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TECHNICAL MEMORANDUM

To:	Mitchell Katz, PhD Water Sciences, Orange County Environmental Protection
From:	Lance Lumbard; Mary Szafraniec, PhD; Nirjhar Shah, PE, PhD Wood Environment & Infrastructure Solutions, Inc.
Date:	September 8, 2021
Re:	Wekiva Springshed Groundwater Monitoring & Data Analysis Summary
Wood Project No:	600478.25 (PO #C16903B019)

EXECUTIVE SUMMARY

Water quality impairments associated with elevated nutrient concentrations (specifically nitrate and total phosphorus) and excessive algal growth within the Wekiva River have prompted the development of Total Maximum Daily Loads (TMDLs) and Basin Management Action Plans (BMAPs), which include a regulatory requirement to reduce more than 200,000 pounds of annual nitrogen (N) load to Wekiva and Rock Springs over the next 15 years. According to the Florida Department of Environmental Protection (FDEP), on-site sewage treatment and disposal systems (OSTDS) and urban turfgrass fertilizer represent the two largest sources of N load to the groundwater at 29% (296,984 lb/yr) and 26% (261,552 lb/yr), respectively. Additional sources of fertilizer N include 11% from farm fertilizer (110,089 lb/yr) and 8% from sports turfgrass fertilizer (80,902 lb/yr). The total N reduction necessary to meet the TMDL is 209,428 lb/yr (FDEP, 2018). To manage nitrate loading to Wekiwa and Rock Springs, Orange County implemented a Fertilizer Ordinance in 2017 which placed restrictions on residential turfgrass fertilizer application. Concurrently, Orange County initiated additional groundwater monitoring efforts involving a variety of innovative analytical techniques necessary to guide future management efforts associated with the Fertilizer Ordinance. The primary objective of this study is to evaluate if turfgrass fertilizer can be identified as an ongoing source of groundwater nitrate and if additional

measures are necessary to reduce the amount of turfgrass fertilizer loading within the Wekiva Springshed.

Wood Environment & Infrastructure Solutions (Wood) provided sampling and analytical assistance beginning in 2017 for 22 groundwater monitoring wells and Wekiwa Spring. Wood utilized these data and additional data collected from the same wells by Orange County and others since 2008 to develop a substantial database which Wood has also used to evaluate potential nitrate sources within the monitoring area.

Most wells selected for the monitoring program were located within residential areas that did not have septic, wastewater, or agricultural operations nearby that could serve as confounding sources of nitrate. Median nitrate concentrations in all wells were generally less than 2 mg/L. The highest nitrate concentrations were observed in one well that was adjacent to a golf course. Since 2008, median nitrate concentrations within the aquifers, in order of highest to lowest values, were: Intermediate Aquifer (1.420 mg/L), Surficial Aquifer (0.813 mg/L), and the Upper Floridan Aquifer (0.018 mg/L). The median nitrate concentration at Wekiva Spring was 1.100 mg/L.

Wood evaluated stable isotopes of nitrogen and oxygen to assess and attribute nitrate sources in a subset of wells with sufficient nitrate concentration and at the Wekiva Spring vent. Stable isotope data collected from the wells in the Wekiva Springshed suggests that NH_4^+(ammonia)/urea-based fertilizers are likely the primary source of nitrate within the sample areas. Other geochemical tracers were used to help evaluate nitrogen sources as well. Boron isotope results were similar to nitrogen and oxygen isotope data and suggest that NH_4^+/urea-fertilizers are a likely source of nitrate to groundwater.

Bayesian modeling (a type of probabilistic modeling) was conducted to estimate the proportional contributions of different N sources based on isotopic signature and probability ranges obtained from literature values. NH_4^+/urea-based turfgrass fertilizer represented between 40% and 70% within most of the wells analyzed with synthetic nitrate representing the dominant source in only one well. Stable isotope signatures suggest manure or wastewater represents less than 40% of all wells studied within the Surficial Aquifer. Denitrification normally represented less than 20% of the nitrate.

The statistical water quality analyses and modeling results suggest that the primary source of nitrate in the study area is associated with fertilizer. Isotopic ratio results varied, but most samples were associated with the typical range of nitrate from mineralized NH_4^+/urea fertilizers or denitrified nitrate that likely originated from the NH_4^+/urea fertilizer source. Water quality data from the Wekiwa Spring vent indicates a complex mixture of groundwater inputs, legacy nitrogen sources, and biogeochemical processes reflecting denitrification along vertical and horizontal groundwater gradients.

The available groundwater data appears sufficient to justify additional efforts to reduce nitrogen loading to the groundwater from fertilizer applications during the wet season when mass transport to the aquifer is the highest. Future sampling and analyses will continue to supplement the increasingly robust database. This will improve the understanding of nitrate sources within the

Wekiva Springshed and will ultimately allow Orange County to better manage the significant challenge of achieving the load reduction requirements of the Wekiva and Rock Springs BMAP and beyond.

1.0 INTRODUCTION

1.1 Scope of Work

The Orange County Environmental Protection Division (County) contracted Wood to evaluate potential sources of nitrogen in the Wekiva Springshed. This project includes groundwater well installation, groundwater monitoring, and groundwater quality analysis from samples collected from different regions of the aquifer system (i.e., Surficial, Intermediate, and Upper Floridan). Analyses included physicochemical measurements, and routine water quality chemistry analyses, as well as wastewater tracers and stable isotopes of nitrogen (N), oxygen (O), and boron (B) to identify and partition the contribution of sources of nitrate (NO_3) within the springshed. The overall project also includes processing and integration of data collected by Wood and others, statistical analyses, and updates to the County's technical team.

This Technical Memorandum describes the results of groundwater quality analyses from Wood's sampling efforts beginning in November 2017 and continuing through June 2021. Groundwater nitrogen source proportions were estimated using Bayesian modeling analysis of $\delta^{15}N$ and $\delta^{18}O$ stable isotope data. In addition, $\delta^{11}B$, chloride-bromide ratios, and other anthropogenic tracers (related to wastewater sources) are also described. This memo presents the results of additional analyses including a GIS-based assessment of land-use coverage proximal to each monitoring well and multivariate analyses (principal component analysis, PCA) of selected water quality parameters. Ongoing groundwater monitoring efforts may result in future updates to the findings reported in this Technical Memorandum.

1.2 Background and Objectives

The Wekiva River system is an important resource for Orange County (as well as Seminole and Lake Counties) and has been designated as an Outstanding Florida Water and a National Wild and Scenic River. The Wekiva River system is fed primarily by a unique assemblage of relatively large springs connected directly to a portion of the Floridan aquifer underlying much of western Orange County. This springshed within Orange County supplies the groundwater recharge necessary for spring discharge.

The Wekiva River (WBID 2956, 2956A, and 2956C) as well as Rock Springs Run (WBID 2967) were designated as impaired for nutrients (nitrate and total phosphorus) and a Total Maximum Daily Load (TMDL) was established for nitrate (286 µg/L) and total phosphorus (65 µg/L) by the Florida Department of Environmental Protection (FDEP) in 2008. The FDEP has determined that the Wekiva River is impaired by nutrients and a Basin Management Action Plan (BMAP) was adopted in 2015 to address potential pollutant sources. An additional BMAP specific to Wekiwa Spring and Rock Springs was adopted in 2018. Orange County is a member of the BMAP working group and has committed to identifying projects and programs such as this current project, aimed at achieving the TMDLs for nitrate and total phosphorus.

Nitrogen and phosphorus are the primary limiting nutrients controlling the growth of algae in springs and other surface waters. Nitrogen and phosphorus are essential elements of cellular structure and metabolism and are generally complexed within biological organisms where they tend to remain sequestered. However, bioavailable forms of nitrogen (including ammonia and nitrate) and phosphorus (including orthophosphate) are more mobile, particularly within surface waters. Nutrient loading to a springshed is influenced by multiple factors including rainfall, land use, and soil type. Nitrate tends to leach through soils more readily than dissolved forms of phosphorus so source determination and load management will be a key step toward achieving the nitrate TMDL.

Nitrate enters groundwater through a variety of sources including fertilizers, septic tanks, reclaimed wastewater, manure, and negligible amounts from natural soil mineralization. As groundwater moves from the Surficial Aquifer into the Intermediate and Upper Floridan Aquifers, differentiation of individual nitrate sources becomes more difficult. Therefore, the Surficial Aquifer system generally provides the greatest opportunity to discretely identify specific nitrate sources, particularly if septic, reclaimed wastewater, and turfgrass fertilizer sources can be individually isolated within the study area.

Nitrate can enter the Surficial Aquifer from direct application of fertilizers containing synthetic (industrially manufactured) nitrate or from nitrification of ammonia which may be applied directly or generated through the hydrolysis of synthetic urea which is often the primary ingredient of residential turfgrass fertilizers. For clarity, urea-based residential turfgrass fertilizers will be identified hereafter as NH_4^+/urea fertilizers.

Each potential source of nitrate has a specific "signature" that can be identified using a variety of techniques including stable isotopes of nitrogen ($\delta^{15}N$) and oxygen ($\delta^{18}O$). For example, some agricultural fertilizers contain synthetic nitrate which is produced using an industrial process and has a different stable isotope signature than nitrate that has been generated from urea hydrolysis and subsequent nitrification of NH_4^+. Additional nitrate source determination methods include tracers such as sucralose, which are associated with septic and wastewater inputs.

Nitrate is readily utilized by certain chemoheterotrophic bacteria living within the soil matrix. Under certain conditions, nitrate reduction occurs through a biological process known as denitrification. Denitrification involves the conversion of nitrate, through a series of intermediate forms of nitrogen, to gaseous forms of nitrogen (namely N_2O and N_2). Denitrification can provide a significant opportunity for nitrate reduction through off-gassing to the atmosphere (Singleton et al. 2007). The loss of nitrate through denitrification alters the stable isotope signature of groundwater and is an important consideration when assessing a potential contribution source.

Findings from research conducted by Tucker et al. (2014) and others have provided preliminary evidence to indicate turfgrass fertilizers are a significant source of nitrate to the Wekiva springshed. These findings prompted Orange County and other local governments to implement a residential turfgrass fertilizer ordinance that bans phosphorus application unless soils indicate a phosphorus deficiency and places seasonal restrictions on nitrogen application and requires the majority of nitrogen-containing ingredients to be "slow-release". Additional restrictions on residential turfgrass fertilizer application should reduce the amount of nitrate leaching to the groundwater and ultimately the Wekiva River as evidenced by the additional data collected by Orange County over the past 13 years.

The objectives of Orange County's efforts to identify potential sources of nitrate loading to the Wekiva springshed include the following:

1) Demonstrate Orange County's involvement as a stakeholder as established in the Wekiva River and Wekiwa Spring and Rock Spring BMAPs
2) Develop a robust and statistically defensible database that can be used to test scientific hypotheses including whether or not nitrate loading can be attributed to the seasonal application of residential turfgrass fertilizer
3) Provide a data-driven framework for regulatory policy implementation and nutrient-pollutant management
4) Provide a means to measure the effectiveness of regulatory policy changes and implement adaptive management strategies

2.0 DATA COLLECTION

In November 2017, Wood began collecting groundwater samples from 22 existing groundwater monitoring wells and at the Wekiwa Spring vent (**Figure 1** and **Table 1)**. The location of each site in reference to the groundwater recharge area is shown in **Figure 2.** The recharge areas consist of three categories: low (1 to 5 in/yr), medium (5-15 in/yr), or high (>= 15 in/yr) (**SJRWMD Technical Fact Sheet SJ2016-FS1**)

All well locations are within Orange County's portion of the Wekiva springshed. Eleven of the 22 monitoring wells were installed in 2008 as part of the SJRWMD nitrate source tracking study (Tucker et al. 2014). The remaining wells were installed by the Florida Department of Environmental Protection or Orange County to create well clusters designed to sample from the three aquifers. For brevity, the well or sample location names are described using the code shown in **Table 1**.

Table 1 – Sample Sites, Depths, Aquifers and GPS Coordinates

Location Code	Location Name	Total Well Depth (Ft)	Aquifer Monitored	Latitude (Deg-N)	Longitude (Deg-W)
XWEKIVASW01	SW01	Spring	NA	28.711909	-81.460448
XWEKIVABW02	BW02	12	Surficial	28.723673	-81.472965
XWEKIVAMW01	MW01	14	Surficial	28.709476	-81.504323
XWEKIVAMW02	MW02	30	Intermediate	28.679405	-81.499191
XWEKIVAMW04	MW04	48	Surficial	28.702733	-81.461825
XWEKIVAMW04R	MW04R	48	Surficial	20.702575	-81.461771
XWEKIVAMW06	MW06	20	Surficial	28.679283	-81.478404
XWEKIVAMW07	MW07	20	Surficial	28.679028	-81.478232
XWEKIVAMW11	MW11	35	Intermediate	28.69358	-81.46239
XWEKIVAMW14	MW14	15	Surficial	28.652062	-81.475606
XWEKIVAMW15	MW15	32	Intermediate	28.665336	-81.470997
XWEKIVAMW17	MW17	15	Intermediate	28.678423	-81.500142
XWEKIVAMW20	MW20	20	Surficial	28.662893	-81.471873
XWEKIVAMW22	MW22	27	Intermediate	28.653581	-81.489653
XDEPFLD	XDEPFLD	110	Upper Floridan	28.766667	-81.520278
XDEPPBD	XDEPPBD	210	Upper Floridan	28.700975	-81.478851
XDEPPBS	XDEPPBS	34	Surficial	28.700989	-81.478852
XWEKIVAMWAI	MWAI	75	Intermediate	28.709167	-81.462778
XWEKIVAMWBU	MWBU	135	Upper Floridan	28.694444	-81.465833
XWEKIVAMWBS	MWBS	40	Surficial	28.694447	-81.465816
XWEKIVAMWBSR	MWBSR	40	Surficial	28.694447	-81.465816
XWEKIVAMWCI	MWCI	90	Upper Floridan	28.68333	-81.47639
XWEKIVAMWDU	MWDU	155	Upper Floridan	28.771389	-81.555278
XWEKIVAMWDS	MWDS	40	Surficial	28.771389	-81.555278
XWEKIVAMWEU	MWEU	90	Upper Floridan	28.7025	-81.493056

Note: Well XWEKVAMW04 was properly abandoned and replaced with XWEKIVAMW04R on March 14, 2019. Well XWEKIVAMWBS was properly abandoned and replaced with XWEKIVAMWBSR on October 14, 2020.

Figure 1 - Aerial Image of Well Locations and Spring Vent (SW01), Coded by Aquifer

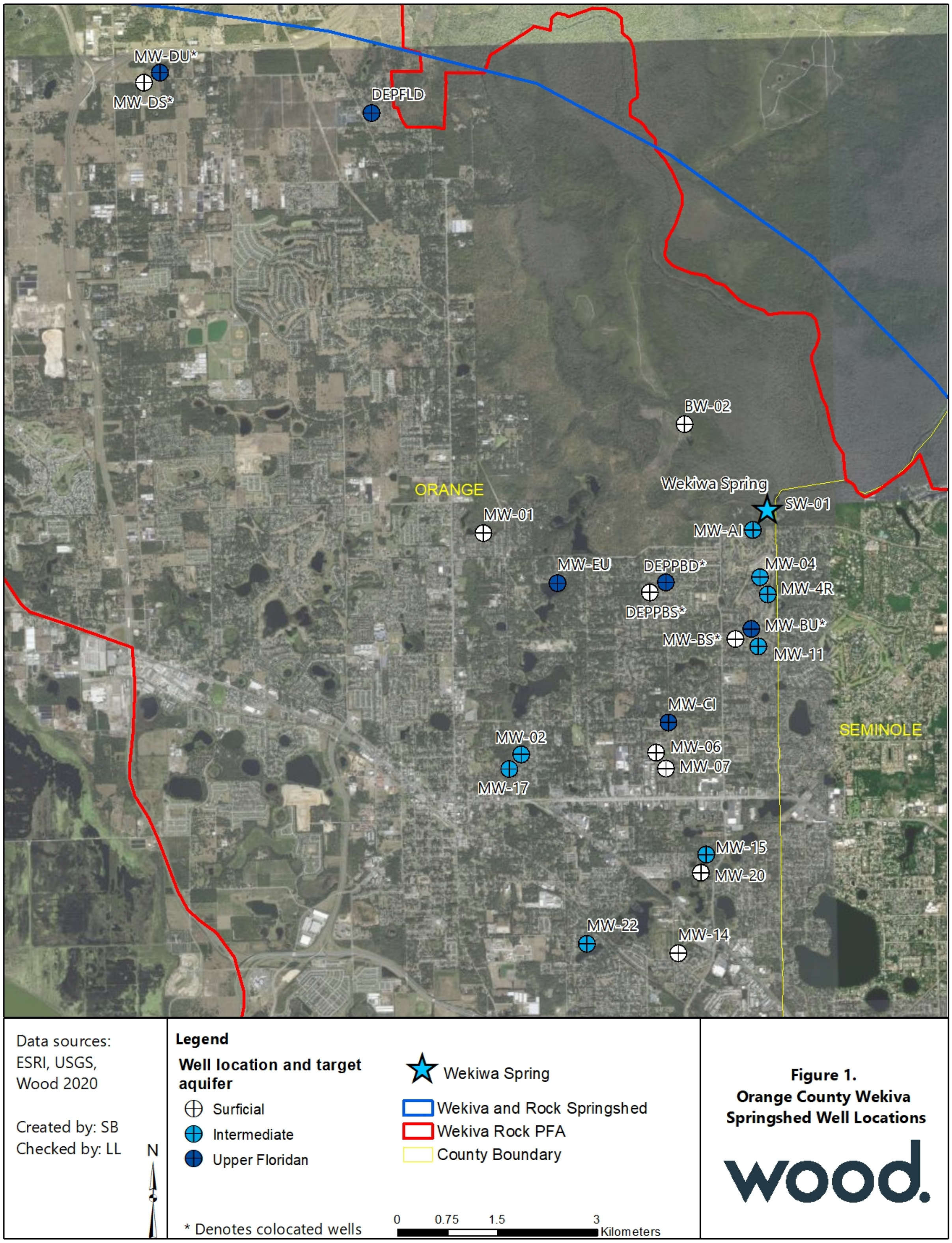
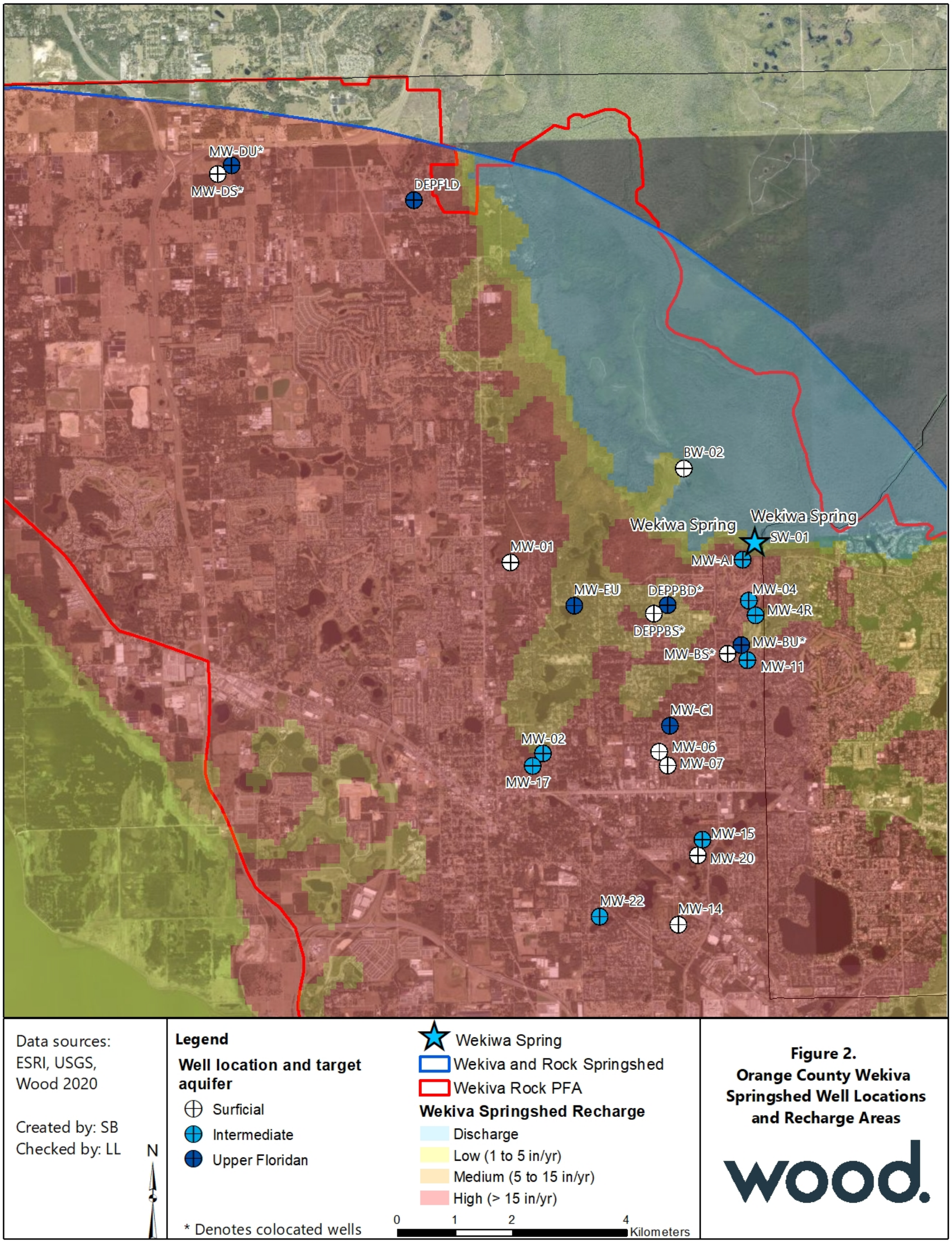


Figure 2 – Map of Sample Locations Within the Wekiva Springshed and Groundwater Recharge Areas (Recharge information from SJRWMD Technical Fact Sheet SJ2016-FS1)



All sample locations were sampled for the physicochemical parameters listed in **Table 2**. Twelve of these locations including MWBS, MWDS, DEPPBS, MW02, MW04, MW07, MW11, MW17, MW20, MW22, MW17, and SW01 were selected for further analysis using stable isotope analyses ($\delta^{15}N$, $\delta^{18}O$, and $\delta^{11}B$) and anthropogenic tracers (caffeine/sucrose/sucralose/aspartame). The twelve locations were selected based on their higher nitrate concentrations.

Table 2 – Water Quality Physicochemical Parameters

<table>
<tr><th>Parameter</th><th>Method</th><th>Container</th><th>Preservation</th><th>Performed By</th></tr>
<tr><td>pH</td><td>FDEP SOP FT 1100</td><td>NA-DM</td><td>NA-DM</td><td>Wood</td></tr>
<tr><td>Specific Conductance</td><td>FDEP SOP FT 1200</td><td>NA-DM</td><td>NA-DM</td><td>Wood</td></tr>
<tr><td>Dissolved Oxygen</td><td>FDEP SOP FT 1500</td><td>NA-DM</td><td>NA-DM</td><td>Wood</td></tr>
<tr><td>Temperature</td><td>FDEP SOP FT 1400</td><td>NA-DM</td><td>NA-DM</td><td>Wood</td></tr>
<tr><td>Turbidity</td><td>FDEP SOP FT-1600</td><td>NA-DM</td><td>NA-DM</td><td>Wood</td></tr>
<tr><td>Chloride</td><td>300.1</td><td rowspan="10">Polyethylene or glass bottle</td><td rowspan="3">4°C</td><td rowspan="10">OC Utilities Lab</td></tr>
<tr><td>Bromide</td><td>300.1</td></tr>
<tr><td>Alkalinity</td><td>310.1</td></tr>
<tr><td>Ammonia (N)</td><td>350.1</td><td rowspan="4">Sulfuric Acid to pH<2, 4°C</td></tr>
<tr><td>Nitrate+Nitrite (N)</td><td>353.2</td></tr>
<tr><td>Nitrate (N)</td><td>353.2</td></tr>
<tr><td>Total Kjeldahl Nitrogen</td><td>353.1</td></tr>
<tr><td>Orthophosphate</td><td rowspan="2">365.1</td><td>Filter, 4°C</td></tr>
<tr><td>Total Phosphorus</td><td>Sulfuric Acid to pH<2, 4°C</td></tr>
<tr><td>Total Organic Carbon</td><td>SU5310C</td><td>Hydrochloric Acid to pH<2, 4°C</td></tr>
<tr><td>Caffeine/Sucrose/ Sucralose/Aspartame</td><td>1694</td><td>Polyethylene or glass bottle</td><td>4°C</td><td>TestAmerica</td></tr>
<tr><td>δ^{15}Nitrogen</td><td>Ion Exchange</td><td>Polyethylene or glass bottle</td><td>4°C</td><td>Isotech</td></tr>
<tr><td>δ^{18}Oxygen</td><td>Ion Exchange</td><td>Polyethylene or glass bottle</td><td>4°C</td><td>Isotech</td></tr>
<tr><td>δ^{11}Boron</td><td>Thermal Ionization MS</td><td>Polyethylene or glass bottle</td><td>4°C</td><td>TestAmerica</td></tr>
</table>

Between November 2017 and June 2021, Wood completed 14 sampling events, based on the OCEPD intent to sample three times per year before, during and after fertilizer application season (**Table 3**). Wood received samples for stable isotope analyses and other water quality data from four additional sample events collected by another firm (Environmental Research & Design, ERD) in April 2019, August 2019, November 2019, and January 2020. Water quality and stable isotope results from ERD were compiled into Wood's overall database for a total of 18 combined sampling events between 2017 and 2021.

Monitoring wells MW04 and MWBS were consistently unproductive, with slow recharge rates. Under the supervision of a Professional Geotechnical Engineer licensed in the State of Florida, Wood installed a new well to replace MW04 on March 26, 2019. This new well, MW04R, is located approximately 50 feet southeast of the original well in a public right-of-way. The depths and screened intervals for both wells are similar and both are in the Surficial Aquifer. Well MW04 was installed to a depth of 48 feet with a screened interval from 33 to 48 feet. Well MW04R was installed to a depth of 47.5 feet with a screened interval from 32.5 to 47.5 feet Wood replaced well MWBS with MWBSR on October 14, 2020 at the same location and depth.

