP-MS (μg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Swine Primary Lagoon	LAG1	04/18/07	308,000	130,000	32,700	6.7	0.036	0.523	0.145	0.036	9.76
Swine Primary Lagoon	LAG2	04/18/07	122,000	58,100	29,200	9.3	0.030	0.249	0.164	0.030	5.24
Swine Primary Lagoon	LAG3	04/18/07	56,500	18,700	73,800	6.7	0.030	0.528	0.163	0.039	7.32
Swine Primary Lagoon	LAG4	04/18/07	55,600	45,700	29,500	9.9	0.034	0.741	0.189	0.042	10.3
Monitoring Well[a]	MW1	06/09/09	0	0	0	3.5	< 0.006[b]	< 0.001	< 0.003	0.009	< 0.040
Monitoring Well	MW2	06/08/09	0	0	0	0.6	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	MW3	06/08/09	0	0	1	0.7	< 0.006	< 0.001	< 0.003	0.008	< 0.040
Monitoring Well	CA1A	06/08/09	0	0	1	0.5	< 0.006	< 0.001	0.003	0.006	< 0.040
Monitoring Well	CA1B	06/08/09	0	0	0	0.8	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA2A	06/08/09	0	0	2	0.6	< 0.006	< 0.001	< 0.003	0.006	< 0.040
Monitoring Well	CA2B	06/08/09	0	0	1	1.3	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA3A	06/08/09	0	0	3	0.8	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA3B	06/08/09	0	0	0	1.0	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA4A	06/08/09	0	0	0	0.6	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA4B	06/08/09	0	0	26	0.9	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA5	06/08/09	0	0	0	1.5	< 0.006	< 0.001	0.004	0.014	< 0.040
Monitoring Well	CA5A	06/08/09	0	0	0	1.4	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA5B	06/08/09	0	0	0	1.1	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA6A	06/08/09	0	0	0	0.8	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA6B	06/08/09	0	0	0	1.1	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA7A	06/09/09	0	0	1	1.5	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA7B	06/09/09	0	0	0	1.6	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA8A	06/09/09	0	0	0	0.2	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA8B	06/09/09	0	0	0	1.5	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA9A	06/08/09	0	0	0	1.2	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA9B	06/08/09	0	0	0	1.2	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA10A	06/08/09	0	0	0	0.5	< 0.006	< 0.001	< 0.003	0.007	< 0.040
Monitoring Well	CA10B	06/08/09	0	0	0	1.1	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA11A	06/09/09	0	0	0	2.0	< 0.006	0.002	< 0.003	0.046	< 0.040
Monitoring Well	CA11B	06/09/09	0	0	0	0.9	< 0.006	< 0.001	< 0.003	< 0.005	< 0.040
Monitoring Well	CA12A	06/09/09	0	0	0	2.3	< 0.006	0.001	< 0.003	0.058	< 0.040
Monitoring Well	CA12B	06/09/09	0	0	0	0.9	< 0.006	< 0.001	< 0.003	0.007	< 0.040
Pond Water Well	CAW	06/09/09	0	0	0	1.0	< 0.006	< 0.001	< 0.003	0.010	< 0.040

[a] For monitoring well samples, microbial counts were not done in 2009, data for those samples are from April 2011 sampling event

[b] Values reported as "<" are below detection limits

selenium which was found at low levels (46-58 µg/L) in ground water samples from the same two wells (CA11A, CA12A) having the abnormally high chloride and sulfate levels noted earlier (Table 14). Antibiotics were analyzed in these nursery swine lagoons in 2002 as well as in 2007 and were found at relatively high levels (Table 15), similar to what was observed for the CAFO Site #1 finisher swine operation. However, there were major differences in the types of antibiotics found and the ranges in concentrations from one sample event to the next, and in some cases there was considerable variation between the four lagoons even within the same sampling event (Table 15). These data imply that

Table 15. CAFO Site #3 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date	TYL[b] (ng/L)	SMZN (ng/L)	STHZ (ng/L)	CTET (ng/L)	ICTET (ng/L)	EICTET (ng/L)	OTET (ng/L)	TET (ng/L)	ETET (ng/L)	LINC (ng/L)
Swine Primary Lagoon	LAG1	07/10/02	< 20[c]	< 10	< 50	5,000	NA	NA	15,000	< 20	NA	< 10
Swine Primary Lagoon	LAG2	07/10/02	< 20	1,000	240,000	7,000	NA	NA	27,000	< 20	NA	< 10
Swine Primary Lagoon	LAG3	07/10/02	< 20	170	1,700	9,000	NA	NA	70,000	< 20	NA	< 10
Swine Primary Lagoon	LAG4	07/10/02	< 20	220,000	1,900,000	160,000	NA	NA	200,000	< 20	NA	< 10
Monitoring Well	CA1A	07/09/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA1B	07/09/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA2A	07/09/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA2B	07/09/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA2B(FD)[a]	07/09/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA3A	07/15/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA3B	07/15/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA4A	07/15/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA4B	07/15/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Monitoring Well	CA4B(FD)	07/15/02	< 20	< 10	< 50	< 20	NA	NA	< 50	< 20	NA	< 10
Swine Primary Lagoon	LAG1	04/18/07	548	1,720	7,570	< 10	282,000	396,000	2,150,000	15,000	5,530	82,000
Swine Primary Lagoon	LAG2	04/18/07	< 5	1,940	14,000	< 10	216,000	243,000	1,450,000	12,000	3,030	89,000
Swine Primary Lagoon	LAG3	04/18/07	< 5	1,910	9,510	< 10	269,000	243,000	663,000	9,620	2,680	77,000
Swine Primary Lagoon	LAG4	04/18/07	< 5	1,480	7,830	< 10	879,000	326,000	1,050,000	13,000	4,240	71,000
Monitoring Well	CA1A	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA1B	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA2A	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA2B	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA3A	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA3B	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA4A	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA4B	04/18/07	< 5	< 5	< 20	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	MW2	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	MW3	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA1B	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA2B	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA3B	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA4B	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA9B	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5
Monitoring Well	CA10B	06/08/09	< 10	< 5	< 50	< 10	< 10	< 10	< 10	< 10	< 10	< 5

[a] FD is field duplicate

[b] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table were not detected in any of these samples

[c] Values reported as "<" are below reporting limits

Table 16. CAFO Site #3 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17α-Ethynylestradiol (ng/L)	Estriol (ng/L)
Swine Primary Lagoon	LAG1	07/10/02	392	NA	48.0	< 40.0	208
Swine Primary Lagoon	LAG2	07/10/02	576	NA	40.0	< 40.0	186
Swine Primary Lagoon	LAG3	07/10/02	530	NA	50.0	< 40.0	175
Swine Primary Lagoon	LAG4	07/10/02	623	NA	48.0	< 40.0	220
Monitoring Well	MW2	07/15/02	< 1.0[b]	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	MW3	07/09/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA1A	07/09/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA1B	07/09/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA2A	07/09/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA2B	07/09/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA2B(FD)[a]	07/09/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA3A	07/15/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA3B	07/15/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA4A	07/15/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA4B	07/15/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CA4B(FD)	07/15/02	< 1.0	NA	< 1.0	< 1.0	< 1.0
Swine Primary Lagoon	LAG1	04/18/07	357	53.7	< 30.0	< 30.0	81.2
Swine Primary Lagoon	LAG2	04/18/07	366	49.8	< 30.0	< 30.0	141
Swine Primary Lagoon	LAG3	04/18/07	285	36.9	< 30.0	< 30.0	67.8
Swine Primary Lagoon	LAG4	04/18/07	307	47.7	< 30.0	< 30.0	58.8
Monitoring Well	CA1A	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA1B	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA2A	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA2B	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA3A	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA3B	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA4A	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA4B	04/16/07	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	MW2	06/08/09	2.4	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	MW3	06/08/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA1B	06/08/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA2B	06/08/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA3B	06/08/09	1.6	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA4B	06/08/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA9B	06/08/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CA10B	06/08/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	MW2	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	MW3	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA1A	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA1B	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA2A	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA2B	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA3A	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA3B	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA4A	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA4B	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA4B(FD)	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA9A	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA9B	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA10A	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CA10B	03/29/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

[a] FD is field duplicate

[b] Values reported as "<" are below quantitation limits

multiple sample events are needed to get good estimates of the loading of antibiotics during land application of swine CAFO wastes. Regardless, none of these or any other antibiotics were detected in the ground water samples collected during the sampling events in 2002, 2007, and 2009 from selected wells expected to represent either background or the most impacted ground water conditions (Table 15). Estrogen hormones were also detected in these lagoons, although at relatively low levels, and these levels were fairly consistent from one sample event to the next (Table 16). These concentrations are similar to what was observed for a separate swine nursery (Hutchins et al., 2007). However, none of these hormones were detected in selected ground water samples from four separate sampling events, with the exception of low levels of estrone that were detected (1.6-2.4 ng/L) in downgradient wells MW2 and CA3B in 2009 (Table 16). These wells were again sampled the following year and estrone was not detected in any of these wells (Table 16). Hence, even though ground water was contaminated with nitrate from land application of CAFO waste at this site, there was little evidence of ground water contamination by the other stressors measured in this study.

3.4 CAFO Site 4 – Dairy

Case Study Summary. This is another site where land application of CAFO waste resulted in contamination of ground water by nitrate, this time through over-application of dairy lagoon effluent. Ground water nitrate concentrations ranged up to 150 mg/L NO_3-N, and over half of these samples showed animal waste signatures ($\delta^{15}N > +10‰$). There was only one complete sampling event for this site, and in most cases again there were few additional stressors besides nitrate detected in the impacted wells. However, there were isolated cases where other stressors were detected, sometimes at significant levels. This was true primarily for ammonium (up to 1.6 mg/L NH_4-N) and microbial indicators (up to > 2,420 cells/100 mL). The antibiotic sulfamethazine was found in one ground water sample at a low level of 11 ng/L, which is slightly above the reporting limit (5 ng/L). Estrone was found in two ground water samples at very low levels of 1.0-1.4 ng/L, which are at or just above the quantitation limit (1.0 ng/L). When these latter two wells were sampled again two years later, estrone was not found (< 0.3 ng/L).

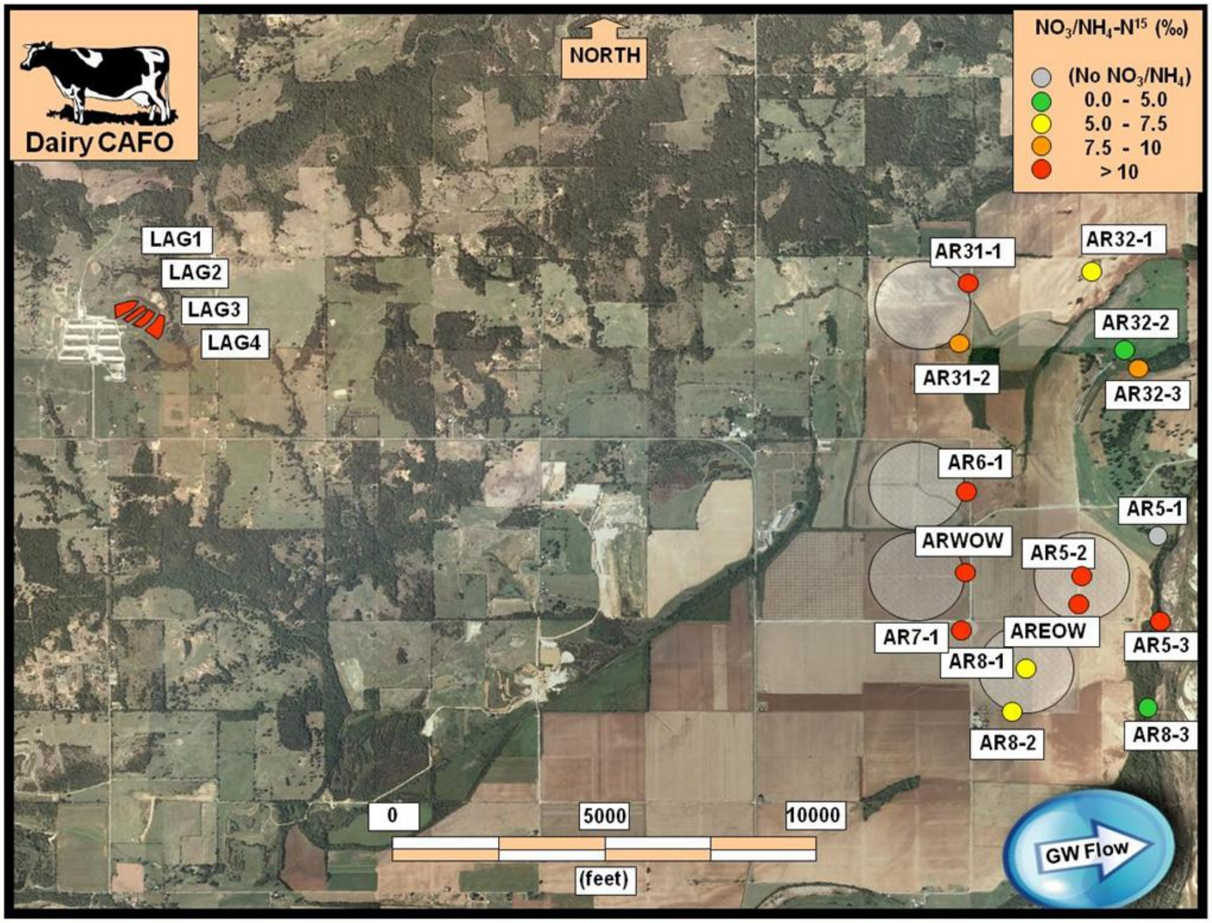


Figure 7. CAFO Site #4 schematic (dairy operation). Colors of lagoons and wells correspond to ranges of $\delta^{15}N$ of nitrate or ammonium as shown in legend at upper right, based on 2009 data.

Site Description. CAFO Site 4 is a dairy operation that was permitted in May 1999 for 3,500 dairy cows, along with 5,000 dry cows and calves. Manure is flushed through two sand filters and then sequentially through four lagoons. The first three lagoons (LAG1-3) are unlined, and effluent was originally pumped from LAG3 several miles east to center pivots for irrigating corn, soybean, and other crops used for feed (Figure 7). The facility actually used only about 600 acres of the 3,625 acres permitted for land application, and ground water was subsequently impacted through over-application. Corrective actions were taken and a fourth lagoon (LAG4) was constructed with a synthetic liner and used to bring total storage into compliance. Starting in June 2006, effluent application to these fields was discontinued. The topsoil in these fields is a mixture of sands and clays, and the aquifer consists of shallow unconsolidated riverine sands associated with an adjacent river, and is underlain by hard shale bedrock approximately 40 ft below ground surface. Ground water flow is generally to the east towards the river, which is located just off of the map shown in Figure 7, and the river flows towards the north. Compared to the previous case studies, this site covers a very large area and in this case the direction of ground water flow becomes more uncertain further away from the land application areas. Well construction logs were not available for this site, but the monitoring wells were reported to have 15-ft to 20-ft screens and were screened across the water table. Two irrigation wells were also sampled, one of which (ARWOW) was clearly poorly sealed and subject to surface effects.

General Chemistry and Stable Isotope Interpretation. General chemistry parameters, reactive nitrogen, and stable isotope data are shown in Tables 17-18. These dairy lagoons had moderately high levels of ammonium (124-203 mg/L NH_4-N), with concentrations dropping as lagoon effluent flowed from LAG1 to LAG4 (Table 18). Correspondingly, δ^{15}N-NH_4 values increased from +10.7‰ to +14.0‰ due to preferential volatilization of the lighter nitrogen isotope (Table 18). Most of the wells within and adjacent to the land

Table 17. CAFO Site #4 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[a]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Dairy Primary Lagoon	LAG1	05/13/09	NA	NA	NA[b]	10.3	74.4	5.5	46.8	342	51.4	435	829
Dairy Secondary Lagoon	LAG2	05/13/09	NA	NA	NA	1.15	68.0	26.2	37.0	280	38.4	199	610
Dairy Tertiary Lagoon	LAG3	05/13/09	NA	NA	NA	3.85	66.9	26.3	30.4	251	33.2	200	505
Dairy Quatenary Lagoon	LAG4	05/13/09	NA	NA	NA	2.85	53.0	7.1	25.3	180	25.8	468	364
Monitoring Well	AR5-1	06/23/09	20.80	NA	4.0	0.04	30.2	66.2	0.047	0.52	0.153	1.6	151
Monitoring Well	AR5-2	05/18/09	9.90	NA	NA	< 0.01[c]	53.2	69.9	0.039	0.33	0.063	1.4	142
Monitoring Well	AR5-3	05/18/09	22.56	NA	NA	< 0.01	32.9	162	0.031	0.05	0.075	0.5	112
Monitoring Well	AR6-1	05/18/09	5.22	NA	NA	< 0.01	4.3	41.6	0.026	0.08	0.085	0.4	93.2
Monitoring Well	AR7-1	06/23/09	6.72	NA	4.9	< 0.01	6.0	30.2	0.040	< 0.02	0.042	0.4	89.4
Monitoring Well	AR8-1	06/23/09	11.82	NA	4.8	< 0.01	61.3	102	0.058	< 0.02	0.042	0.7	106
Monitoring Well	AR8-2	05/18/09	11.00	NA	NA	0.01	32.4	52.2	0.054	0.06	0.089	0.3	86.5
Monitoring Well	AR8-3	06/23/09	22.43	NA	2.7	0.01	14.5	38.7	0.051	0.07	0.466	1.7	98.6
Monitoring Well	AR31-1	06/23/09	6.87	NA	2.0	0.01	7.8	49.6	0.265	< 0.02	0.211	3.8	50.0
Monitoring Well	AR31-2	05/18/09	8.70	NA	NA	< 0.01	23.8	89.7	0.055	0.03	0.091	0.6	95.3
Monitoring Well	AR32-1	06/23/09	16.88	NA	6.0	< 0.01	14.7	32.3	0.027	0.14	0.031	0.5	97.1
Monitoring Well	AR32-2	06/23/09	13.63	NA	3.7	< 0.01	53.2	127	0.048	0.71	0.073	4.2	128
Monitoring Well	AR32-3	06/23/09	3.06	NA	3.7	0.20	304	532	0.756	2.79	1.50	9.3	140
Water Well	AREOW	05/18/09	11.44	NA	NA	< 0.01	27.0	111	0.025	0.07	0.083	0.4	111
Water Well	ARWOW	05/18/09	2.00	NA	NA	0.31	36.1	114	0.060	1.08	0.162	1.4	131

[a] Feet from top of casing

[b] DO probe failure at these locations

[c] Values reported as "<" are below detection limits

application areas had moderate to high nitrate values (15-150 mg/l NO_3-N) and retained this animal waste signature (> +10‰ δ^{15}N-NO_3), but there were a few exceptions. Wells further downgradient all had low nitrate values (< 0.01-5.7 mg/L NO_3-N), but exhibited a relatively wide range of δ^{15}N values (Figure 7). Considering all of the site wells, there was no correlation (r^2 = -0.0034) between ground water nitrate and chloride values, but this was predominantly affected by an extremely high chloride result for the ground water sample from well AR32-3 (Table 17). This was a very shallow well, and this ground water sample also had the highest levels of ammonium, orthophosphate, sulfate, and TOC compared to those of all of the other wells (Table 17-18). Excluding this well, there was a weak positive correlation (r^2 = 0.2351) between ground water nitrate and chloride values. These ground water samples generally had moderate to high DO levels and very low TOC concentrations (Table 17) which would not be expected to support significant denitrification, and the concentrations of nitrate and nitrous oxide were also generally quite low (Table 18). There was no clear evidence of denitrification provided by the stable nitrate isotope relationships (Figure 8). There was no correlation (r^2 = 0.0000) between increasing δ^{15}N values and decreasing nitrate concentration which would