Table 3 – Wood and ERD Sampling Events (2017- 2021)

Year	Month	Firm
2017	November	Wood
2018	April	Wood
2018	June	Wood
2018	August	Wood
2018	December	Wood
2019	March	Wood
2019	April	ERD
2019	July	Wood
2019	August	ERD
2019	October	Wood
2019	November	ERD
2020	January	ERD
2020	March	Wood
2020	June	Wood
2020	September	Wood
2020	December	Wood
2021	March	Wood
2021	June	Wood

3.0 DATA ANALYSES RESULTS

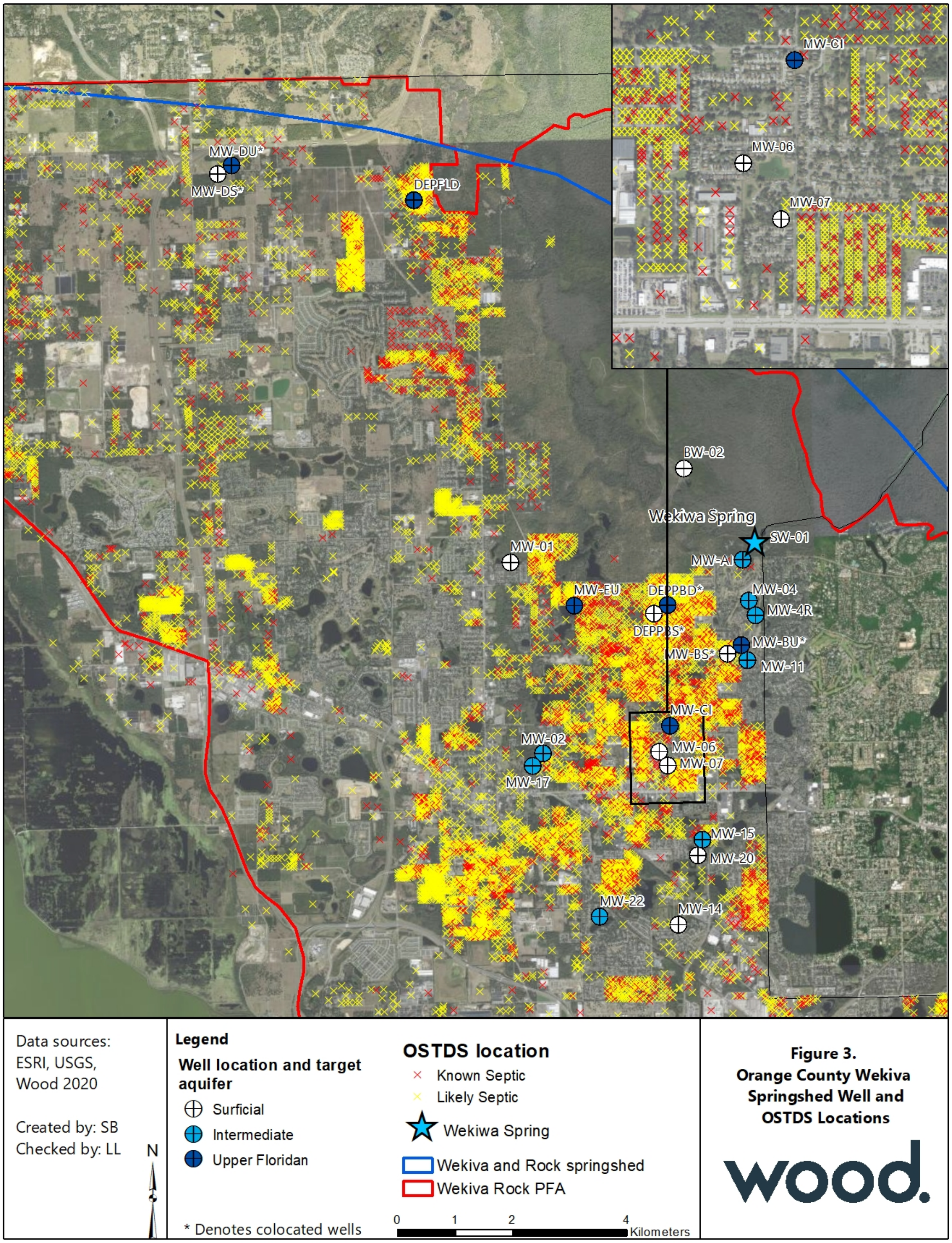
Wood analyzed data collected by Wood and ERD from 2017 through 2021. Wood will continue to update the database as new data are collected. These data were reviewed for quality assurance and quality control (QA/QC) of laboratory analysis reports, calibration records, and well logs in accordance with groundwater sampling methods specified in CH. 62-160, FAC. Sampling locations for stable isotope analyses have varied to prioritize sampling from wells with nitrate levels above method detection limits (MDL) needed for the isotopic analysis methods. Additional water quality data were obtained from the original sampling conducted in 2008 and 2009 by MACTEC (Tucker et al., 2014), and from 2011 through 2016 from the County. These earlier data were used in time-series plots but not in statistical analyses.

Wood first processed and screened the data by standardizing measurement units, removal of data with fatal qualifier codes (Codes: A, F, G, H, K, L, N, O, T, V, Y), and for censored data (non-detects, Code: "U"), Wood used one-half the MDL for censored data. The Grubb's test was used to detect statistical outliers. Three outliers collected on 3/28/2019 for wells MW20, MWBU, and MWBS, with nitrate + nitrite concentrations of 18.1, 14.9, and 15.2 mg/L and were removed. Statistical analyses included calculation of descriptive statistics, creation of time-series plots, multivariate analyses, and preliminary source modeling. Data were first compiled and processed in Excel, then statistical analyses were conducted in Excel, R, or Primer-e. Water quality summary statistics are provided in **Appendix 1**.

3.1 Land Use Analyses

Land use is an important factor that can influence groundwater chemistry. Wood compiled land use and septic tank density information using ArcGIS in a 1-km radius (776.5 acres) circular buffer around each well. Land use and septic tank data were first downloaded from the FDEP geospatial dataset: (https://geodata.dep.state.fl.us/datasets/2f0e5f9a180a412fbd77dc5628f28de3_3), and the Florida Department of Health (FDOH) Onsite Sewage Treatment and Disposal System (OSTDS) (http://ww10.doh.state.fl.us/pub/bos/Inventory/FloridaWaterManagementInventory/), respectively (**Figure 3**). The selection of a 1-km radius circular buffer was based on methods used by Canion, et. al., (2020). The land use cover (FLUCCS II land use codes) was calculated within this area. In addition, the number of septic tanks within this area was also determined by summing the "known" and "likely" septic tanks.

Figure 3 – Location of Wells and Onsite Sewage Treatment & Disposal Systems (OSTDS)



Since many of the wells are relatively close in proximity, their 1-km buffers overlapped. Therefore, the total study area was characterized by ignoring ("dissolving") the interior buffer boundaries resulting in a total area of 9,926 acres. Residential land use (including the sum of low, medium, and high-density residential categories) accounted for the greatest coverage, at 47.7%. The total number of septic tanks over the entire area was 4,269 (0.43/acre, **Appendix 2, Table 1**).

Land use and number of septic tanks were calculated within the 1-km buffer around each well (**Table 4**). These results are presented in **Appendix 2, Table 2**. Residential land use comprised about 55% of the total land use (**Table 4**). All other land use categories contributed less than 10% of the total area within buffer areas. The number of septic systems within the individual buffer areas range from 0 to 1,071 (**Appendix 2, Table 2**). Fertilizer application rate and coverage was assumed to be the same within each well buffer.

Table 4 - Average land use calculated from each well buffer (776.5 acres)

Land Use Category	Mean (%)	St.dev
Residential Low Density	10.4	6.3
Residential Medium Density	38.8	27.0
Residential High Density	6.1	6.8
Total residential	**55.3**	**24.6**
Commercial and Services	**4.8**	**6.7**
Industrial	**0.2**	**0.7**
Institutional	**1.9**	**3.0**
Recreational	**4.7**	**7.2**
Open Land	**0.7**	**1.5**
Cropland and Pastureland	3.9	6.9
Tree Crops	0.6	2.0
Tree Plantations	1.0	2.8
Nurseries and Vineyards	3.4	4.4
Specialty Farms	<0.01	<0.01
Total Agriculture	**8.9**	**14.0**
Other Open Lands <Rural>	0.033	0.1
Total Rural	0.8	1.5
Herbaceous	0.5	1.2
Shrub and Brushland	0.9	3.7

Table 4 - Average land use calculated from each well buffer (776.5 acres) - continued

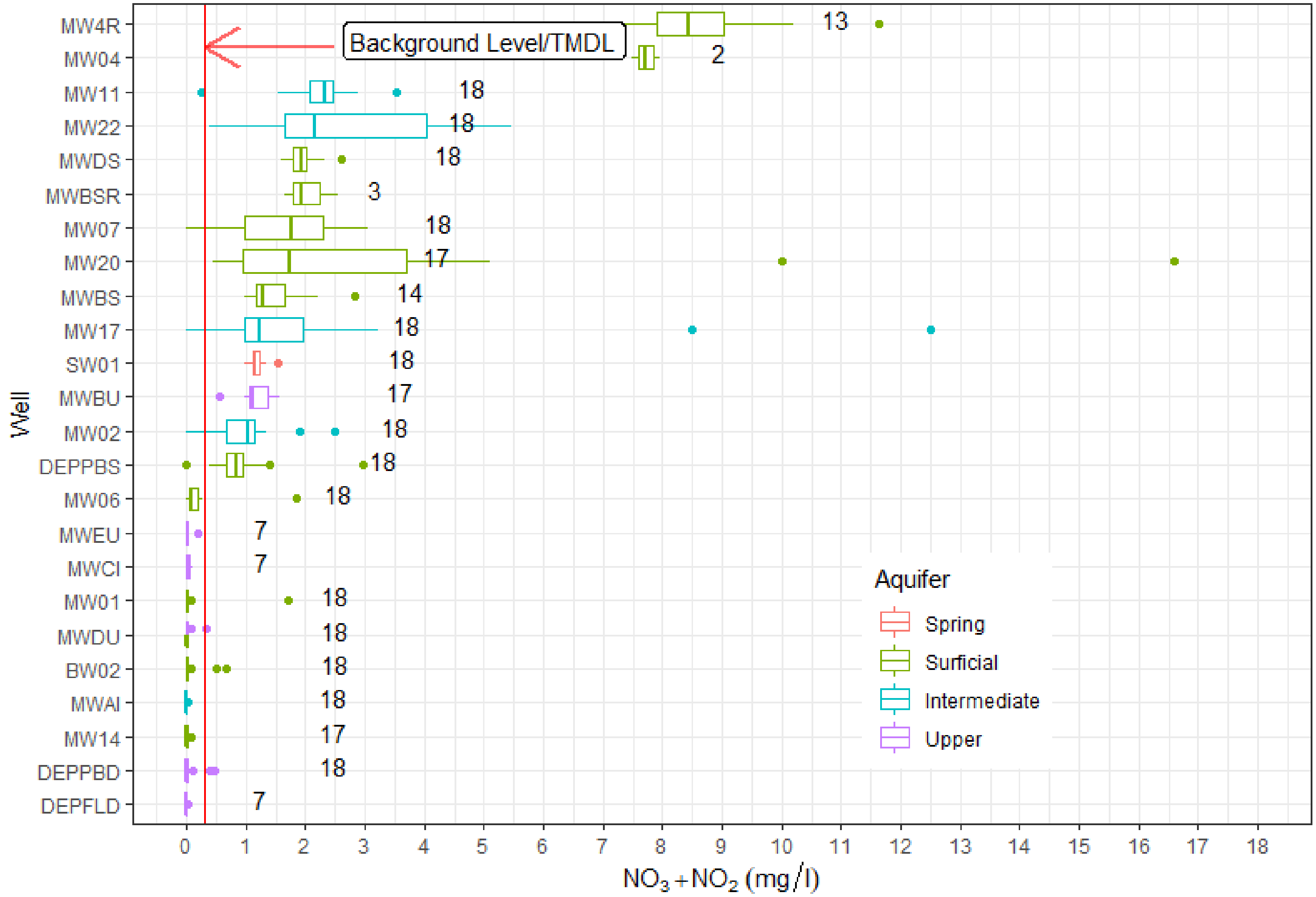
Land Use Category	Mean (%)	St.dev
Mixed Rangeland	0.4	0.9
Total rural, natural shrub and grassland	**2.7**	**4.0**
Upland Coniferous Forests	2.0	4.0
Upland Hardwood Forests	1.4	2.6
Upland Mixed Forests	3.9	4.9
Total Forested	**7.3**	**7.7**
Streams and Waterways	<0.01	<0.01
Lakes	1.5	2.4
Reservoirs	0.8	0.7
Total Lakes	**2.3**	**3.0**
Major Springs	**0.021**	**0.1**
Wetland Hardwood Forests	2.7	8.9
Wetland Forested Mixed	2.7	6.4
Vegetated Non-Forested Wetlands	2.9	3.8
Total Wetlands	**8.3**	**14.7**
Disturbed Lands	**1.1**	**3.4**
Transportation	**1.0**	**1.7**
Communications	**0.1**	**0.3**
Utilities	**1.3**	**1.3**

Note: Values represent the percent total land use within a 776.5-acre buffer area. Means and standard deviation (St.dev) were calculated from the 24 sample locations. Values are rounded and may not total 100%.

3.2 Nitrate Concentrations

Nitrate + nitrite (nitrate hereafter since nitrite is typically undetectable) concentrations ranged from below detection (<0.001 mg/L) to 14.5 mg/L (**Figure 4**). The majority of nitrate median values were below 2 mg/L. Wells generally clustered into three categories including: 1) at or below 1 mg/L, 2) between 1 and 2 mg/L, and 3) above 2 mg/L. The highest median nitrate concentrations between 7.5 and 8.5 mg/L were measured in wells MW04/MW4R. High nitrate levels (average: 10 mg/L) from this

Figure 4 - Boxplots of Nitrate+Nitrite (Nitrate) in Wells and Surface Water



Note: Boxplots are listed in order (from top to bottom) from highest to lowest median nitrate values. Sample dates are from November 2017 through June 2021. Background level is 0.03 mg/L from Tucker et al. (2014). Middle line represents median values. Left and right bounds of boxes are 25th and 75th percentiles. Whiskers are 1.5 x interquartile range. Dots are outliers. Sample sizes are noted to the right of the box plot.

well were reported in 2008 and 2009 (Tucker et al., 2014) This well is located in a residential development that is downgradient of a golf course. Residential land use was dominant in the study area and covered about 70% of the 776.5-acre buffer around this well. Median nitrate values of around 2 mg/L were measured in wells MW07, MWDS, MW22, and MW11. Median nitrate values of around 1 mg/L were measured in MWBU, MWBS, MW17, SW01 (spring vent), MW02, XDEPPBS, and MW20. Nitrate concentrations were relatively low (<1 mg/L) in the remaining wells including MW06, MW01, MWDU, MWC1, BW02, MWAI, MW14, DEPPBD, DEPFLD, and MWEU.

Wood created time-series plots for a subset of locations with data beginning in 2008 (**Appendix 3**). Trend tests were not conducted, as these long-term data were discontinuous. However, it appears that nitrate concentrations decreased between 2008 and 2021 in wells MW04/4R, MW11, MW07, MW20, MW02, and MW06. Patterns were not as clear in well MW22. This may be the result of Orange County's 2017 Fertilizer Ordinance requiring slow-release nitrogen fertilizer and restricting the application of nitrogen-containing fertilizers during the wet season between June 1 and September 30 to licensed applicators. Nitrate concentrations appeared to decrease in well MW17 after 2009, then started to increase again after 2018. Nitrite concentrations appeared to remain unchanged in the Wekiwa Spring vent (SW01).

3.2.1 Relationships Between Nitrate and Water Quality Parameters

Figures 1 through **9** in **Appendix 4** show the relationships between nitrate and selected water quality parameters, and well depth. These figures include nitrate concentration versus: dissolved oxygen (**Figure 1**, **Appendix 4**); total alkalinity (**Figure 2**, **Appendix 4**); depth to water (**Figure 3**, **Appendix 4**); pH (**Figure 4**, **Appendix 4**); specific conductance (**Figure 5**, **Appendix 4**); total nitrogen (**Figure 6**, **Appendix 4**); total phosphorus (**Figure 7**, **Appendix 4**); TSS (**Figure 8**, **Appendix 4**), and well depth (**Figure 9**, **Appendix 4**).

Except for total nitrogen, there was no apparent relationship between nitrate and other water quality parameters or well depth. The linear relationship between total nitrogen and nitrate is expected because nitrate is a large component of the total nitrogen in many of these samples.

3.2.2. Correlations Among Water Quality Parameters and Nitrate Among Wells

Spearman rank correlations among water quality parameters and depth were used to explore relationships among these parameters (**Figure 1**, **Appendix 5**). A total of 293 sampling events were used in this analysis. The strongest correlations were observed between nitrate and total nitrogen ($r = 0.85$). Alkalinity and pH were also strongly correlated ($r = 0.85$). Depth was negatively correlated with total organic carbon (TOC) ($r = -0.63$, $p = 0.001$).

Nitrate concentrations were positively, and significantly correlated with dissolved oxygen ($r = 0.55$, $p = 0.001$). A significant, but weak, correlation was also observed between nitrate and chloride ($r=0.25$, $p = 0.001$). Nitrate and TOC were negatively correlated ($r = -0.11$, $p = 0.05$).

Spearman correlations were also used to compare nitrate concentrations among wells (**Figures 2** and **3**, **Appendix 5**). These analyses require a complete dataset, with no missing values; this resulted in 16 wells and the spring vent having complete nitrate data for all sampling events. The strongest positive correlation was between well MWAI and BW02 ($r = 0.67$) and between the spring vent (SW01) and MW11 ($r = 0.64$). The strongest negative correlations were found between the spring vent (SW01) and well MW20 ($r = -0.77$) and between wells MWBS and MW02 ($r = -0.67$).

3.3 Multivariate Analyses

Wood also used a multivariate approach to reduce the dimensionality of the data to explore similarity in water quality among sampling locations. Therefore, a Principal Components Analysis (PCA) was performed. The PCA requires a complete dataset, (i.e., no missing data). This resulted in a total of 23 sample locations with 12 physicochemical water quality parameters and depth. Water quality parameters included: alkalinity, nitrate, ammonia-N, chloride, dissolved oxygen concentration, pH, specific conductance, total organic carbon (TOC), total phosphorus, turbidity, and sulfate. Data from well MW04 and MW04R were combined. Isotopes were not used in these analyses since they were only sampled from a subset of wells and isotope values were frequently below detection.

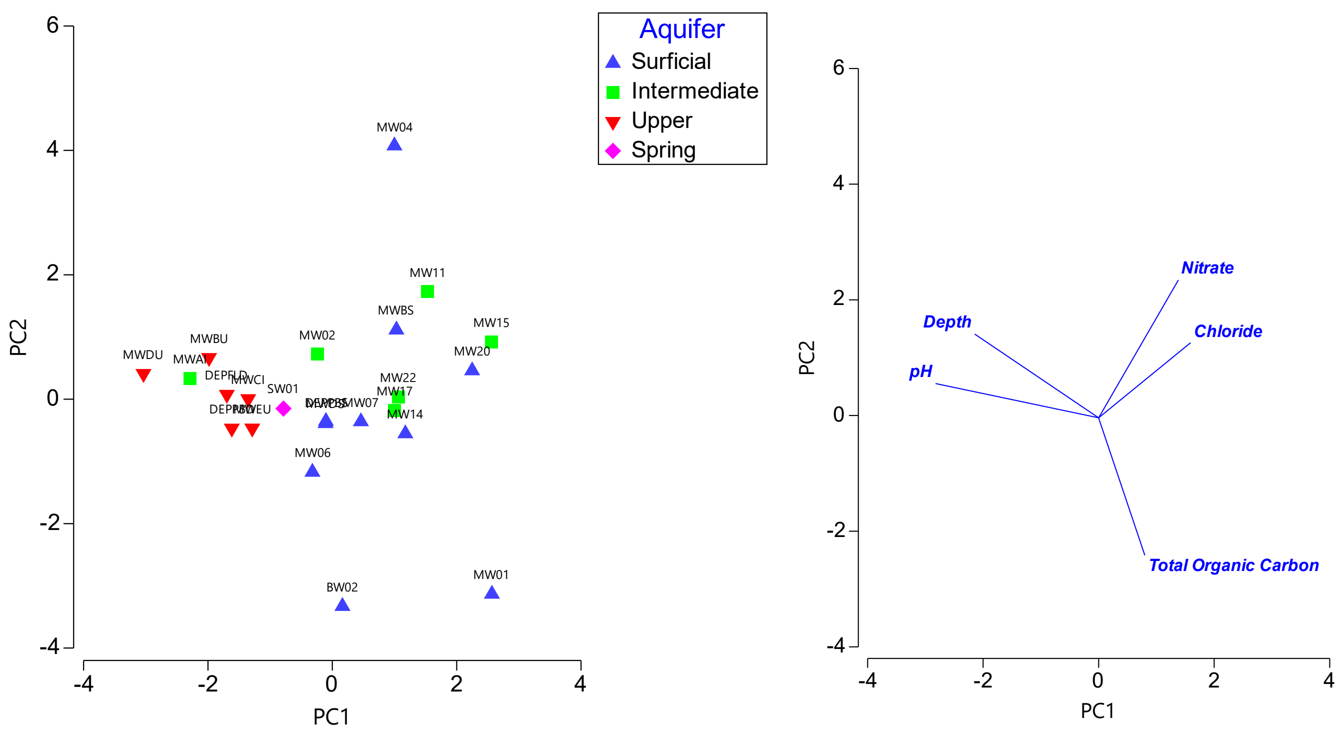
The PCA analysis results are shown in **Figure 5**. Water quality parameters were first averaged across all sampling events. Then, the relationships among parameters were examined using sman plots and correlations. Highly correlated parameters (correlations of $r > 0.70$) were removed. Total nitrogen (TN) was removed as it was correlated with nitrate ($r = 0.98$), and total alkalinity was removed as it was correlated with pH ($r= 0.79$). Next, since the water quality data are reported on varying scales, these data were normalized to a mean of zero and standard deviation of one. Next, a matrix of similarity/dissimilarity values based on the water quality variables was created by calculating the Euclidean distance between sample sites. Finally, a PCA ordination plot was created using these Euclidean distances to display the results. Vectors representing the water quality variables that explained the greatest variability were overlain on a separate PCA figure for clarity. These analyses were completed using Primer-e software (Ver. 7.0).

The PCA ordination attempts to project a high-dimensional data structure onto a two-dimensional plane with individual planes referred to as PC1 and PC2. The PCA analyses of the Wekiva well data resulted in the first two axes explaining 41.8% of the variance in the water quality multivariate structure, with the two axes explaining similar amounts of variability (PC1: 22.5% and PC2: 19.3%). The PCA program outputs are shown in **Appendix 6**. The PC1 axis appears to be separating wells based on greater depths and pH versus wells with higher nitrate and chloride values. The second axis, PC2, appears to be separating wells with higher TOC (wells BW02 and MW01), versus nitrate with well MW04 as an example.

The distances between the wells on **Figure 5**, indicate the degree of similarity (or conversely, dissimilarity). Wells closer together have more similar water quality and depth characteristics. The majority of wells clustering in the center of the figure suggests that water quality is relatively similar, but also indicates a depth and pH versus chloride and nitrate gradient as noted earlier. The Upper Floridan Aquifer wells appear to cluster closer together suggesting similar water quality and depth characteristics. In contrast, the Intermediate and Surficial Aquifers are further apart on the PCA graph, suggesting these wells have higher variability in water quality characteristics and depths. The water quality from the spring vent fell near the center of the plot, reflecting the likely mixture of source waters.

The first PCA axis appears to be separating wells based on a gradient of greater depth and pH versus wells with higher nitrate and chloride values. Two wells at the bottom of the PCA figure (BW02 and MW01) are separated by higher total organic carbon concentrations.

Figure 5 - Principal Component Analysis (PCA) Comparing Similarity in Water Quality Among Sample Sites



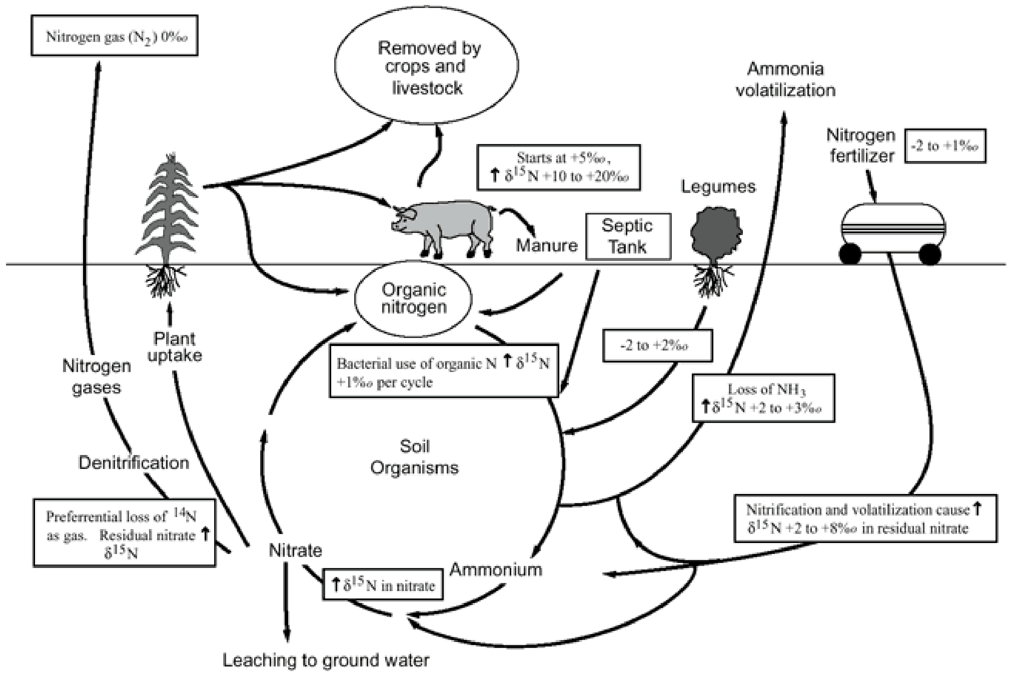
Note: Left plot represents PCA scores based on mean values for each sample location. Data from wells MW04 and MW04R were combined and averaged. Right plot shows vectors of water quality variables accounting for the greatest differences along the PCA axes with correlations > 0.6. The length of the lines corresponds to the relative strength of the loading. Axis PC1 accounted for 22.5% of the variation in the data, and PC2 accounted for 19.3% of the variation.

3.4 Stable Isotope Data and Nitrate Source Signatures

Isotopic signatures can be used for identifying sources of water and pollution (Kendall 1998). In groundwater pollution studies. A commonly used method is to compare the dual nitrate isotopes of $\delta^{15}N$ and $\delta^{18}O$ (e.g., Kendall 1998; Roadcap et al. 2002; Tucker et al. 2014; Reddy et al. 2017; Canion et al. 2020, etc.). Certain ratios of $\delta^{15}N$ and $\delta^{18}O$ in groundwater can indicate nitrogen sources including synthetic fertilizer, mineralized fertilizer, soil organic nitrogen, manure, and/or wastewater (including septic waste). In addition, $\delta^{15}N$ and $\delta^{18}O$ can be evaluated separately in comparison with other water quality parameters including nitrate. Increasing $\delta^{15}N$ enrichment suggests greater synthetic nitrate contributions and increasing $\delta^{18}O$ enrichment suggests greater wastewater contributions. It should be noted that there are limitations to these characterizations, especially in areas with different levels of confinement, degree of denitrification, age of and depth to groundwater, and in areas with a mixture of nitrogen sources (Xue et al., 2009; Canion et al., 2020).