Table 18. CAFO Site #4 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	N_2O-N (mg/L)	δ^2H-H_2O (‰)	$\delta^{18}O$-H_2O (‰)	$\delta^{15}N$-NO_3 (‰)	$\delta^{18}O$-NO_3 (‰)	$\delta^{15}N$-NH_4 (‰)
Dairy Primary Lagoon	LAG1	05/13/09	203	0.49	0.74	< 0.01	-16.5	-2.2	NA	NA	+10.7
Dairy Secondary Lagoon	LAG2	05/13/09	195	0.42	0.60	< 0.01	-17.2	-2.6	NA	NA	+11.2
Dairy Tertiary Lagoon	LAG3	05/13/09	177	0.39	0.58	< 0.01	-13.7	-2.2	NA	NA	+12.4
Dairy Quatenary Lagoon	LAG4	05/13/09	124	0.35	0.43	< 0.01	-6.7	-0.5	NA	NA	+14.0
Monitoring Well	AR5-1	06/23/09	0.33	< 0.01	< 0.01	< 0.01	-24.5	-4.3	NA	NA	NA
Monitoring Well	AR5-2	05/18/09	< 0.02[a]	151	0.01	0.04	-21.2	-3.4	+13.0	+44.5	NA
Monitoring Well	AR5-3	05/18/09	< 0.02	0.93	0.01	< 0.01	-30.0	-4.3	+15.7	+49.6	NA
Monitoring Well	AR6-1	05/18/09	< 0.02	18.6	0.01	< 0.01	-23.0	-3.6	+15.8	+30.4	NA
Monitoring Well	AR7-1	06/23/09	< 0.02	5.22	0.01	0.01	-24.9	-3.8	+15.4	+45.4	NA
Monitoring Well	AR8-1	06/23/09	< 0.02	56.7	< 0.01	0.09	-27.4	-4.1	+6.4	+36.4	NA
Monitoring Well	AR8-2	05/18/09	0.02	69.7	0.03	0.01	-29.6	-4.6	+7.3	+18.8	NA
Monitoring Well	AR8-3	06/23/09	0.14	5.72	0.01	< 0.01	-24.7	-4.0	+3.0	+16.9	NA
Monitoring Well	AR31-1	06/23/09	< 0.02	14.6	0.47	0.11	-24.8	-4.0	+18.4	+34.5	NA
Monitoring Well	AR31-2	05/18/09	< 0.02	46.7	0.01	0.01	-26.9	-4.5	+9.9	+21.3	NA
Monitoring Well	AR32-1	06/23/09	< 0.02	1.56	< 0.01	0.01	-22.7	-4.2	+5.4	+33.0	NA
Monitoring Well	AR32-2	06/23/09	0.25	0.92	< 0.01	< 0.01	-22.8	-3.9	+2.1	+26.3	NA
Monitoring Well	AR32-3	06/23/09	1.57	< 0.01	< 0.01	< 0.01	-23.1	-3.6	NA	NA	+8.5
Water Well	AREOW	05/18/09	0.02	29.3	0.09	0.04	-25.2	-4.5	+11.3	+27.0	NA
Water Well	ARWOW	05/18/09	0.71	15.6	0.45	0.01	-24.6	-4.4	+21.1	+36.0	NA

[a] Values reported as "<" are below detection limits

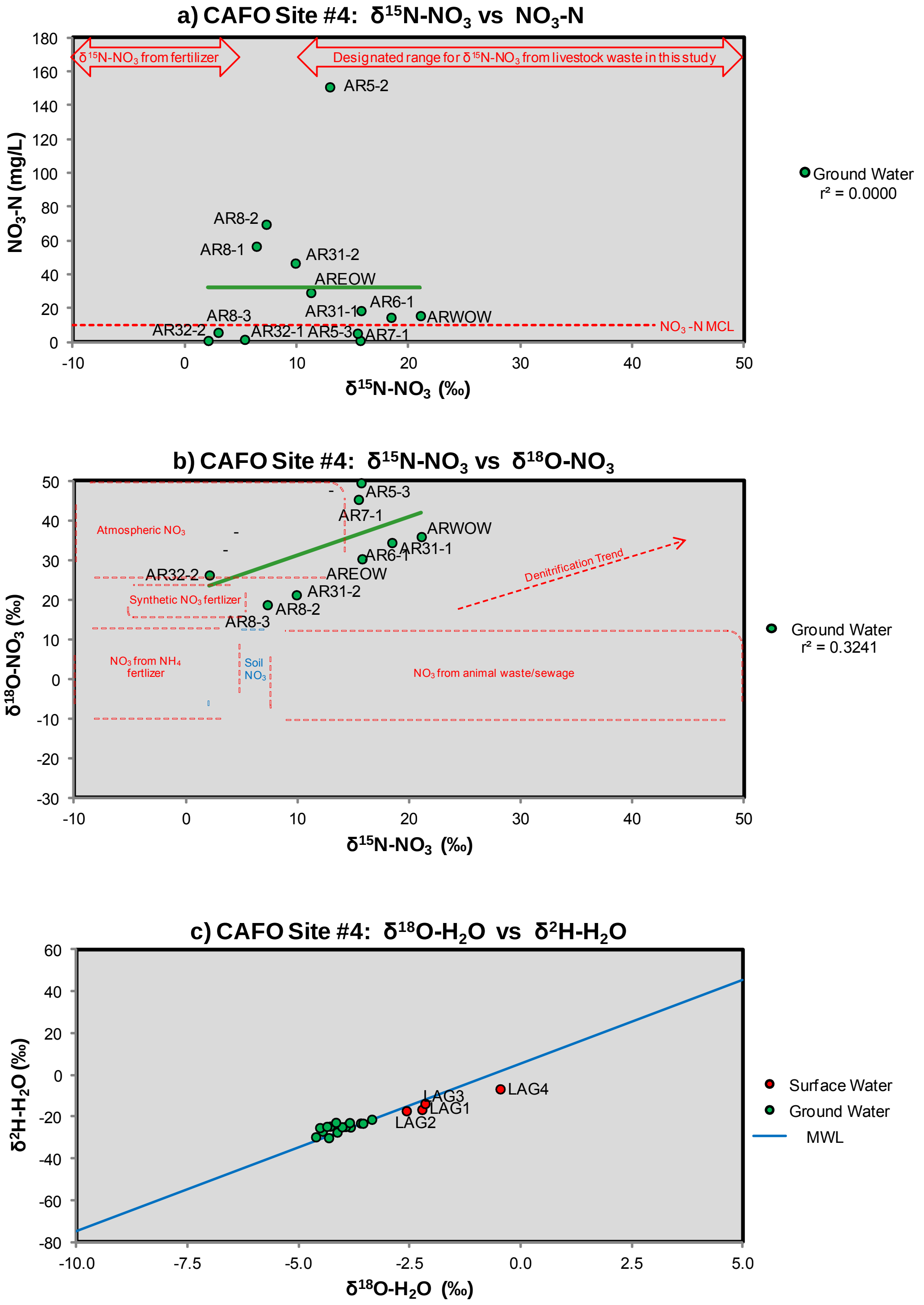


Figure 8. CAFO Site #4 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for NO_3-N. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

indicate denitrification (Figure 8a). It is possible that denitrification is occurring, but the nitrate sources are so varied that this relationship is masked. There was a moderate positive correlation ($r^2 = 0.3241$) between increasing $\delta^{18}O-NO_3$ values and $\delta^{15}N-NO_3$ values in these ground water samples that would tend to indicate denitrification (Figure 8b), but the data are too variable to draw any substantive conclusions. Interestingly, the ground water $\delta^{18}O-NO_3$ values are all very high at this site, and because denitrification would be expected to enrich $\delta^{18}O-NO_3$ values, this might provide some additional indication of denitrification. Somewhat surprisingly, all of the wells at this site seemed to have similar recharge histories, as shown by water stable isotope data grouping fairly well around the MWL, with little relative variation in $\delta^{18}O-H_2O$ values (Figure 8c). Unfortunately, there were no suitable wells upgradient of the land application areas, and so it is difficult to conclusively link the high nitrate values with land application of dairy waste and not fertilizer. However, given the amounts of effluent that were applied, the sandy nature of the soil, and the high $\delta^{15}N$ values within and immediately adjacent to the land application areas, the evidence points to the dairy lagoon effluent as the source of high nitrate in the ground water at this site.

Evaluation of Additional Stressor Impact. As with the previous site that focused on land application, there was little consistent evidence showing that the additional stressors monitored in this study were also being transported past the active soil zone along with nitrate, although there were isolated occurrences that appeared to be linked to land application of dairy wastes. Ground water orthophosphate concentrations, with two exceptions, were generally low (0.025 – 0.060 mg/L PO_4-P), and were not correlated with nitrate concentrations ($r^2 = -0.0377$). Ground water samples from wells AR31-1 and AR32-3, however, showed elevated levels of 0.265 and 0.756 mg/L PO_4-P, respectively (Table 17). As discussed previously, the ground water sample from well AR32-3 had very unusual chemistry, and the high level of orthophosphate may not be derived from land application of dairy waste. Conversely, well AR31-1 is located adjacent to a center pivot (Figure 7) and the ground water sample from this well had a nitrate concentration of 14.6 mg/L NO_3-N with a relatively strong animal waste signature of +18.4‰ $\delta^{15}N-NO_3$ (Table18), and so land application of dairy waste could be the source of orthophosphate

Table 19. CAFO Site #4 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococc (cells per 100 mL)	As by ICP-MS (µg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Dairy Primary Lagoon	LAG1	05/13/09	1,960,500	1,232,500	124,500	NA	< 0.006[a]	0.035	0.011	0.109	0.055
Dairy Secondary Lagoon	LAG2	05/13/09	218,000	135,000	141,400	NA	< 0.006	0.030	0.010	0.079	0.046
Dairy Tertiary Lagoon	LAG3	05/13/09	213,000	121,000	130,000	NA	< 0.006	0.025	0.009	0.108	0.042
Dairy Quatenary Lagoon	LAG4	05/13/09	19,890	15,650	17,250	NA	< 0.006	0.017	0.008	0.062	0.043
Monitoring Well	AR5-1	06/23/09	7	0	1	NA	< 0.006	< 0.004	< 0.003	0.059	< 0.040
Monitoring Well	AR5-2	05/18/09	1	0	0	NA	< 0.006	< 0.004	< 0.003	0.079	< 0.040
Monitoring Well	AR5-3	05/18/09	NA	NA	NA	NA	< 0.006	< 0.004	< 0.003	0.032	< 0.040
Monitoring Well	AR6-1	05/18/09	186	7	13	NA	< 0.006	< 0.004	< 0.003	0.034	< 0.040
Monitoring Well	AR7-1	06/23/09	1	0	0	NA	< 0.006	0.005	< 0.003	0.036	< 0.040
Monitoring Well	AR8-1	06/23/09	2	0	3	NA	< 0.006	< 0.004	< 0.003	0.049	< 0.040
Monitoring Well	AR8-2	05/18/09	31	6	4	NA	< 0.006	< 0.004	< 0.003	0.048	< 0.040
Monitoring Well	AR8-3	06/23/09	0	0	1	NA	< 0.006	< 0.004	< 0.003	0.040	< 0.040
Monitoring Well	AR31-1	06/23/09	816	3	27	NA	< 0.006	0.005	< 0.003	0.021	< 0.040
Monitoring Well	AR31-2	05/18/09	0	0	2	NA	< 0.006	< 0.004	< 0.003	0.040	< 0.040
Monitoring Well	AR32-1	06/23/09	3	0	13	NA	< 0.006	< 0.004	< 0.003	0.047	0.110
Monitoring Well	AR32-2	06/23/09	> 2,420	219	0	NA	< 0.006	< 0.004	< 0.003	0.043	< 0.040
Monitoring Well	AR32-3	06/23/09	365	261	5	NA	< 0.006	< 0.004	< 0.003	0.050	< 0.040
Water Well	AREOW	05/18/09	122	0	18	NA	< 0.006	< 0.004	0.004	0.036	< 0.040
Water Well	ARWOW	05/18/09	> 2,420	> 2,420	> 2,420	NA	< 0.006	< 0.004	< 0.003	0.070	< 0.040

[a] Values reported as "<" are below detection limits

in this sample. Interestingly, the ground water sample from this well also showed the highest levels of nitrite and nitrous oxide detected in any of these samples, indicating that denitrification either had occurred or was occurring in the ground water at this location. Microbial indicators were sporadically detected in moderate to high numbers in both non-impacted and impacted ground water samples (Table 19), and there was no correlation between nitrate and total coliform numbers ($r^2 = -0.0787$). For example, total coliform numbers were > 2,420 cells/100 mL in both the non-impacted well AR32-2 (0.92 mg/L NO_3-N) and the impacted well ARWOW (15.6 mg/L NO_3-N). The greatest numbers of all three microbial indicators were found in the ground water sample from well ARWOW, which had a poor seal (i.e., a barrel over the top) and coincidentally also contained a floating dead rat. For this particular well, these numbers can be readily attributed to an artifact of well integrity rather than a significant ground water contamination event. Arsenic, copper, nickel, and zinc levels were all relatively low in the dairy lagoons and were near or below detection limits in the ground water samples (Table 19). Selenium concentrations were moderate in the dairy lagoons (62-109 µg/L) and appeared to be uniformly distributed in ground water samples (21-79 µg/L), although there was a weak positive correlation ($r^2 = 0.2943$) between ground water nitrate and selenium levels.

Table 20. CAFO Site #4 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date	SDMX[b] (ng/L)	SMZN (ng/L)	ICTET (ng/L)	EICTET (ng/L)	OTET (ng/L)	TET (ng/L)	LINC (ng/L)
Dairy Primary Lagoon	LAG1	05/13/09	354	964	< 10	< 10	2,170	76	< 5
Dairy Primary Lagoon	LAG1(FD)[a]	05/13/09	188	255	57	33	557	< 10	< 5
Dairy Secondary Lagoon	LAG2	05/13/09	620	989	< 10	< 10	2,820	< 10	< 5
Dairy Tertiary Lagoon	LAG3	05/13/09	1,060	1,240	< 10	< 10	1,030	< 10	< 5
Dairy Quaternary Lagoon	LAG4	05/13/09	14,000	599	78	55	375	< 10	117
Monitoring Well	AR5-2	05/18/09	< 5[c]	11	< 10	< 10	< 10	< 10	< 5
Monitoring Well	AR5-3	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Monitoring Well	AR6-1	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Monitoring Well	AR6-1(FD)	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Monitoring Well	AR8-1	06/23/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Monitoring Well	AR8-2	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Monitoring Well	AR31-2	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Water Well	AREOW	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5
Water Well	ARWOW	05/18/09	< 5	< 5	< 10	< 10	< 10	< 10	< 5

[a] FD is field duplicate

[b] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table were not detected in any of these

[c] Values reported as "<" are below reporting limits

Antibiotics, primarily tetracyclines and sulfonamides, were detected in the dairy lagoons, but at levels much less than those observed in swine lagoons (Table 20). Of these, the only antibiotic found in ground water was sulfamethazine, and this was detected in only one sample at a low level (11 ng/L) that was only about twice that of the reporting limit (Table 20). Still, this detection is plausible, since this ground water sample had very high levels of nitrate (Table 18) and in a separate study sulfamethazine was also detected in ground water downgradient from dairy lagoons (Watanabe et al., 2010). Estrogen hormones were found at relatively low concentrations in these dairy lagoons, and were rarely detected in ground water (Table 21). Estrone was detected at very low levels (1.0-1.4 ng/L) in AR8-1 and ARWOW, and each of these wells was located within or adjacent to a center pivot and both were impacted by nitrate (15.6-56.7 mg/L NO_3-N), although the ground water sample from well AR8-1 did not show an animal waste signature (Figure 7). These wells were again sampled in 2011, and no estrogens were detected (< 0.3 ng/L). Collectively, these data show that even though ground water contamination by nitrate occurred due to land application of dairy lagoon effluent at this site, there were few additional stressors associated with the CAFO dairy waste that could consistently be found at high levels along with the correspondingly elevated levels of nitrate.

Table 21. CAFO Site #4 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17α-Ethynylestradiol (ng/L)	Estriol (ng/L)
Dairy Primary Lagoon	LAG1	05/13/09	656	267	107	< 40.0	< 40.0
Dairy Primary Lagoon	LAG1(FD)[a]	05/13/09	670	261	88.0	< 40.0	< 40.0
Dairy Secondary Lagoon	LAG2	05/13/09	613	125	< 40.0	< 40.0	< 40.0
Dairy Tertiary Lagoon	LAG3	05/13/09	549	102	< 40.0	< 40.0	< 40.0
Dairy Quaternary Lagoon	LAG4	05/13/09	452	118	68.6	< 40.0	< 40.0
Monitoring Well	AR5-2	05/18/09	< 1.0[b]	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	AR5-3	05/18/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	AR6-1	05/18/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	AR6-1(FD)	05/18/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	AR8-1	06/23/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	AR8-2	05/18/09	1.4	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	AR31-2	05/18/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	AREOW	05/18/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	ARWOW	05/18/09	1.0	< 1.0	< 1.0	< 1.0	< 1.0
Dairy Primary Lagoon	LAG1	07/12/11	1,450	86.5	101	< 10.0	< 10.0
Dairy Quatenary Lagoon	LAG4	07/12/11	723	< 10.0	< 10.0	< 10.0	< 10.0
Monitoring Well	AR8-2	07/12/11	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Water Well	ARWOW	07/12/11	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

[a] FD is field duplicate

[b] Values reported as "<" are below quantitation limits

3.5 CAFO Site 5 – Swine Combined (Closed)

Case Study Summary. This is the only site that we have studied to date where ground water has been significantly and consistently impacted by contaminants other than nutrients from a CAFO, and in this case represents a very problematic scenario where swine waste from a leaking lagoon has directly contaminated shallow ground water with high levels of ammonium (up to 390 mg/L NH_4-N), nitrate (up to 78 mg/L NO_3-N), orthophosphate (up to 61 mg/L PO_4-P), estrogen hormones (up to 23,800 ng/L estrone), and arsenic (up to 540 µg/L arsenic). We have been monitoring remediation efforts for nutrients at this site for the past nine years, and those results will be published separately. Analytical data for sampling events in 2003, 2005, 2007, 2009, and 2011 are presented in Appendix A.

Site Description. CAFO Site 5 was a farrow-to-wean combined swine facility that has since been closed. Operations began in November 1992 with four sets of barns and a central lagoon. There were two sow/boar units and two nursery units, with a combined

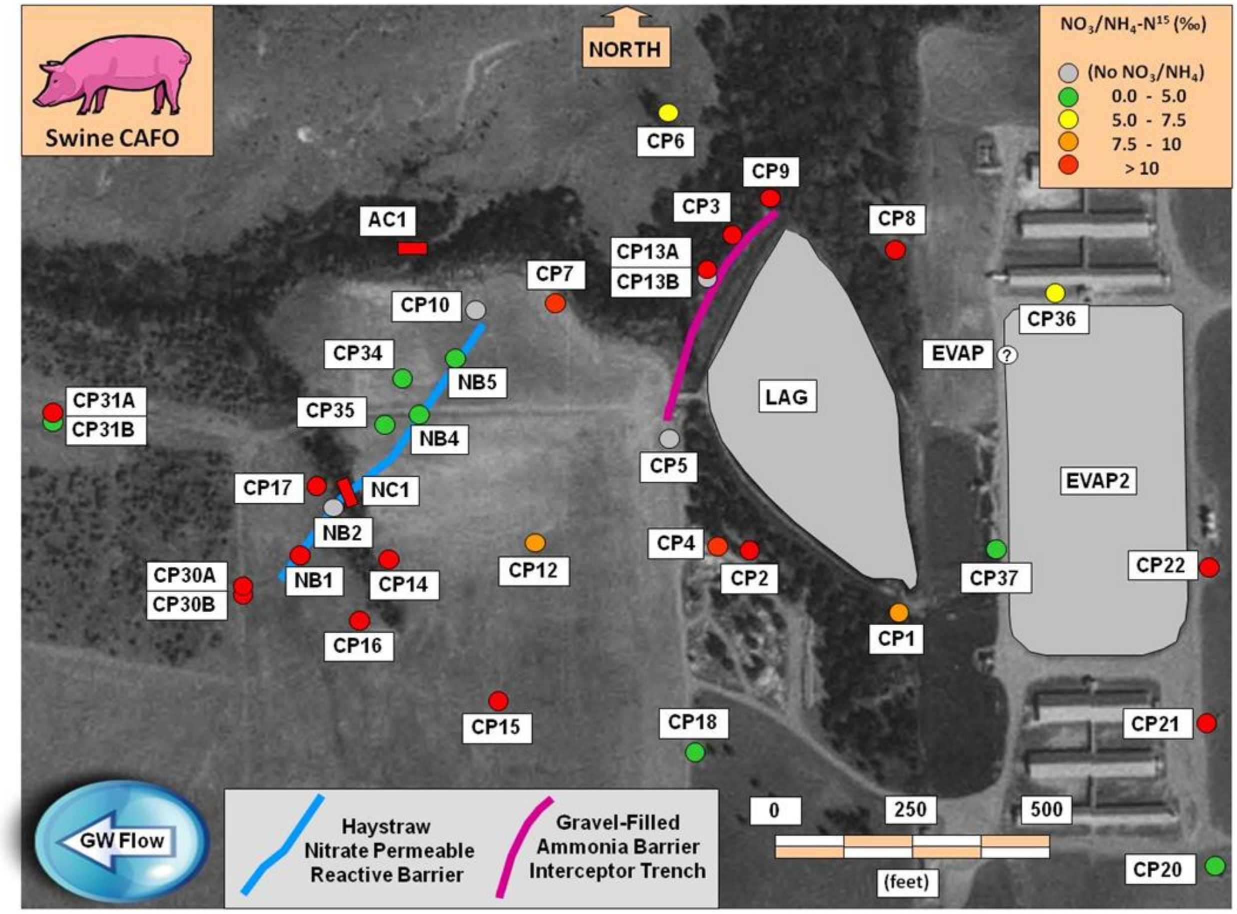


Figure 9. CAFO Site #5 schematic (swine combined operation, since closed). Colors of lagoons and wells correspond to ranges of $\delta^{15}N$ of nitrate or ammonia as shown in legend at upper right, based on 2009 data. Source water (EVAP) to the evaporation basin could not be sampled in 2009 because the recovery wells were not operating at the time.

total of 7,200 feeder pigs, 2,400 sows, and 120 boars. Wastes from the units were pumped into a large lagoon which reportedly had a compacted soil liner and were land applied at various locations around the facility (Figure 9). There are conflicting reports as to the integrity of this liner, but regardless substantial leakage did occur, and in fact so much so that reportedly little land application of the effluent was observed during site operation. Ground water contamination ensued with high levels of ammonium in ground water adjacent to the lagoon and high concentrations of nitrate in ground water further away from the lagoon (unpublished data). The impacted aquifer is a shallow conglomerate of fractured sandstone with interspersed clay and sand lenses, and is underlain by a thick layer of shale approximately 25-50 ft below ground surface. The facility was closed and the animals removed in November 1999, in part because of the ground water contamination issue and in part due to an outbreak of disease among the animals. The lagoon was emptied and the contents were land-applied north of the facility. A remediation action plan was put into place in 2002-2003, and consisted of an interceptor trench for ammonium and a haystraw permeable reactive barrier (PRB) for nitrate. Ammonium was captured using five pumping wells within the gravel-filled interceptor trench to pump ground water contaminated with ammonium to a large evaporation basin (Figure 9), and these pumping wells were started in January 2003 at a combined rate of 7-8 GPM. An additional pumping well was constructed in April 2006 between wells CP2 and CP4 and was operated at 2 GPM to try to reduce high ammonium concentrations at that location. The nitrate PRB was designed to promote in situ denitrification for removal of nitrate and was constructed in January 2003, just four months prior to our first sample event. We have been monitoring this site annually to not only assess long-term performance of the PRB, but also to evaluate ground water quality trends across the site.

<u>General Chemistry and Stable Isotope Interpretation.</u> No original swine lagoon matrix was available for sampling at the start of this study, and so the concentrations of stressors in the source material are unknown. However, these are expected to be similar to the types and concentrations of stressors found in other swine lagoons. In contrast to what was found with the other site operations, ammonium concentrations were quite high in several of these ground water samples, with five wells ranging from 74-361 mg/L NH_4-N in 2003, 80-354 mg/L NH_4-N in 2005, 44-161 mg/L NH_4-N in 2007, 22-74 mg/L NH_4-N in 2009, and 25-137 mg/L NH_4-N in 2011. Full data sets for these sample events are provided in Appendix A, and Tables 22-23 provide example data from the 2009 sampling event for general chemistry parameters, reactive nitrogen, and stable isotopes. With the exception of well CP18 and several wells affected by the denitrifying PRB, all wells showing detectable levels of ammonium had animal waste signatures as evidenced by δ^{15}N-NH_4 values exceeding +10‰ (Figure 9, Table 23). One reason why the δ^{15}N-NH_4 values may have been less in ground water samples taken within and downgradient off the denitrifying PRB is that the ammonium in these samples was not derived from animal wastes, but from ammonification of organic nitrogen within the reactive straw matrix. With few exceptions, nitrate in ground water samples downgradient of the lagoon also

Table 22. CAFO Site #5 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[b]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Swine Primary "Lagoon" [a]	LAG	06/17/09	NA	NA	8.6	0.01	8.3	6.5	0.711	1.37	0.966	8.72	34.8
Evaporation Basin	EVAP2	06/17/09	NA	NA	13.9	0.21	206	< 0.1	0.845	28.6	2.74	147	142
Creek	NC1	06/17/09	NA	NA	3.7	0.01	47.3	7.0	0.051	2.54	0.100	5.01	71.8
Creek	AC1	06/17/09	NA	NA	3.3	0.09	27.1	13.0	0.845	6.47	1.02	6.55	64.9
Monitoring Well	CP1	06/15/09	11.80	24-59	1.4	0.26	30.8	16.3	0.046	0.23	0.085	0.96	60.9
Monitoring Well	CP2	06/15/09	16.26	20-40	2.2	8.17	42.6	29.2	21.3	92.3	20.9	14.3	113
Monitoring Well	CP3	06/15/09	9.23	10-20	0.9	0.04	15.6	6.8	3.26	30.8	3.21	5.21	45.6
Monitoring Well	CP4	06/15/09	19.31	12-42	1.7	1.40	38.2	29.4	12.3	65.4	13.1	11.4	88.9
Monitoring Well	CP5	06/15/09	15.50	12-37	1.8	1.74	156	10.1	0.060	1.39	0.214	7.37	186
Monitoring Well	CP6	06/17/09	19.26	12-32	3.0	0.14	88.2	98.3	0.045	0.61	0.057	2.31	95.4
Monitoring Well	CP7	06/15/09	11.86	10-25	7.2	0.04	118	29.5	0.082	82.4	0.107	10.6	154
Monitoring Well	CP8	06/15/09	11.23	11-36	5.5	< 0.01[c]	25.6	79.7	0.058	0.19	0.082	0.97	47.7
Monitoring Well	CP9	06/15/09	11.39	10-25	1.2	< 0.01	18.0	17.7	0.041	3.31	0.056	1.85	57.4
Monitoring Well	CP10	06/15/09	10.10	8-13	3.2	0.12	20.8	18.6	0.213	0.13	0.140	2.29	33.4
Monitoring Well	CP12	06/15/09	16.81	15-35	1.7	0.20	48.1	21.8	0.068	0.93	0.143	2.51	94.6
Monitoring Well	CP13A	06/15/09	7.85	10-25	0.8	2.59	9.7	3.3	4.15	33.5	5.68	8.95	45.9
Monitoring Well	CP13B	06/15/09	22.08	33-38	6.5	0.07	7.6	9.3	0.201	0.25	0.567	0.24	47.3
Monitoring Well	CP14	06/16/09	11.14	10-30	5.1	< 0.01	24.6	22.3	0.139	0.19	0.143	1.14	50.7
Monitoring Well	CP15	06/16/09	18.53	9-24	7.7	0.06	111	17.0	0.160	0.51	0.214	0.62	53.8
Monitoring Well	CP16	06/16/09	11.63	9-29	5.3	< 0.01	20.4	22.6	0.148	0.08	0.170	1.17	35.9
Monitoring Well	CP17	06/16/09	9.94	9-24	4.7	0.06	29.2	15.4	0.056	0.09	0.056	0.34	51.5
Monitoring Well	CP18	06/17/09	29.46	34-49	9.1	0.41	53.6	37.8	0.224	4.36	0.511	0.73	56.6
Monitoring Well	CP20	06/17/09	13.22	14-24	7.9	< 0.01	6.1	23.0	0.109	< 0.02	0.124	0.26	24.7
Monitoring Well	CP21	06/17/09	14.13	12-22	7.7	< 0.01	52.0	26.2	0.063	< 0.02	0.068	0.37	27.9
Monitoring Well	CP22	06/17/09	15.00	14-24	7.1	< 0.01	75.9	51.0	0.046	0.10	0.067	0.72	42.2
Monitoring Well	CP30A	06/16/09	19.95	26-36	6.7	< 0.01	53.3	20.8	0.100	0.10	0.126	0.36	41.9
Monitoring Well	CP30B	06/16/09	21.57	41-51	7.0	< 0.01	35.5	18.6	0.032	0.27	0.242	0.57	46.4
Monitoring Well	CP31A	06/16/09	15.67	26-36	9.2	0.13	26.2	19.0	0.148	0.10	0.148	0.21	38.4
Monitoring Well	CP31B	06/16/09	28.51	43-53	6.1	0.01	6.8	26.5	0.285	0.37	0.620	0.42	32.2
Monitoring Well	CP34	06/15/09	14.65	8-28	0.8	15.0	106	< 0.1	0.134	17.1	2.40	28.5	163
Monitoring Well	CP35	06/15/09	12.83	8-28	0.7	8.70	98.7	3.5	0.065	11.2	1.06	14.8	122
Monitoring Well	CP36	06/17/09	12.80	24-39	4.8	< 0.01	9.1	23.1	0.097	0.15	0.100	0.46	33.9
Monitoring Well	CP37	06/15/09	9.80	21-36	4.5	0.01	14.6	31.6	0.083	0.09	0.102	0.39	32.7
Nitrate Barrier Well	NB1	06/16/09	14.48	10-25	0.4	3.51	36.6	21.9	0.033	0.54	0.048	1.89	71.7
Nitrate Barrier Well	NB2	06/16/09	4.72	10-25	0.5	11.1	39.5	11.8	0.031	0.46	0.151	3.08	82.8
Nitrate Barrier Well	NB4	06/16/09	12.85	10-25	0.6	14.8	139	2.6	0.060	3.40	0.129	7.81	151
Nitrate Barrier Well	NB5	06/16/09	NA	10-25	0.5	12.9	18.1	1.3	0.034	3.89	1.19	13.9	83.2

[a] Lagoon was emptied in 1999; matrix is primarily runoff and rainfall

[b] Feet from top of casing

[c] Values reported as "<" are below detection limits

showed an animal waste signature (> +10‰ $\delta^{15}N\text{-}NO_3$), although this also occurred in some of the ground water samples taken upgradient of the lagoon (eg, wells CP21 and CP22, Figure 9). In some cases the observed $\delta^{15}N\text{-}NO_3$ values were surely enriched due to denitrification (especially within and downgradient of the denitrifying PRB), but overall the data were too variable to discern general trends of denitrification across the site. This may be because the 2009 sample data reflect site conditions after several years of operation of the denitrifying PRB, and it is no longer as active as it had been in the past. Parts of the PRB in the southwest section are becoming depleted in organic carbon,

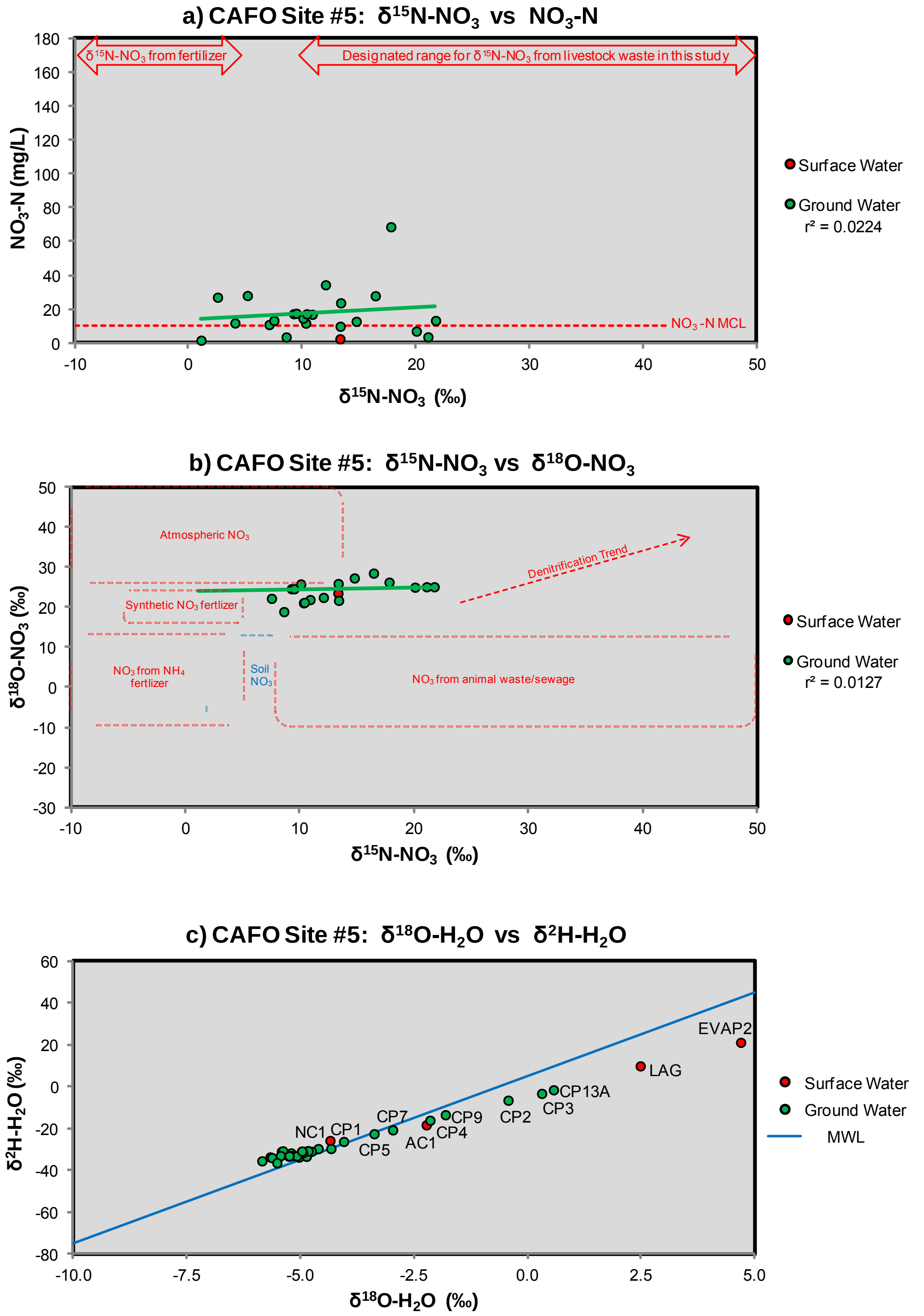
Table 23. CAFO Site #5 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	N_2O-N (mg/L)	$\delta^{2}H$-H_2O (‰)	$\delta^{18}O$-H_2O (‰)	$\delta^{15}N$-NO_3 (‰)	$\delta^{18}O$-NO_3 (‰)	$\delta^{15}N$-NH_4 (‰)
Swine Primary "Lagoon" [a]	LAG	06/17/09	0.02	0.05	0.02	< 0.01	+9.9	+2.5	NA	NA	NA
Evaporation Basin	EVAP2	06/17/09	0.19	0.18	0.08	< 0.01	+21.2	+4.7	NA	NA	NA
Creek	NC1	06/17/09	1.24	0.13	0.15	< 0.01	-25.7	-4.4	NA	NA	+12.5
Creek	AC1	06/17/09	3.50	2.55	0.43	0.01	-18.3	-2.2	+13.3	+23.5	+30.6
Monitoring Well	CP1	06/15/09	< 0.02[b]	3.68	0.02	< 0.01	-26.2	-4.1	+8.6	+18.9	NA
Monitoring Well	CP2	06/15/09	72.8	0.04	0.03	< 0.01	-6.5	-0.4	NA	NA	+13.8
Monitoring Well	CP3	06/15/09	22.4	0.16	0.01	< 0.01	-3.3	+0.3	NA	NA	+18.8
Monitoring Well	CP4	06/15/09	60.1	17.4	0.09	0.11	-16.1	-2.2	+9.3	+24.6	+17.3
Monitoring Well	CP5	06/15/09	0.21	0.03	0.01	< 0.01	-22.5	-3.4	NA	NA	NA
Monitoring Well	CP6	06/17/09	< 0.02	11.0	0.01	0.02	-35.5	-5.9	+7.1	+28.4	NA
Monitoring Well	CP7	06/15/09	73.5	11.8	< 0.01	< 0.01	-20.7	-3.0	+10.3	+21.1	+13.2
Monitoring Well	CP8	06/15/09	< 0.02	17.0	0.04	0.09	-33.6	-5.7	+10.9	+21.9	NA
Monitoring Well	CP9	06/15/09	2.24	7.12	< 0.01	0.01	-13.4	-1.8	+20.1	+25.0	+15.5
Monitoring Well	CP10	06/15/09	0.02	0.02	< 0.01	< 0.01	-29.6	-4.3	NA	NA	NA
Monitoring Well	CP12	06/15/09	0.19	17.6	0.03	0.07	-31.7	-5.2	+9.5	+24.6	NA
Monitoring Well	CP13A	06/15/09	24.6	0.01	0.01	< 0.01	-1.5	+0.6	NA	NA	+17.1
Monitoring Well	CP13B	06/15/09	0.05	0.74	0.08	< 0.01	-31.0	-5.4	NA	NA	NA
Monitoring Well	CP14	06/16/09	< 0.02	9.96	0.03	0.02	-30.7	-5.4	+13.4	+25.9	NA
Monitoring Well	CP15	06/16/09	< 0.02	68.7	0.05	0.03	-32.9	-5.2	+17.8	+26.3	NA
Monitoring Well	CP16	06/16/09	0.05	12.9	0.04	0.02	-33.0	-5.4	+14.8	+27.3	NA
Monitoring Well	CP17	06/16/09	< 0.02	14.7	0.01	0.01	-33.9	-5.6	+10.1	+25.8	NA
Monitoring Well	CP18	06/17/09	3.14	28.1	0.07	0.10	-29.6	-4.6	+5.2	+28.0	-2.3
Monitoring Well	CP20	06/17/09	< 0.02	27.1	0.03	0.01	-31.1	-4.9	+2.6	+30.4	NA
Monitoring Well	CP21	06/17/09	< 0.02	28.0	0.02	0.02	-31.8	-4.9	+16.4	+28.5	NA
Monitoring Well	CP22	06/17/09	< 0.02	13.4	0.01	0.02	-31.1	-5.0	+21.7	+25.1	NA
Monitoring Well	CP30A	06/16/09	< 0.02	34.5	0.02	0.05	-33.7	-5.0	+12.1	+22.5	NA
Monitoring Well	CP30B	06/16/09	0.02	23.8	0.03	0.06	-33.3	-4.9	+13.4	+21.7	NA
Monitoring Well	CP31A	06/16/09	< 0.02	17.3	0.02	0.02	-36.4	-5.5	+10.4	+21.2	NA
Monitoring Well	CP31B	06/16/09	0.03	1.73	0.19	0.01	-33.1	-5.1	+1.1	+21.3	NA
Monitoring Well	CP34	06/15/09	10.2	0.09	0.02	< 0.01	-30.9	-4.8	NA	NA	+3.7
Monitoring Well	CP35	06/15/09	6.22	0.01	0.08	< 0.01	-30.6	-4.8	NA	NA	+4.8
Monitoring Well	CP36	06/17/09	< 0.02	13.4	0.05	0.03	-30.7	-4.8	+7.5	+22.2	NA
Monitoring Well	CP37	06/15/09	< 0.02	11.9	0.04	0.01	-31.6	-5.0	+4.1	+22.5	NA
Nitrate Barrier Well	NB1	06/16/09	< 0.02	3.71	< 0.01	0.10	-33.2	-5.3	+21.1	+25.1	NA
Nitrate Barrier Well	NB2	06/16/09	< 0.02	< 0.01	< 0.01	< 0.01	-33.1	-5.1	NA	NA	NA
Nitrate Barrier Well	NB4	06/16/09	1.50	0.01	< 0.01	< 0.01	-30.8	-5.0	NA	NA	-2.4
Nitrate Barrier Well	NB5	06/16/09	1.67	< 0.01	0.05	< 0.01	-33.1	-5.3	NA	NA	+2.7