The isotopic signature of nitrate can be altered by biogeochemical processes such as denitrification. In some areas of the Wekiva Springshed, there is a relatively thick confining layer that can slow infiltration to the deeper aquifer. This can result in environmental conditions that favor denitrification. In addition, shallow water tables are likely to have higher total organic carbon in the soil and reducing environments, which also favors denitrification processes resulting in lower nitrate concentrations, regardless of fertilizer inputs (Tucker et al. 2014). Several other biotic and abiotic processes can also influence the isotopic signatures as shown in **Figure 6**, which was excerpted from Townsend (2008). Thus, multiple methods and lines of evidence such as nitrate isotopes, boron isotopes, wastewater tracers, and ion ratios should be used in conjunction to support identification of nitrogen sources (Fenech et al. 2012).

Figure 6 - Influences of the Nitrogen Cycle Processes on $\delta^{15}N$



From Townsend 2008, http://www.kgs.ku.edu/Hydro/Publications/2008/OFR08_31/, accessed 3 February 2020

3.4.1 Comparison of Nitrate and Isotopes Among Aquifers

Nitrate concentrations were evaluated for different aquifers and were compared to $\delta^{15}N$ stable isotope results among aquifers (**Appendix 7**). Median nitrate concentrations were highest and similar in samples collected from the spring vent (1.15 mg/L), and from wells in the Intermediate Aquifer (1.13 mg/L). Lower median nitrate concentrations were measured from wells in the Surficial Aquifer (0.85), with the lowest median nitrate concentration observed in wells sampling the Upper Floridan Aquifer (0.018 mg/L) as shown in **Figure 1**, **Appendix 7**. **Figure 2**, **Appendix 7** shows the most enriched $\delta^{15}N$ isotopes were measured in the Upper Floridan Aquifer suggesting much of the nitrate has been denitrified as it travels downward through the aquifer systems. Less enriched $\delta^{15}N$ isotopes were measured in samples from the Surficial and Intermediate Aquifers where the denitrification process is not as pronounced. $\delta^{15}N$ isotope ratios in the spring vent water were intermediate, reflecting the mixture of waters.

3.4.2 Comparison of Groundwater Gradients, Nitrate and Isotopes between Deep and Shallow Paired Wells

Groundwater gradients were estimated for the three sets of paired wells using the well screen mid-point values using a calculator from the USEPA website (https://www3.epa.gov/ceampubl/learn2model/part-two/onsite/vgradient.html). The results are shown in **Table 5**. Groundwater flow gradients were downward and ranged from 0.0044 to 0.38, reflecting groundwater recharge potential and the effects of confining layers. The lowest gradient was measured in the pair with the deepest well.

Table 5 – Mean Vertical Groundwater Gradients for Paired Wells (n=14 sample events, 2017 through 2020)

Well Pair	Surface Elevation (ft)	Well Screen Interval (ft)	Mean Depth to Water (ft)	Mean Hydrologic Gradient	Flow Direction
MWBS	86.56	25'-40'	29.73	0.26	Down
MWBU	86.28	115'-135'	53.78		
DEPPBS	59.29	24'-34'	20.92	0.0044	Down
DEPPBD	59.09	200'-210'	21.49		
MWDS	127.11	25'-40'	25.80	0.38	Down
MWDU	125.46	160'-180	78.51		

Note: Gradients calculated using well screen mid-point values using the calculator from the USEPA website: https://www3.epa.gov/ceampubl/learn2model/part-two/onsite/vgradient.html

Nitrate concentrations and $\delta^{15}N$ isotopes were compared between the three pairs of shallow and deep wells are shown in **Appendix 8, Figures 3** through **6**. Nitrate concentrations were higher in the Surficial Aquifer than in the Upper Floridan Aquifer for two of the three paired wells (DEPPBS and DEPPBD, and MWDS and MWDU). Nitrate concentrations were similar between paired wells: MWBS and MWBU. $\delta^{15}N$ isotopes were measured in paired wells MWBS and MWBU. These results suggest that at this location, the Upper Floridan Aquifer water is more enriched in $\delta^{15}N$ than the Surficial aquifer which suggests denitrification is occurring as nitrate is transported to the Upper Floridan Aquifer.

Nitrogen and oxygen stable isotope concentrations were each plotted against nitrate concentrations as shown in **Figures 7** and **8**. No statistically significant relationships were observed between either the nitrogen or oxygen stable isotopes and nitrate concentrations.

3.4.3 Nitrate Source Determination using Dual Nitrate Isotopes

Isotope values for the twelve sample sites with stable isotope data were also plotted in a biplot shown in **Figure 9** with source bounding boxes that were adapted from Canion et al. (2020). Bounding boxes for synthetic NO_3-based fertilizer, NH_4^+/urea fertilizer, soil organic matter, and wastewater/septic were included. Similar variations of bounding box distributions have been reported in the literature by several others. Multiple variations of similar biplots have been reviewed for this project and it is apparent that there are slight shifts in bounding values for different sources, even for studies that include data from Florida-specific groundwater wells. Based on a review of the literature, these types of boxes appear to be somewhat regionally specific for some of the sources. A denitrification zone (moving from the bottom left to the top right of the figure), represents an approximate trajectory of denitrification, and is overlaid for reference on the biplot.

It appears that many of the wells fall within the NH_4^+/urea range. No isotopes fell in the synthetic NO_3 fertilizer range of values. Many samples fell along the denitrification line. Many samples plot within the overlapping NH_4^+/urea and manure/wastewater zones. However, other values were observed outside the source boxes but within the denitrification zone suggesting that this nitrate may have originated from reduced NH_4^+/urea fertilizer (Tucker et al. 2014). This is more frequently the case for shallow wells in Surficial Aquifer that may be influenced by leaching of soil and fertilizer nitrogen.

These results suggest a mixture of nitrogen sources based on the data available through June 2021. More specifically, samples collected from XDEPPBS, MW07, MW04, MW10, MW22 have $\delta^{15}N$ and $\delta^{18}O$ stable isotope values suggest that NH_4^+/urea fertilizers could be a significant source of n nitrate to the groundwater in these areas. It should be noted that values along the denitrification line (**Figure 9**) suggest that denitrification of nitrate could be the source of $\delta^{15}N$ and $\delta^{18}O$ enrichment as well. This inference is supported by reduced conditions within the Wekiva Springshed that may be facilitating denitrification and other nitrate reduction reactions.

Figure 7 - Bivariate Plot of Nitrate Concentrations and δ^{15}N Isotope Concentrations

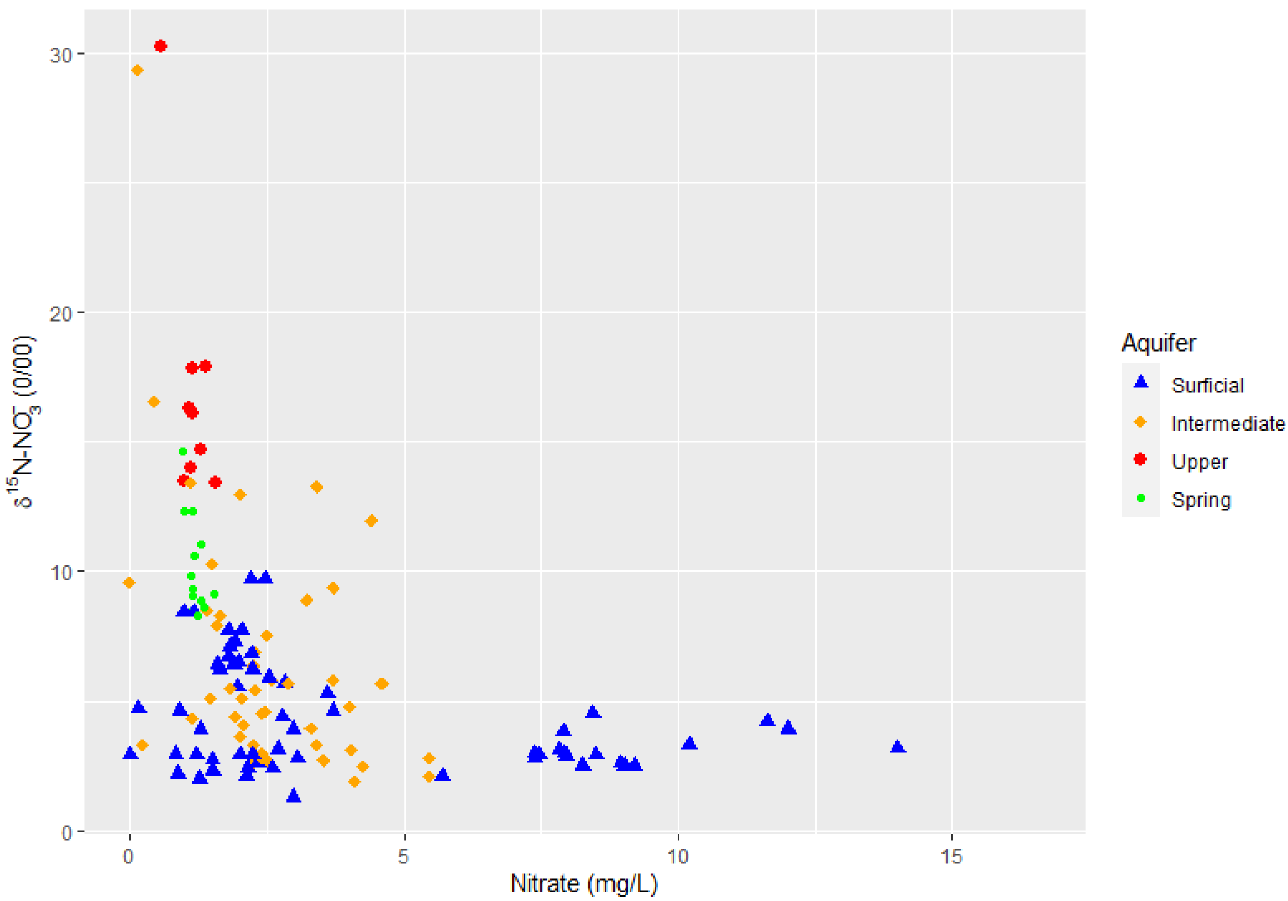


Figure 8 - Bivariate Plot of Nitrate Concentrations and $\delta^{18}O$ Isotope Concentrations

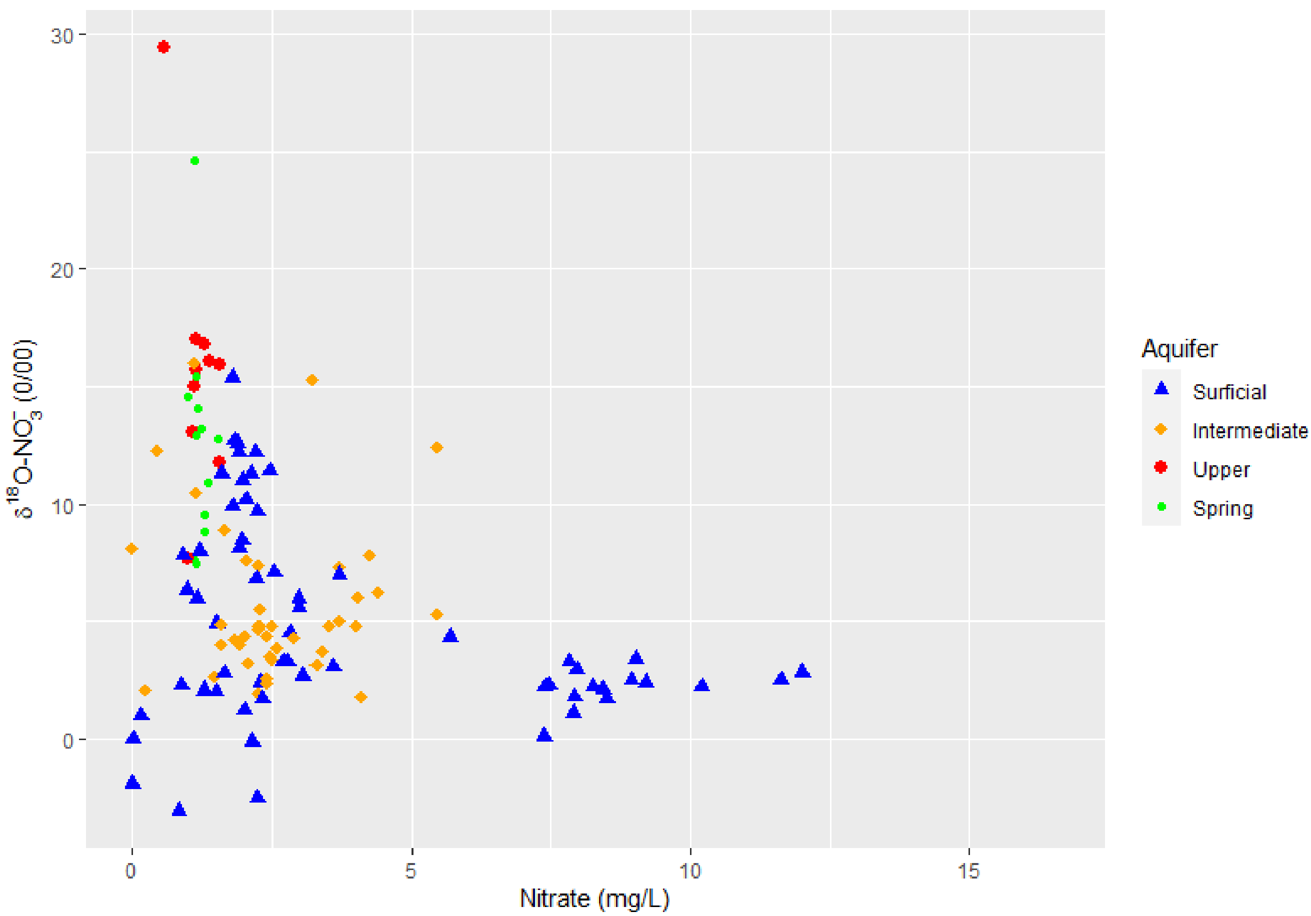
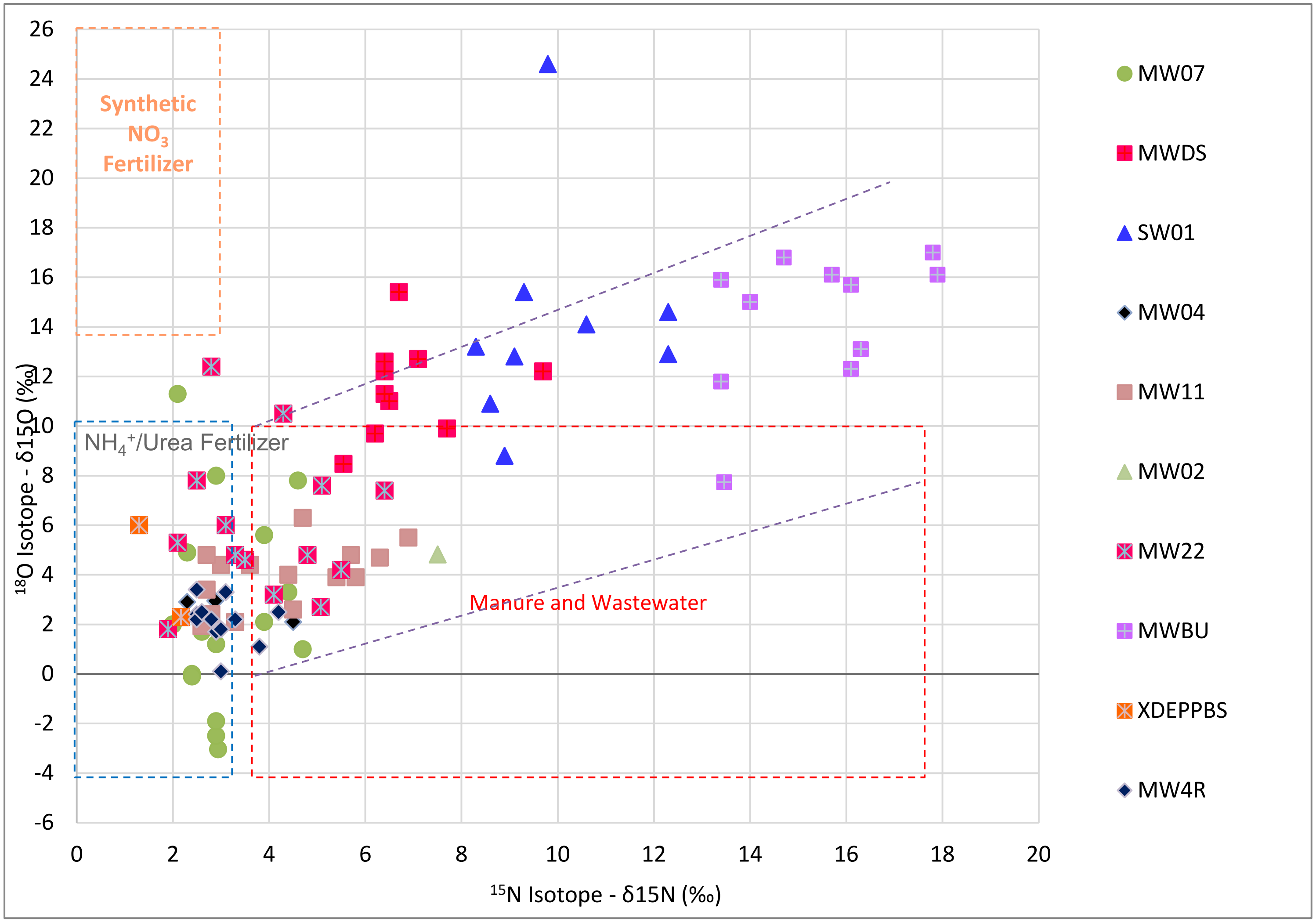


Figure 9 - Nitrogen and Oxygen Stable Isotope Bivariate Plot with Source Categories (2017 through 2021)



Note: Source isotopic ranges are represented using boxes bounded by values adapted from Canion et al. 2020. Purple diagonal lines indicate the approximate trajectory of denitrification (as shown by Canion et al. 2020). Data are from Nov 2017 through June 2021.

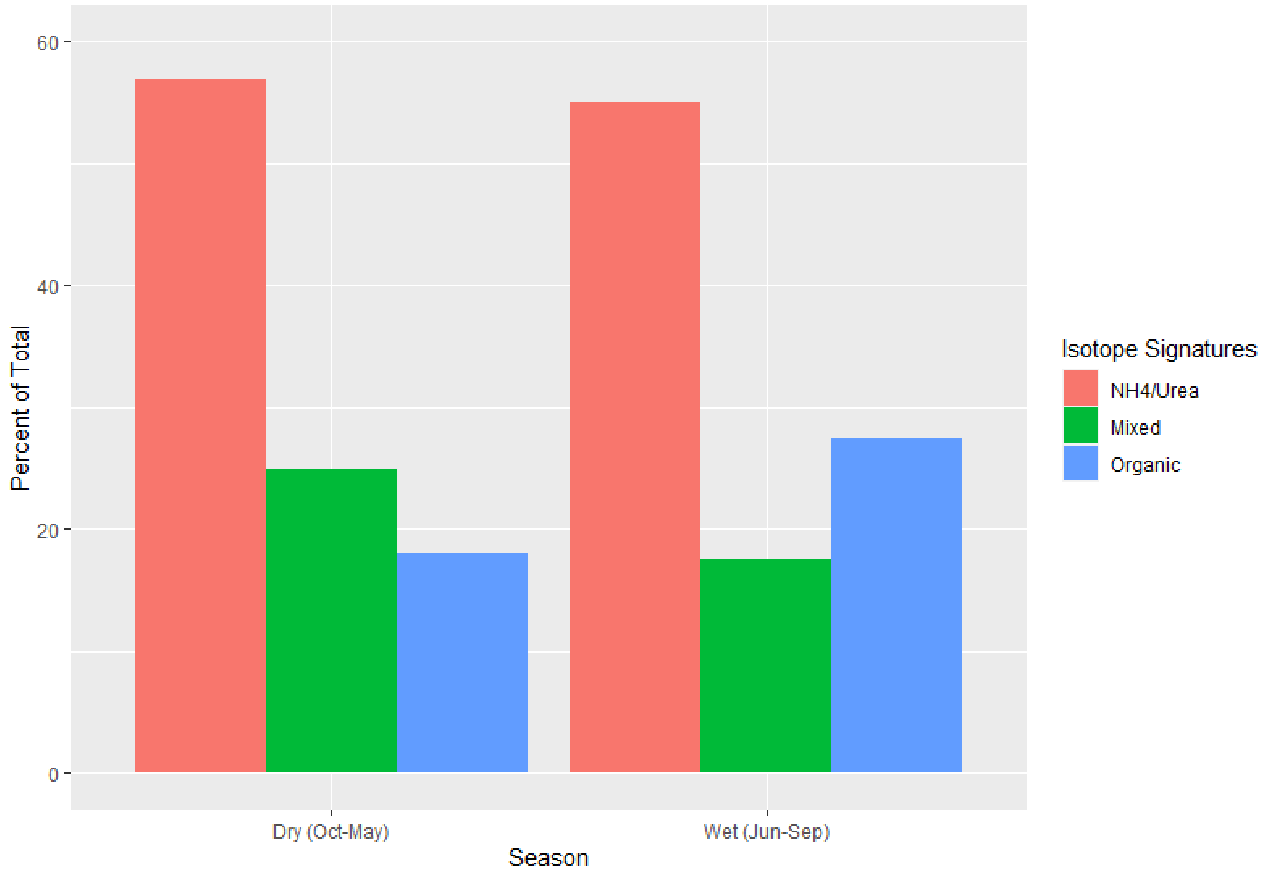
3.5 Seasonal Differences in Stable Isotopes

To further examine the isotopic signatures of potential sources of nitrogen, Wood compared the data distributions of $\delta^{15}N$ isotopic values in an approach similar to Heffernan et al. (2012). Heffernan et al. (2012) classified nitrogen sources into three categories: synthetic fertilizer in Heffernan et al., 2012, defined by < 6 $\delta^{15}N$ ‰), mixed (between 6 $\delta^{15}N$ ‰ and 9 $\delta^{15}N$ ‰), and organic (> 9 $\delta^{15}N$ ‰). Wood then compared distributions between wet and dry seasons. The wet season was defined as the four months from June through September and the dry season as the eight months from October through May. Because of the longer dry season, sample sizes in the dry season were about two times greater than wet season sampling (n = 55 and 24, respectively).

The greatest number of samples (78.5%) fell within the synthetic fertilizer (NH_4^+/Urea or nitrate-based) and mixed zones after combining all isotopic results. (**Figure 10**). These results are similar to the findings of Heffernan et al. (2012), who reported that for water collected from 113 spring vents in Florida, 88.2% of the isotope signatures suggested a mixture of synthetic fertilizers and mixed nitrate sources.

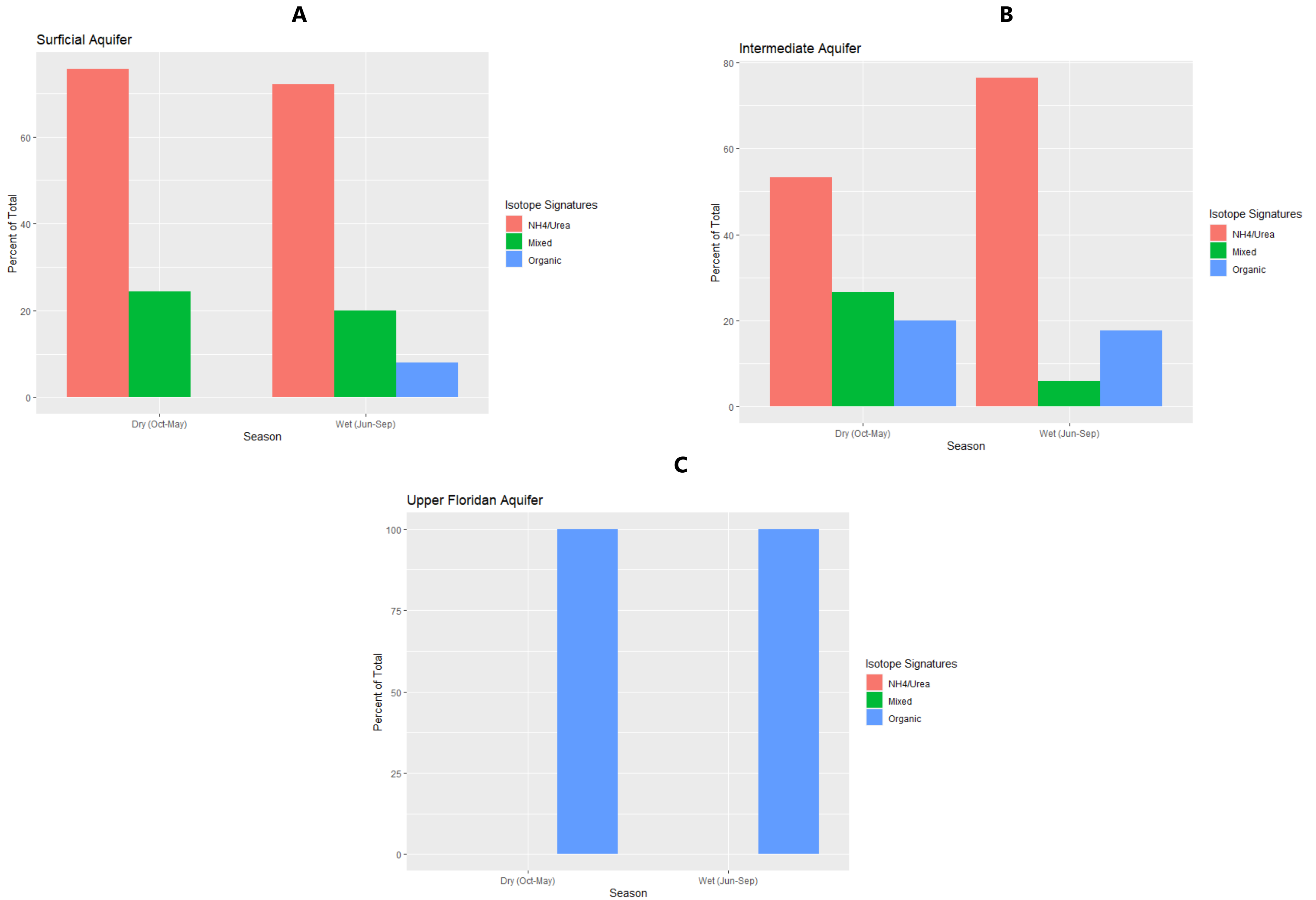
The greatest number of samples from the Surficial and Intermediate Aquifer wells are within the "NH_4^+/Urea" and "Mixed" isotopes categories (**Figures 11A** and **11B**). During the wet season, $\delta^{15}N$ was less enriched than the dry season within the Intermediate Aquifer suggesting that nitrate sources from NH_4^+/Urea fertilizers represent a greater proportion of the overall nitrate contribution. All isotopic ratios were enriched in the Upper Florida Aquifer, and are within the "Organic" range (**Figure 11C**). This indicates that nitrate in the Upper Florida Aquifer is likely influenced by denitrification where the nitrate may have originated from an inorganic source such as fertilizer. Additional sampling frequency throughout an entire year may help resolve seasonal differences in isotopic signatures.

Figure 10 - δ^{15}N Values for all Samples in the Dry Season (October through May) and Wet Season (June through September).



Note: three potential nitrate sources include NH_4^+/urea, mixed, and organic pools. These sources are defined as: NH_4/urea (<6 δ^{15}N ‰), mixed (between 6 δ^{15}N ‰ and 9 δ^{15}N ‰), and organic (>9 δ^{15}N ‰).

Figure 11 - $\delta^{15}N$ Values for Surficial (A), Intermediate (B) and Upper Floridan (C) Aquifer Wells in the Dry Season (October through May) and Wet Season (June through September)



3.6 Bayesian Mixing Model Analysis of Groundwater Well Data

Bayesian mixing models have been used to help identify probable sources of nitrate. The Bayesian mass balance mixing model simmr was used to evaluate Wekiva well data (Parnel, 2021). The simmr package is part of the MixSIAR framework, and is an upgrade to the SIAR package (Parnell, and Inger, 2021, (https://cran.r-project.org/web/packages/simmr/vignettes/simmr.html,). The simmr package is designed to solve mixing equations for stable isotopes, and can help estimate the fractional contribution of the four nitrogen sources including NH_4^+/urea, synthetic nitrate fertilizer, septic/manure, and denitrification. This model was run using the dual nitrate ($\delta^{15}N$ and $\delta^{18}O$) stable isotope data from seven wells including MW04/4R, MW07, MW11, MW17, MW22, MWBS, and MWDS. These wells were selected for analysis because of their similar locations within the Surficial Aquifer and data sufficiency.

Mixing models are dependent on establishing informative Dirichlet priors. Priors are used to establish, *a priori*, the probability of the distribution of the sources. However, establishing "informative" priors can be problematic without a good understanding of the relative proportions of the nitrate sources which can affect the output. Thus, for our modeling efforts, we used "uninformative" priors with the assumption that all nitrate sources could contribute equally.

The Bayesian model also requires isotopic ranges (means and standard deviations) for the modeled system. These values can be derived from previous studies or published data. Wood obtained the mean and standard deviation (SD) values from Canion et al. (2020) for mineralized, synthetic nitrate, and manure/wastewater. Denitrification mean and SD values were obtained from Drummond Carpenter (2021). The values used in the modeling analyses are provided in **Table 6**.

The first step in the modeling is to plot the well within the isotopic range boundaries. These ranges are called "convex hulls" or "mixing polygons" and are used to check the assumption that observed isotope values fall within the mixing region and that mixture components can therefore be reasonably inferred from the Bayesian model. The traditional method constructs the mixing polygon using the source means as vertices. However, because source means are inherently uncertain, a more robust method uses a Monte Carlo approach to estimate the probability that data fall within the mixing polygon, by simulating thousands of mixing polygons whose vertices are sampled from the normal distributions given by the source means and standard deviations (Smith et al. 2013). The result is visualized on the isotope biplot as contour lines of equal probability.

Using the traditional convex hull method, many samples fell outside the mixing polygon (**Figure 12**). However, the alternative Monte Carlo method indicated that the majority of the data likely fell within the mixing polygon if one accounts for the uncertainty in the source mean. The isotope data are enclosed within the 95% mixing region indicated by the outermost contour (**Figure 13**).

Table 6 - Mean and Standard Deviations of Nitrogen and Oxygen Isotope Values Associated with Selected Nitrogen Sources

Source	$\delta^{15}N$ (‰) Mean	$\delta^{15}N$ (‰) Standard Deviation	$\delta^{18}O$ (‰) Mean	$\delta^{18}O$ (‰) Standard Deviation
NH_4^+ /Urea Fertilizer) (Mfert)	-0.9	2.07	4.18	2.87
Septic/Manure	12.8	6.1	4.18	2.87
Synthetic (NO_3 Fertilizer) (Sfert)	0.65	81.75	8.54	2.92
Denitrification*	25.0	5.0	25.0	5.0

*Note - Drummond Carpenter (2021)

Next, the Bayesian model simmr was run using data from November 2017 through June 2021 to estimate the proportion of nitrogen sources for the seven wells described previously. Specifically, the simmr_mcmc routine with 3,600 iterations was used. The initial model output suggested poor performance with this dataset. Post-hoc tests conducted on the Bayesian model output to verify convergence and other model diagnostics indicated that even though the model runs converged onto a stable solution, the standard deviations associated with the results were found to be very large and can potentially influence the results. However, as noted, the Monte Carlo modeling demonstrated a good fit. Therefore, to test if small sample size presents an issue, Wood also conducted a bootstrapping exercise using means and standard deviations from the individual wells to increase sample size to n = 99. This resulted in a better model, with no error warning. This implies that sample size is limited.

Nonetheless, assuming these data are adequate, the simmr model outputs depicting the estimated proportion of the potential nitrate sources for wells MW4, MW7, MW11, MW17, MW22, MWBS and MWDS are shown in **Figure 14**. These results suggest that the nitrate sources are variable, but NH_4^+/urea fertilizer accounted for 40% or more of the nitrate source in five of the seven wells evaluated. Well MWDS had the lowest nitrate contribution from NH_4^+/urea fertilizer (15%) and the highest proportion of synthetic nitrate fertilizer of around 50%. The remaining wells had synthetic nitrate contributions of 20% or less. Septic and manure sources represented less than 20% of the nitrate in five of the seven wells. Wells MW11 and MWBS had a slightly higher nitrate contribution from septic and manure of about 30%. The denitrification process appears to represent between 5 and 10% of nitrate within most wells with higher denitrification contribution of 25% at well MWDS and almost 40% of the nitrate at well MW17

These Bayesian model results correspond well with the data shown in the isotopic biplot (**Figure 9**) and are similar to the findings of Drummond Carpenter (2021). The lack of ideal fit for this model is potentially caused by the presence of multiple nitrogen sources which may be influencing groundwater quality in these wells.

Figure 12 - Nitrogen and Oxygen Isotope Convex Hulls Derived from Bayesian Modeling

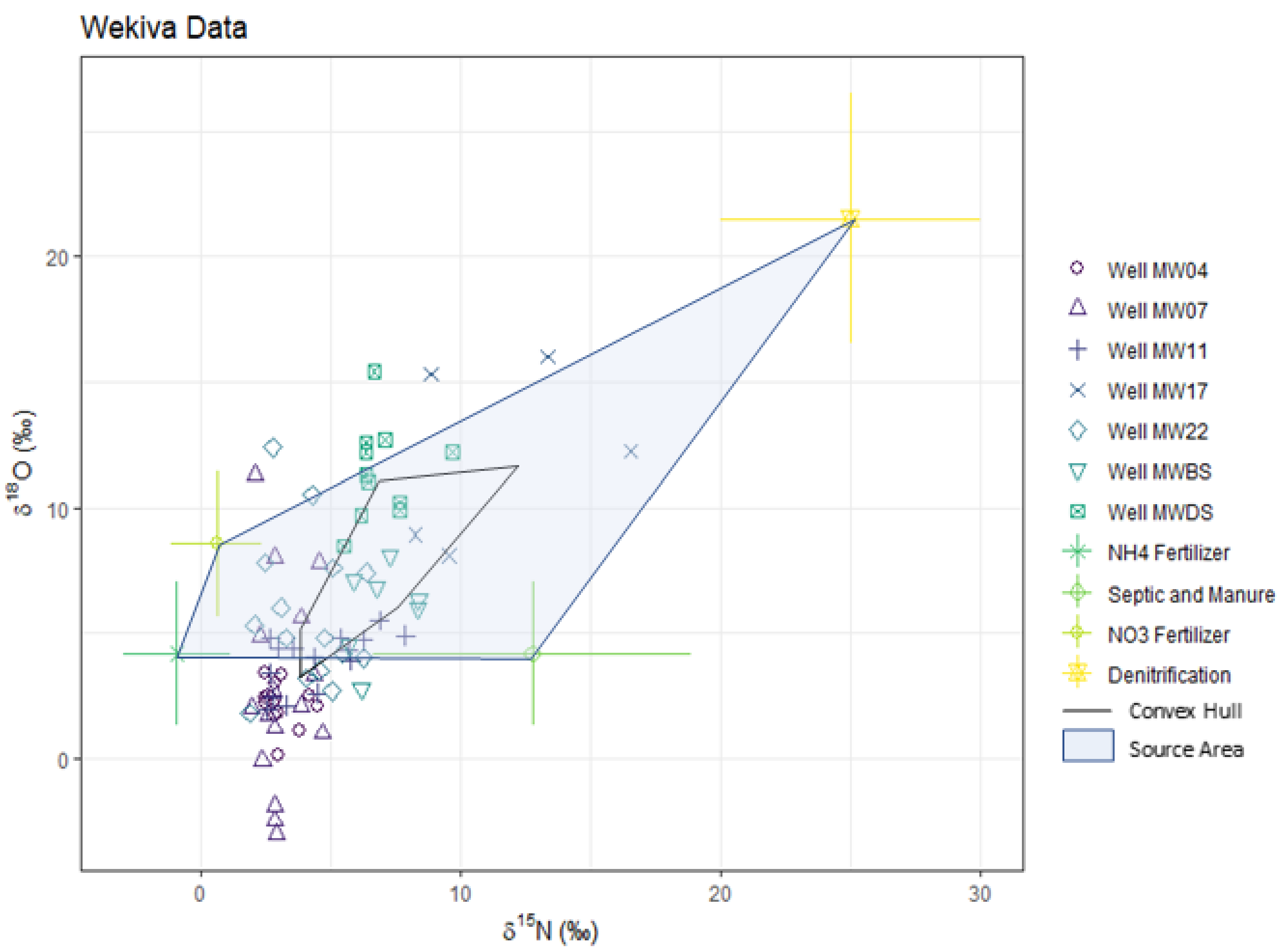
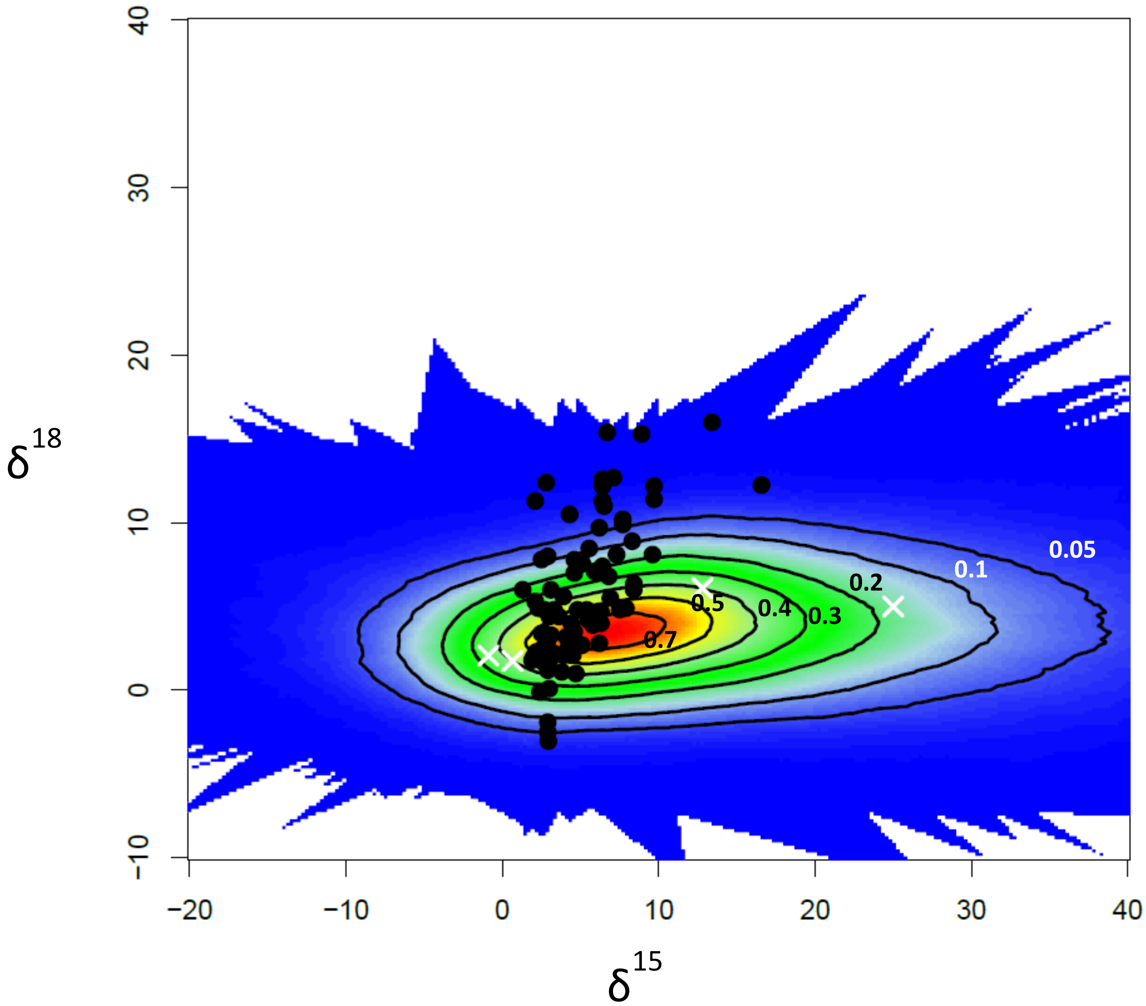


Figure 13 - Mixing Region Based on Monte Carlo Derived Probabilities



Note: Contour lines represent equal probabilities. Black circles represent isotopic data from current sampling event from 2017 through June 2021. White crosses represent source (i.e., NH_4^+/urea fertilizer, NO_3 fertilizer, septic and manure, and denitrification).

Figure 14 – Output from simmr Bayesian Mixing Model Analyses. Box plots represent the fractional proportion of potential nitrate sources.

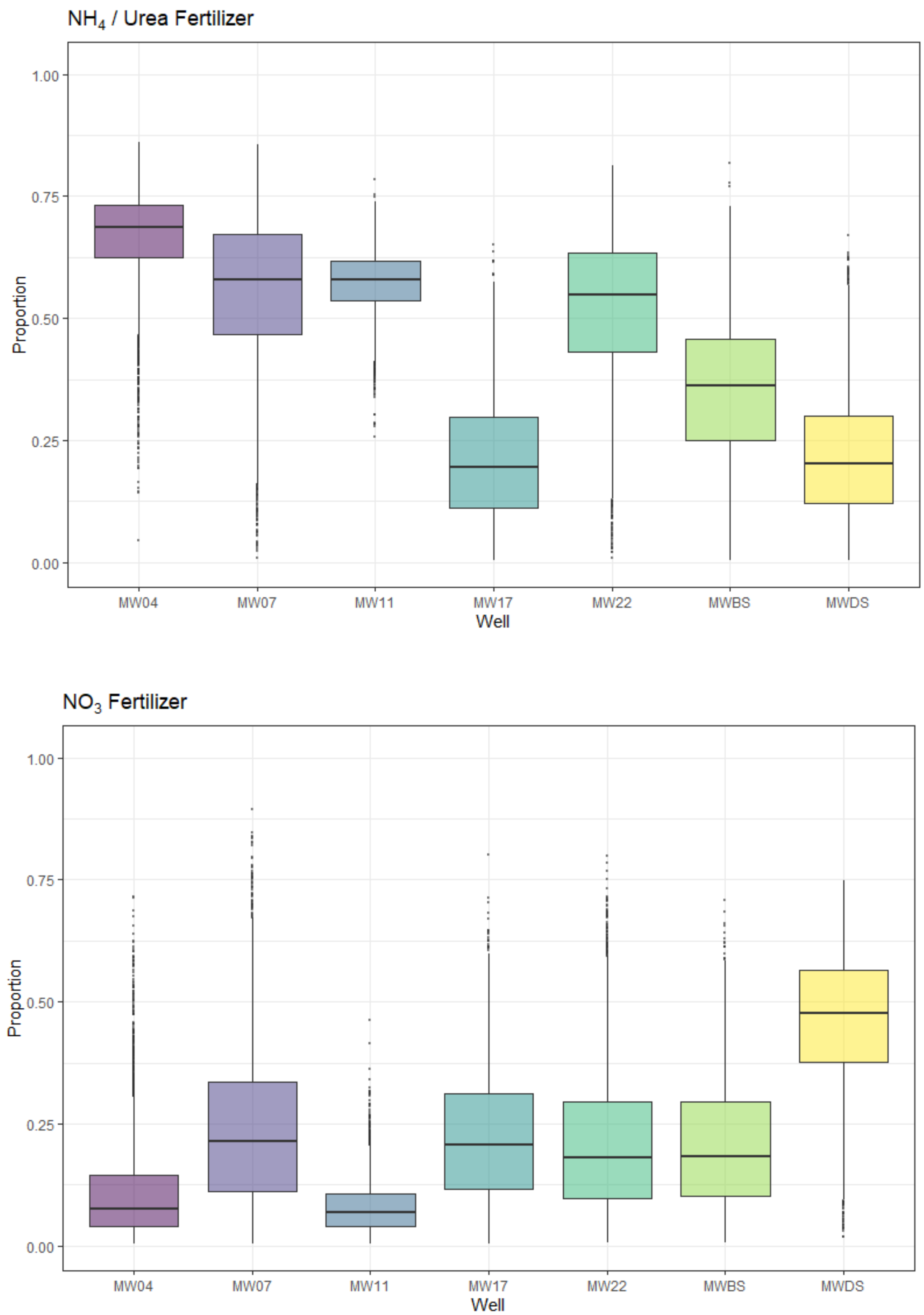
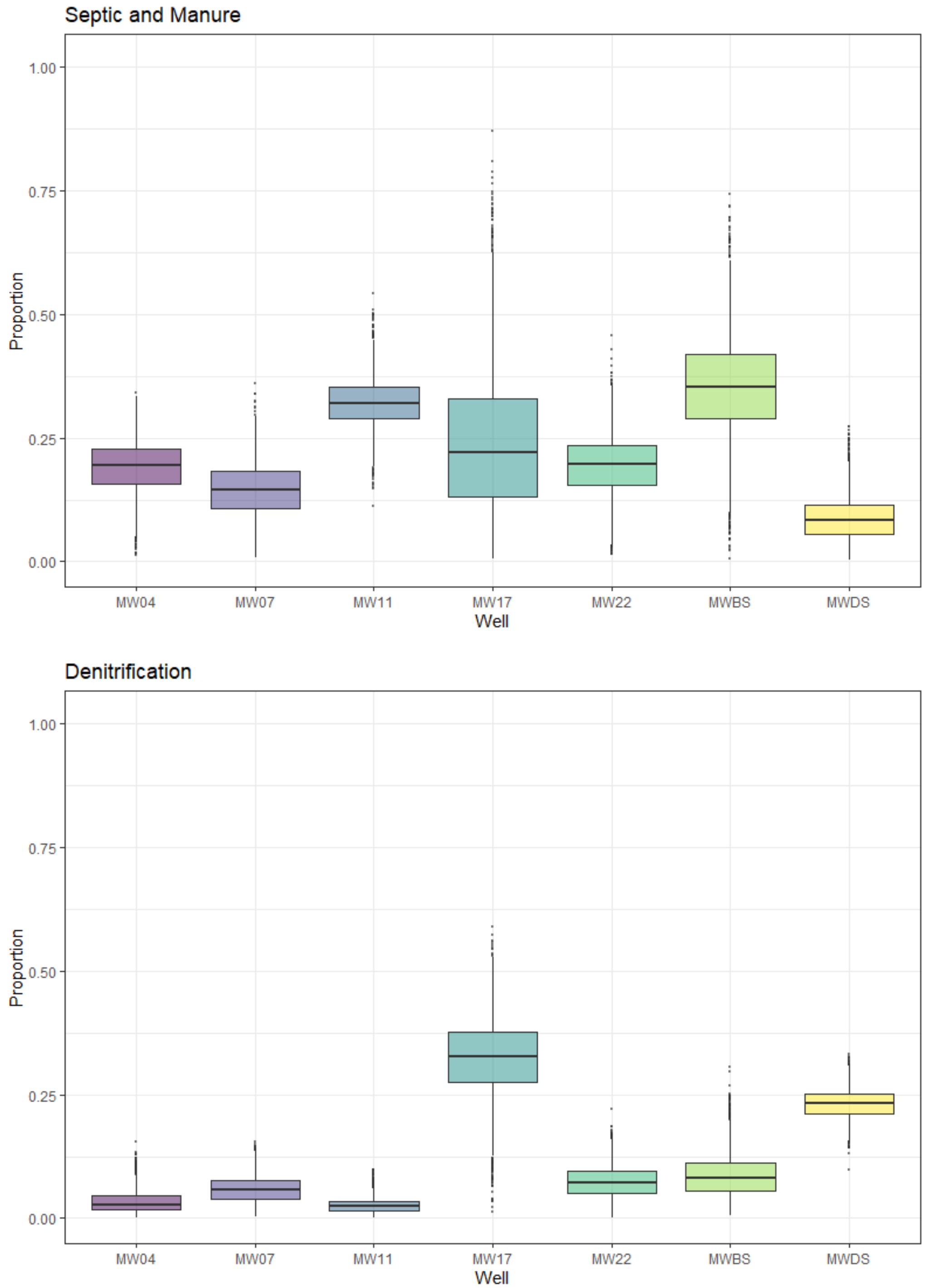


Figure 14 - Output from simmr Bayesian Mixing Model Analyses (continued)



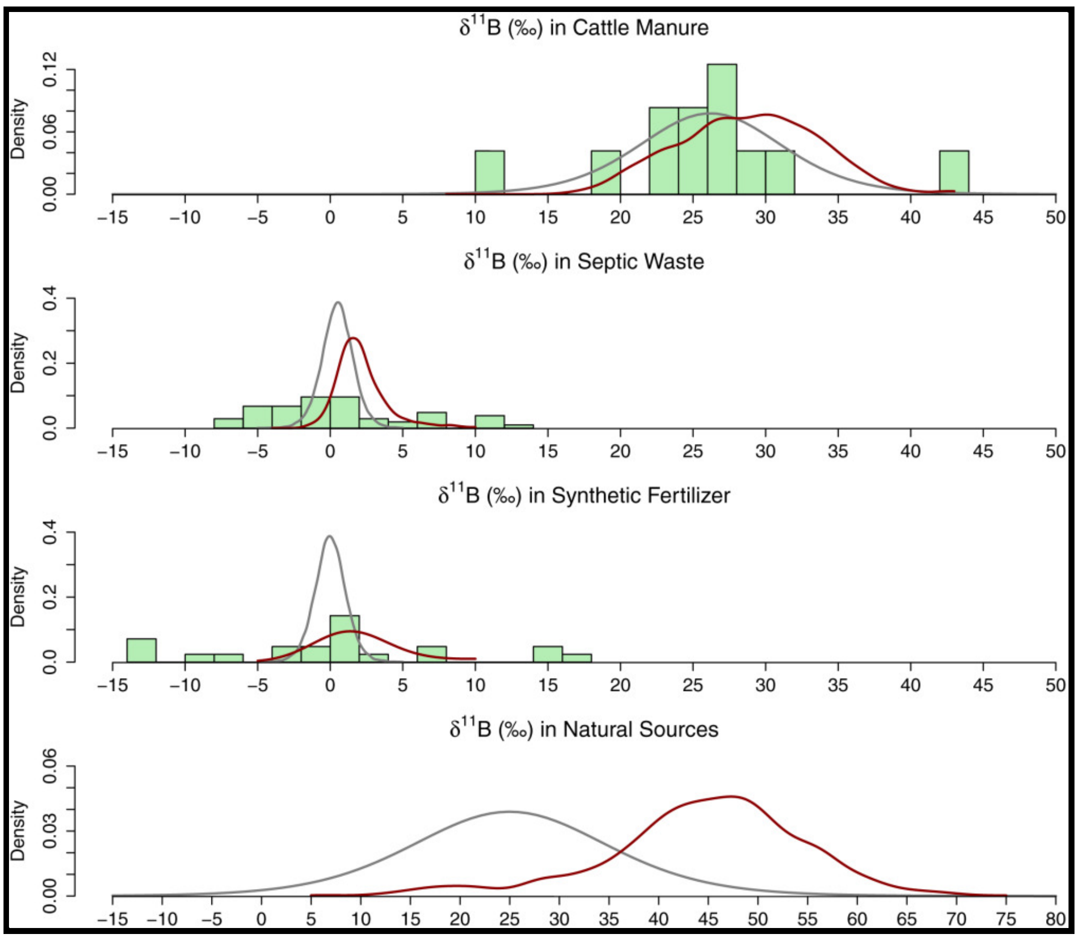
Note: Box plots created from simmr mixing model outputs (middle line represents median, the bottom and the top of the boxes are 25th and 75th quantiles. Whiskers represent values 1.5 times the interquartile ranges. Dots represent outliers. Uninformed priors were used, meaning all sources have equal probabilities (e.g., 1,1,1,1).

3.7 Geochemical Tracers

3.7.1 Boron Isotopes

In addition to N and O isotopes, Wood also collected samples for analysis of boron isotopes (^{11}B) as another line of evidence to assist in the assessment of N sources contributing to the wells in the Wekiva Springshed. Boron stable isotopes have been used by some researchers for source tracking with mixed results. Boron is a component of many, but not all detergents (Katz et al. 2011). Boron was considered because it is not affected by the denitrification process, is relatively conservative, and is rare in the natural environment. Thus, the boron isotope is a potentially viable tracer for nitrogen pollution and mineralized fertilizer (Bronders et al. 2012, Ransom et al. 2016). **Figure 15** illustrates the ranges in $\delta^{11}B$ values that have been measured in groundwaters contaminated by various sources of nitrogen. There is a wide range in the Boron isotope values, overlapping across fertilizer sources, natural sources, and organic waste (manure and septic), however, the highest ranges were found associated with cattle manure.