[a] Lagoon was emptied in 1999; matrix is primarily runoff and rainfall

[b] Values reported as "<" are below detection limits

which is why ground water samples from well CP17 show moderate DO, moderate nitrate levels, and low TOC values, whereas ground water samples from wells CP34 and CP35 show low DO, low nitrate levels, and high TOC values (Tables 22-23). Regardless, concentrations of nitrite and nitrous oxide were low in almost all of the ground water samples, including those taken from locations which were within or downgradient of the denitrifying PRB (Table 23). This observation illustrates that the absence of detection of these transient intermediates of denitrification does not necessarily mean that this process is not occurring. However, there was also little supporting evidence for denitrification provided by stable isotope relationships (Figure 10). There was no correlation of



a) CAFO Site #5: $\delta^{15}N\text{-}NO_3$ vs $NO_3\text{-}N$

b) CAFO Site #5: $\delta^{15}N\text{-}NO_3$ vs $\delta^{18}O\text{-}NO_3$

c) CAFO Site #5: $\delta^{18}O\text{-}H_2O$ vs $\delta^{2}H\text{-}H_2O$

Figure 10. CAFO Site #5 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for $NO_3\text{-}N$. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

increasing δ^{15}N-NO_3 values with decreasing nitrate concentrations ($r^2 = 0.0224$, Figure 10a), or of increasing δ^{18}O-NO_3 values with increasing δ^{15}N-NO_3 values ($r^2 = 0.0127$, Figure 10b), both of which would indicate denitrification. The δ^{18}O-NO_3 values were also very high in ground water at this site, similar to what was observed for Site #4, and the reason for this is again unclear. But regardless of whether denitrification is enriching δ^{15}N-NO_3 values, the linkage between the lagoon CAFO wastes as the original source and the increased reactive nitrogen concentrations downgradient of the lagoon is unmistakable. Additional evidence for the direct hydraulic connection between the leaking lagoon and the shallow aquifer is provided by water stable isotope data, which show ground water in wells immediately downgradient of the lagoon (CP2, CP3, CP4, CP9, and CP13A) have water stable isotope ratios closer to that of the surface water lagoon compared to that of the other wells (Figure 10c). Interestingly, water stable isotope ratios also provide some insights into the nature of the surface water streams in this area. For example, the surface water sample AC1 shows contamination by ammonium (3.50 mg/L NH_4-N) and it also contains nitrate (2.55 mg/L NO_3-N), both of which have an animal waste signature as shown by δ^{15}N values in excess of +10‰ (Table 23). Although this stream sample does show somewhat of an evaporative signature, its water stable isotope ratio is more closely associated with the impacted ground water wells than with the other surface water bodies (Figure 10c), which indicates that ground water is probably discharging into this stream. This is much more apparent for the stream location NC1 (Figure 10c), which has been observed to emanate directly from ground water discharge during dry weather periods.

Evaluation of Additional Stressor Impact. In contrast to the other sites in this study, very high levels of orthophosphate (3.26-21.3 mg/L PO_4-P) were found in ground water samples from several wells (wells CP2, CP3, CP4, and CP13A) immediately adjacent to the leaking lagoon, and ground water samples from these wells also showed very high ammonium levels which were strongly correlated ($r^2 = 0.9406$) with orthophosphate levels (Tables 22-23). Ground water orthophosphate concentrations ranged from 0.031-0.285 mg/L PO_4-P for the other wells sampled in 2009, and did not correlate with either nitrate ($r^2 = 0.0128$) or ammonium ($r^2 = -0.0028$). Microbial indicator counts were

Table 24. CAFO Site #5 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococci (cells per 100 mL)	As by ICP-MS (µg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Swine Primary "Lagoon" [a]	LAG	06/17/09	> 2,420	3	73	8.0	0.010	< 0.004	< 0.003	< 0.005	< 0.040
Evaporation Basin	EVAP2	06/17/09	> 2,420	26	> 2,420	118	0.140	< 0.004	0.006	0.032	< 0.040
Creek	NC1	06/17/09	> 2,420	1,530	1,990	12.5	< 0.006[b]	< 0.004	< 0.003	0.020	< 0.040
Creek	AC1	06/17/09	> 2,420	866	980	27.3	0.022	< 0.004	< 0.003	0.020	< 0.040
Monitoring Well	CP1	06/15/09	0	0	3	1.2	< 0.006	< 0.004	< 0.003	0.038	< 0.040
Monitoring Well	CP2	06/15/09	0	0	6	122	0.144	< 0.004	0.016	0.059	< 0.040
Monitoring Well	CP3	06/15/09	0	0	0	126	0.129	< 0.004	0.004	0.016	< 0.040
Monitoring Well	CP4	06/15/09	0	0	1	84.4	0.098	< 0.004	0.003	0.142	< 0.040
Monitoring Well	CP5	06/15/09	0	0	0	9.2	0.046	< 0.004	0.016	0.062	< 0.040
Monitoring Well	CP6	06/17/09	3	0	3	1.3	< 0.006	< 0.004	< 0.003	0.050	< 0.040
Monitoring Well	CP7	06/15/09	0	0	0	3.1	0.027	< 0.004	0.012	0.083	< 0.040
Monitoring Well	CP8	06/15/09	194	0	11	1.8	< 0.006	< 0.004	< 0.003	0.014	< 0.040
Monitoring Well	CP9	06/15/09	0	0	0	1.9	< 0.006	< 0.004	< 0.003	0.021	< 0.040
Monitoring Well	CP10	06/15/09	0	0	0	2.6	0.015	0.004	0.003	0.005	0.040
Monitoring Well	CP12	06/15/09	0	0	0	1.5	< 0.006	< 0.004	< 0.003	0.058	< 0.040
Monitoring Well	CP13A	06/15/09	0	0	0	90.8	0.091	< 0.004	< 0.003	0.016	< 0.040
Monitoring Well	CP13B	06/15/09	0	0	0	9.5	0.014	< 0.004	< 0.003	0.020	< 0.040
Monitoring Well	CP14	06/16/09	1	0	0	1.5	< 0.006	< 0.004	< 0.003	0.017	< 0.040
Monitoring Well	CP15	06/16/09	0	0	0	1.4	< 0.006	< 0.004	< 0.003	0.028	< 0.040
Monitoring Well	CP16	06/16/09	0	0	0	1.5	< 0.006	< 0.004	< 0.003	0.013	< 0.040
Monitoring Well	CP17	06/16/09	46	0	0	0.9	< 0.006	< 0.004	< 0.003	0.011	< 0.040
Monitoring Well	CP18	06/17/09	1	0	0	0.9	< 0.006	< 0.004	< 0.003	0.013	< 0.040
Monitoring Well	CP20	06/17/09	0	0	0	2.1	< 0.006	< 0.004	< 0.003	0.013	< 0.040
Monitoring Well	CP21	06/17/09	140	0	0	0.8	< 0.006	< 0.004	< 0.003	0.011	< 0.040
Monitoring Well	CP22	06/17/09	1	0	0	0.6	< 0.006	< 0.004	< 0.003	0.013	< 0.040
Monitoring Well	CP30A	06/16/09	0	0	2	1.2	< 0.006	< 0.004	< 0.003	0.019	< 0.040
Monitoring Well	CP30B	06/16/09	2	0	7	1.2	< 0.006	< 0.004	< 0.003	0.014	< 0.040
Monitoring Well	CP31A	06/16/09	0	0	0	3.9	< 0.006	< 0.004	< 0.003	< 0.005	< 0.040
Monitoring Well	CP31B	06/16/09	0	0	2	8.5	< 0.006	< 0.004	< 0.003	0.005	< 0.040
Monitoring Well	CP34	06/15/09	0	0	0	152	0.158	0.005	0.045	0.072	< 0.040
Monitoring Well	CP35	06/15/09	0	0	0	129	0.170	< 0.004	0.016	0.110	< 0.040
Monitoring Well	CP36	06/17/09	0	0	2	2.6	< 0.006	< 0.004	< 0.003	0.015	< 0.040
Monitoring Well	CP37	06/15/09	0	0	1	1.1	< 0.006	< 0.004	< 0.003	0.017	< 0.040
Nitrate Barrier Well	NB1	06/16/09	0	0	1	1.1	< 0.006	< 0.004	< 0.003	0.091	< 0.040
Nitrate Barrier Well	NB2	06/16/09	0	0	0	0.8	0.012	< 0.004	< 0.003	0.216	< 0.040
Nitrate Barrier Well	NB4	06/16/09	12	1	0	2.1	0.026	< 0.004	< 0.003	0.098	< 0.040
Nitrate Barrier Well	NB5	06/16/09	> 2,420	488	3	15.6	0.065	< 0.004	< 0.003	0.106	< 0.040

[a] Lagoon was emptied in 1999; matrix is primarily runoff and rainfall

[b] Values reported as "<" are below detection limits

generally low to moderate throughout the study (Appendix A), although there were sporadic high counts in certain wells now and again, as illustrated for the 2009 data in Table 24. Fecal enterococci counts were initially very high (> 2,420 cells/100 mL) in the PRB wells NB1-NB5, presumably due to animal manure that may have been present in the square and round bales used for the PRB, but these values dropped rapidly with time (Appendix A). However, this does indicate that caution should be used in evaluating additional stressors in ground water downgradient of the PRB, since the source of these may not be the original swine CAFO waste. It does seems likely that arsenic, and to a lesser extent selenium, was present at moderate to high concentrations in the original swine waste, because ground water concentrations of these two metalloids in 2009 were elevated in the downgradient wells immediately adjacent to the lagoon (Table 24), and these concentrations were even higher in 2003 (Appendix A). Both of these compounds are listed as feed additives for swine (Luce and Maxwell, 1991), and could be expected to be in the original swine waste. For the 2009 sample analyses, elevated arsenic concentrations as measured by ICP-OES were confirmed by ICP-MS (Table 24). Although swine waste is the most likely source of the elevated arsenic and selenium levels observed in these impacted wells, it is also possible that infiltration of liquid swine waste also affected oxidation-reduction potential and helped mobilize naturally-occurring arsenic and selenium from the subsurface matrix. Surprisingly, there was little evidence of veterinary antibiotics in any of the ground water samples from either the 2003 or 2009 sampling events (Table 25). Sulfamethoxazole was detected in 2003 in one upgradient well (Table 25), but at a level (80 ng/L) close to the reporting limit (50 ng/L), and this finding is somewhat questionable because sulfamethoxazole is not generally used in swine CAFOs (Pruden, 2009b). Lincomycin was detected in 2009 in two impacted wells, although in one case the value (6 ng/L) was close to the detection limit (5 ng/L) here as well. Based on information from site personnel, antibiotics were routinely used in this swine CAFO, but specifics regarding identity and concentration were not available. Conversely, estrogen hormones were routinely detected in the most impacted wells throughout this study, and often at concentrations well above the quantitation limits (Table 26). For the 2009 data, there is a moderate positive correlation between ground water ammonium concentrations and total estrogen levels ($r^2 = 0.4563$), and an even better correlation between ground water ammonium concentrations and logarithmic values of total estrogen levels ($r^2 = 0.8433$). Unlike as has been observed elsewhere (Kolodziej and Sedlak, 2007), these ground water estrogens have been consistently detected and concentrations are generally decreasing with time (Figure 11). Well CP7 is an exception, but this may be because it is further away from the source and is more subject to variations in ground water flow. Ground water flow fluctuates at this site in response to rainfall events, and water table elevations have changed by as much as five feet over the course of this study. In addition, the pumping wells for the ammonium

Table 25. CAFO Site #5 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date One	SMOX[c] (ng/L)	Sample Date Two	LINC (ng/L)
Swine Primary "Lagoon" [a]	LAG	05/22/03	< 50[d]	06/17/09	< 5
Evaporation Basin	EVAP2	NA	NA	06/17/09	< 5
Creek	NC1	05/28/03	< 50	06/17/09	< 5
Creek	AC1	05/28/03	< 50	06/17/09	< 5
Monitoring Well	CP1	05/20/03	< 50	06/15/09	< 5
Monitoring Well	CP2	05/22/03	< 50	06/15/09	< 5
Monitoring Well	CP2(FD)[a]	05/22/03	< 50	NA	NA
Monitoring Well	CP3	05/22/03	< 50	06/15/09	30
Monitoring Well	CP4	05/22/03	< 50	06/15/09	< 5
Monitoring Well	CP5	05/22/03	< 50	06/15/09	6
Monitoring Well	CP6	05/21/03	< 50	NA	NA
Monitoring Well	CP7	05/22/03	< 50	06/15/09	< 5
Monitoring Well	CP8	05/21/03	80	NA	NA
Monitoring Well	CP9	05/21/03	< 50	NA	NA
Monitoring Well	CP10	05/20/03	< 50	NA	NA
Monitoring Well	CP12	05/21/03	< 50	NA	NA
Monitoring Well	CP13A	05/22/03	< 50	06/15/09	< 5
Monitoring Well	CP13B	05/22/03	< 50	NA	NA
Monitoring Well	CP13B(FD)	05/22/03	< 50	NA	NA
Monitoring Well	CP14	05/21/03	< 50	NA	NA
Monitoring Well	CP15	05/21/03	< 50	NA	NA
Monitoring Well	CP16	05/21/03	< 50	NA	NA
Monitoring Well	CP17	05/21/03	< 50	NA	NA
Monitoring Well	CP18	05/21/03	< 50	NA	NA
Monitoring Well	CP20	05/20/03	< 50	NA	NA
Monitoring Well	CP21	05/20/03	< 50	NA	NA
Monitoring Well	CP22	05/21/03	< 50	NA	NA
Monitoring Well	CP30A	05/19/03	< 50	NA	NA
Monitoring Well	CP30B	05/19/03	< 50	NA	NA
Monitoring Well	CP31A	05/19/03	< 50	NA	NA
Monitoring Well	CP31B	05/19/03	< 50	NA	NA
Monitoring Well	CP34	05/21/03	< 50	NA	NA
Monitoring Well	CP35	05/21/03	< 50	NA	NA
Nitrate Barrier Well	NB1	05/28/03	< 50	NA	NA
Nitrate Barrier Well	NB2	05/28/03	< 50	NA	NA
Nitrate Barrier Well	NB4	05/28/03	< 50	06/16/09	< 5
Nitrate Barrier Well	NB5	05/28/03	< 50	06/16/09	< 5

[a] Lagoon was emptied in 1999; matrix is primarily runoff and rainfall

[b] FD is field duplicate

[c] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table were not detected in any of these samples

[d] Values reported as "<" are below reporting limits

interceptor trench were shut down for about a year starting in mid-July 2007. Although estrogen levels are dropping, it may be several years before 17β-estradiol and estrone drop below the PNECs of 1.0 ng/L and 3-5 ng/L, respectively, as established by Young et al. (2004). In summary, there is good evidence that this swine operation resulted in contamination of ground water by not only nitrate and ammonium, but also by orthophosphate, arsenic, and estrogen hormones. It should be emphasized that this occurred primarily through a direct hydraulic connection between the leaking lagoon and

Table 26. CAFO Site #5 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17α-Ethynylestradiol (ng/L)	Estriol (ng/L)
Swine Primary "Lagoon" [a]	LAG	05/22/03	< 1.0[c]	NA	< 1.0	< 1.0	< 1.0
Evaporation Basin	EVAP2	05/22/03	NA	NA	NA	NA	NA
Creek	NC1	05/28/03	< 1.0	NA	< 1.0	< 1.0	< 1.0
Creek	AC1	05/28/03	< 20	NA	< 20	< 20	< 20
Monitoring Well	CP1	05/20/03	22.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CP2	05/22/03	11,200	NA	40.9	< 20	824
Monitoring Well	CP2(FD)[b]	05/22/03	3,800	NA	24.0	< 20	732
Monitoring Well	CP3	05/22/03	1,020	NA	< 20	< 20	283
Monitoring Well	CP4	05/22/03	1,310	NA	20.8	< 20	597
Monitoring Well	CP7	05/22/03	47.8	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CP13A	05/22/03	941	NA	21.9	< 20	195
Monitoring Well	CP34	05/21/03	< 1.0	NA	< 1.0	< 1.0	< 1.0
Monitoring Well	CP35	05/21/03	< 1.0	NA	< 1.0	< 1.0	< 1.0
Swine Primary "Lagoon"	LAG	06/17/09	1.2	< 1.0	< 1.0	< 1.0	< 1.0
Evaporation Basin	EVAP2	06/17/09	13.8	< 2.0	< 2.0	< 2.0	< 2.0
Creek	NC1	06/17/09	39.0	< 1.0	< 1.0	NC (< 1.6)	< 1.0
Creek	AC1	06/17/09	7.1	< 1.0	1.5	< 1.0	< 1.0
Monitoring Well	CP1	06/15/09	6.9	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CP2	06/15/09	622	< 2.0	4.9	NC (< 4.6)	41.5
Monitoring Well	CP3	06/15/09	1.4	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	CP4	06/15/09	152	< 1.0	3.1	NC (< 3.2)	23.6
Monitoring Well	CP7	06/15/09	73.2	< 1.0	6.9	< 1.0	11.3
Monitoring Well	CP13A	06/15/09	19.1	< 1.0	1.1	NC (< 2.1)	6.4
Monitoring Well	CP34	06/15/09	11.2	< 1.0	1.4	NC (< 14.6)	8.9
Monitoring Well	CP35	06/15/09	NA	7.9	2.5	NC (< 3.4)	8.7
Swine Primary "Lagoon"	LAG	06/20/11	NC (< 4.5)[d]	< 0.3	< 0.3	< 0.3	< 0.3
Evaporation Basin	EVAP2	06/20/11	4.3	< 0.3	< 0.3	< 0.3	< 0.3
Creek	NC1	NA	NA	NA	NA	NA	NA
Creek	AC1	06/20/11	3.8	NC (< 1.0)	NC (< 1.2)	< 0.3	< 0.3
Monitoring Well	CP1	06/21/11	1.5	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CP2	06/21/11	216	NC (< 14.8)	NC (< 1.8)	< 0.3	< 0.3
Monitoring Well	CP3	06/21/11	1.6	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CP4	06/21/11	388	NC (< 5.7)	NC (< 2.1)	NC (< 1.0)	< 0.3
Monitoring Well	CP4(FD)	06/21/11	388	NC (< 5.7)	NC (< 1.8)	NC (< 1.0)	< 0.3
Monitoring Well	CP7	06/21/11	22.9	NC (< 3.4)	< 0.3	< 0.3	< 0.3
Monitoring Well	CP13A	06/21/11	4.7	< 0.3	< 0.3	< 0.3	< 0.3
Monitoring Well	CP34	06/20/11	12.0	< 0.3	NC (< 0.4)	< 0.3	< 0.3
Monitoring Well	CP34(FD)	06/20/11	12.1	< 0.3	NC (< 0.5)	< 0.3	< 0.3
Monitoring Well	CP35	06/20/11	98.9	1.9	NC (< 1.5)	< 0.3	< 0.3

[a] Lagoon was emptied in 1999; matrix is primarily runoff and rainfall

[b] FD is field duplicate

[c] Values reported as "<" are below quantitation limits

[d] NC is not confirmed due to interference; if analyte is present, it is below estimate shown in parantheses

ground water, and these results may not hold true for other sites where ground water quality is only impacted through over-application of CAFO wastes.