Figure 15 - $\delta^{11}B$ Values Measured in Manure, Septic Waste Fertilizer, and Natural Sources

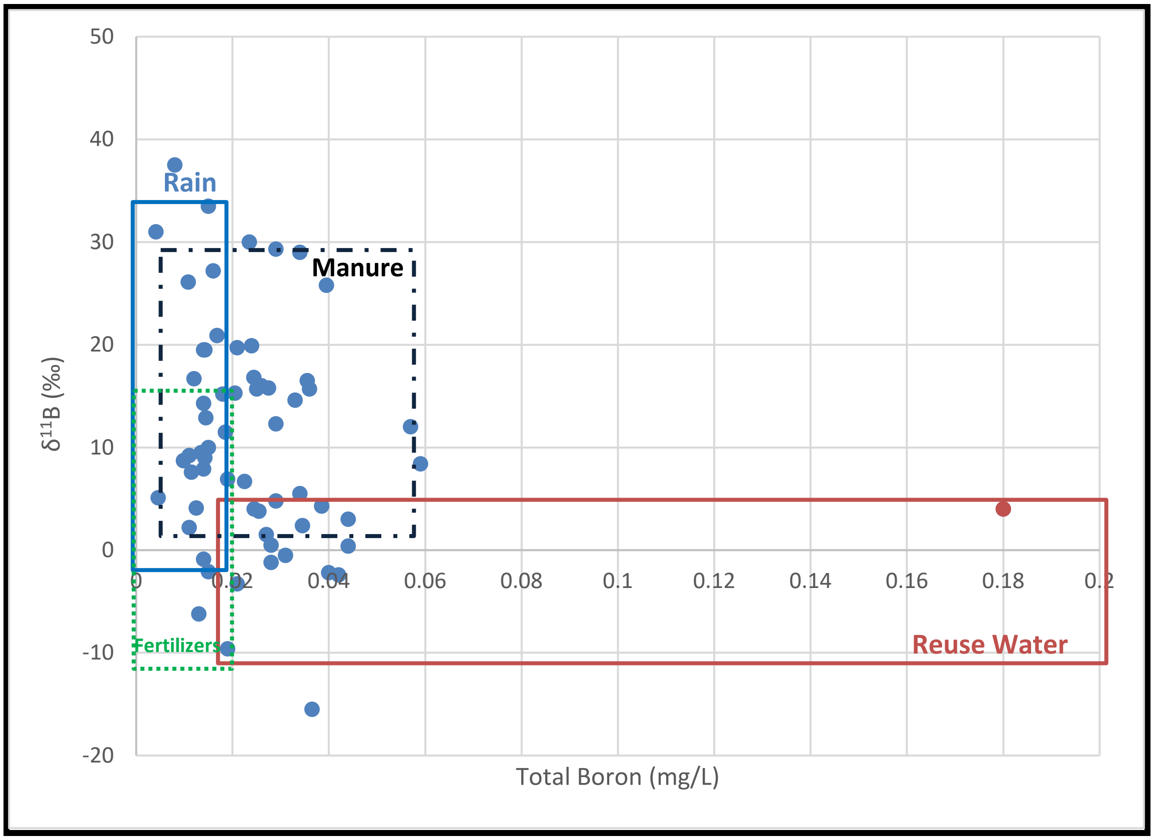


Note: Source of figure is from Ransom et al. (2016). $\delta^{11}B$ measured in dairy manure, septic waste, synthetic fertilizers, and natural sources as compiled from literature sources (green bars), prior probability density used in the analysis (grey line), and posterior probability density predicted by the model (red line). If no green bars are shown, values were given as a range only (Table 2 in Ransom et al. 2016).

Comparing the amount of total boron, $\delta^{11}B$, and $\delta^{15}N$ can also provide another method to identify potential sources of nitrate (Reed and Duranceau 2016). Available $\delta^{11}B$ stable isotope and total boron data are plotted in **Figure 16** which shows bounding boxes adapted from Briand et al. (2017). For reference, Wood collected a reuse water sample for boron and $\delta^{11}B$ stable isotope analyses. The reuse water sample fit the appropriate nitrate and plotted on the far-right side of the reuse water bounding box. The remainder of the well and spring samples were variable with relatively low boron concentrations and slightly enriched $\delta^{11}B$ ratios. Many of the sample results fell within overlapping ranges and outside of the fertilizer range which appears to be a limitation of the available scientific literature. Canion et al (2020) confirm the limitation of using a boron versus $\delta^{11}B$ analysis indicating that it is used primarily to help differentiate manure and wastewater in areas with agriculture. The $\delta^{11}B$, and $\delta^{15}N$ biplot does appear to indicate that the wells sampled for $\delta^{11}B$ stable isotope are not particularly influenced by reuse water.

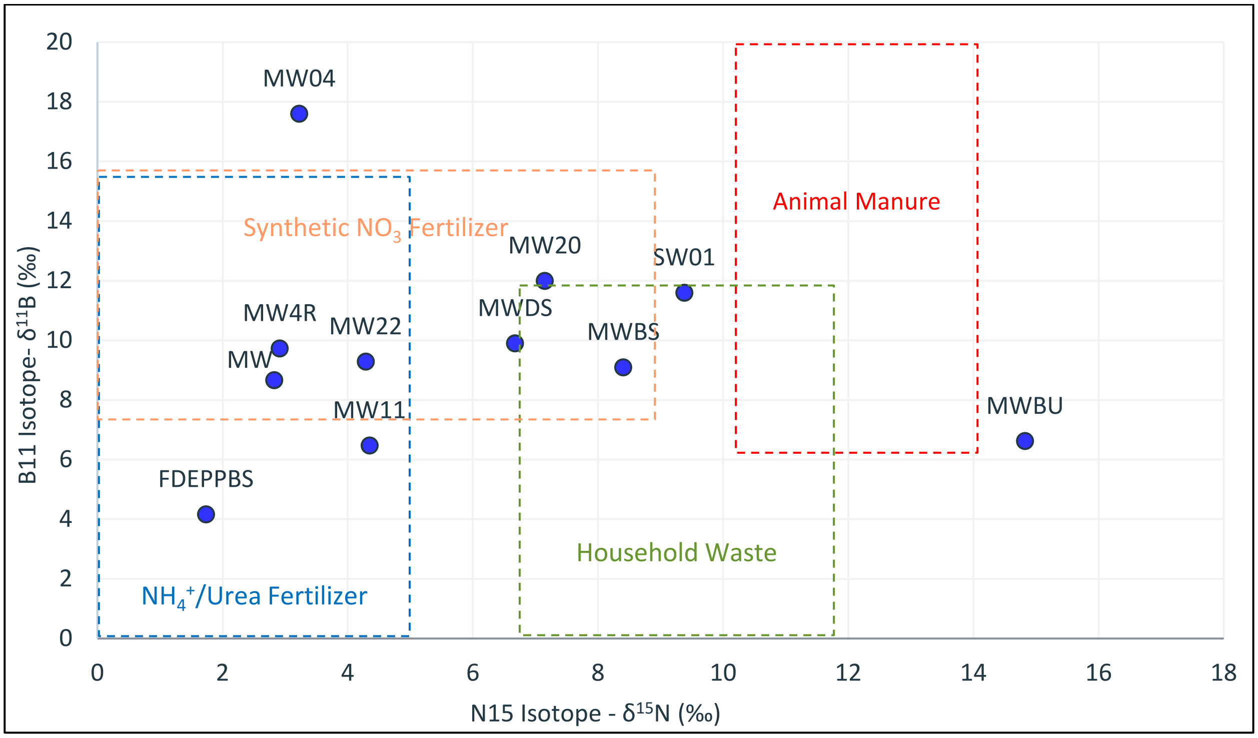
Available $\delta^{15}N$ and $\delta^{11}B$ data are plotted in **Figure 17** which includes nitrate source bounding boxes adapted from Briand et. al (2017). The results from **Figure 17** are more consistent with the results of the $\delta^{15}N$ and $\delta^{18}O$ stable isotope biplot with most of the data falling within fertilizer bounding boxes. The spring vent value falls between all of the bounding boxes suggesting a mixture of sources. Interestingly, well MW04 had the highest ratio of $\delta^{11}B$ which may be related to the location of the adjacent golf course.

Figure 16 - Biplot of total boron versus δ11B. Boxes encompass typical ratios for Manure (black box), rain (blue box), and red box (reuse water)



Note: Bounding boxes adapted from Briand et al. (2017). Data include samples from April 2017 through November 2019, for this initial exploratory analysis, all total boron values were used (e.g., qualified values, mostly "I", were used to create this biplot)

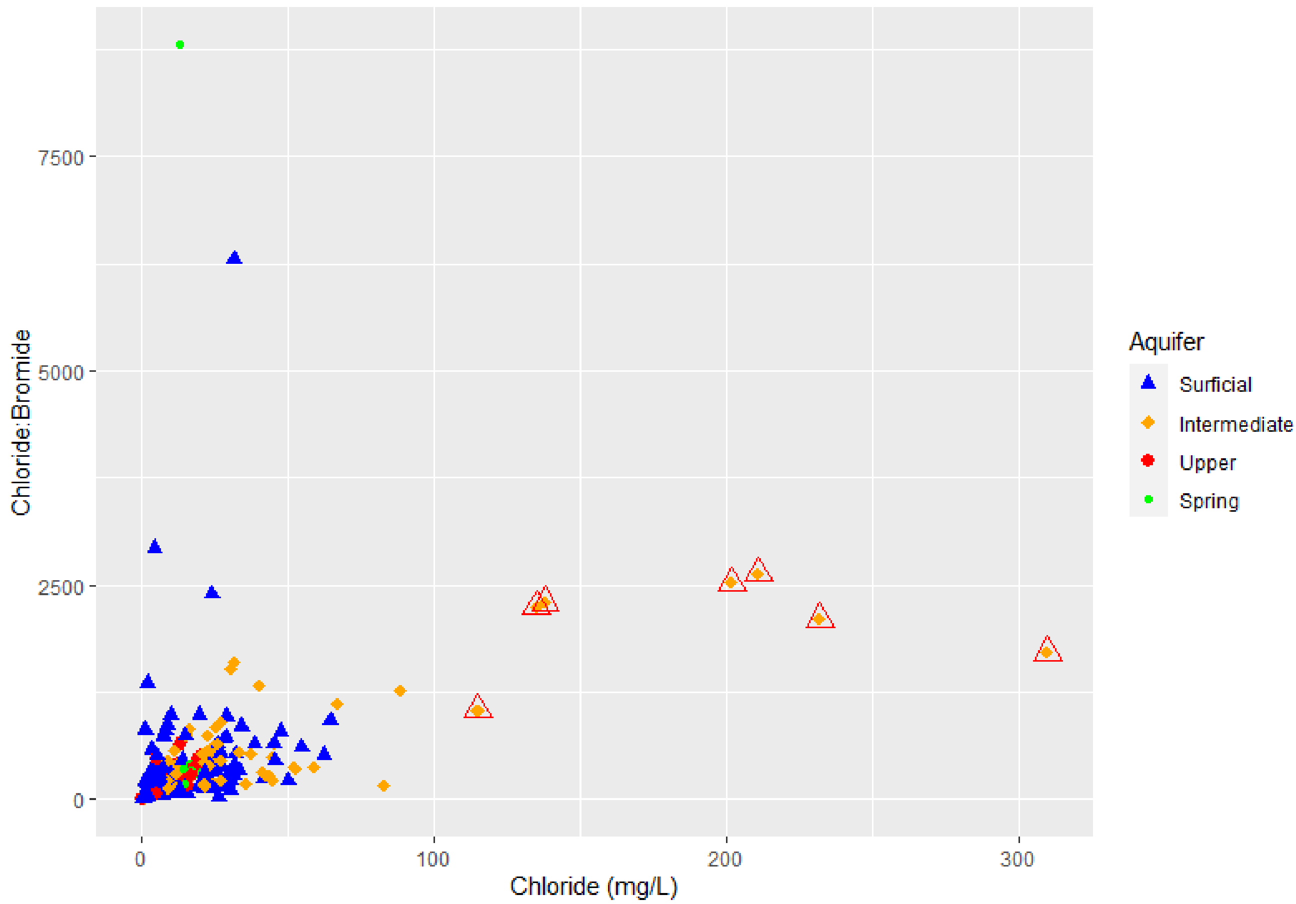
Figure 17 - Biplot of $\delta^{15}N$ versus $\delta^{11}B$



3.7.2 Chloride to Bromide Ratios

The ratio of geochemical tracers such as chloride (Cl^-) to bromide (Br^-) can indicate pollution (Seiler 2005; Katz et al. 2011). These constituents are relatively conservative, and human activities can increase their levels. Wastewater effluents including septic tanks, animal waste, and sewage tend to have higher Cl:Br ratio values (>400 are indicative of wastewater sources, Canion et al. 2020). As shown in **Figure 18**, several wells had Cl:Br ratios greater than 400: specifically, MW02 and MW11 with the highest ratio values (>1000). The MW11 data are highlighted in red. Both of these wells are in urban land use areas and have mid-range in nitrate levels although MW11 may also be influenced by a nearby golf course.

Figure 18 - Chloride vs Cl:Br Ratios by Mass



Note: Red triangles represent well W11.

3.8 Anthropogenic Tracers

Anthropogenic tracers were used by Wood to help further identify wells that may be impacted by wastewater. The tracers include the sweeteners: aspartame, sucrose and sucralose, and the stimulant caffeine. These are conservative tracers and have slow breakdown rates in the environment. Wood collected wastewater tracers at the wells and spring vent.

Tracers were not normally detected but were observed in five wells including BW02, MW07, and MWBS, MW11, and MW22 and the spring (**Table 7**). Out of the limited number of tracer observations, sucrose was detected most often. The highest sucrose level was found in MW22. It is important to note that both sucrose and caffeine have potential natural sources. Sucralose was detected only at well BW02 which is a shallow (12 ft) background well in Wekiva State Park. This well was used as a natural reference site in Tucker et al. (2014). Well BW02 is within about 5 feet from a parking lot and 20 feet from a picnic table and may have been contaminated as a result. The reported sucralose concentration was relatively low and was not found again at this or any other well location. Aspartame was not detected in any well or spring samples.

Table 7 - Summary of Wastewater Tracer Results at Wells and Wekiwa Spring.

Location	Date Collected	Tracer	Result	Qualifier	Unit
SW01	21-Jun-18	Sucralose	1.15	I	ug/L
MW22	25-Mar-19	Sucrose	13.4		ug/L
BW02	27-Mar-19	Sucrose	5.64		ug/L
MW11	28-Mar-19	Sucrose	6.13		ug/L
MWBS	28-Mar-19	Sucrose	12		ug/L
MW07	23-Jul-19	Sucrose	4.91		ug/L
BW02	20-Aug-19	Sucralose	31.5		ug/L
MW07	20-Aug-19	Caffeine	2.21		ug/L
MW07	20-Aug-19	Sucrose	5.46		ug/L
MW22	20-Aug-19	Caffeine	2.1		ug/L

Note: Because of limited detections, sampling for tracers was discontinued after August 2019.

4.0 CONCLUSIONS AND RECOMMENDATIONS

Wood assessed potential sources of nitrate in groundwater using multiple lines of evidence including direct measurement of water quality parameters and other source tracking methods such as stable isotopes, geochemical tracers, and anthropogenic markers. These data were then used to develop statistical analyses and models which provided additional information regarding nitrate sources within the study area.

Nitrate is the primary parameter of concern assessed in this study. Median nitrate values were generally less than 2.0 mg/L. Higher nitrate concentrations were measured in wells MW04 and the replacement well MW4R. This well is located in a residential area adjacent to a golf course, and high nitrate concentrations at this location have been previously reported by Tucker et al. (2014). Median nitrate concentrations in the aquifers, in order of highest to lowest, values were: Intermediate Aquifer (1.13 mg/L), Surficial Aquifer (0.85 mg/L), and the Upper Floridan Aquifer (0.018 mg/L). Nitrate concentrations within the Intermediate and Surficial Aquifers were elevated compared to natural groundwater conditions of 0.3 mg/L (MACTEC 2010).

Nitrate concentration appears to have decreased over time for wells with data available since 2008. These wells were located within areas selected to exclude potential sources of nitrate other than turfgrass fertilizer and would presumably contain lower nitrate concentrations than a similar area with septic or sewer. Nitrate concentrations in the Upper Floridan Aquifer were two orders of magnitude lower than the Surficial and Intermediate Aquifers suggesting that factors including denitrification, dilution, and dispersion may be contributing.

A principal components analysis (PCA) identified two primary water quality gradients including a nitrate versus TOC gradient, and a chloride versus depth and pH gradient. The PCA showed that the deep Upper Floridan wells appeared to cluster together and were primarily differentiated by depth and higher pH. The water quality in the Intermediate and Surficial aquifers were more variable, and the wells did not cluster on the PCA figure. Some wells separated in PCA space because of high TOC (BW02 and MW01) and high nitrate (MW04). The water from the spring vent appeared to be in the center of the plot, reflecting the mixture of water sources. Wells MWBU and MW02 were closest in PCA space to SW01, suggesting that these wells were most similar in water quality to the spring vent water.

Land use in 1-km-radius (776.5 acres) buffers around the wells demonstrated that these wells are primarily in residential land uses, covering about 55%, on average of the buffer areas. Given the large distance between septic tanks and monitoring wells, there was no apparent relationship between the amount of residential land use area or septic tank density and groundwater nitrate concentrations.

Stable isotopes of nitrogen, oxygen, and boron were analyzed to assess and attribute nitrate sources in the wells and the spring vent. A biplot of stable isotopes strongly indicates that NH_4^+/urea-based fertilizers are the primary source of nitrate. Stable isotope data for $\delta^{15}N$ and $\delta^{18}O$ clearly follows a denitrification trend suggesting that data points outside of the NH_4^+/urea bounding box are likely to be derived from NH_4^+/urea fertilizer.

Bayesian modeling was conducted to estimate the fractional contributions of different nitrate sources based on isotopic signatures. These results suggest that within most of shallow wells that were measured for isotopes, NH_4^+/urea fertilizers are responsible for at least 40% of the nitrate contribution. Modeling also indicated that one well may be influenced by synthetic nitrate fertilizer. Manure/septic typically accounted for 40% of the nitrate or less. Denitrification was evident as a significant source of nitrate for one well, but appeared to account for less than 15% of the nitrate in the remaining wells.

Statistical water quality analyses and modeling efforts suggest that the turfgrass fertilizer comprises a significant proportion of potential nitrate sources. Isotopic ratios varied, but most samples indicated nitrate signatures from mineralized NH_4^+/urea fertilizers or enriched denitrified nitrate from the same NH_4^+/urea fertilizer source. Water quality data from the Wekiwa Spring vent indicates a complex mixture of groundwater inputs, legacy nitrogen sources (Canion et al., 2020), and biogeochemical processes reflecting denitrification along vertical and horizontal groundwater gradients (FDEP, 2018).

Canion et al. (2020) reported that groundwater in Upper Floridan Aquifer within the Wekiva springshed generally has DO concentrations of less than 1 mg/L. Low DO conditions in aquifers have been found to produce a reduced environment that may be influencing denitrification and possibly other mechanisms that reduce nitrogen such as dissimilatory nitrogen reduction to ammonium (Heffernan et al. 2012; Canion et al. 2020).

Other geochemical tracers were used to evaluate potential nitrogen sources. Boron isotope results were somewhat variable but results from the $\delta^{15}N$ and $\delta^{11}B$ isotope biplot are similar to $\delta^{15}N$ and $\delta^{18}O$ isotope biplot suggesting that NH_4^+/urea-fertilizers are a likely source of nitrate.

Chloride to bromide ratio was also used to evaluate potential nitrate sources within the Wekiva Springshed. The Cl:Br ratio for the current study suggests that two wells (MW02 and MW11) may have some influence from wastewater. Anthropogenic tracers were rarely detected but were found in some of the wells, suggesting minor influences of wastewater. Well BW02 is a background well, that appears to be contaminated with artificial sweeteners.

Water quality results and modeling efforts indicate that turfgrass fertilizer appears to be a significant source of nitrate within the study area as demonstrated in other research (e.g., Tucker 2014). Available data appears sufficient to justify additional efforts to reduce nitrogen loading to the groundwater, particularly during the wet season when nitrate mass transport to groundwater is highest. Based on the findings of the nitrate source data, implementing a "summer blackout" period for fertilizer application between June 1 through September 30 may assist Orange County with their efforts to control nitrate loading to both Wekiwa Spring and Wekiva River in accordance with the adopted BMAPs. Future sampling and analyses will continue to supplement the increasingly robust database and will improve the understanding of nitrate sources to the Wekiva Springshed and will ultimately allow Orange County to better manage the significant challenge of achieving the load reduction requirements of the Wekiwa and Rock Springs BMAP.

4.1 Future Work Efforts

Wekiva springshed data will continue to be collected, QA/QC checked, and compiled for future work efforts. The database will continue to be updated. The following specific items should be completed as part of the next work effort to produce an updated technical memorandum:

- Based on results from the GIS spatial analysis, and the water quality data, create a denitrification layer based on environmental conditions (i.e., create Potential Denitrification Zones).
- Adjust land use versus nitrate analyses based on smaller buffers, depth of well and potential flow paths.
- Continue to update the Bayesian model with an increased number of samples.
- Identify laboratories with an improved limit of detection for isotopes and tracers.
- Increase sample frequency for a subset of hi-priority wells to potentially identify patterns in fertilizer application.
- Install additional wells. One well should be placed between well MW4R and the Wekiwa Spring vent (perhaps along Wekiva Springs Rd.) to determine groundwater differences between well MW04R and the spring vent.

Appendices

1. Water Quality Summary Statistics
2. Land Use and Septic Tank Data for 1-km Well Buffer Zones
3. Time-series Plots of Nitrate + Nitrite Concentrations for Locations with Data from October 2008 through June 2021
4. Figures Examining Relationships between Selected Parameters and Nitrate Concentrations
5. Correlation Matrices for Comparison among Water Quality Variables and Nitrate Concentrations among Wells
6. Output from Principal Components Analyses of Water Quality for 18 Sample Sites
7. Comparison of Nitrate Concentrations and δ15N Stable Isotope Ratios by Aquifer
8. Nitrate + Nitrite Concentrations and δ15N Stable Isotope Ratios by Aquifer for Septic Tank Density and Acres of Residential Land Use Within 1-km Monitoring Well Buffers

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APPENDIX 1

Water Quality Summary Statistics from October 2008 through June 2021

Appendix 1. Water Quality Summary Stats						
October 2008 Through June 2021						
Total Nitrogen (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW02	36	0.652	0.310	0.608	0.170	1.590
DEPFLD	7	0.012	0.009	0.012	0.003	0.022
DEPPBD	18	0.181	0.129	0.160	0.000	0.510
DEPPBS	18	0.960	0.626	0.855	0.000	3.090
MW01	36	2.516	0.608	2.565	0.540	4.090
MW02	34	1.489	1.515	1.270	0.000	9.180
MW03	2	0.470	0.354	0.470	0.220	0.720
MW04	13	11.354	3.909	12.700	1.320	14.800
MW06	30	0.822	1.191	0.275	0.000	4.400
MW07	35	2.428	1.548	1.950	0.000	7.830
MW11	35	2.698	0.595	2.650	1.190	4.050
MW14	32	0.240	0.384	0.141	0.003	2.070
MW15	4	1.066	0.261	1.057	0.758	1.391
MW17	34	1.649	2.971	0.819	0.000	13.000
MW20	33	3.972	3.389	3.140	0.580	16.900
MW22	33	2.879	1.639	2.380	0.416	6.380
MW4R	13	9.072	1.408	8.609	7.500	11.785
MWAI	18	0.091	0.047	0.100	0.005	0.180
MWBS	14	1.631	0.545	1.391	0.980	2.990
MWBSR	3	2.207	0.627	2.100	1.640	2.880
MWBU	17	1.349	0.359	1.300	0.690	2.189
MWCI	7	0.221	0.233	0.127	0.018	0.569
MWDS	18	2.652	2.264	2.000	1.590	11.600
MWDU	18	0.094	0.107	0.081	0.005	0.445
MWEU	7	0.535	0.048	0.537	0.450	0.609
SW01	35	1.121	0.277	1.140	0.120	1.765
Total Phosphorus (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	tp	4	0.094	0.082	0.087	0.011
BW02	tp	40	0.051	0.040	0.035	0.008
DEPFLD	tp	8	0.058	0.029	0.049	0.040
DEPPBD	tp	19	0.066	0.024	0.061	0.000
DEPPBS	tp	19	0.167	0.407	0.048	0.000

MW01	tp	40	0.013	0.013	0.010	0.002
MW02	tp	38	0.442	0.177	0.415	0.000
MW03	tp	6	0.715	1.227	0.155	0.081
MW04	tp	17	0.722	2.292	0.168	0.071
MW05	tp	4	0.808	0.777	0.565	0.200
MW06	tp	34	0.083	0.132	0.038	0.000
MW07	tp	39	0.215	0.234	0.144	0.000
MW08	tp	4	0.188	0.040	0.190	0.140
MW09	tp	4	0.139	0.070	0.150	0.046
MW10	tp	4	0.010	0.015	0.003	0.003
MW11	tp	39	0.183	0.326	0.048	0.001
MW12	tp	1	0.003	NA	0.003	0.003
MW13	tp	4	0.295	0.208	0.250	0.110
MW14	tp	36	0.524	1.008	0.200	0.003
MW15	tp	8	1.196	0.117	1.208	1.033
MW16	tp	4	0.007	0.007	0.004	0.003
MW17	tp	38	0.121	0.176	0.079	0.000
MW18	tp	1	0.053	NA	0.053	0.053
MW19	tp	1	0.380	NA	0.380	0.380
MW20	tp	38	0.038	0.098	0.015	0.003
MW21	tp	4	0.165	0.075	0.160	0.078
MW22	tp	37	0.069	0.126	0.037	0.003
MW23	tp	1	0.003	NA	0.003	0.003
MW24	tp	1	0.011	NA	0.011	0.011
MW4R	tp	13	0.151	0.089	0.121	0.090
MWAI	tp	18	0.076	0.040	0.078	0.016
MWBS	tp	15	0.543	0.537	0.210	0.042
MWBSR	tp	3	0.281	0.314	0.143	0.059
MWBU	tp	18	0.100	0.035	0.092	0.041
MWCI	tp	7	0.538	0.092	0.507	0.443
MWDS	tp	18	0.228	0.624	0.040	0.003
MWDU	tp	18	0.100	0.058	0.081	0.058
MWEU	tp	7	0.129	0.007	0.131	0.117
OR548	tp	1	0.120	NA	0.120	0.120
OR893	tp	1	0.230	NA	0.230	0.230
SW01	tp	39	0.118	0.022	0.121	0.003
Total Kjeldahl Nitrogen (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	4	0.512	0.316	0.640	0.048	0.720
BW02	36	0.650	0.311	0.645	0.150	1.200

DEPFLD	4	0.077	0.069	0.043	0.040	0.180
DEPPBD	15	0.144	0.061	0.160	0.000	0.250
DEPPBS	15	0.078	0.048	0.045	0.000	0.180
MW01	36	2.409	0.630	2.420	0.520	4.060
MW02	34	0.110	0.078	0.100	0.000	0.320
MW03	6	0.199	0.223	0.114	0.048	0.620
MW04	17	0.178	0.300	0.110	0.035	1.300
MW05	4	1.820	1.173	1.550	0.780	3.400
MW06	30	0.163	0.115	0.140	0.000	0.560
MW07	35	0.428	0.245	0.400	0.000	1.110
MW08	4	1.575	0.275	1.550	1.300	1.900
MW09	4	0.042	0.012	0.048	0.024	0.048
MW10	4	0.042	0.012	0.048	0.024	0.048
MW11	35	0.205	0.159	0.180	0.024	0.920
MW12	1	0.680	NA	0.680	0.680	0.680
MW13	4	1.578	0.581	1.550	0.910	2.300
MW14	32	0.175	0.140	0.135	0.024	0.660
MW15	4	0.945	0.342	0.880	0.620	1.400
MW16	4	0.764	0.498	0.965	0.024	1.100
MW17	34	0.192	0.161	0.160	0.000	0.920
MW18	1	0.024	NA	0.024	0.024	0.024
MW19	1	1.300	NA	1.300	1.300	1.300
MW20	34	0.233	0.218	0.185	0.024	1.290
MW21	4	1.350	0.191	1.300	1.200	1.600
MW22	33	0.084	0.044	0.080	0.024	0.180
MW23	1	0.760	NA	0.760	0.760	0.760
MW24	1	0.024	NA	0.024	0.024	0.024
MW4R	9	0.128	0.044	0.140	0.045	0.200
MWAI	14	0.111	0.037	0.110	0.045	0.180
MWBS	11	0.125	0.071	0.140	0.042	0.210
MWBSR	3	0.188	0.148	0.180	0.045	0.340
MWBU	14	0.094	0.050	0.100	0.042	0.180
MWCI	3	0.147	0.042	0.160	0.100	0.180
MWDS	14	0.099	0.059	0.090	0.045	0.220
MWDU	14	0.091	0.064	0.045	0.042	0.220
MWEU	3	0.513	0.057	0.530	0.450	0.560
OR548	1	0.250	NA	0.250	0.250	0.250
OR893	1	2.000	NA	2.000	2.000	2.000
SW01	35	0.089	0.052	0.090	0.024	0.220

Nitrate + Nitrite (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	4	0.493	0.439	0.530	0.043	0.870
BW02	40	0.077	0.191	0.009	0.001	0.753
DEPFLD	8	0.004	0.006	0.003	0.001	0.018
DEPPBD	19	0.058	0.134	0.005	0.000	0.480
DEPPBS	19	0.919	0.596	0.848	0.000	2.980
MW01	40	0.063	0.266	0.018	0.001	1.698
MW02	38	1.617	1.725	1.125	0.000	9.180
MW03	6	0.040	0.038	0.035	0.005	0.104
MW04	17	10.926	4.250	12.000	0.018	14.500
MW05	4	1.935	1.380	1.700	0.540	3.800
MW06	34	0.641	0.978	0.242	0.000	3.930
MW07	39	2.132	1.611	1.700	0.000	7.320
MW08	4	0.048	0.040	0.043	0.005	0.100
MW09	4	0.352	0.248	0.335	0.098	0.640
MW10	4	3.700	1.499	3.350	2.300	5.800
MW11	39	2.604	0.885	2.405	0.240	4.600
MW12	1	0.002	NA	0.002	0.002	0.002
MW13	4	1.928	2.672	0.740	0.330	5.900
MW14	36	0.394	0.983	0.010	0.003	4.200
MW15	8	2.053	1.996	1.826	0.157	4.400
MW16	4	1.930	2.021	1.740	0.140	4.100
MW17	38	1.441	2.435	0.841	0.000	12.500
MW18	1	0.240	NA	0.240	0.240	0.240
MW19	1	0.086	NA	0.086	0.086	0.086
MW20	37	3.599	3.189	2.800	0.440	16.600
MW21	4	0.771	0.813	0.690	0.005	1.700
MW22	37	2.816	1.639	2.380	0.190	6.400
MW23	1	1.900	NA	1.900	1.900	1.900
MW24	1	0.260	NA	0.260	0.260	0.260
MW4R	13	8.661	1.191	8.440	7.380	11.630
MWAI	18	0.008	0.008	0.005	0.001	0.032
MWBS	14	1.494	0.504	1.281	0.982	2.820
MWBSR	3	2.033	0.461	1.920	1.640	2.540
MWBU	17	1.208	0.266	1.130	0.553	1.580
MWCI	7	0.032	0.036	0.018	0.004	0.096
MWDS	18	1.949	0.258	1.925	1.590	2.590
MWDU	18	0.037	0.079	0.017	0.001	0.345
MWEU	7	0.044	0.073	0.018	0.001	0.206

OR548	1	0.005	NA	0.005	0.005	0.005
OR893	1	0.032	NA	0.032	0.032	0.032
SW01	39	1.064	0.243	1.100	0.007	1.528
NH4-N (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	4	0.065	0.066	0.049	0.013	0.150
BW02	26	0.190	0.206	0.100	0.005	0.690
DEPFLD	1	0.009	NA	0.009	0.009	0.009
DEPPBD	5	0.031	0.040	0.005	0.000	0.093
DEPPBS	5	0.004	0.002	0.005	0.000	0.005
MW01	26	2.053	0.608	2.070	0.010	3.570
MW02	24	0.013	0.006	0.013	0.000	0.020
MW03	6	0.078	0.158	0.013	0.010	0.400
MW04	15	0.014	0.005	0.015	0.005	0.020
MW05	4	0.043	0.041	0.029	0.013	0.100
MW06	20	0.015	0.012	0.013	0.000	0.060
MW07	25	0.027	0.035	0.015	0.000	0.140
MW08	4	0.583	0.345	0.415	0.400	1.100
MW09	4	0.033	0.024	0.031	0.013	0.058
MW10	4	0.010	0.004	0.011	0.005	0.013
MW11	25	0.036	0.041	0.020	0.005	0.140
MW12	1	0.320	NA	0.320	0.320	0.320
MW13	4	0.063	0.062	0.049	0.013	0.140
MW14	23	0.013	0.005	0.013	0.005	0.020
MW15	4	2.150	0.342	2.100	1.800	2.600
MW16	4	0.201	0.236	0.146	0.010	0.500
MW17	24	0.085	0.166	0.055	0.000	0.850
MW18	1	0.010	NA	0.010	0.010	0.010
MW19	1	0.230	NA	0.230	0.230	0.230
MW20	24	0.018	0.024	0.015	0.005	0.130
MW21	4	0.131	0.168	0.065	0.013	0.380
MW22	23	0.016	0.010	0.015	0.005	0.051
MW23	1	0.310	NA	0.310	0.310	0.310
MW24	1	0.098	NA	0.098	0.098	0.098
MW4R	4	0.005	0.000	0.005	0.005	0.005
MWAI	4	0.025	0.013	0.025	0.010	0.040
MWBS	1	0.005	NA	0.005	0.005	0.005
MWBSR	3	0.005	0.000	0.005	0.005	0.005
MWBU	4	0.005	0.000	0.005	0.005	0.005
MWDS	4	0.005	0.000	0.005	0.005	0.005

MWDU	4	0.005	0.000	0.005	0.005	0.005
OR548	1	0.052	NA	0.052	0.052	0.052
OR893	1	1.800	NA	1.800	1.800	1.800
SW01	25	0.013	0.006	0.015	0.005	0.020

Dissolved Oxygen (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW02	35	1.2	1.2	0.7	0.1	4.6
DEPFLD	8	0.1	0.1	0.0	0.0	0.4
DEPPBD	19	0.4	0.5	0.1	0.0	1.8
DEPPBS	19	7.0	1.2	7.6	5.1	8.3
MW01	36	0.6	0.6	0.5	0.0	3.5
MW02	33	1.2	1.0	1.1	0.0	5.8
MW03	2	5.4	0.6	5.4	4.9	5.8
MW04	12	6.0	0.7	6.3	4.7	6.8
MW06	30	1.7	1.2	1.5	0.2	5.1
MW07	34	5.6	1.5	5.8	1.3	7.5
MW11	35	3.5	1.6	3.3	1.1	7.4
MW14	31	2.7	1.4	2.5	0.6	5.7
MW15	4	0.7	0.1	0.7	0.6	0.9
MW17	33	1.1	0.7	1.1	0.2	3.8
MW20	34	2.7	1.7	2.1	0.4	6.8
MW22	33	6.7	0.9	6.9	4.9	8.9
MW4R	13	5.2	0.6	5.1	4.4	6.1
MWAI	18	0.3	0.4	0.2	0.0	1.7
MWBS	14	3.2	1.0	3.2	1.6	4.8
MWBSR	3	4.4	1.6	3.8	3.3	6.2
MWBU	18	0.2	0.2	0.1	0.0	0.5
MWCI	7	0.5	0.3	0.5	0.1	1.0
MWDS	18	1.9	1.0	1.6	1.3	5.5
MWDU	18	0.4	0.9	0.2	0.0	4.1
MWEU	7	0.0	0.0	0.0	0.0	0.1
SW01	34	0.7	0.6	0.5	0.0	2.6
Alkalinity (mg/L as CaCO3)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	4	18	14	18	1	34
BW02	22	63	51	54	1	194
DEPFLD	7	96	7	97	83	102

DEPPBD	18	103	27	111	0	121
DEPPBS	18	12	10	9	0	30
MW01	22	21	36	11	1	174
MW02	22	118	45	131	0	170
MW03	4	42	10	42	30	54
MW04	6	165	12	170	150	180
MW05	4	63	13	60	50	81
MW06	22	39	81	19	0	380
MW07	22	26	9	28	0	44
MW08	4	24	6	25	16	30
MW09	4	28	13	27	16	41
MW10	4	1	0	1	1	1
MW11	23	9	8	5	1	28
MW12	1	110	NA	110	110	110
MW13	4	25	3	25	21	27
MW14	21	1	1	1	0	6
MW15	8	19	6	17	14	31
MW16	4	53	6	54	45	59
MW17	22	8	8	5	0	28
MW18	1	19	NA	19	19	19
MW19	1	10	NA	10	10	10
MW20	23	9	9	7	1	28
MW21	4	61	10	60	51	71
MW22	22	9	35	1	0	166
MW23	1	88	NA	88	88	88
MW24	1	13	NA	13	13	13
MW4R	13	196	27	203	148	228
MWAI	18	102	5	102	97	113
MWBS	15	9	6	6	3	21
MWBSR	3	5	1	4	4	6
MWBU	18	134	18	138	73	156
MWCI	7	32	10	38	15	41
MWDS	18	4	3	3	1	15
MWDU	18	104	5	104	96	118
MWEU	7	207	13	204	190	226
OR548	1	120	NA	120	120	120
OR893	1	260	NA	260	260	260
SW01	27	126	26	132	32	143
Specific Conductance (umhos/cm)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**

BW01	4	107	54	116	45	153
BW02	38	161	81	152	52	381
DEPFLD	8	346	27	348	305	390
DEPPBD	19	290	19	295	257	337
DEPPBS	19	61	17	52	38	87
MW01	40	224	100	174	115	589
MW02	37	417	66	424	299	548
MW03	6	181	56	181	85	251
MW04	16	504	120	535	145	618
MW05	4	257	18	255	240	280
MW06	34	88	45	79	58	305
MW07	38	95	27	94	53	175
MW08	4	225	23	233	192	241
MW09	4	180	80	203	64	249
MW10	4	121	20	115	104	149
MW11	39	313	251	211	42	1172
MW12	1	385	NA	385	385	385
MW13	4	147	82	142	69	237
MW14	35	145	33	135	95	238
MW15	8	352	70	342	279	434
MW16	4	374	80	358	294	485
MW17	37	289	60	276	227	508
MW18	1	169	NA	169	169	169
MW19	1	280	NA	280	280	280
MW20	37	349	105	336	43	615
MW21	4	430	32	423	400	474
MW22	37	122	37	117	57	212
MW23	1	628	NA	628	628	628
MW24	1	77	NA	77	77	77
MW4R	13	630	100	644	418	772
MWAI	18	307	13	312	281	328
MWBS	14	149	11	149	124	167
MWBSR	3	145	18	136	134	166
MWBU	18	365	53	382	225	407
MWCI	7	139	24	147	104	163
MWDS	18	104	89	83	70	461
MWDU	18	253	123	229	207	744
MWEU	7	413	18	419	374	429
OR548	1	319	NA	319	319	319
OR893	1	554	NA	554	554	554
SW01	38	365	17	364	340	428

Chloride (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	4	4.5	2.6	3.8	2.3	8.3
BW02	22	4.0	2.4	3.6	0.6	7.8
DEPFLD	8	7.9	6.4	12.0	0.1	13.5
DEPPBD	18	4.9	2.7	5.9	0.1	7.4
DEPPBS	18	2.7	1.7	2.6	0.1	5.2
MW01	22	24.4	9.4	22.0	12.1	50.0
MW02	21	21.7	13.7	16.4	0.1	51.7
MW03	4	11.1	5.3	13.5	3.2	14.0
MW04	6	23.3	3.2	22.5	19.9	28.8
MW05	4	11.0	3.3	10.8	7.4	15.0
MW06	21	5.5	3.7	5.3	0.1	14.9
MW07	21	4.3	5.2	2.6	0.2	24.0
MW08	4	34.8	4.6	35.0	29.0	40.0
MW09	4	12.3	8.6	10.8	3.5	24.0
MW10	4	20.3	3.6	19.5	17.0	25.0
MW11	22	101.3	82.2	63.1	13.0	310.0
MW12	1	27.0	NA	27.0	27.0	27.0
MW13	4	15.4	12.1	12.5	5.7	31.0
MW14	21	12.2	3.8	12.3	7.1	21.0
MW15	8	35.3	11.3	30.7	25.0	51.6
MW16	4	40.0	18.1	36.5	22.0	65.0
MW17	21	32.0	16.9	27.0	0.1	82.7
MW18	1	18.0	NA	18.0	18.0	18.0
MW19	1	28.0	NA	28.0	28.0	28.0
MW20	22	32.6	14.1	30.7	17.0	73.4
MW21	4	73.5	6.0	75.0	65.0	79.0
MW22	22	11.6	4.9	10.8	6.0	23.6
MW23	1	64.0	NA	64.0	64.0	64.0
MW24	1	10.0	NA	10.0	10.0	10.0
MW4R	12	22.1	17.1	22.2	0.1	54.6
MWAI	17	19.6	9.4	22.8	0.2	27.1
MWBS	14	29.1	10.0	27.5	21.8	62.4
MWBSR	3	18.7	3.3	19.5	15.1	21.6
MWBU	17	14.9	8.3	18.4	0.1	22.7
MWCI	7	7.5	6.4	11.6	0.3	14.0
MWDS	18	6.8	1.3	6.7	5.0	9.4
MWDU	18	5.0	2.4	5.6	0.1	7.6

MWEU	7	6.5	6.0	10.6	0.1	12.3
OR548	1	11.0	NA	11.0	11.0	11.0
OR893	1	12.0	NA	12.0	12.0	12.0
SW01	26	14.6	6.4	17.1	0.0	19.5
Total Organic Carbon (mg/L)						
Well	**N**	**Mean**	**SD**	**Median**	**Min**	**Max**
BW01	4	5.4	3.8	4.9	1.7	10.0
BW02	21	21.9	11.9	21.5	5.2	40.9
DEPFLD	7	0.5	0.3	0.7	0.0	0.8
DEPPBD	17	1.0	0.2	1.0	0.7	1.5
DEPPBS	17	0.7	0.4	0.7	0.0	1.5
MW01	21	14.0	5.1	14.0	0.0	23.7
MW02	20	1.2	0.4	1.2	0.3	2.0
MW03	4	0.2	0.0	0.2	0.2	0.3
MW04	6	0.3	0.2	0.3	0.2	0.8
MW05	4	12.3	6.8	11.5	5.3	21.0
MW06	20	2.0	1.5	1.7	0.3	6.5
MW07	20	5.5	1.7	5.4	2.6	7.9
MW08	4	23.8	7.8	25.0	14.0	31.0
MW09	4	3.7	1.3	4.2	1.8	4.6
MW10	4	0.2	0.0	0.2	0.2	0.3
MW11	21	0.8	0.4	0.7	0.2	1.8
MW12	1	14.0	NA	14.0	14.0	14.0
MW13	4	19.2	10.6	16.5	9.8	34.0
MW14	20	1.4	0.6	1.4	0.8	3.0
MW15	7	1.1	1.1	1.2	0.1	2.9
MW16	4	6.5	1.3	7.1	4.6	7.2
MW17	20	1.4	0.6	1.3	0.2	2.9
MW18	1	1.5	NA	1.5	1.5	1.5
MW19	1	24.0	NA	24.0	24.0	24.0
MW20	21	2.7	0.9	2.8	1.2	5.4
MW21	4	23.5	9.0	21.0	16.0	36.0
MW22	21	0.5	0.3	0.4	0.2	1.1
MW23	1	4.5	NA	4.5	4.5	4.5
MW24	1	4.3	NA	4.3	4.3	4.3
MW4R	12	0.7	0.4	0.6	0.2	1.5
MWAI	17	0.7	0.3	0.6	0.4	1.4
MWBS	14	0.7	0.5	0.5	0.2	2.0
MWBSR	3	0.6	0.4	0.5	0.4	1.1
MWBU	17	0.8	0.2	0.8	0.6	1.4

MWCI	6	1.3	1.0	1.0	0.4	2.9
MWDS	17	0.8	0.6	0.6	0.3	2.5
MWDU	17	0.7	0.3	0.6	0.4	1.6
MWEU	6	2.6	0.5	2.6	2.1	3.2
OR548	1	0.2	NA	0.2	0.2	0.2
OR893	1	8.9	NA	8.9	8.9	8.9
SW01	21	0.9	0.3	0.9	0.2	1.4

APPENDIX 2

Land Use and Septic Tank Data for 1-km Well Buffer Zones

Tale 1. Number of septic tanks and land use categories for entire study area, created by merging ("dissolving") overlapping well areas. Note: recreational FLUCCS Class includes both forested areas, as well as golf course. This information is noted in the Recreational Land Use Details. OSTDS is the number of septic tanks.

OSTDS Category	Number
Known Septic	1165
Likely Septic	3104
Grand Total	**4269**

Level 2 Code	Level 2 Description	Area (acres)	% of Total
1100	Residential Low Density	1003.4	10.1
1200	Residential Medium Density	3118.6	31.4
1300	Residential High Density	611.0	6.2
1400	Commercial and Services	516.6	5.2
1500	Industrial	42.3	0.4
1700	Institutional	172.4	1.7
1800	Recreational	316.2	3.2
1900	Open Land	72.8	0.7
2100	Cropland and Pastureland	411.2	4.1
2200	Tree Crops	52.7	0.5
2400	Nurseries and Vineyards	367.0	3.7
2500	Specialty Farms	0.0	0.0
2600	Other Open Lands <Rural>	4.9	0.0
3100	Herbaceous	76.0	0.8
3200	Shrub and Brushland	157.0	1.6
3300	Mixed Rangeland	56.7	0.6
4100	Upland Coniferous Forests	288.4	2.9
4200	Upland Hardwood Forests	158.7	1.6
4300	Upland Mixed Forests	466.3	4.7
4400	Tree Plantations	132.7	1.3
5100	Streams and Waterways	3.3	0.0
5200	Lakes	135.7	1.4
5300	Reservoirs	71.5	0.7
5500	Major Springs	3.7	0.0
6100	Wetland Hardwood Forests	610.1	6.1
6300	Wetland Forested Mixed	424.9	4.3
6400	Vegetated Non-Forested Wetlands	309.3	3.1

7400	Disturbed Lands	96.8	1.0
8100	Transportation	101.5	1.0
8200	Communications	9.5	0.1
8300	Utilities	135.3	1.4
Total		**9926.6**	100.0

Table 2. Land use and number of septic tanks for each sample site. Note: recreational FLUCCS Class includes both forested areas, as well as golf course. This information is noted in the Recreational Land Use Details. OSTDS is the number of septic tanks.

FLUCCS Level II Class	FLUCCS Level II Name	Acres	% [a]	Recreational Land Use Details
BW-02				
6100	Wetland Hardwood Forests	286	37%	
6300	Wetland Forested Mixed	230	30%	
3200	Shrub and Brushland	137	18%	
4100	Upland Coniferous Forests	76	10%	
4300	Upland Mixed Forests	44	6%	
NA	Other	3	0.4%	
5200	Lakes	2	0%	
6400	Vegetated Non-Forested Wetlands	1	0%	
BW-02 Total		776	100%	
No. of OSTDS			0	
DEPFLD				
4300	Upland Mixed Forests	128	16%	
1200	Residential Medium Density	121	16%	
1100	Residential Low Density	100	13%	
4400	Tree Plantations	87	11%	
2400	Nurseries and Vineyards	84	11%	
4200	Upland Hardwood Forests	70	9%	
2100	Cropland and Pastureland	60	8%	
3100	Herbaceous	40	5%	
6400	Vegetated Non-Forested Wetlands	39	5%	
NA	Other	47	6%	
3300	Mixed Rangeland	29	4%	
8300	Utilities	15	2%	
5200	Lakes	3	0%	
DEPFLD Total		776	100%	
No. of OSTDS			372	
DEPPBD				
1200	Residential Medium Density	647	83%	
4100	Upland Coniferous Forests	66	8%	
1800	Recreational	39	5%	Forested area

NA	Other	24	3%	
8300	Utilities	19	2%	
1400	Commercial and Services	2	0%	
1100	Residential Low Density	2	0%	
6400	Vegetated Non-Forested Wetlands	1	0%	
4300	Upland Mixed Forests	1	0%	
5300	Reservoirs	0	0%	
DEPPBD Total		776	100%	
No. of OSTDS			1026	
DEPPBS				
1200	Residential Medium Density	647	83%	
4100	Upland Coniferous Forests	66	9%	
1800	Recreational	39	5%	Forested area
NA	Other	24	3%	
8300	Utilities	19	2%	
1400	Commercial and Services	2	0%	
1100	Residential Low Density	2	0%	
6400	Vegetated Non-Forested Wetlands	1	0%	
4300	Upland Mixed Forests	1	0%	
5300	Reservoirs	0	0%	
DEPPBS Total		776	100%	
No. of OSTDS			1025	
MW-01				
1200	Residential Medium Density	189	24%	
4100	Upland Coniferous Forests	108	14%	
1300	Residential High Density	91	12%	
2400	Nurseries and Vineyards	86	11%	
1100	Residential Low Density	83	11%	
6300	Wetland Forested Mixed	62	8%	
1400	Commercial and Services	43	6%	
4300	Upland Mixed Forests	35	5%	
NA	Other	80	10%	
5300	Reservoirs	15	2%	
3200	Shrub and Brushland	13	2%	
1700	Institutional	10	1%	
3100	Herbaceous	9	1%	
1500	Industrial	8	1%	
8300	Utilities	8	1%	
6400	Vegetated Non-Forested Wetlands	7	1%	
1800	Recreational	5	1%	
1900	Open Land	4	0%	

5200	Lakes	1	0%	
MW-01 Total		776	100%	
No. of OSTDS			249	
MW-02				
1200	Residential Medium Density	195	25%	
1400	Commercial and Services	103	13%	
1300	Residential High Density	79	10%	
1700	Institutional	78	10%	
1100	Residential Low Density	74	10%	
6400	Vegetated Non-Forested Wetlands	43	5%	
6300	Wetland Forested Mixed	41	5%	
NA	Other	163	21%	
5200	Lakes	32	4%	
2400	Nurseries and Vineyards	30	4%	
4300	Upland Mixed Forests	26	3%	
8100	Transportation	23	3%	
1800	Recreational	20	3%	
4200	Upland Hardwood Forests	19	2%	
5300	Reservoirs	12	2%	
2600	Other Open Lands <Rural>	1	0%	
MW-02 Total		776	100%	
No. of OSTDS			300	
MW-04				
1200	Residential Medium Density	347	45%	
1100	Residential Low Density	190	24%	
1800	Recreational	168	22%	Golf course and forested area
NA	Other	71	9%	
1300	Residential High Density	33	4%	
4300	Upland Mixed Forests	15	2%	
8300	Utilities	12	2%	
5300	Reservoirs	6	1%	
1700	Institutional	5	1%	
4100	Upland Coniferous Forests	2	0%	
6300	Wetland Forested Mixed	0	0%	
MW-04 Total		776	100%	
No. of OSTDS			136	
MW-06				
1200	Residential Medium Density	330	42%	
1400	Commercial and Services	122	16%	

1100	Residential Low Density	72	9%	
2400	Nurseries and Vineyards	68	9%	
1300	Residential High Density	59	8%	
1900	Open Land	39	5%	
NA	Other	88	11%	
2100	Cropland and Pastureland	27	4%	
4300	Upland Mixed Forests	24	3%	
8100	Transportation	14	2%	
8300	Utilities	7	1%	
3200	Shrub and Brushland	6	1%	
5300	Reservoirs	5	1%	
4200	Upland Hardwood Forests	3	0%	
1700	Institutional	2	0%	
MW-06 Total		776	100%	
No. of OSTDS			892	
MW-07				
1200	Residential Medium Density	322	42%	
1400	Commercial and Services	127	16%	
1100	Residential Low Density	72	9%	
2400	Nurseries and Vineyards	67	9%	
1300	Residential High Density	61	8%	
1900	Open Land	38	5%	
NA	Other	90	12%	
2100	Cropland and Pastureland	25	3%	
4300	Upland Mixed Forests	25	3%	
8100	Transportation	14	2%	
8300	Utilities	7	1%	
3200	Shrub and Brushland	7	1%	
5300	Reservoirs	6	1%	
4200	Upland Hardwood Forests	3	0%	
1700	Institutional	2	0%	
MW-07 Total		776	100%	
No. of OSTDS			873	
MW-11				
1200	Residential Medium Density	588	76%	
1100	Residential Low Density	60	8%	
1800	Recreational	55	7%	Golf course
NA	Other	73	9%	
1700	Institutional	20	3%	
8300	Utilities	10	1%	
5200	Lakes	10	1%	

4300	Upland Mixed Forests	10	1%	
1300	Residential High Density	9	1%	
6400	Vegetated Non-Forested Wetlands	6	1%	
5300	Reservoirs	6	1%	
3100	Herbaceous	1	0%	
MW-11 Total		776	100%	
No. of OSTDS			306	
MW-14				
1300	Residential High Density	116	15%	
1400	Commercial and Services	111	14%	
6400	Vegetated Non-Forested Wetlands	103	13%	
1200	Residential Medium Density	95	12%	
1100	Residential Low Density	81	10%	
5200	Lakes	40	5%	
8100	Transportation	38	5%	
1700	Institutional	36	5%	
NA	Other	156	20%	
6300	Wetland Forested Mixed	32	4%	
2100	Cropland and Pastureland	23	3%	
1900	Open Land	21	3%	
3300	Mixed Rangeland	16	2%	
8300	Utilities	16	2%	
4300	Upland Mixed Forests	12	2%	
1500	Industrial	11	1%	
4200	Upland Hardwood Forests	9	1%	
5300	Reservoirs	6	1%	
6100	Wetland Hardwood Forests	6	1%	
3100	Herbaceous	3	0%	
	MW-14 Total	776	100%	
No. of OSTDS			73	
MW-15				
1200	Residential Medium Density	200	26%	
1400	Commercial and Services	125	16%	
1300	Residential High Density	117	15%	
1100	Residential Low Density	87	11%	
6400	Vegetated Non-Forested Wetlands	62	8%	
5200	Lakes	59	8%	
NA	Other	126	16%	
8100	Transportation	33	4%	
2100	Cropland and Pastureland	25	3%	
5300	Reservoirs	17	2%	