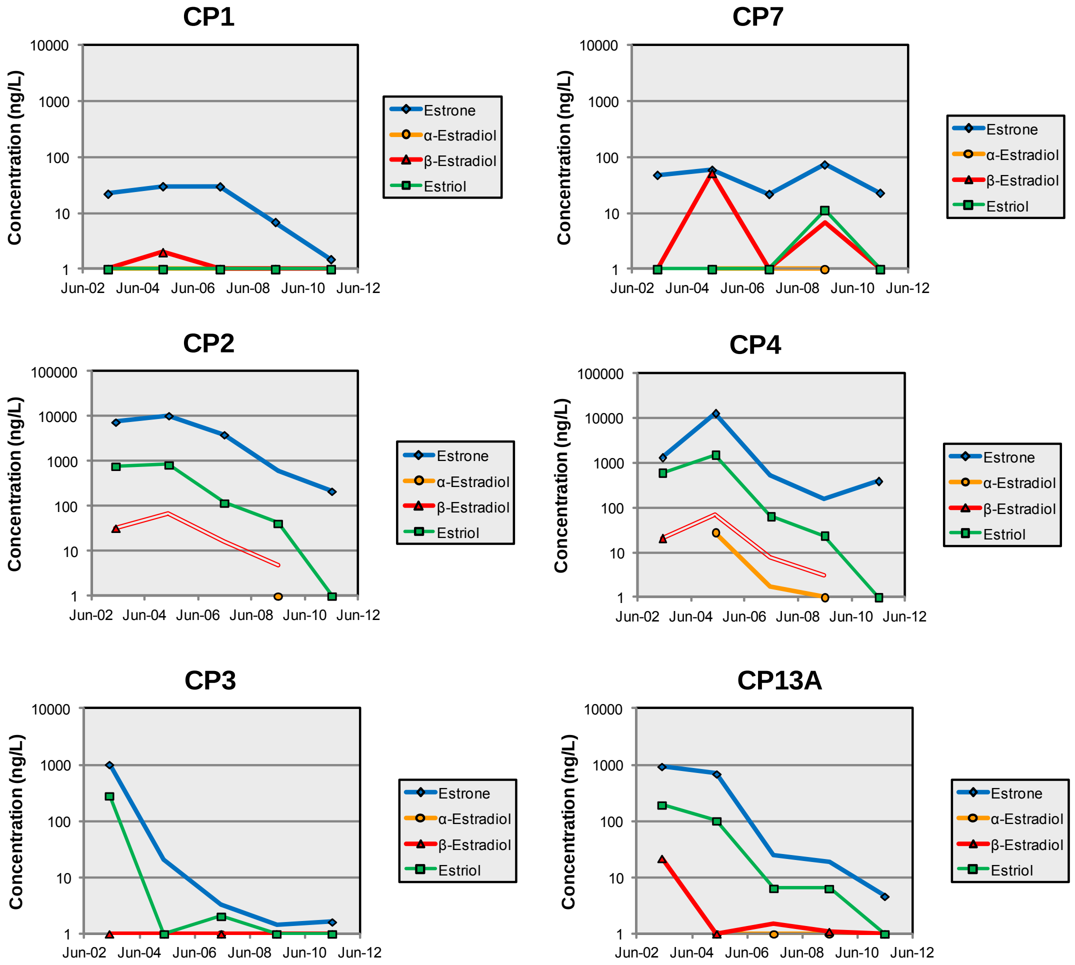


Figure 11. CAFO Site #5 ground water estrogen concentrations at selected well locations over time. Concentrations less than 1 ng/L were set at 1 ng/L to accommodate logarithmic scale.

3.6 CAFO Site 6 - Beef Feedlot

Case Study Summary. Nitrate contamination is pervasive in the ground water at this beef feedlot site, with an average concentration of 64 mg/L NO_3-N (29 wells) and a maximum value of 167 mg/L NO_3-N. Nitrate most likely derives from several CAFO waste sources at this site, including the feedlot, lagoons, land application areas, and underground piping. However, we found very little evidence of ground water contaminants other than nitrate (and occasionally ammonium). In a few impacted wells there were slightly elevated

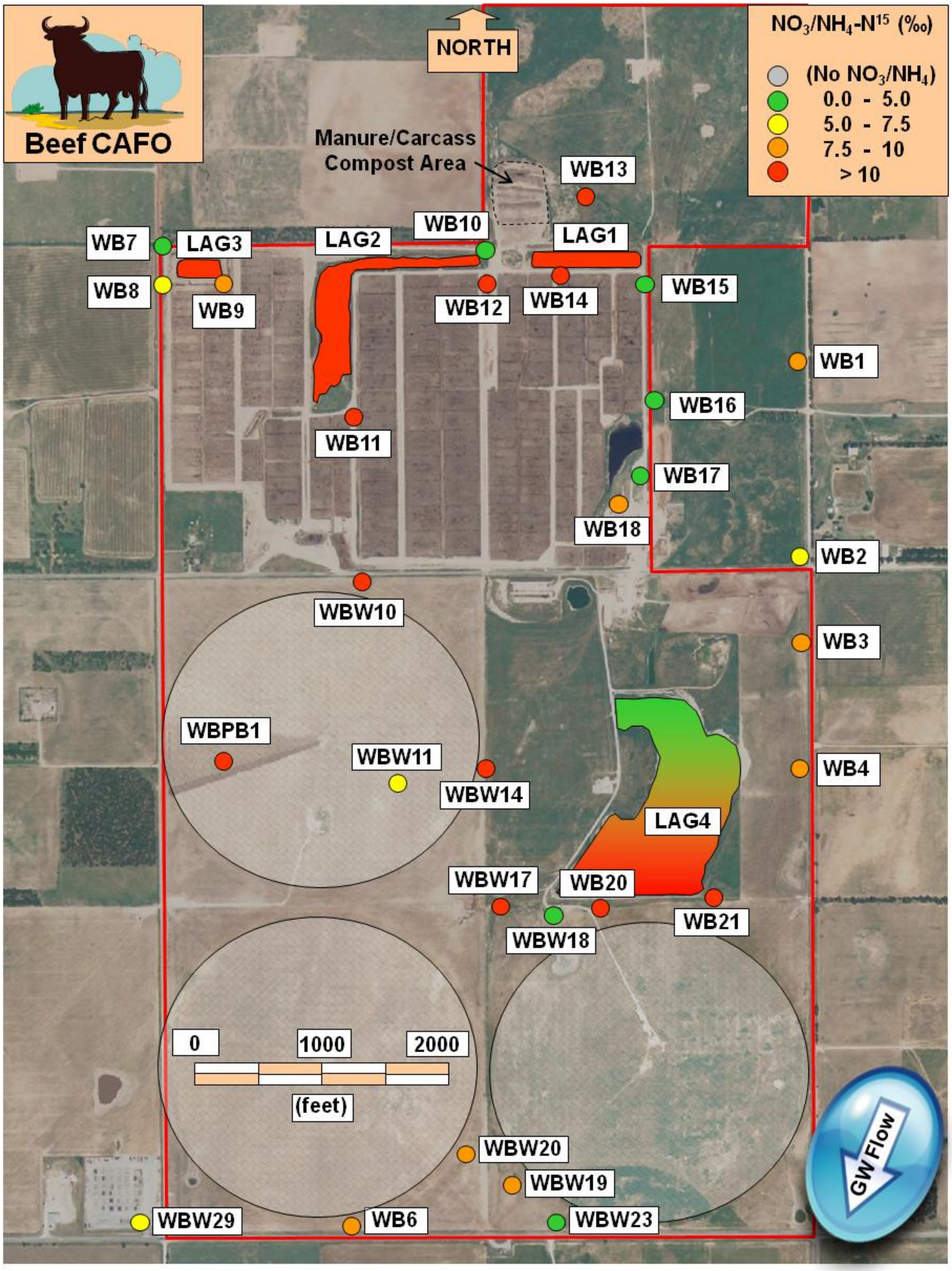


Figure 12. CAFO Site #6 schematic (beef feedlot operation). Colors of lagoons (2006-2009) and wells (2009) correspond to ranges of δ15N of nitrate or ammonia as shown in legend at upper right. Lagoon LAG4 shows gradient fill based on three sample events (2006, 2008, and 2009).

levels of nickel and selenium, but there was no consistent animal waste signature based on the stable nitrate isotope data for these samples. In ground water samples obtained from one poorly-sealed well the antibiotic sulfamethazine was detected at very low levels (14-17 ng/L) in both field duplicates. Estrone was also detected in this same well at very low levels (1.8-2.3 ng/L) in both field duplicate ground water samples, and a follow-up sample event two years later again showed that this well contained estrone, this time at 1.1 ng/L in each field duplicate ground water sample.

Site Description. CAFO Field Site 6 is a beef feedlot operation that was started in the 1940's, but at a much smaller scale at that time. Since that time it has expanded to include about 27,000 cattle. There is an area set aside for composting of manure and carcasses, and there are unlined lagoons constructed around the pens which function more like runoff retention ponds rather than anaerobic lagoons (Figure 12). In fact some of these lagoons are supersaturated in dissolved oxygen (12-22 mg/L O_2) due to the presence of algae. These lagoons (LAG1-3) receive runoff from the pens and are pumped to a large secondary lagoon (LAG4) located south of the feedlot. LAG4 also receives ground water as make-up water so that this lagoon effluent can be used for irrigation. Effluent is also pumped from lagoons LAG2 and LAG3 to center pivots and land applied to irrigate and fertilize grasses and corn crops that are grown for grazing and/or feed. The topsoil is sandy, with mixed sands and clay lenses in the vadose zone grading to general sands in the aquifer, underlain by red bedrock at about 65 ft.

General Chemistry and Stable Isotope Interpretation. The exact source of CAFO-derived nitrate contamination in the ground water is difficult to ascertain at this site, because there were many possible avenues, including manure/carcass composting, leaking runoff retention ponds and lagoons, leaking piping infrastructure, infiltration from the feedlot itself, and over-application of solids as well as lagoon effluent. This may be why there appeared to be localized trends in the ground water chemistry, but no overall correlation of chloride, sulfate, TOC, or $\delta^{15}N$ values with nitrate concentrations (Tables 27-28). For example, ground water samples from the impacted wells WB11-WB14 had moderate to high nitrate concentrations (29-148 mg/L NO_3-N) and high chloride levels (138-666

Table 27. CAFO Site #6 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[b]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Beef Secondary Lagoon	LAG4-1[a]	04/10/06	NA	NA	0.1	0.34	365	275	8.53	62.7	13.9	155	226
Beef Secondary Lagoon	LAG4-2	04/10/06	NA	NA	0.1	0.34	321	249	7.28	63.6	13.5	149	230
Beef Secondary Lagoon	LAG4-3	04/10/06	NA	NA	0.2	0.35	314	239	7.77	63.5	13.7	160	235
Beef Primary Lagoon	LAG1	02/12/08	NA	NA	1.7	0.33	160	262	7.70	49.6	14.7	81.0	138
Beef Secondary Lagoon	LAG4	02/12/08	NA	NA	3.1	< 0.01[c]	330	277	8.01	46.8	13.9	141	176
Beef Primary Lagoon	LAG1	05/26/09	NA	NA	22.0	1.05	166	175	9.65	16.9	105	107	273
Beef Primary Lagoon	LAG2	05/26/09	NA	NA	8.3	0.39	294	298	0.808	84.3	9.96	161	239
Beef Primary Lagoon	LAG3	05/26/09	NA	NA	1.8	2.25	414	13.3	22.3	159	24.1	582	310
Beef Secondary Lagoon	LAG4	05/26/09	NA	NA	11.9	0.14	387	376	5.66	55.5	7.14	136	147
Monitoring Well	WB1	05/26/09	27.78	31-45	6.8	0.04	10.8	1,170	0.120	0.70	0.287	1.98	7.3
Monitoring Well	WB2	05/26/09	26.12	34-48	5.3	< 0.01	18.5	275	0.084	0.53	0.267	1.08	12.6
Monitoring Well	WB3	05/26/09	27.19	33-47	8.1	< 0.01	22.9	119	0.048	0.42	0.090	1.28	14.2
Monitoring Well	WB4	05/26/09	22.80	32-46	6.2	< 0.01	80.1	138	0.089	0.82	0.302	2.07	27.2
Monitoring Well	WB6	05/26/09	20.77	12-27	8.6	< 0.01	153	213	0.228	0.87	0.283	3.87	13.2
Monitoring Well	WB7	05/27/09	29.37	31-45	7.0	< 0.01	67.0	601	0.109	0.64	0.380	1.90	15.6
Monitoring Well	WB8	05/27/09	21.44	21-36	8.2	< 0.01	32.8	594	0.100	0.56	0.285	0.95	10.5
Monitoring Well	WB9	05/27/09	21.48	17-32	2.9	0.25	1,260	1,170	0.095	1.11	0.304	4.01	26.3
Monitoring Well	WB10	05/27/09	27.26	26-40	6.7	0.01	73.1	68.2	0.082	0.75	0.106	4.61	29.3
Monitoring Well	WB11	05/27/09	24.60	27-42	5.3	0.02	139	85.2	0.096	0.71	0.133	2.49	19.6
Monitoring Well	WB12	05/27/09	24.27	28-43	3.1	0.18	200	963	0.038	1.00	0.090	5.09	35.2
Monitoring Well	WB13	05/27/09	23.87	22-36	7.4	0.01	138	107	0.068	0.75	0.110	3.72	46.8
Monitoring Well	WB14	05/27/09	23.70	22-37	1.9	4.73	666	1,720	0.068	3.35	0.175	27.4	96.2
Monitoring Well	WB15	05/27/09	27.27	25-39	3.8	0.02	61.0	490	0.152	0.47	0.148	1.38	38.2
Monitoring Well	WB16	05/27/09	26.29	33-47	4.1	< 0.01	89.1	1,140	0.119	0.76	0.214	2.77	18.0
Monitoring Well	WB17	05/27/09	30.00	32-47	0.6	0.02	183	110	0.333	0.58	0.334	1.82	48.5
Monitoring Well	WB18	05/27/09	27.68	27-41	0.8	0.02	251	616	0.122	0.60	0.132	2.15	41.6
Monitoring Well	WB20	05/28/09	18.50	8-22	2.3	0.13	370	75.5	0.394	15.4	0.434	45.7	151
Monitoring Well	WB21	05/27/09	28.16	28-42	5.8	< 0.01	107	740	0.172	0.89	0.188	3.63	43.9
Pivot Boring	WBPB1	05/28/09	25.10	28-42	5.5	< 0.01	130	286	0.214	0.65	0.196	2.04	17.6
Water Well	WBW10	05/28/09	30.85	NA	0.2	12.2	265	< 0.1	0.090	20.3	0.136	4.73	35.1
Water Well	WBW11	05/30/09	21.69	NA	3.9	0.01	348	339	0.205	0.64	0.194	2.43	13.1
Water Well	WBW14	05/28/09	NA	NA	1.6	< 0.01	247	274	0.051	1.01	0.085	5.39	54.7
Water Well	WBW17	05/30/09	15.00	NA	2.9	0.24	118	173	0.074	0.42	0.102	1.51	34.6
Water Well	WBW18	05/28/09	22.01	NA	0.3	0.31	282	1.0	0.162	2.22	0.195	9.05	93.9
Water Well	WBW19	05/28/09	NA	NA	7.5	< 0.01	227	236	0.056	0.16	0.082	2.67	15.4
Water Well	WBW20	05/28/09	NA	NA	2.2	< 0.01	267	259	0.077	0.69	0.089	2.62	20.8
Water Well	WBW23	05/28/09	20.11	NA	5.2	< 0.01	248	256	0.076	0.60	0.114	2.33	19.0
Water Well	WBW29	05/28/09	NA	NA	1.2	< 0.01	240	325	0.039	0.72	0.081	2.81	23.7

[a] One of three sampling locations in this lagoon

[b] Feet from top of casing

[c] Values reported as "<" are below detection limits

mg/L Cl) and showed an animal waste signature (δ^{15}N-NO_3 > +10‰), as might be expected. But ground water samples from other impacted wells in this area (WB10, WB15-WB17) also had very high nitrate concentrations (78-167 mg/L NO_3-N), and yet had generally lower chloride levels (61-183 mg/L Cl) and no animal waste signature (δ^{15}N-NO_3 < 5‰). Another example is that ground water samples from wells WB9 and WB14 had very high levels of sulfate (1,170-1,720 mg/L SO_4) and chloride (66-1,260

Table 28. CAFO Site #6 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N	NO_3-N	NO_2-N	N_2O-N	$\delta^{2}H$-H_2O	$\delta^{18}O$-H_2O	$\delta^{15}N$-NO_3	$\delta^{18}O$-NO_3	$\delta^{15}N$-NH_4
			(mg/L)	(mg/L)	(mg/L)	(mg/L)	(‰)	(‰)	(‰)	(‰)	(‰)
Beef Secondary Lagoon	LAG4-1[a]	04/10/06	30.5	< 0.01	0.24	< 0.01	-16.6	-2.1	NA	NA	+25.4
Beef Secondary Lagoon	LAG4-2	04/10/06	35.5	< 0.01	0.21	< 0.01	-16.8	-1.7	NA	NA	+26.1
Beef Secondary Lagoon	LAG4-3	04/10/06	32.1	< 0.01	0.24	< 0.01	-16.2	-1.7	NA	NA	+25.9
Beef Primary Lagoon	LAG1	02/12/08	31.3	8.08	0.13	< 0.01	-31.7	-5.0	+14.2	7.5	+19.8
Beef Secondary Lagoon	LAG4	02/12/08	21.0	0.58	0.33	< 0.01	-26.7	-3.9	NA	NA	+20.4
Beef Primary Lagoon	LAG1	05/26/09	48.3	0.46	0.43	< 0.01	-17.1	-1.2	NA	NA	+17.6
Beef Primary Lagoon	LAG2	05/26/09	49.6	0.14	0.17	< 0.01	-16.2	-0.9	NA	NA	+26.6
Beef Primary Lagoon	LAG3	05/26/09	90.6	0.22	0.23	< 0.01	-18.3	-1.1	NA	NA	+20.6
Beef Secondary Lagoon	LAG4	05/26/09	19.2	0.05	0.12	< 0.01	-12.8	-0.3	NA	NA	+3.8
Monitoring Well	WB1	05/26/09	0.09	34.6	0.08	< 0.01	-38.0	-5.6	+8.7	+10.6	NA
Monitoring Well	WB2	05/26/09	0.05	35.9	0.06	< 0.01	-36.9	-5.2	+5.0	-5.1	NA
Monitoring Well	WB3	05/26/09	0.03	74.7	0.02	0.01	-27.3	-3.5	+8.1	+3.1	NA
Monitoring Well	WB4	05/26/09	0.07	70.8	0.07	0.03	-36.1	-5.1	+9.7	+2.0	NA
Monitoring Well	WB6	05/26/09	0.06	81.7	0.12	0.01	-28.6	-3.7	+9.5	-16.6	NA
Monitoring Well	WB7	05/27/09	0.13	15.0	0.05	0.01	-41.5	-5.6	+3.4	+7.6	NA
Monitoring Well	WB8	05/27/09	0.05	50.4	0.10	0.01	-33.0	-5.3	+6.1	+15.2	NA
Monitoring Well	WB9	05/27/09	0.05	73.5	0.02	0.18	-33.9	-4.7	+7.8	+16.8	NA
Monitoring Well	WB10	05/27/09	0.04	78.3	0.06	0.04	-36.4	-4.9	+4.1	+20.7	NA
Monitoring Well	WB11	05/27/09	0.02	29.0	0.01	0.02	-35.4	-4.2	+13.5	+1.3	NA
Monitoring Well	WB12	05/27/09	< 0.02[b]	88.6	0.02	< 0.01	-35.7	-4.5	+15.8	+17.8	NA
Monitoring Well	WB13	05/27/09	0.03	82.1	0.07	0.07	-39.3	-5.0	+18.2	+18.8	NA
Monitoring Well	WB14	05/27/09	0.07	148	0.08	0.08	-30.4	-3.2	+22.3	+12.2	NA
Monitoring Well	WB15	05/27/09	0.05	91.6	0.07	0.03	-44.3	-6.1	+4.9	+6.8	NA
Monitoring Well	WB16	05/27/09	< 0.02	167	0.05	0.01	-37.7	-5.0	+4.7	+11.5	NA
Monitoring Well	WB17	05/27/09	< 0.02	97.3	0.11	0.07	-39.3	-5.1	+2.4	+11.6	NA
Monitoring Well	WB18	05/27/09	< 0.02	140	0.03	0.29	-39.8	-4.9	+9.3	+1.3	NA
Monitoring Well	WB20	05/28/09	8.94	40.3	0.29	0.01	-18.0	-1.1	+39.9	+16.8	+30.8
Monitoring Well	WB21	05/27/09	< 0.02	43.6	0.09	0.03	-31.8	-4.2	+10.2	+4.1	NA
Pivot Boring	WBPB1	05/28/09	< 0.02	23.0	0.10	0.02	-23.2	-3.0	+18.2	+5.1	NA
Water Well	WBW10	05/28/09	20.3	0.05	0.06	< 0.01	-31.1	-4.1	NA	NA	+11.6
Water Well	WBW11	05/30/09	< 0.02	30.9	< 0.01	0.01	-29.9	-4.6	+5.3	-2.2	NA
Water Well	WBW14	05/28/09	< 0.02	60.6	0.02	0.04	-32.9	-4.0	+30.0	+10.7	NA
Water Well	WBW17	05/30/09	< 0.02	30.9	0.01	0.17	-34.3	-4.6	+10.8	+5.8	NA
Water Well	WBW18	05/28/09	0.75	28.5	0.03	0.27	-26.6	-3.0	+4.2	+13.4	NA
Water Well	WBW19	05/28/09	< 0.02	66.0	0.01	0.06	-30.3	-4.0	+9.0	-5.3	NA
Water Well	WBW20	05/28/09	< 0.02	60.1	0.01	0.16	-31.0	-3.8	+9.1	+11.1	NA
Water Well	WBW23	05/28/09	< 0.02	69.0	0.01	0.08	-32.0	-4.1	+4.4	+4.6	NA
Water Well	WBW29	05/28/09	< 0.02	46.3	0.01	0.06	-29.0	-3.8	+5.7	+8.2	NA