1900	Open Land	11	1%	
3300	Mixed Rangeland	9	1%	
8300	Utilities	7	1%	
4200	Upland Hardwood Forests	6	1%	
6100	Wetland Hardwood Forests	6	1%	
1700	Institutional	6	1%	
2400	Nurseries and Vineyards	4	1%	
3200	Shrub and Brushland	2	0%	
MW-15 Total		776	100%	
No. of OSTDS			148	
MW-17				
1200	Residential Medium Density	197	25%	
1400	Commercial and Services	126	16%	
1700	Institutional	89	11%	
1300	Residential High Density	77	10%	
1100	Residential Low Density	61	8%	
6400	Vegetated Non-Forested Wetlands	42	5%	
NA	Other	184	24%	
6300	Wetland Forested Mixed	31	4%	
8100	Transportation	30	4%	
4300	Upland Mixed Forests	26	3%	
2400	Nurseries and Vineyards	21	3%	
1800	Recreational	20	3%	
5200	Lakes	19	2%	
4200	Upland Hardwood Forests	19	2%	
5300	Reservoirs	12	2%	
2600	Other Open Lands <Rural>	5	1%	
4400	Tree Plantations	1	0%	
	MW-17 Total	776	100%	
No. of OSTDS			280	
MW-20				
1200	Residential Medium Density	198	26%	
1300	Residential High Density	113	15%	
1100	Residential Low Density	94	12%	
6400	Vegetated Non-Forested Wetlands	76	10%	
1400	Commercial and Services	69	9%	
5200	Lakes	63	8%	
2100	Cropland and Pastureland	47	6%	
NA	Other	115	15%	
8100	Transportation	30	4%	
1700	Institutional	17	2%	

4200	Upland Hardwood Forests	15	2%	
5300	Reservoirs	14	2%	
8300	Utilities	11	1%	
1900	Open Land	10	1%	
3300	Mixed Rangeland	9	1%	
6100	Wetland Hardwood Forests	6	1%	
2400	Nurseries and Vineyards	4	1%	
6300	Wetland Forested Mixed	0	0%	
MW-20 Total		776	100%	
No. of OSTDS			135	
MW-22				
1200	Residential Medium Density	261	34%	
1300	Residential High Density	196	25%	
1100	Residential Low Density	70	9%	
8300	Utilities	47	6%	
6400	Vegetated Non-Forested Wetlands	46	6%	
NA	Other	157	20%	
4300	Upland Mixed Forests	26	3%	
1500	Industrial	23	3%	
2100	Cropland and Pastureland	22	3%	
5200	Lakes	18	2%	
5300	Reservoirs	13	2%	
1400	Commercial and Services	10	1%	
3100	Herbaceous	10	1%	
3300	Mixed Rangeland	10	1%	
8200	Communications	10	1%	
1700	Institutional	7	1%	
2400	Nurseries and Vineyards	5	1%	
7400	Disturbed Lands	4	0%	
1900	Open Land	1	0%	
MW-22 Total		776	100%	
No. of OSTDS			298	
MW-4R				
1200	Residential Medium Density	352	45%	
1100	Residential Low Density	191	25%	
1800	Recreational	164	21%	Golf course and forested area
NA	Other	69	9%	
1300	Residential High Density	33	4%	
4300	Upland Mixed Forests	14	2%	
8300	Utilities	12	1%	

5300	Reservoirs	6	1%	
1700	Institutional	5	1%	
4100	Upland Coniferous Forests	1	0%	
MW-4R Total		776	100%	
No. of OSTDS			136	
MW-AI				
6100	Wetland Hardwood Forests	183	24%	
1800	Recreational	165	21%	Golf course and forested area
1200	Residential Medium Density	163	21%	
1100	Residential Low Density	119	15%	
6300	Wetland Forested Mixed	54	7%	
NA	Other	93	12%	
4100	Upland Coniferous Forests	33	4%	
1300	Residential High Density	27	3%	
4300	Upland Mixed Forests	18	2%	
1700	Institutional	5	1%	
5500	Major Springs	4	0%	
8300	Utilities	3	0%	
5100	Streams and Waterways	2	0%	
5300	Reservoirs	1	0%	
MW-AI Total		776	100%	
No. of OSTDS			48	
MW-BS				
1200	Residential Medium Density	596	77%	
1800	Recreational	52	7%	Golf course
1100	Residential Low Density	45	6%	
NA	Other	83	11%	
1700	Institutional	20	3%	
8300	Utilities	15	2%	
6400	Vegetated Non-Forested Wetlands	12	1%	
5200	Lakes	11	1%	
1300	Residential High Density	9	1%	
4300	Upland Mixed Forests	8	1%	
5300	Reservoirs	6	1%	
3100	Herbaceous	1	0%	
MW-BS Total		776	100%	
No. of OSTDS			500	
MW-BU				
1200	Residential Medium Density	596	77%	

1800	Recreational	52	7%	Golf course
1100	Residential Low Density	45	6%	
NA	Other	83	11%	
1700	Institutional	20	3%	
8300	Utilities	15	2%	
6400	Vegetated Non-Forested Wetlands	12	1%	
5200	Lakes	11	1%	
1300	Residential High Density	9	1%	
4300	Upland Mixed Forests	8	1%	
5300	Reservoirs	6	1%	
3100	Herbaceous	1	0%	
MW-BU Total		776	100%	
No. of OSTDS			500	
MW-CI				
1200	Residential Medium Density	494	64%	
1100	Residential Low Density	71	9%	
1300	Residential High Density	55	7%	
2400	Nurseries and Vineyards	51	7%	
NA	Other	105	14%	
2100	Cropland and Pastureland	29	4%	
1400	Commercial and Services	24	3%	
4300	Upland Mixed Forests	12	2%	
1900	Open Land	10	1%	
6400	Vegetated Non-Forested Wetlands	9	1%	
8300	Utilities	7	1%	
5200	Lakes	5	1%	
4200	Upland Hardwood Forests	3	0%	
5300	Reservoirs	3	0%	
1700	Institutional	2	0%	
3100	Herbaceous	1	0%	
MW-CI Total		776	100%	
No. of OSTDS			1071	
MW-DS				
2100	Cropland and Pastureland	188	24%	
1100	Residential Low Density	125	16%	
4300	Upland Mixed Forests	118	15%	
7400	Disturbed Lands	93	12%	
2400	Nurseries and Vineyards	82	11%	
2200	Tree Crops	53	7%	
4200	Upland Hardwood Forests	52	7%	
4400	Tree Plantations	45	6%	

NA	Other	21	3%	
3100	Herbaceous	16	2%	
8300	Utilities	4	0%	
4100	Upland Coniferous Forests	1	0%	
6400	Vegetated Non-Forested Wetlands	0	0%	
2500	Specialty Farms	0	0%	
MW-DS Total		776	100%	
No. of OSTDS			72	
MW-DU				
2100	Cropland and Pastureland	188	24%	
1100	Residential Low Density	125	16%	
4300	Upland Mixed Forests	118	15%	
7400	Disturbed Lands	93	12%	
2400	Nurseries and Vineyards	82	11%	
2200	Tree Crops	53	7%	
4200	Upland Hardwood Forests	52	7%	
4400	Tree Plantations	45	6%	
NA	Other	21	3%	
3100	Herbaceous	16	2%	
8300	Utilities	4	0%	
4100	Upland Coniferous Forests	1	0%	
6400	Vegetated Non-Forested Wetlands	0	0%	
2500	Specialty Farms	0	0%	
MW-DU Total		776	100%	
No. of OSTDS			72	
MW-EU				
1200	Residential Medium Density	391	50%	
1100	Residential Low Density	99	13%	
1800	Recreational	66	8%	Forested area
2100	Cropland and Pastureland	63	8%	
6400	Vegetated Non-Forested Wetlands	54	7%	
NA	Other	105	13%	
2400	Nurseries and Vineyards	31	4%	
4300	Upland Mixed Forests	28	4%	
6300	Wetland Forested Mixed	25	3%	
1700	Institutional	11	1%	
4100	Upland Coniferous Forests	5	1%	
8300	Utilities	3	0%	
1400	Commercial and Services	2	0%	
5200	Lakes	1	0%	
MW-EU Total		776	100%	

No. of OSTDS			500	
SW-01				
6100	Wetland Hardwood Forests	347	45%	
1800	Recreational	136	18%	Golf course and forested area
1100	Residential Low Density	107	14%	
1200	Residential Medium Density	58	7%	
6300	Wetland Forested Mixed	36	5%	
NA	Other	92	12%	
4100	Upland Coniferous Forests	28	4%	
4300	Upland Mixed Forests	27	3%	
1300	Residential High Density	22	3%	
1700	Institutional	4	1%	
5500	Major Springs	4	0%	
5100	Streams and Waterways	3	0%	
8300	Utilities	3	0%	
5300	Reservoirs	1	0%	
	SW-01 Total	776	100%	
No. of OSTDS			7	
[a] Land uses with <5% summed and included as "Other".				

APPENDIX 3

Time-series Plots of Nitrate + Nitrite Concentrations for Locations with Data from October 2008 through June 2021

Figure 1 – Nitrate + Nitrite Concentration Versus Time at Wells MW04 and MW4R (Data not available for years 2010, 2015 and 2016)

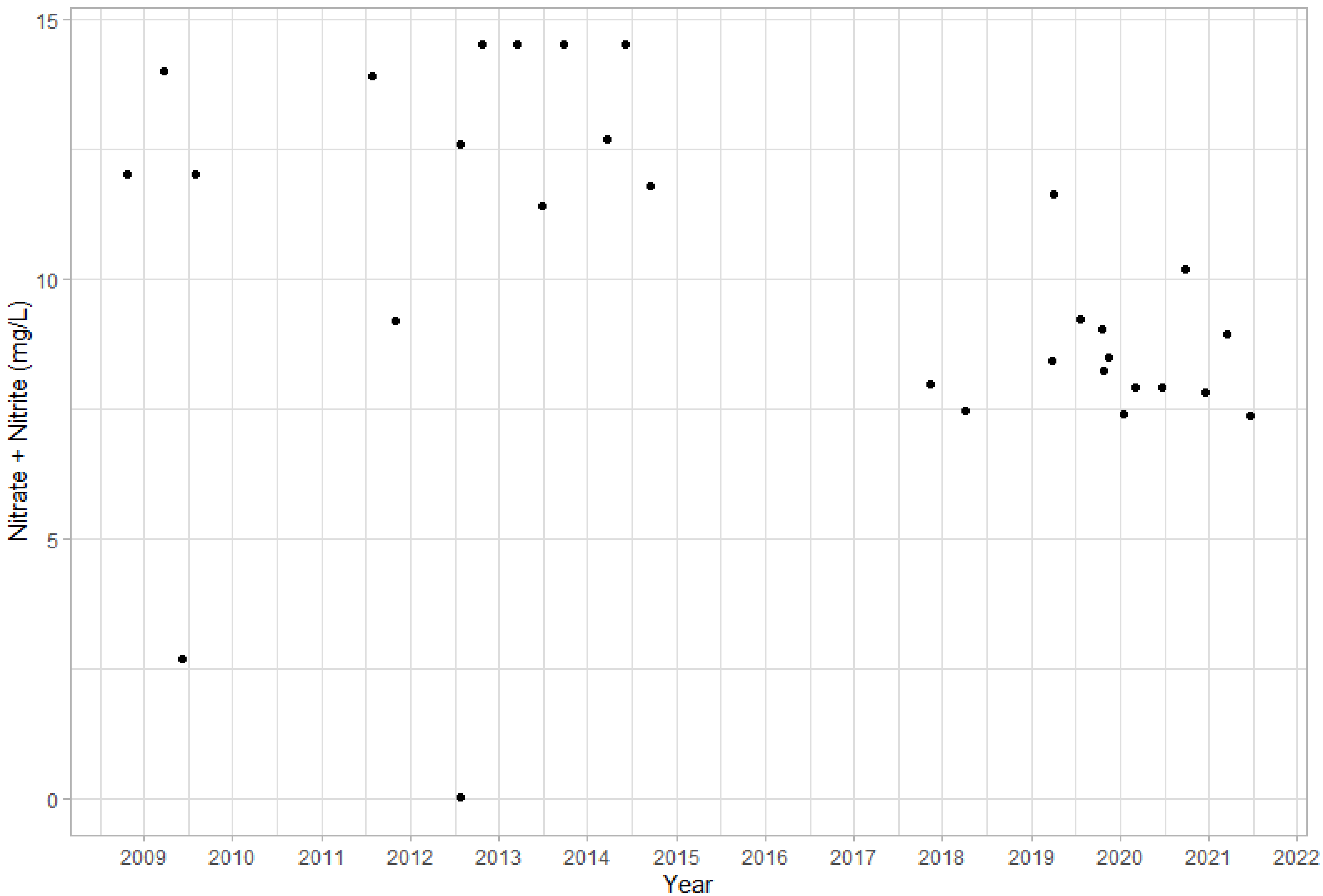


Figure 2 - Nitrate + Nitrite Concentration Versus Time at Well MW11 (Data not available for year 2010)

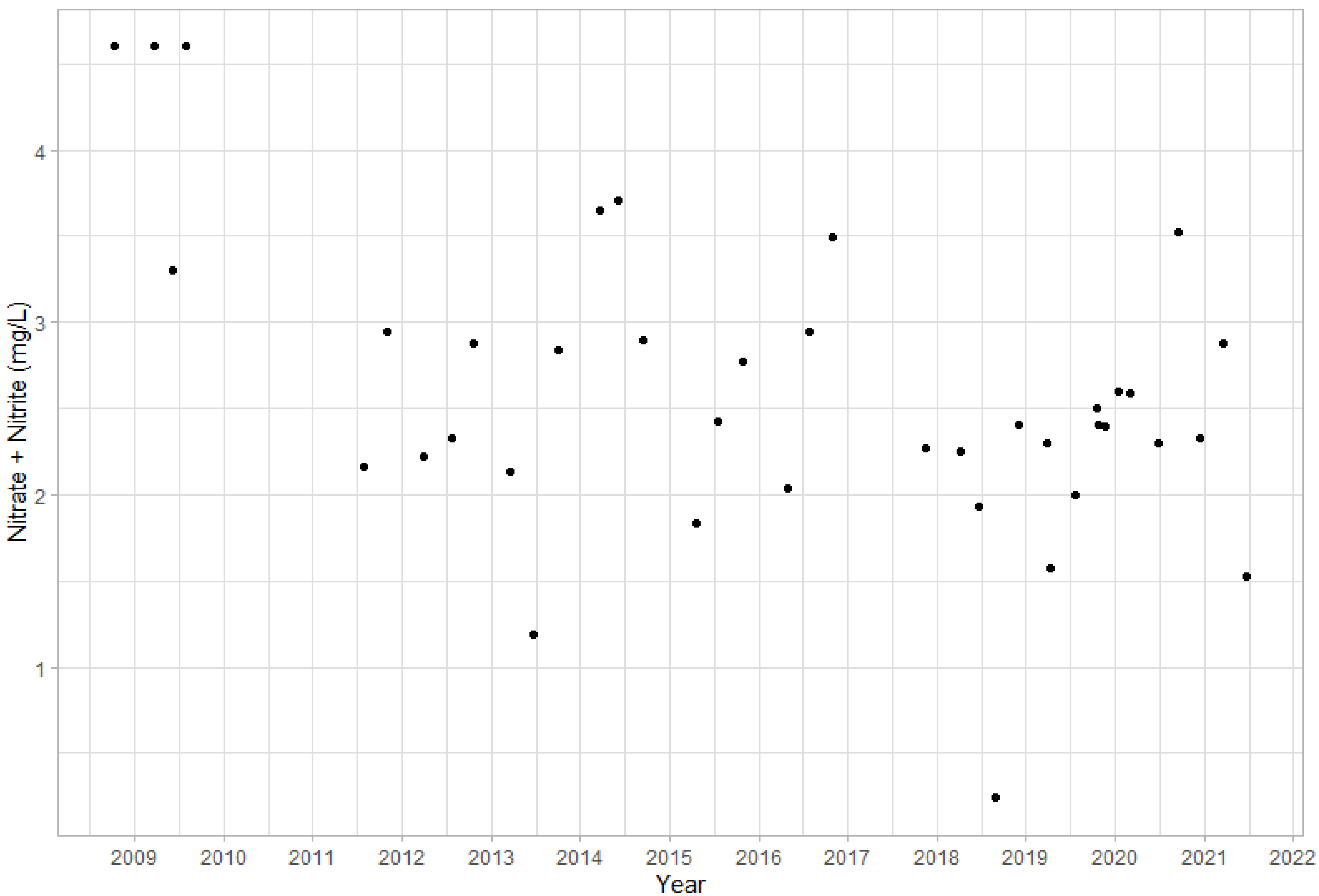


Figure 3 - Nitrate + Nitrite Concentration Versus Time at Well MW07 (Data not available for year 2010)

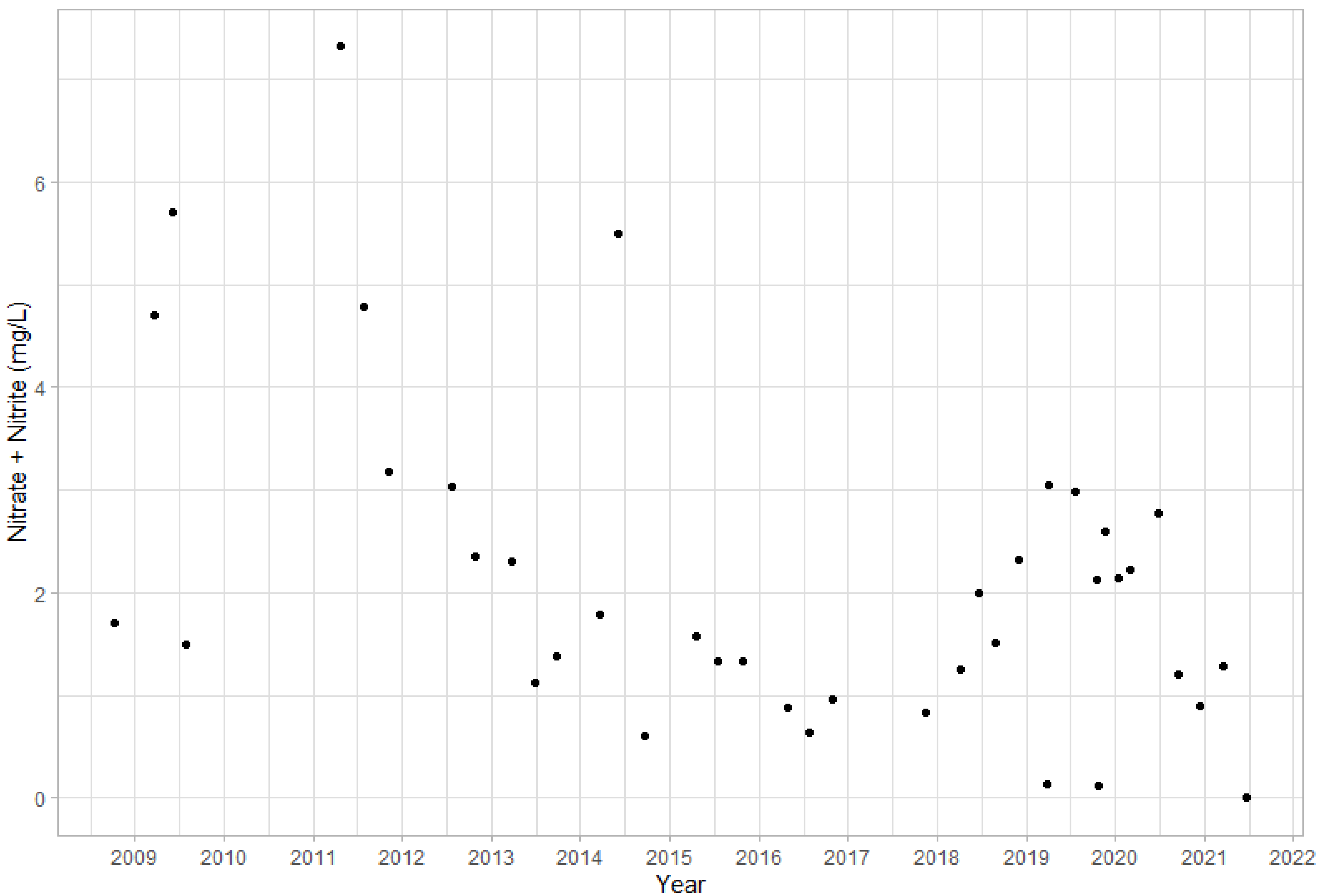


Figure 4 - Nitrate + Nitrite Concentration Versus Time at Well MW22 (Data not available for year 2010)

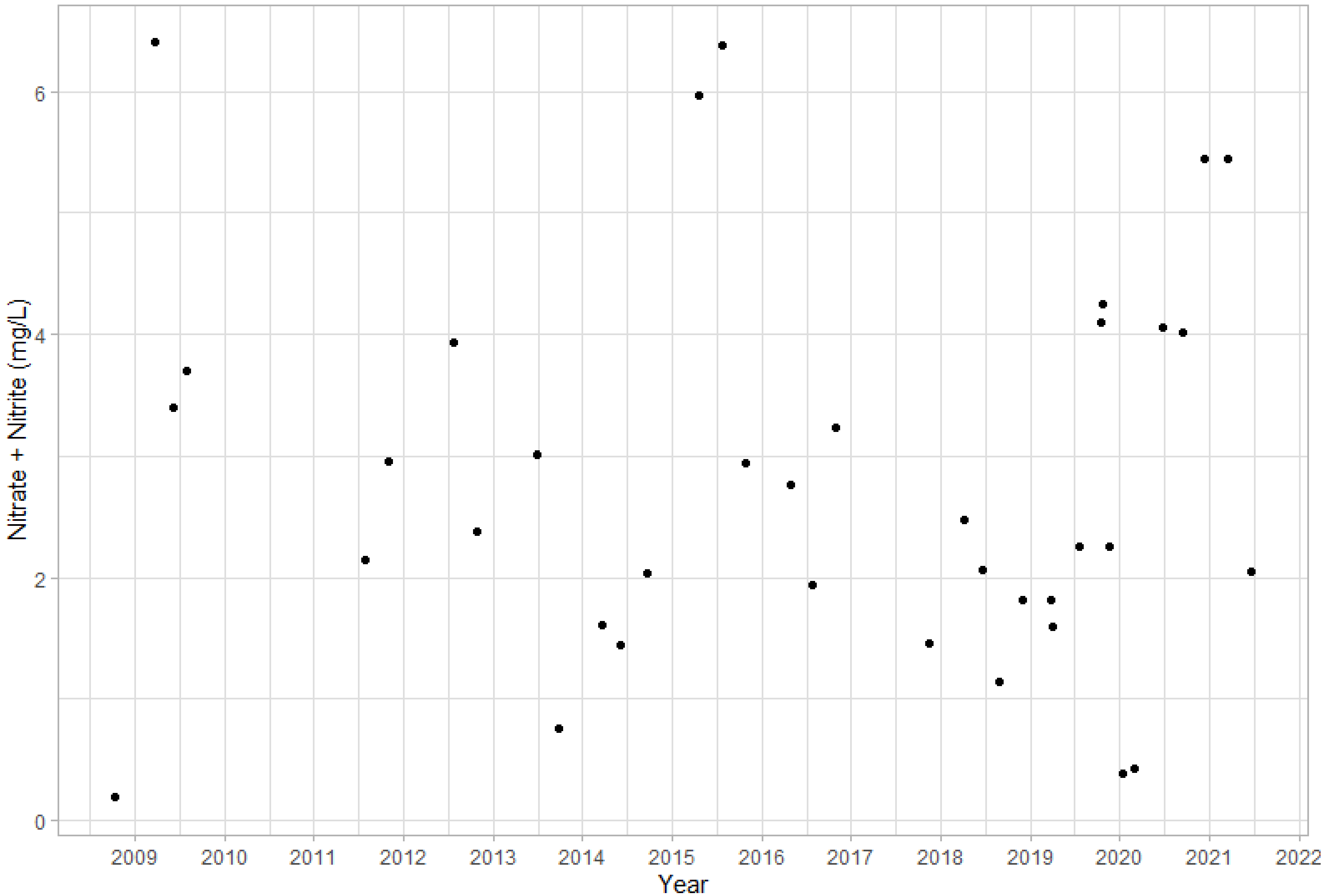


Figure 5 - Nitrate + Nitrite Concentration Versus Time at Well MW20 (Data not available for year 2010)

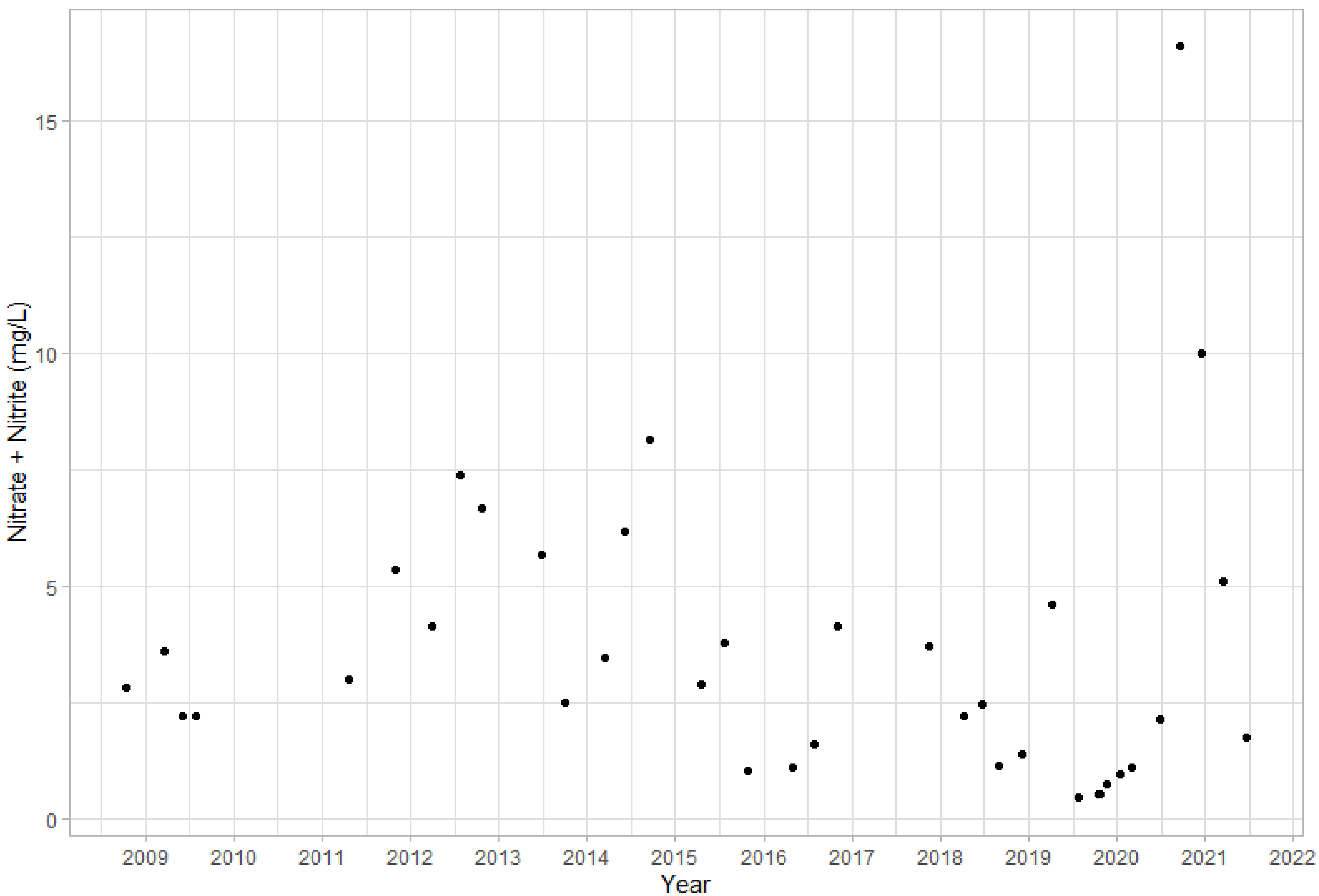


Figure 6 - Nitrate + Nitrite Concentration Versus Time at Well MW17 (Data not available for year 2010)