[a] One of three sampling locations in this lagoon

[b] Values reported as "<" are below detection limits

mg/L Cl), whereas ground water samples from wells WB1 and WB16 also showed very high sulfate levels (1,140-1,170 mg/L SO_4) and very low chloride levels (11-89 mg/L Cl) compared to those of most of the other wells (Table 27). There was also no correlation between ground water nitrate and chloride levels ($r^2 = 0.0259$). Of the 28 wells with ground water nitrate exceeding the MCL of 10 mg/L NO_3-N, about 25% had borderline animal waste signatures ($\delta^{15}N$ = +8‰ to +10‰) and about 30% had definitive animal waste signatures ($\delta^{15}N > +10‰$). One well (WBW10) represented an anomaly in that the ground water sample from this well had very low nitrate (0.05 mg/L NO_3-N), but had the highest levels of ammonium (20.3 mg/L NH_4-N) and methane (12.2 mg/L) compared to those of any of the other wells (Tables 27-28). Because this well is not located adjacent to a lagoon (Figure 12), it is difficult to understand why this ammonium level would be so high, since these levels are typically seen only in ground water directly impacted by leaking lagoons. Furthermore, the ground water samples from wells WB11 and WB12, located between the upgradient lagoon LAG2 and this well, had very low ammonium levels (< 0.03 mg/L NH_4-N). Well WBW10 was a large-diameter well with a loose fitting steel plate as a cover, and site personnel suspect that an animal might have fallen into this well in the past. But it is also possible that there could have been leakage somewhere along the pipeline transferring effluent from lagoons LAG2 and LAG3 to the land application areas.

The impact of denitrification is also hard to assess at this site. Several ground water samples had low but definitive levels of nitrite and nitrous oxide (e.g., > 0.10 mg/L as N) showing that denitrification was occurring (Table 28). However, DO values were moderate to high and TOC values were less than 5 mg/L in over 80% of the ground water samples (Table 27), and so it is unlikely that denitrification would have reduced nitrate concentrations or enriched $\delta^{15}N$ values to any significant extent. This variability which potentially arises from multiple source terms also complicates the interpretation of stable isotope relationships, which seemed to provide little evidence of denitrification (Figure 13). There is no correlation ($r^2 = -0.0003$) between ground water $\delta^{15}N$-NO_3 values and nitrate concentrations (Figure 13a), and the positive correlation between ground water $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values (Figure 13b) is very weak ($r^2 = 0.0541$). The observed variability in the ground water chemistry is exacerbated by the variability in ground water age among the wells sampled, as shown by the wide range of $\delta^{18}O$-H_2O values in these samples (Figure 13c). Interestingly, well WB20 is more closely grouped with the surface water lagoons than with any of the other wells and shows a definitive evaporative signature (Figure 13c). This well has a very shallow screen interval compared to the other wells (Table 27) and is located adjacent to LAG4 (Figure 12), and its ground water

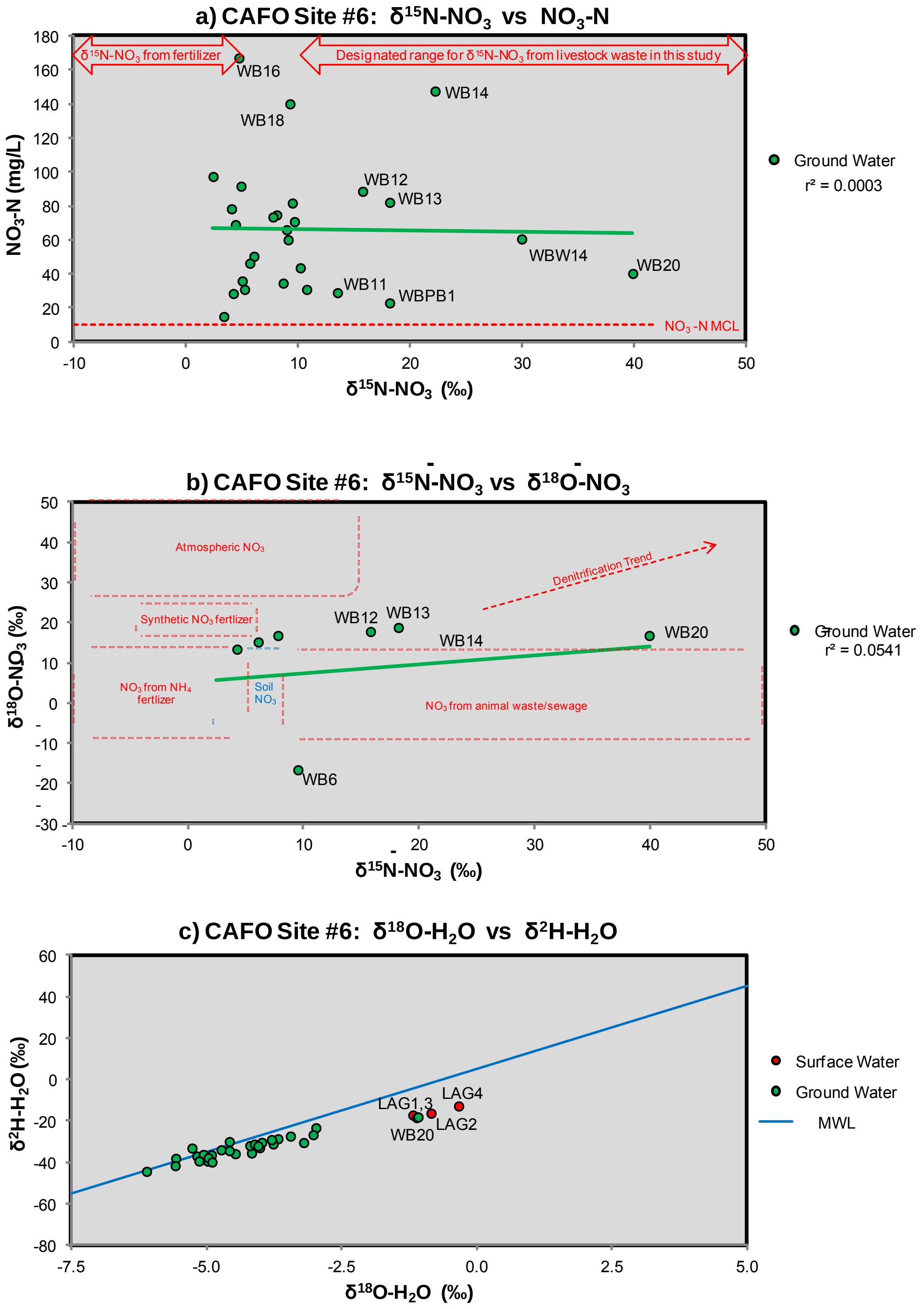


Figure 13. CAFO Site #6 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for NO_3-N. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

sample shows a strong impact from animal waste as represented by high concentrations of ammonium (8.94 mg/L NH_4-N), nitrate (40.3 mg/L NO_3-N) and TOC (45.7 mg/L), as well as strong animal signatures from ammonium (+30.8‰ δ^{15}N-NH_4) and nitrate (+39.9‰ δ^{15}N-NO_3). These observations indicate that this well is probably being directly impacted by leakage from lagoon LAG4. In summary, regardless of the variability in ground water chemistry at this site, most of the evidence shows that ground water contamination by nitrate is derived from CAFO wastes, and that denitrification processes are not likely enriching δ^{15}N values to the extent necessary to lead to misinterpretations of stable nitrogen isotope data.

Evaluation of Additional Stressor Impact. Ground water orthophosphate concentrations ranged from 0.038–0.394 mg/L PO_4-P in these wells, and again there was no correlation with nitrate concentrations (r^2 = -0.0062). Although there was little evidence of additional stressors other than nitrate in these impacted wells, what few detections occurred were generally found in those ground water samples showing an animal waste signature. Total coliform counts were low (< 20 cells/100 mL) in all ground water samples except for that taken from well WB20, which yielded 816 cells/100 mL (Table 29). As discussed earlier, this well was most likely directly impacted by leakage from the adjacent lagoon LAG4. Fecal coliforms were not found in any of the ground water samples, but fecal enterococci were detected in several ground water samples, with the highest numbers (91-108 cells/100 mL) found in ground water samples from wells WB11 and WB14 (Table 29). These two wells were located adjacent to the feedlot and lagoons (Figure 12), and ground water samples from each well showed animal waste signatures with δ^{15}N-NO_3 values ranging from +13.5‰ to +22.3‰ (Table 28). Regarding metals and metalloids, ground water concentrations of arsenic, copper, and zinc were either below detection limits or were at very low levels in all of the ground water samples, with the exceptions of those from well WBW17 and WBW29, which contained 32 µg/L Cu and 433 µg/L Zn, respectively (Table 29). These are both water wells with submerged metal pumps, which may be the cause of these detections. There was some evidence of elevated nickel and selenium levels in several of the ground water samples, but the concentrations were still quite low (Table 29). Ground water samples showing the

Table 29. CAFO Site #6 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococci (cells per 100 mL)	As by ICP-MS (μg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Beef Secondary Lagoon	LAG4-1[a]	04/10/06	2,910	1,310	1,780	NA	0.020	< 0.007	0.009	0.009	0.171
Beef Secondary Lagoon	LAG4-2	04/10/06	3,870	1,860	3,170	NA	0.023	< 0.007	0.009	0.009	0.186
Beef Secondary Lagoon	LAG4-3	04/10/06	4,880	1,540	3,870	NA	0.025	< 0.007	0.008	0.008	0.141
Beef Secondary Lagoon	LAG1	02/12/08	798	219	1,350	NA	< 0.009[b]	0.004	< 0.007	< 0.008	0.156
Beef Secondary Lagoon	LAG4	02/12/08	1,480	35	748	NA	< 0.009	0.013	0.008	< 0.008	0.260
Beef Primary Lagoon	LAG1	05/26/09	116,000	16,500	10,400	NA	< 0.006	< 0.004	0.005	0.062	< 0.040
Beef Primary Lagoon	LAG2	05/26/09	12,600	1,300	310	NA	< 0.006	< 0.004	0.011	0.077	< 0.040
Beef Primary Lagoon	LAG3	05/26/09	12,400	3,270	8,520	NA	< 0.006	< 0.004	0.022	0.367	< 0.040
Beef Secondary Lagoon	LAG4	05/26/09	> 242,000	58	300	NA	< 0.006	< 0.004	0.012	0.054	< 0.040
Monitoring Well	WB1	05/26/09	0	0	1	NA	< 0.006	< 0.004	0.013	< 0.005	< 0.040
Monitoring Well	WB2	05/26/09	0	0	0	NA	< 0.006	< 0.004	< 0.003	< 0.005	< 0.040
Monitoring Well	WB3	05/26/09	0	0	0	NA	< 0.006	< 0.004	< 0.003	< 0.005	< 0.040
Monitoring Well	WB4	05/26/09	0	0	2	NA	< 0.006	< 0.004	< 0.003	< 0.005	< 0.040
Monitoring Well	WB6	05/26/09	0	0	7	NA	< 0.006	< 0.004	0.006	< 0.005	< 0.040
Monitoring Well	WB7	05/27/09	0	0	3	NA	< 0.006	< 0.004	0.004	0.009	< 0.040
Monitoring Well	WB8	05/27/09	12	0	0	NA	< 0.006	< 0.004	< 0.003	< 0.005	< 0.040
Monitoring Well	WB9	05/27/09	5	0	0	NA	< 0.006	< 0.004	0.042	0.057	< 0.040
Monitoring Well	WB10	05/27/09	0	0	15	NA	< 0.006	< 0.004	0.007	0.019	< 0.040
Monitoring Well	WB11	05/27/09	1	0	91	NA	< 0.006	< 0.004	0.005	0.010	< 0.040
Monitoring Well	WB12	05/27/09	0	0	1	NA	< 0.006	< 0.004	0.018	0.037	< 0.040
Monitoring Well	WB13	05/27/09	4	0	1	NA	< 0.006	< 0.004	< 0.003	0.013	< 0.040
Monitoring Well	WB14	05/27/09	5	0	108	NA	< 0.006	0.009	0.028	0.080	< 0.040
Monitoring Well	WB15	05/27/09	3	0	1	NA	< 0.006	< 0.004	0.003	0.008	< 0.040
Monitoring Well	WB16	05/27/09	10	0	0	NA	< 0.006	< 0.004	0.006	< 0.005	< 0.040
Monitoring Well	WB17	05/27/09	6	0	3	NA	< 0.006	< 0.004	0.008	0.019	< 0.040
Monitoring Well	WB18	05/27/09	16	0	0	NA	< 0.006	< 0.004	0.010	0.007	< 0.040
Monitoring Well	WB20	05/28/09	816	0	8	NA	< 0.006	0.008	0.022	0.122	< 0.040
Monitoring Well	WB21	05/27/09	17	0	1	NA	< 0.006	< 0.004	< 0.003	0.045	< 0.040
Pivot Boring	WBPB1	05/28/09	0	0	9	NA	< 0.006	0.004	< 0.003	0.040	< 0.040
Water Well	WBW10	05/28/09	0	0	0	NA	< 0.006	< 0.004	< 0.003	0.038	< 0.040
Water Well	WBW11	05/30/09	2	0	0	NA	< 0.006	< 0.004	0.007	0.018	< 0.040
Water Well	WBW14	05/28/09	0	0	0	NA	< 0.006	< 0.004	0.011	0.033	< 0.040
Water Well	WBW17	05/30/09	0	0	10	NA	< 0.006	0.032	0.003	0.006	< 0.040
Water Well	WBW18	05/28/09	0	0	0	NA	< 0.006	0.006	0.019	0.087	< 0.040
Water Well	WBW19	05/28/09	1	0	0	NA	< 0.006	< 0.004	0.006	0.019	< 0.040
Water Well	WBW20	05/28/09	0	0	0	NA	< 0.006	0.007	0.005	< 0.005	< 0.040
Water Well	WBW23	05/28/09	2	0	0	NA	< 0.006	< 0.004	0.004	0.047	< 0.040
Water Well	WBW29	05/28/09	0	0	0	NA	< 0.006	0.009	0.006	0.019	0.433

[a] One of three sampling locations in this lagoon

[b] Values reported as "<" are below detection limits

highest concentrations of nickel (18-42 µg/L Ni) also showed the highest concentrations of selenium (37-122 µg/L Se). Even though these levels were found in ground waters with high nitrate values (29-148 mg/L NO_3-N), some ground water samples did not show animal waste signatures (+4.2 to +7.8‰ δ^{15}N, wells WB9 andWBW18) whereas other ground water samples did show animal waste signatures (+15.8 to +39.9‰ δ^{15}N, wells WB12, WB14, and WB20), and so it is inconclusive whether CAFO wastes were the

Table 30. CAFO Site #6 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date	TYL[c] (ng/L)	SCLP (ng/L)	SMZN (ng/L)	ICTET (ng/L)	EICTET (ng/L)	TET (ng/L)
Beef Secondary Lagoon	LAG4-1[a]	04/10/06	< 5[d]	< 5	< 5	< 10	< 10	< 10
Beef Secondary Lagoon	LAG4-2	04/10/06	< 5	756	< 5	< 10	< 10	< 10
Beef Secondary Lagoon	LAG4-3	04/10/06	136	< 5	< 5	< 10	< 10	< 10
Beef Primary Lagoon	LAG1	05/26/09	< 10	< 5	< 5	10,000	6,990	126
Beef Primary Lagoon	LAG1(FD)[b]	05/26/09	< 10	< 5	< 5	8,270	4,740	143
Beef Primary Lagoon	LAG2	05/26/09	< 10	< 5	< 5	2,040	1,260	180
Beef Primary Lagoon	LAG3	05/26/09	< 10	< 5	< 5	< 10	< 10	< 10
Beef Secondary Lagoon	LAG4	05/26/09	< 10	< 5	< 5	85	61	< 10
Monitoring Well	WB6	05/26/09	< 10	< 5	< 5	< 10	< 10	< 10
Monitoring Well	WB14	05/27/09	< 10	< 5	< 5	< 10	< 10	< 10
Monitoring Well	WB14(FD)	05/27/09	< 10	< 5	< 5	< 10	< 10	< 10
Monitoring Well	WB16	05/27/09	< 10	< 5	< 5	< 10	< 10	< 10
Monitoring Well	WB20	05/28/09	< 10	< 5	< 5	< 10	< 10	< 10
Pivot Boring	WBPB1	05/28/09	< 10	< 5	< 5	< 10	< 10	< 10
Water Well	WBW10	05/28/09	< 10	< 5	17	< 10	< 10	< 10
Water Well	WBW10(FD)	05/28/09	< 10	< 5	14	< 10	< 10	< 10
Water Well	WBW19	05/28/09	< 10	< 5	< 5	< 10	< 10	< 10
Water Well	WBW20	05/28/09	< 10	< 5	< 5	< 10	< 10	< 10
Water Well	WBW23	05/28/09	< 10	< 5	< 5	< 10	< 10	< 10
Water Well	WBW29	05/28/09	< 10	< 5	< 5	< 10	< 10	< 10

[a] One of three sampling locations in this lagoon

[b] FD is field duplicate

[c] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table were not detected in any of these samples

[d] Values reported as "<" are below reporting limits

sources of these stressors. However, elevated concentrations of copper and nickel have been found underneath unlined cattle slurry lagoons elsewhere (Gooddy et al., 2002). The lagoons at this beef feedlot site had relatively low concentrations of antibiotics (Table 30) and estrogen hormones (Table 31) compared to lagoons associated with other types of CAFOs, and so perhaps it is not surprising that these stressors were generally not detected in these ground water samples despite the relatively high levels of nitrate. The one exception was observed for well WBW10, the somewhat anomalous well described earlier that was poorly-sealed and which was contaminated with ammonium (20.3 mg/L NH$_4$-N) instead of nitrate. The antibiotic sulfamethazine was detected at very low levels (14-17 ng/L) in both field duplicate ground water samples from this well (Table 30), and this antibiotic has been detected in ground water downgradient of beef feedlots elsewhere (Batt et al., 2006). Estrone was also detected in this both field duplicate ground water samples from this well, but the concentrations were low (1.8-2.3 ng/L) and close to the quantitation limit of 1.0 ng/L (Table 31). A follow-up sampling in 2011 confirmed the

Table 31. CAFO Site #6 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17β-Ethynylestradiol (ng/L)	Estriol (ng/L)
Beef Secondary Lagoon	LAG4-1[a]	04/10/06	< 40.0[c]	< 20.0	< 60.0	< 60.0	< 20.0
Beef Secondary Lagoon	LAG4-2	04/10/06	< 40.0	< 20.0	< 60.0	< 60.0	< 20.0
Beef Secondary Lagoon	LAG4-3	04/10/06	< 40.0	< 20.0	< 60.0	< 60.0	< 20.0
Beef Primary Lagoon	LAG1	02/12/08	31.8	< 20.0	28.6	< 20.0	< 20.0
Beef Secondary Lagoon	LAG4	02/12/08	< 20.0	< 20.0	38.0	< 20.0	< 20.0
Beef Primary Lagoon	LAG1	05/26/09	< 40.0	< 40.0	< 40.0	< 40.0	< 40.0
Beef Primary Lagoon	LAG1(FD)[b]	05/26/09	< 40.0	< 40.0	< 40.0	< 40.0	< 40.0
Beef Primary Lagoon	LAG2	05/26/09	< 40.0	< 40.0	< 40.0	< 40.0	< 40.0
Beef Primary Lagoon	LAG3	05/26/09	62.0	41.8	57.1	< 40.0	< 40.0
Beef Secondary Lagoon	LAG4	05/26/09	< 40.0	< 40.0	< 40.0	< 40.0	< 40.0
Monitoring Well	WB6	05/26/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	WB14	05/27/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	WB14(FD)	05/27/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	WB16	05/27/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Monitoring Well	WB20	05/28/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Pivot Boring	WBPB1	05/28/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	WBW10	05/28/09	1.8	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	WBW10(FD)	05/28/09	2.3	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	WBW19	05/28/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	WBW20	05/28/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Water Well	WBW29	05/28/09	< 1.0	< 1.0	< 1.0	< 1.0	< 1.0
Beef Primary Lagoon	LAG3	05/18/11	< 25.0	< 25.0	< 25.0	< 25.0	< 25.0
Water Well	WBW10	04/20/11	1.1	< 0.3	< 0.3	< 0.3	< 0.3
Water Well	WBW10(FD)	04/20/11	1.1	< 0.3	< 0.3	< 0.3	NC (< 0.4)[d]

[a] One of three sampling locations in this lagoon

[b] FD is field duplicate

[c] Values reported as "<" are below quantitation limits

[d] NC is not confirmed due to interference; if analyte is present, it is below estimate shown in parantheses

detection of estrone in this well, but again at a very low concentration (1.1 ng/L). As observed earlier, site personnel have speculated that the source of contamination in this well might have been due to the death of an animal that became trapped within the well, but it is also possible that leakage from a lagoon or a pipeline carrying lagoon effluent for land application could also be the cause. In summary, even though ground water contamination by nitrate is extensive at this site and probably derives from several avenues of transport of reactive nitrogen from CAFO waste, there was little evidence of consistent ground water contamination by the other stressors monitored in this study.

3.7 CAFO Site 7 – Swine Farrowing Sow

Case Study Summary. Unlike the other sites previously discussed, this site was chosen to represent a relatively non-impacted case study. Ground water contamination by nitrate has not occurred in the off-site wells downgradient from this CAFO, and is not expected to happen, if at all, for several years due to the extent of the vadose zone and the depth to ground water. We included this site to document background conditions in the event of future impacts, and to ascertain whether the CAFO waste stressors monitored in this study were already present. Surprisingly, moderate levels of fecal enterococci were detected in these off-site ground water samples, and in one instance estrone was detected at a very low level (0.5 ng/L). The source of these contaminants is unknown, and could possibly be due to grazing livestock in this area.

Site Description. CAFO Site 7 is a farrowing sow swine facility that began operation in May 1999 with about 25,000 swine, including 19,920 gestating sows, 4,980 farrowing sows, and 100 boars. Prior to 2004, swine waste from two large barn complexes was treated using a series of five anaerobic lagoons with liquid manure being first directed to two covered digester lagoons (L1OLD, L2OLD), then to two uncovered primary lagoons (L3OLD, L4OLD), and finally to an uncovered secondary lagoon (L5OLD) before being used for land application (Figure 14). All of these lagoons were lined with synthetic liners. Prior to 2005, the secondary lagoon effluent was pumped to four center pivot locations northeast of the barns and land applied for production of wheat, corn, and alfalfa. The subsurface geology at this site is highly heterogeneous and consists of mixed sands with silt and caliche stringers, and depths to ground water range from 40-130 ft below ground surface. Regional ground water flow is south towards a small river. There were concerns regarding ground water impacts to a wildlife management area about one mile south of the land application area, and five monitoring wells (BV1-BV5) were constructed in July 1999 on private and public lands south of the facility (Figure 14). We conducted quarterly sampling of these five off-site monitoring wells for routine chemical parameters from August 2000 through July 2002, and in late July 2002 were granted access to the facility to sample the five lagoons as well as on-site monitoring wells

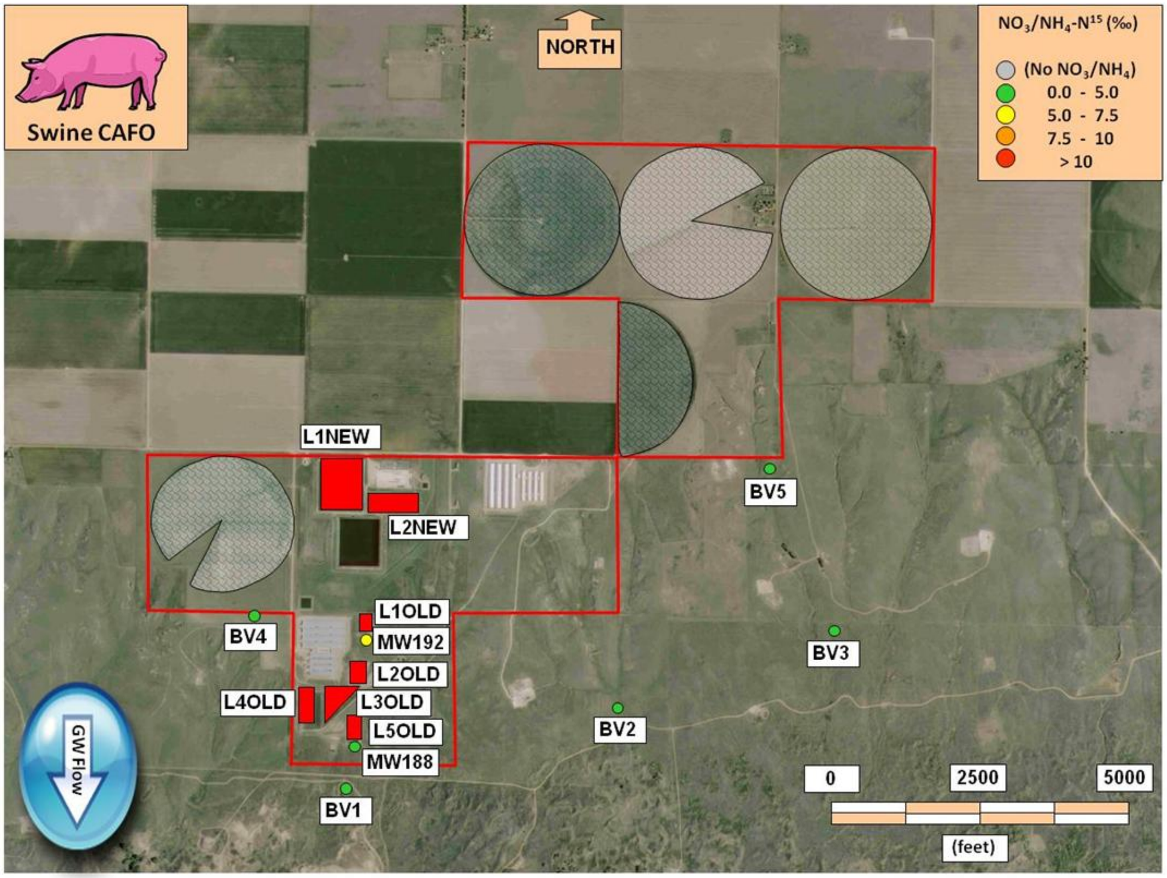


Figure 14. CAFO Site #7 schematic (farrowing sow operation, control site). Colors of lagoons and wells correspond to ranges of δ^{15}N of nitrate or ammonia as shown in legend at upper right, based on 2002 and 2010 data.

adjacent to those lagoons. Because most of those monitoring wells were dry, we were only able to get a complete sample out of one well (MW188) and had to sample multiple times with a dedicated bailer to get a partial sample from another (MW192). This marked the end of our quarterly sampling program. During this time, additional concerns had been brought forth later regarding the potential for over-application of CAFO waste based on the volumes being generated and the amount of land available for irrigation, and a settlement was reached in December 2001 which led to several operating changes at this facility. The old lagoons were subsequently decommissioned, and in 2003 a revised system was brought on-line consisting of two covered settling basins, two mechanical solids separators, a 4-acre concrete pad for composting solids, a primary anaerobic lagoon (L1NEW), a secondary storage lagoon (L2NEW), and an evaporation basin (Figure 14). All of these surface water impoundments were lined with synthetic liners, and the composted solids were commercially sold or otherwise distributed off-site. The net result has been a large reduction in the amount of liquid manure that needs to be land-applied. Additional land area was also subsequently obtained for land application, including a new center pivot area located just west of the new lagoons (Figure 14), and land application began on this area in May 2005. We revisited this facility in September 2010 to sample the new lagoons and to ascertain whether there had been any significant changes in ground water chemistry in the off-site wells during the past eight years.

General Chemistry and Stable Isotope Interpretation. These wells are screened at much deeper intervals those at the sites previously discussed, and the depth to ground water ranges widely (Table 32). Ground water nitrate levels were low (1.48-3.12 mg/L NO_3-N) in these off-site wells in 2002, and there was no evidence of an animal waste signature by δ^{15}N (< +5‰), nor was there any significant increase in ground water nitrate levels even after eight years (Table 33). Ground water samples from the two lagoon wells sampled in 2002 showed contamination by reactive nitrogen, but the sources are uncertain. Ground

Table 32. CAFO Site #7 Sample Locations and General Parameters.

Sample Type	Sample ID	Sample Date	Water Level (ft TOC)[b]	Screen Intvl (ft TOC)	DO (mg/L)	CH_4 (mg/L)	Cl (mg/L)	SO_4 (mg/L)	o-PO_4-P (mg/L)	TKN (mg/L)	TP (mg/L)	TOC (mg/L)	TIC (mg/L)
Old Swine Digester #1	L1OLD	07/30/02	NA	NA	4.4	11.4	488	11.7	168	NA	NA	682	NA
Old Swine Digester #2	L2OLD	07/30/02	NA	NA	3.6	10.8	473	8.9	135	NA	NA	532	NA
Old Swine Primary Lagoon #1	L3OLD	07/30/02	NA	NA	0.4	2.16	551	< 0.1	159	NA	NA	432	NA
Old Swine Primary Lagoon #2	L4OLD	07/30/02	NA	NA	0.5	0.86	586	< 0.1	161	NA	NA	321	NA
Old Swine Secondary Lagoon	L5OLD	07/30/02	NA	NA	2.5	0.22	1,370	97.1	200	NA	NA	278	NA
CAFO Monitoring Well	MW188	07/30/02	100.14	NA	2.0	< 0.01[c]	94.7	27.5	0.030	NA	NA	8.9	NA
CAFO Monitoring Well	MW192	07/30/02	77.54	NA	NA	NA	182	64.7	0.038	NA	NA	2.9	NA
Off-Site Monitoring Well	BV1	07/29/02	95.01	110-120	8.1	< 0.01	46.5	37.9	0.040	NA	NA	1.3	NA
Off-Site Monitoring Well	BV2	07/29/02	42.02	78-88	4.6	< 0.01	63.9	27.6	0.034	NA	NA	1.6	NA
Off-Site Monitoring Well	BV3	07/29/02	46.46	88-98	6.0	< 0.01	57.3	37.2	0.030	NA	NA	1.2	NA
Off-Site Monitoring Well	BV4	07/29/02	126.74	130-140	2.9	< 0.01	54.6	29.6	0.031	NA	NA	0.5	NA
Off-Site Monitoring Well	BV5	07/29/02	90.10	110-120	2.6	< 0.01	11.5	8.2	0.031	NA	NA	< 0.1	NA
New Swine Primary Lagoon	L1NEW-1[a]	09/28/10	NA	NA	0.2	4.70	1,510	1.8	106	1,010	114	953	971
New Swine Primary Lagoon	L1NEW-2	09/28/10	NA	NA	0.7	5.37	1,440	1.6	106	1,040	120	734	1,380
New Swine Secondary Lagoon	L2NEW	09/27/10	NA	NA	0.9	1.32	1,750	< 0.5	177	390	173	922	580
Off-Site Monitoring Well	BV1	09/13/10	97.97	110-120	5.7	< 0.01	43.3	25.6	0.094	0.06	0.118	0.96	43.7
Off-Site Monitoring Well	BV2	09/14/10	43.62	78-88	4.8	< 0.01	41.7	27.0	0.103	0.27	0.540	1.16	51.1
Off-Site Monitoring Well	BV3	09/14/10	50.32	88-98	5.7	< 0.01	62.2	39.9	0.111	0.12	0.155	0.65	44.8
Off-Site Monitoring Well	BV4	09/14/10	130.37	130-140	7.1	< 0.01	57.2	28.7	0.074	0.11	0.166	0.96	43.4
Off-Site Monitoring Well	BV5	09/14/10	95.90	110-120	6.5	< 0.01	14.3	8.0	0.043	0.05	0.084	0.47	53.6

[a] One of two sampling locations in this lagoon
[b] Feet from top of casing
[c] Values reported as "<" are below detection limits

Table 33. CAFO Site #7 Reactive Nitrogen and Stable Isotopes.

Sample Type	Sample ID	Sample Date	NH_4-N (mg/L)	NO_3-N (mg/L)	NO_2-N (mg/L)	N_2O-N (mg/L)	δ^2H-H_2O (‰)	$\delta^{18}O$-H_2O (‰)	$\delta^{15}N$-NO_3 (‰)	$\delta^{18}O$-NO_3 (‰)	$\delta^{15}N$-NH_4 (‰)
Old Swine Digester #1	L1OLD	07/30/02	1,250	< 0.01	0.25	< 0.01	-20.4	-4.3	NA	NA	+9.9
Old Swine Digester #2	L2OLD	07/30/02	1,030	< 0.01	0.18	< 0.01	-22.2	-4.3	NA	NA	+8.7
Old Swine Primary Lagoon #1	L3OLD	07/30/02	1,130	< 0.01	0.22	< 0.01	-22.2	-2.9	NA	NA	+15.9
Old Swine Primary Lagoon #2	L4OLD	07/30/02	714	< 0.01	0.14	< 0.01	-15.4	-1.3	NA	NA	+26.3
Old Swine Secondary Lagoon	L5OLD	07/30/02	122	< 0.01	0.35	< 0.01	+15.6	+5.1	NA	NA	+19.5
CAFO Monitoring Well	MW188	07/30/02	13.3	5.20	< 0.01	0.01	-52.6	-7.4	+2.7	NA	NA
CAFO Monitoring Well	MW192	07/30/02	< 0.02[b]	56.7	0.14	NA	-49.1	-7.1	+6.0	NA	NA
Off-Site Monitoring Well	BV1	07/29/02	< 0.02	3.12	< 0.01	< 0.01	-50.5	-7.2	+2.9	NA	NA
Off-Site Monitoring Well	BV2	07/29/02	< 0.02	2.61	< 0.01	< 0.01	-46.4	-6.9	+2.1	NA	NA
Off-Site Monitoring Well	BV3	07/29/02	< 0.02	1.51	< 0.01	< 0.01	-56.3	-7.6	+4.6	NA	NA
Off-Site Monitoring Well	BV4	07/29/02	< 0.02	2.25	< 0.01	0.01	-68.5	-9.6	+4.3	NA	NA
Off-Site Monitoring Well	BV5	07/29/02	< 0.02	1.48	< 0.01	0.01	-53.1	-7.7	+3.4	NA	NA
New Swine Primary Lagoon	L1NEW-1[a]	09/28/10	836	0.07	0.98	< 0.01	-5.7	+1.3	NA	NA	+31.7
New Swine Primary Lagoon	L1NEW-2	09/28/10	808	0.08	0.98	< 0.01	-6.1	+0.9	NA	NA	+25.9
New Swine Secondary Lagoon	L2NEW	09/27/10	279	0.12	0.67	< 0.01	-0.6	+2.3	NA	NA	+60.0
Off-Site Monitoring Well	BV1	09/13/10	< 0.02	2.71	0.01	< 0.01	-51.6	-7.0	+0.6	+19.2	NA
Off-Site Monitoring Well	BV2	09/14/10	< 0.02	3.67	0.01	< 0.01	-47.3	-7.0	-0.1	+18.9	NA
Off-Site Monitoring Well	BV3	09/14/10	< 0.02	1.54	0.03	< 0.01	-53.4	-7.2	-0.2	+22.7	NA
Off-Site Monitoring Well	BV4	09/14/10	< 0.02	2.34	0.00	< 0.01	-63.0	-8.8	+1.2	+33.9	NA
Off-Site Monitoring Well	BV5	09/14/10	< 0.02	1.75	0.01	< 0.01	-51.2	-7.2	+1.0	+29.3	NA

[a] One of two sampling locations in this lagoon

[b] Values reported as "<" are below detection limits

water from well MW188 had a high level of ammonium (13.3 mg/L NH_4-N) and a low level of nitrate (5.20 mg/L NO_3-N), whereas ground water from well 192 showed only a high level of nitrate (56.7 mg/L NO_3-N). In both cases there was no animal waste signature by $\delta^{15}N$-NO_3, and unfortunately the ammonium found in MW188 was not characterized for $\delta^{15}N$-NH_4. Ground water DO levels are moderate and TOC levels were very low at this site (Table 32), and ground water nitrite and nitrous oxide concentrations were also very low (Table 33). It seems therefore unlikely that denitrification was occurring to any significant extent, and the nitrate stable isotope relationships do not provide any evidence to show otherwise (Figure 15). There was no correlation (r^2 = -0.0394) between increasing ground water $\delta^{15}N$-NO_3 values and decreasing ground water nitrate concentrations that would indicate denitrification (Figure 15a), although there was a positive correlation ($r^2 = 0.5832$) between ground water $\delta^{15}N$-NO_3 and $\delta^{18}O$-NO_3 values that could support this (Figure 15b). However, if denitrification were responsible for this positive correlation, the slope of the line would theoretically be between one and two (Kendall et al., 2008), and the slope of this trend line is around eight. Regardless, the

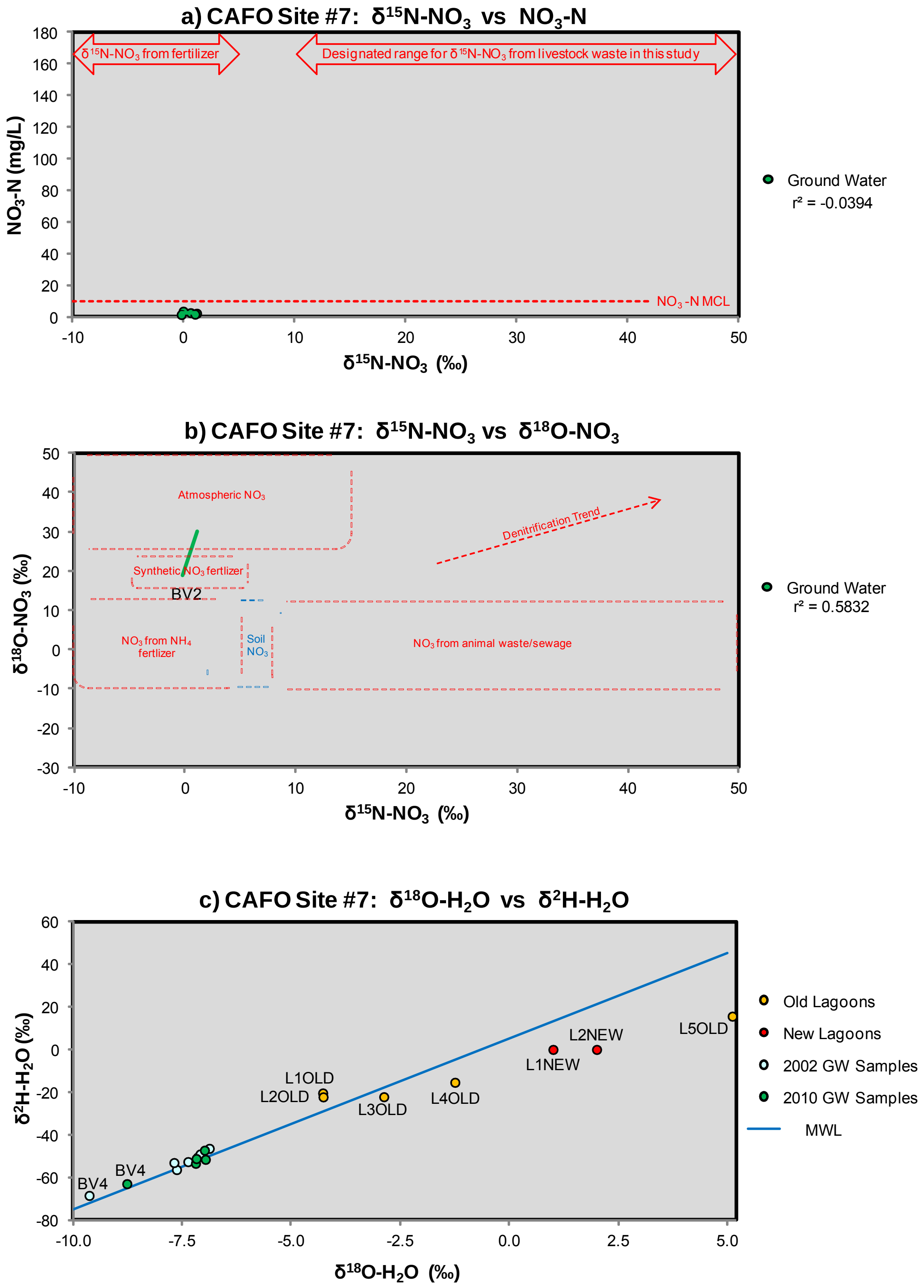


Figure 15. CAFO Site #7 isotope data relationships. Selected sample locations are identified. Green trend lines are linear correlations. The red dashed line shown in (a) is the MCL for NO_3-N. The ranges shown in (b) are adapted from Silva et al. (2002b) and Kendall et al. (2008). The MWL shown in (c) is the meteoric water line as described by Taylor (1974).

data are too few to attach much significance to the interpretation of these stable nitrate isotope relationships. In contrast, stable water isotope ratios were consistent with expectations regarding the surface water samples, with the covered digesters and the secondary lagoons showing the least and greatest evaporative signatures, respectively (Figure 15c). In addition, the stable water isotope ratios for the covered digesters were above the MWL, as would be expected due to the enrichment of deuterium by methanogenesis (Hackley et al., 1996), and methane concentrations were much higher in these covered digesters compared to the other lagoons (Table 32). Ground waters from these deeper wells were less enriched in $\delta^{18}O$-H_2O than the shallower ground waters associated with the other sites, and in this case the $\delta^{18}O$-H_2O values were especially low in ground water samples from well BV4 from both sample events (Figure 15c).

Evaluation of Additional Stressor Impact. Nitrate concentrations were low in ground water samples from these off-site wells and few additional stressors were expected to be present. Orthophosphate concentrations in ground water were moderately low and ranged from 0.030 to 0.111 mg/L PO_4-P (Table 32). Surprisingly, moderate levels of fecal enterococci were detected in ground water samples from these off-site wells (Table 34). The source(s) of these are unknown, although there is grazing livestock in this area. The moderately high level of fecal enterococci observed in the ground water sample from well BV4 in 2002 was not related to land application of CAFO waste, because the center pivot in the land application area just north and upgradient of this well (Figure 14) was not yet in operation. The highest level of fecal enterococci was found in ground water from the CAFO lagoon well MW188, which also had a relatively high ammonium level (Table 33), and again may indicate some contamination of ground water from the very high levels of fecal enterococci found in lagoon L5OLD (Table 34). In contrast, metal and metalloid concentrations were either very low or were not detected in these well samples. Ground water arsenic concentrations, as confirmed by ICP-MS, ranged from 5.1-21.4 µg/L in these off-site wells, with As (V) being the predominant species in all wells (Appendix A). The two new lagoons had arsenic concentrations ranging from 95-106 µg/L As (as measured by ICP-OES), and arsenic was again mostly present as As (V), although in this case there was also some evidence of Roxarsone in L2NEW, albeit at

Table 34. CAFO Site #7 Microbial Indicators, Metals, and Metalloids.

Sample Type	Sample ID	Sample Date	Total Coliforms (cells per 100 mL)	Fecal Coliforms (cells per 100 mL)	Fecal Enterococci (cells per 100 mL)	As by ICP-MS (µg/L)	As (mg/L)	Cu (mg/L)	Ni (mg/L)	Se (mg/L)	Zn (mg/L)
Old Swine Digester #1	L1OLD	07/30/02	12,600	0[b]	199,000	NA	< 0.033[c]	0.023	0.134	< 0.030	0.081
Old Swine Digester #2	L2OLD	07/30/02	24,100	0	120,000	NA	< 0.033	0.085	0.157	< 0.030	0.419
Old Swine Primary Lagoon #1	L3OLD	07/30/02	37,200	0	48,800	NA	< 0.033	0.013	0.162	< 0.030	0.068
Old Swine Primary Lagoon #2	L4OLD	07/30/02	52,000	0	7,030	NA	< 0.033	0.030	0.143	< 0.030	0.190
Old Swine Secondary Lagoon	L5OLD	07/30/02	388,000	0	17,300,000	NA	< 0.033	0.076	0.256	< 0.030	0.299
CAFO Monitoring Well	MW188	07/30/02	117	0	> 2,420	NA	< 0.033	0.011	0.007	< 0.030	< 0.040
CAFO Monitoring Well	MW192	07/30/02	9	0	18	NA	< 0.033	< 0.011	0.007	< 0.030	0.056
Off-Site Monitoring Well	BV1	07/29/02	21	0	35	NA	< 0.033	< 0.011	0.005	< 0.030	0.090
Off-Site Monitoring Well	BV2	07/29/02	61	0	61	NA	< 0.033	0.015	0.005	< 0.030	< 0.040
Off-Site Monitoring Well	BV3	07/29/02	64	0	83	NA	< 0.033	0.015	< 0.004	< 0.030	< 0.040
Off-Site Monitoring Well	BV4	07/29/02	35	0	138	NA	< 0.033	< 0.011	0.007	< 0.030	< 0.040
Off-Site Monitoring Well	BV5	07/29/02	15	0	88	NA	< 0.033	< 0.011	< 0.004	< 0.030	< 0.040
New Swine Primary Lagoon	L1NEW-1[a]	09/28/10	61,300	46,100	26,100	NA	0.099	0.025	0.159	0.126	0.130
New Swine Primary Lagoon	L1NEW-2	09/28/10	48,800	43,500	19,400	NA	0.095	0.020	0.156	0.122	0.119
New Swine Secondary Lagoon	L2NEW	09/27/10	> 2,420	472	> 2,420	NA	0.106	0.016	0.181	0.115	0.171
Off-Site Monitoring Well	BV1	09/13/10	0	0	4	21.4	0.015	0.003	0.005	0.005	0.061
Off-Site Monitoring Well	BV2	09/14/10	> 2,420	0	29	11.0	< 0.015	< 0.003	0.005	0.007	< 0.040
Off-Site Monitoring Well	BV3	09/14/10	1	0	3	11.3	< 0.015	< 0.003	0.005	0.010	< 0.040
Off-Site Monitoring Well	BV4	09/14/10	7	0	0	5.7	< 0.015	< 0.003	0.003	0.009	< 0.040
Off-Site Monitoring Well	BV5	09/14/10	0	0	0	5.1	< 0.015	< 0.003	0.001	0.013	< 0.040

[a] One of two sampling locations in this lagoon

[b] The 2002 lagoon samples showed no fecal coliforms detected; these data questionable

[c] Values reported as "<" are below detection limits

Table 35. CAFO Site #7 Veterinary Antibiotics.

Sample Type	Sample ID	Sample Date	ICTET[c] (ng/L)	OTET (ng/L)	LINC (ng/L)
New Swine Primary Lagoon	L1NEW-1[a]	09/28/10	< 10	< 10	> 50,000
New Swine Primary Lagoon	L1NEW-2	09/28/10	1,700	330	> 50,000
New Swine Secondary Lagoon	L2NEW	09/27/10	< 10	< 10	47,000
New Swine Secondary Lagoon	L2NEW(FD)[b]	09/27/10	< 10	< 10	100,000
Off-Site Monitoring Well	BV1	09/13/10	< 10	< 10	< 5
Off-Site Monitoring Well	BV2	09/14/10	< 10	< 10	< 5
Off-Site Monitoring Well	BV3	09/14/10	< 10	< 10	< 5
Off-Site Monitoring Well	BV3(FD)	09/14/10	< 10	< 10	< 5
Off-Site Monitoring Well	BV4	09/14/10	< 10	< 10	< 5
Off-Site Monitoring Well	BV5	09/14/10	< 10	< 10	< 5

[a] One of two sampling locations in this lagoon

[b] FD is field duplicate

[c] See Table 1 for abbreviations. Antibiotics listed in Table 1 that are not shown in this table were not detected in any of these samples

[d] Values reported as "<" are below reporting limits

very low levels (Appendix A). As noted earlier, Roxarsone is occasionally used in swine operations, and has been detected in swine lagoons (Makris et al., 2008). Ground water samples from well BV1 also had very low but detectable concentrations of zinc (61-90 µg/L Zn) in both 2002 and 2010 (Table 34). Although this well is immediately downgradient of L5OLD, there are no additional indicators of impact from CAFO waste based on general chemistry parameters (Table 32) or stable isotopes (Table 33), and ground water chloride concentrations have remained low (40-50 mg/L) throughout the 2000-2002 sampling period (unpublished data). A few veterinary antibiotics were detected in the two new swine lagoons at this site (Table 35), but the numbers and concentrations were generally less than those observed for other swine lagoons. Veterinary antibiotics were not detected in ground water samples collected from the off-site monitoring wells. As expected, estrogen concentrations were quite high in these swine lagoons, but only estrone was detected in one well, and at a very low level of 0.5 ng/L (Table 36). Interestingly, this again was well BV1, which compared to the other

Table 36. CAFO Site #7 Estrogen Hormones.

Sample Type	Sample ID	Sample Date	Estrone (ng/L)	17α-Estradiol (ng/L)	17β-Estradiol (ng/L)	17α-Ethynylestradiol (ng/L)	Estriol (ng/L)
Old Swine Digester #1	L1OLD	07/30/02	16,900	NA	3,000	< 40.0	8,070
Old Swine Digester #2	L2OLD	07/30/02	24,900	NA	2,190	< 40.0	10,400
Old Swine Primary Lagoon #1	L3OLD	07/30/02	19,100	NA	2,400	< 40.0	7,810
Old Swine Primary Lagoon #2	L4OLD	07/30/02	9,590	NA	2,250	< 40.0	5,030
Old Swine Secondary Lagoon	L5OLD	07/30/02	< 40.0[c]	NA	< 40.0	< 40.0	< 40.0
CAFO Monitoring Well	MW188	07/30/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
CAFO Monitoring Well	MW192	07/30/02	NA	NA	NA	NA	NA
Off-Site Monitoring Well	BV1	07/29/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
Off-Site Monitoring Well	BV2	07/29/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
Off-Site Monitoring Well	BV3	07/29/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
Off-Site Monitoring Well	BV4	07/29/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
Off-Site Monitoring Well	BV4(FD)[a]	07/29/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
Off-Site Monitoring Well	BV5	07/29/02	< 2.0	NA	< 2.0	< 2.0	< 2.0
New Swine Primary Lagoon	L1NEW-1[b]	09/28/10	19,200	1,130	763	< 3.0	21,200
New Swine Primary Lagoon	L1NEW-2	09/28/10	20,100	1,150	950	< 3.0	20,000
New Swine Secondary Lagoon	L2NEW	09/27/10	10,300	1,260	340	< 3.0	11,500
New Swine Secondary Lagoon	L2NEW(FD)	09/27/10	10,400	1,310	361	< 3.0	13,600
Off-Site Monitoring Well	BV1	09/13/10	0.5	< 0.3	< 0.3	< 0.3	< 0.3
Off-Site Monitoring Well	BV2	09/14/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Off-Site Monitoring Well	BV3	09/14/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Off-Site Monitoring Well	BV3(FD)	09/14/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Off-Site Monitoring Well	BV4	09/14/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3
Off-Site Monitoring Well	BV5	09/14/10	< 0.3	< 0.3	< 0.3	< 0.3	< 0.3

[a] FD is field duplicate
[b] One of two sampling locations in this lagoon
[c] Values reported as "<" are below quantitation limits

off-site wells had the slightly higher levels of arsenic and zinc reported earlier. The analytical data and quality control measures were especially good in this case and support this as a true detection in this ground water sample, but the concentration was still less than 1 ng/L and was also very close to the quantitation limit (0.3 ng/L). Considering the data obtained to date, and with the exception of fecal enterococci, these off-site wells show little impact by nitrate or any other stressors related to CAFO waste. These data will provide background information to assess any future impacts from this facility, especially in the location of BV4 where land application of swine waste commenced in 2005 just upgradient of this well and affords the greatest potential for ground water contamination by nitrate and perhaps other CAFO stressors.

4.0 Summary and Recommendations

Collectively, these data show that ground water contamination by nitrate or ammonium can occur at very different types of CAFOs, whether through leaking lagoons, leaking pipes or infrastructure, land application of wastes in excess of agronomic needs, or other factors. Because of limited site access and resource constraints, it was beyond the scope of this study to evaluate the relative contributions of the individual sources leading to ground water contamination by nutrients at each of these study sites, or to ascertain whether adequate management strategies were either in place or being rigorously followed. But in some cases it is clear that leaking lagoons (Sites #2 and #5) and excessive land application (Sites #3 and #4) were the sources for the high ground water nitrate or ammonium levels observed in the sampled wells, and actions have been taken to mitigate or eliminate these source terms at these sites.

Generally, ammonium was observed in ground water only at those sites where there appeared to be a direct hydrologic connection between the sampled wells and leaking lagoons (Sites #2 and #5). These two sites have since been closed and the lagoons have been decommissioned. The lagoons at these two sites were not constructed with synthetic liners, nor was there any evidence that clay or other fill material had been brought in to construct liners. Although NPDES regulations do not specifically require the use of liners at CAFO lagoons, permitting authorities have the discretion to include additional special conditions such as liners where there is a potential to discharge to ground water that has a direct hydrological connection to waters of the U.S. (USEPA, 2012d). It has been estimated that in this region, which includes the study area, roughly 80% of the CAFO lagoons rely on in situ clay for bases or have clay liners, and about 15% (mostly swine lagoons) use synthetic liners. The remaining 5%, including the two study sites listed above, are either unlined or have no available construction information.

Leaking lagoons would be expected to have a more direct impact on ground water quality than land application of CAFO wastes, especially in situations where there are elevated water tables and leakage occurs throughout the lagoon bottom or sidewalls. In reality, all CAFO lagoons do in fact leak, at least to some extent, but with properly-constructed lagoons the leakage rates are supposed to be low enough to preclude adverse environmental impacts. More information is needed on whether design leakage rates are acceptable in relation to diverse hydrogeological settings, and whether additional guidance is needed for lagoon construction. Land application, on the other hand, uses a very active surface soil matrix and leads to more extensive removal of contaminants from water infiltrating through the soil profile due to enhanced volatilization, photolysis, and aerobic biodegradation. Even so, land application can lead to a larger areal extent of ground water contamination, especially in locations with multiple center pivot sprinklers (e.g., Site #4).

We found little evidence of significant ground water contamination by stressors other than nitrate or ammonium at these sites, except in those cases where CAFO wastes leaked directly from the lagoons into associated aquifers. Even in those cases, where ground water nitrate concentrations greatly exceeded the MCLs and even moderate to high levels of ammonium could also be detected, the other stressor concentrations were generally quite low. This suggests, but does not necessarily imply, that if CAFOs were properly managed so as to preclude ground water contamination by nitrate in excess of the MCL, then the other stressors associated with CAFO wastes in this study (microbial indicators, metals and metalloids, antibiotics, and estrogen hormones) might also be attenuated to acceptable risk levels. Additional field studies are needed to test this hypothesis, preferably with more frequent sampling events to account for seasonal variations and long-term effects. For most of the sites in this study (Sites #1, #2, #4, and #6), the sampling frequency was very limited (one to three sample events over several years), and it is possible that there could have been pulse events of ground water contamination by these other stressors caused by seasonal variations or intermittent discharges that could have been missed. But this possibility is very site-specific and would not be expected to be an issue where routine land application or leakage from faulty lagoons generates relatively continuous sources.

It is also important to note that this study did not evaluate true pathogens, other synthetic hormones (e.g., trenbolone), or other antibiotics (e.g., monensin), and additional research is also needed to ascertain whether these stressors would exhibit similar potential for contaminating ground water through leaking lagoons or land application of CAFO wastes. This study also does not address estrogen conjugates, which are expected to be more mobile in the soil than free estrogens, nor does it address long-term effects from the buildup of salts, metals, phosphate, and micronutrients on ground water quality or soil productivity. Finally, the relatively few detections of other stressors in nutrient-impacted ground waters in this study should not obscure the fact that contamination of ground water by nitrate or ammonium is in itself a significant environmental problem, and can lead to legacy impacts on receiving surface waters with direct hydrologic connection to contaminated ground waters.

These are all commercial operations and in many respects are very typical of the types of commercial CAFOs found in the south central United States. But it should be understood that these sites were chosen for this study based on the fact that, with one exception, the operation had already resulted in ground water contamination by nitrate and/or ammonium. We specifically selected these sites for this study because we wanted to determine whether other stressors found in CAFO wastes were also present in the contaminated ground water. In this respect, then, these sites do represent more problematic scenarios, and therefore this suite of case studies does not imply that six of seven CAFOs in the U.S. will have contaminated ground water. However, the design and nutrient management failures responsible for ground water contamination at these sites can and probably do occur at CAFO sites across the U.S., although the extent has not been determined and should be investigated. Finally, much more work is needed to address the efficacy of currently accepted CAFO nutrient management strategies (i.e., BMPs) for ground water protection from contamination by nutrients as well as other stressors, and to ascertain whether additional guidance or regulatory controls are needed.

5.0 Quality Assurance

This project was designed to evaluate CAFO impacts on ground water quality through examination of several commercial field operations as case studies. A Quality Assurance Project Plan (QAPP #G-10033) was followed throughout this study. Field data were obtained as described in this report and met the project's data quality requirements. The only exception to this occurred in the analysis for estrogen hormones by GC/MS/MS during 2009, where problems were encountered during switchover to a new instrument that led to false detections at or near the quantitation limit. For this reason, it was decided to not provide estimates of concentrations of estrogen hormones in samples analyzed in 2009 at values less than the quantitation limit. For consistency, this was done with the estrogen hormone data for the other years as well. This approach was deemed reasonable because these limits are in the very low nanogram per liter range, where the potential for data artifacts caused by matrix interferences becomes much greater. It should be noted that the analysis for antibiotics by LC/MS/MS is subject to similar constraints, which is why the outside laboratory used for antibiotic analyses also does not provide concentration estimates less than the quantitation (reporting) limit. Conclusions and recommendations made in this report are supported by these data, and are scientifically, but not legally defensible. Caution is warranted when extrapolating this information and findings to other CAFO sites.

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