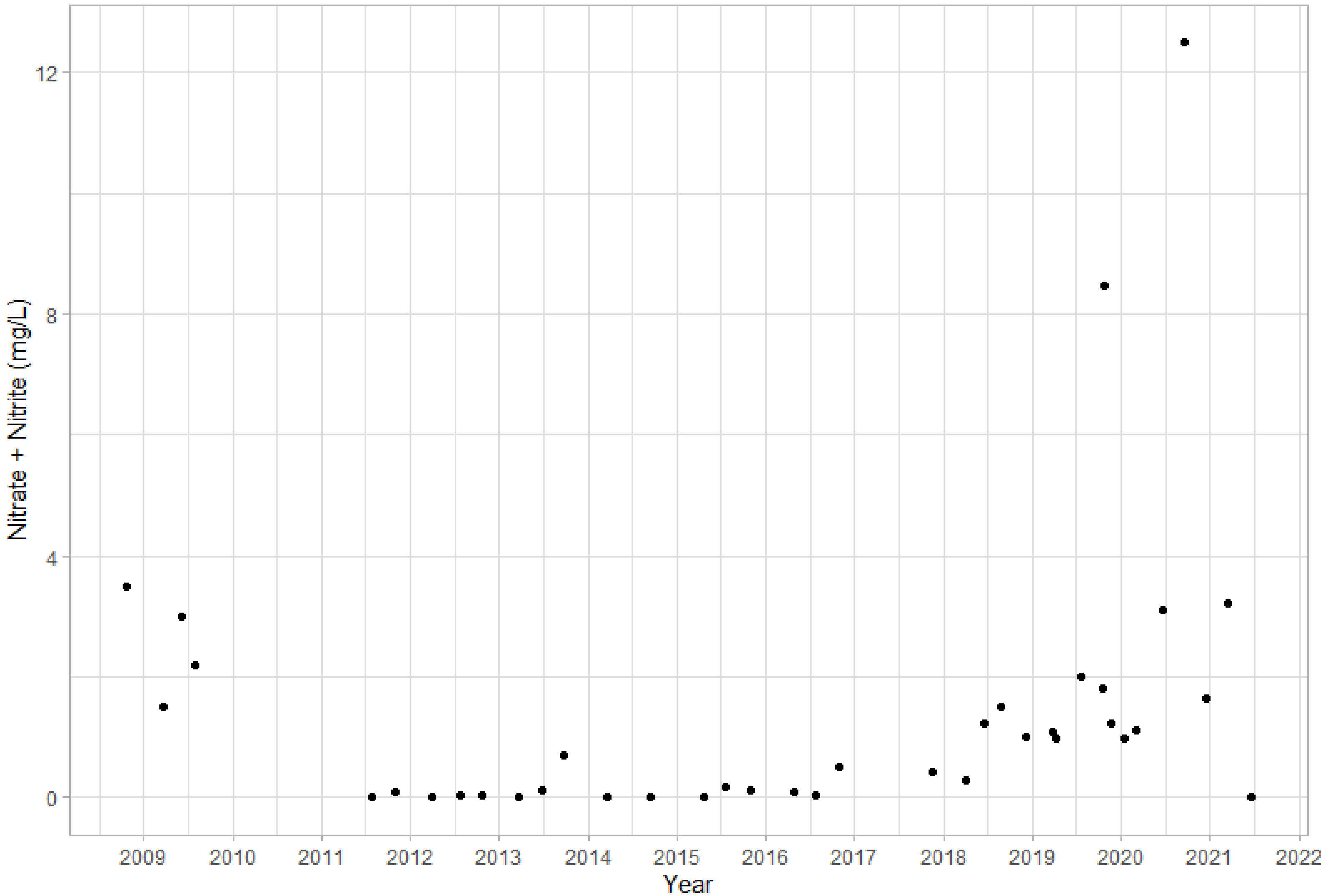


Figure 7 – Nitrate + Nitrite Concentration Versus Time at Spring Vent SW01 (Data not available for year 2010)

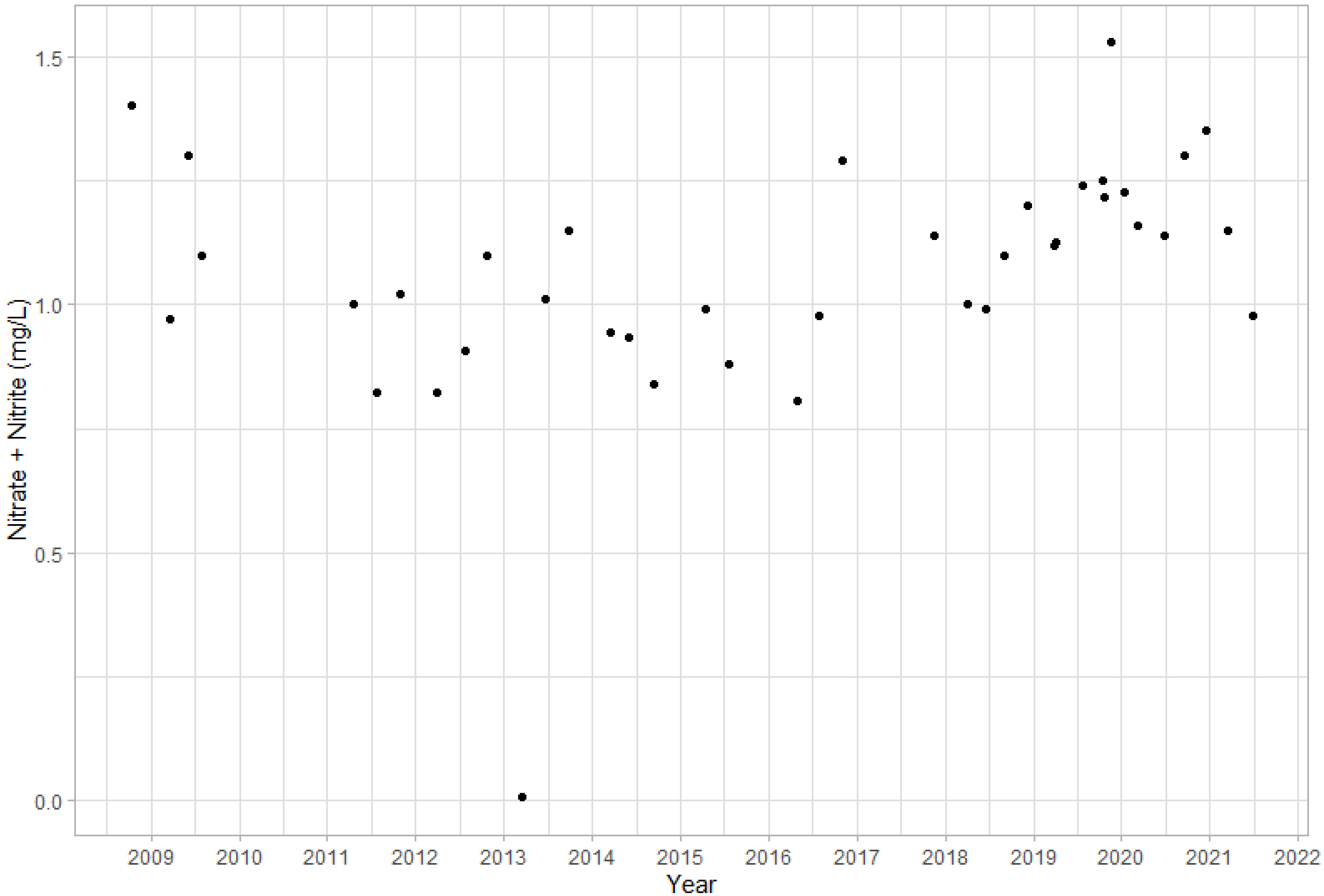


Figure 8 - Nitrate + Nitrite Concentration Versus Time at Well MW02

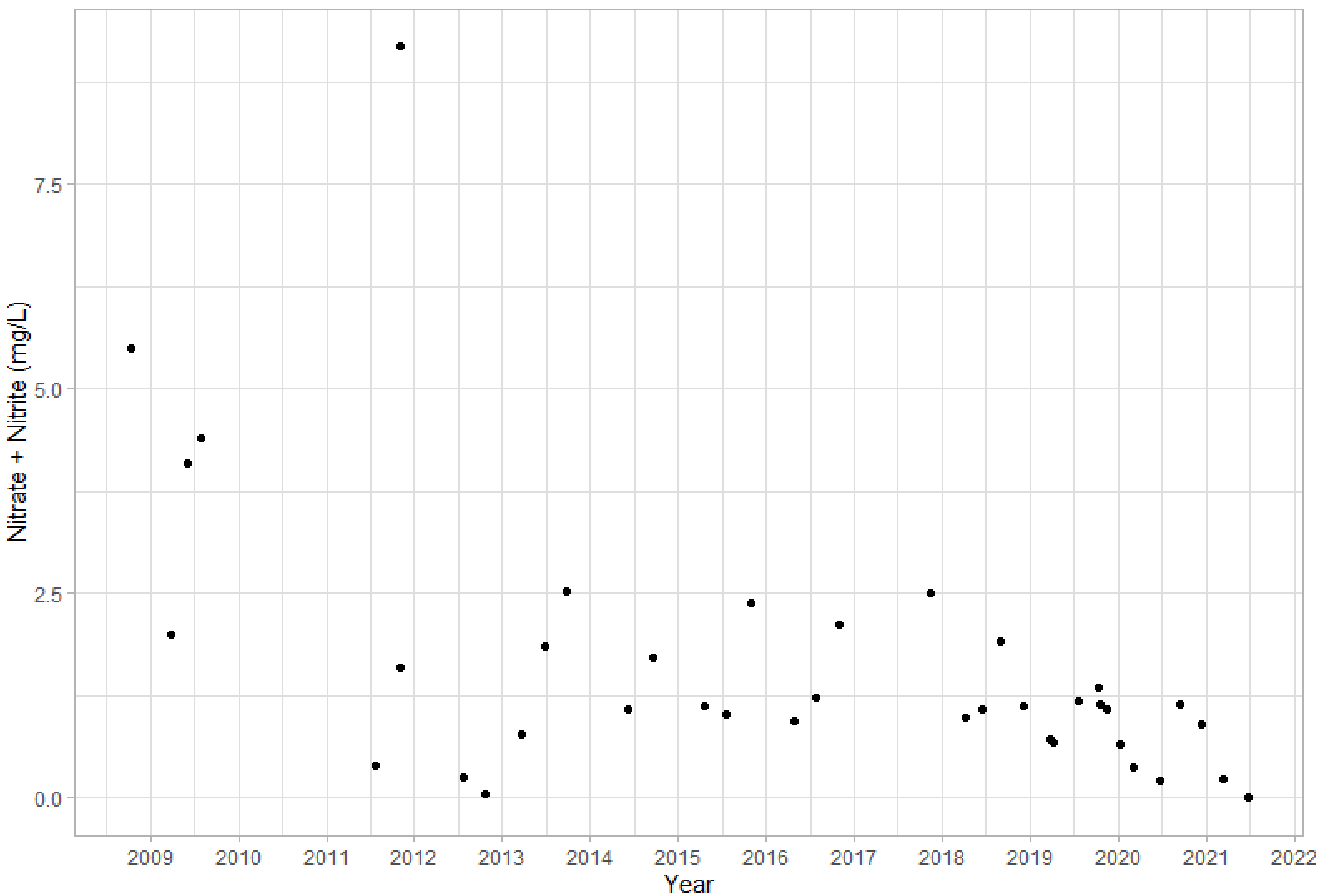
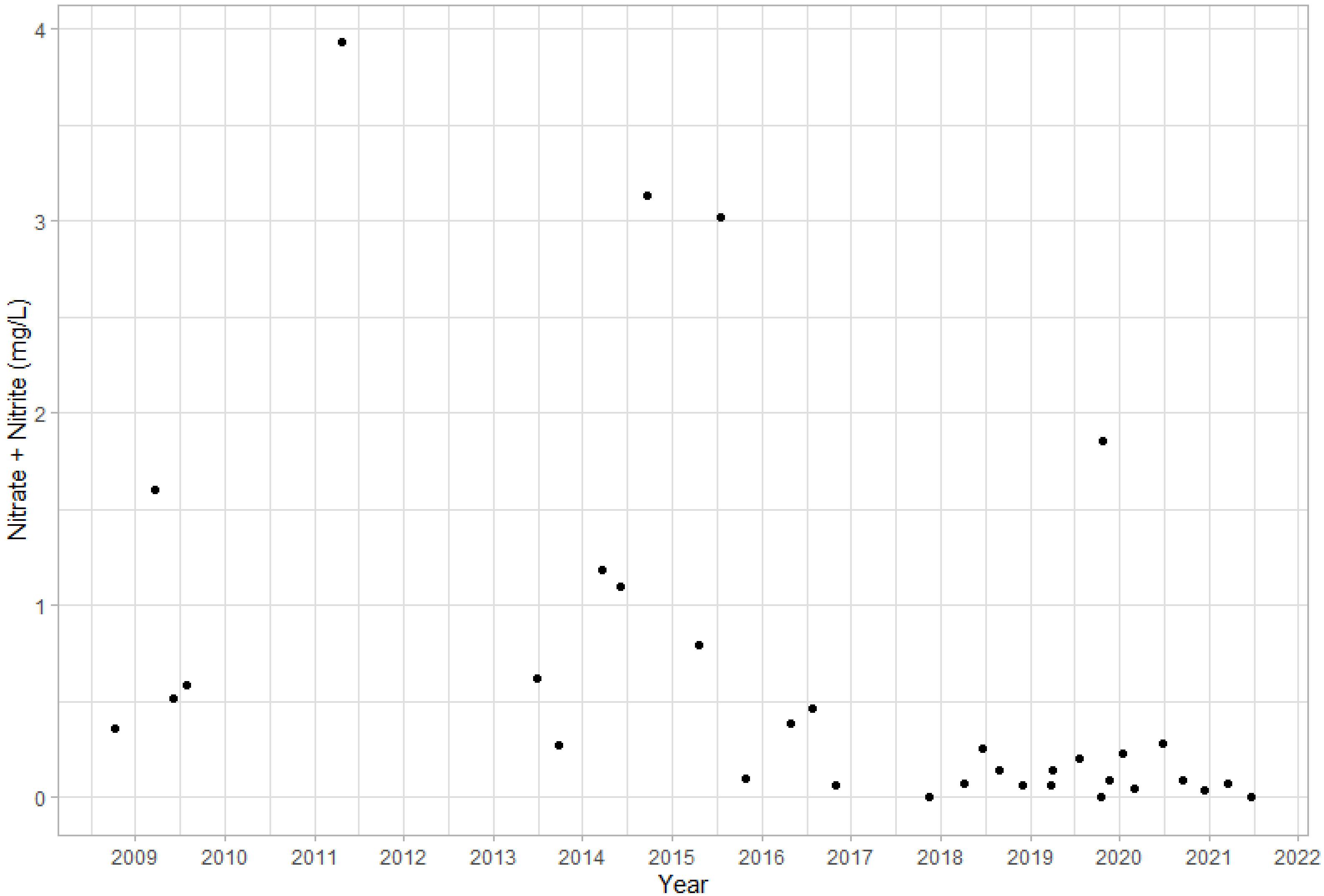


Figure 9 - Nitrate + Nitrite Concentration Versus Time at Well MW06



APPENDIX 4

Figures Examining Relationships between Selected Parameters and Nitrate Concentrations

Figure 1. Dissolved oxygen concentrations versus nitrate.

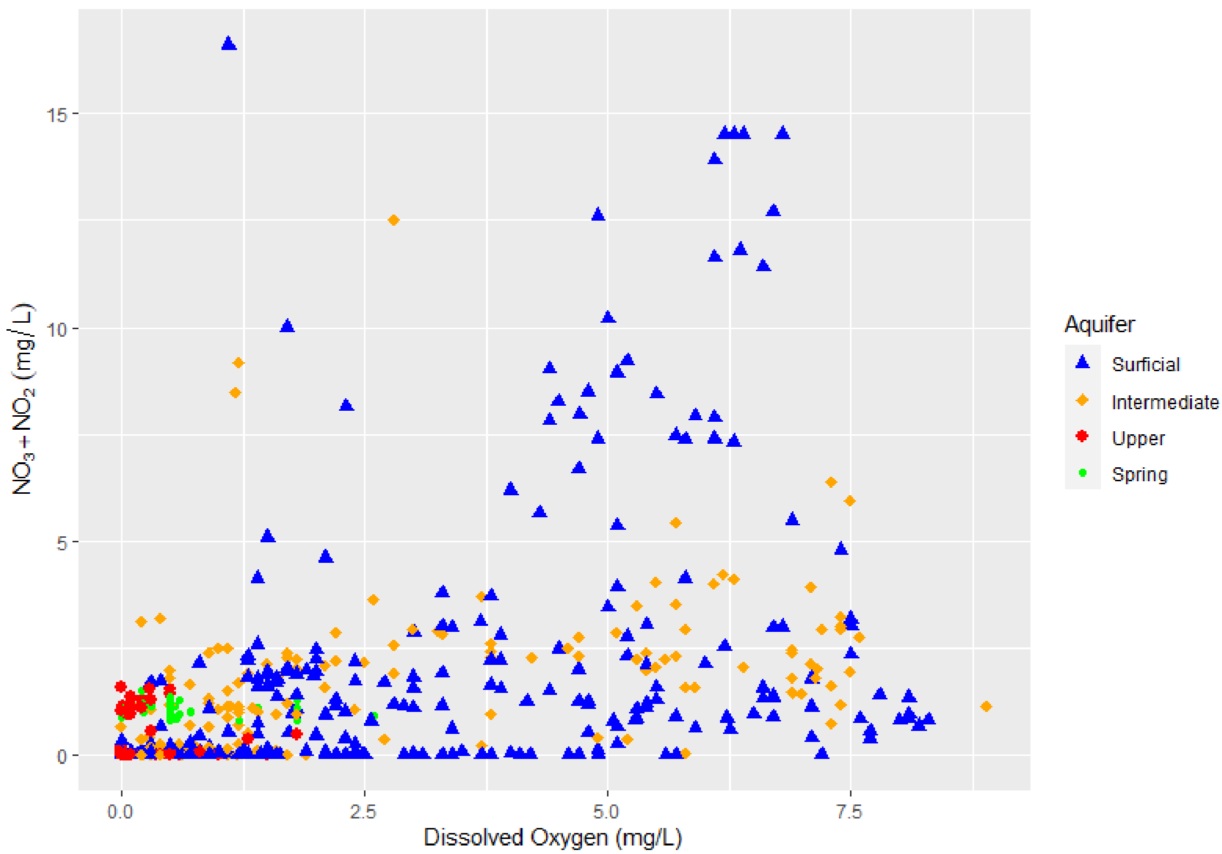


Figure 2. Total alkalinity versus nitrate.

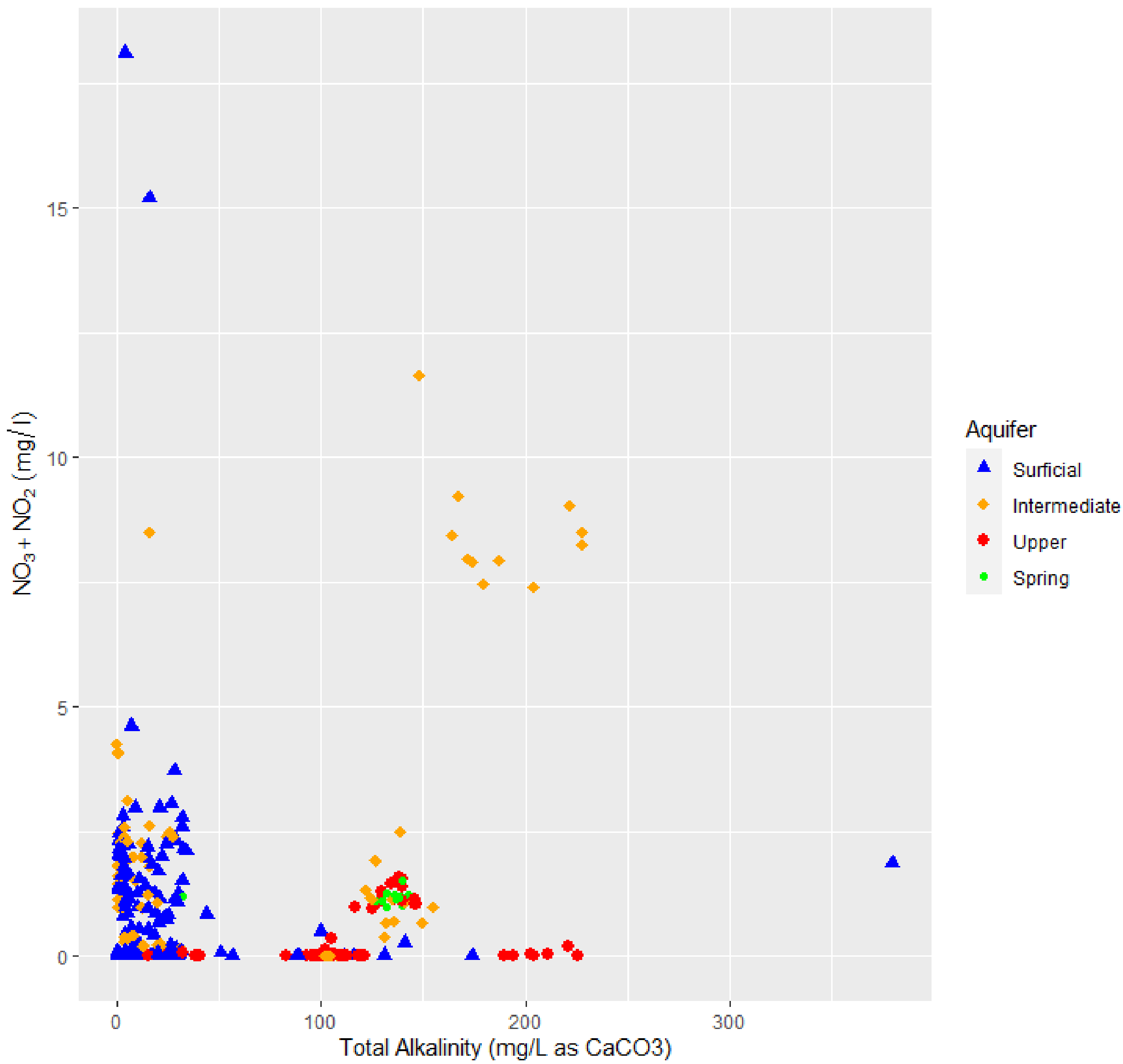


Figure 3. Depth to water versus nitrate.

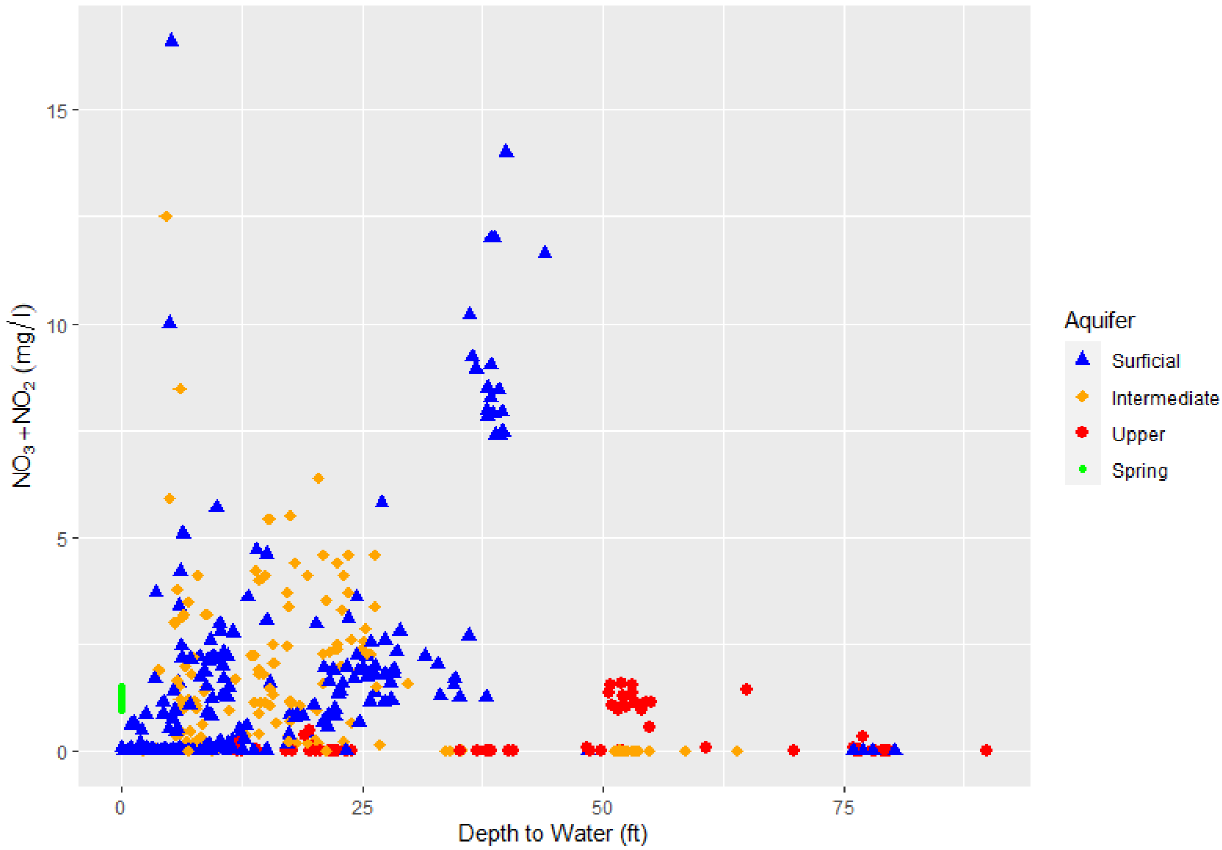


Figure 4. pH versus nitrate

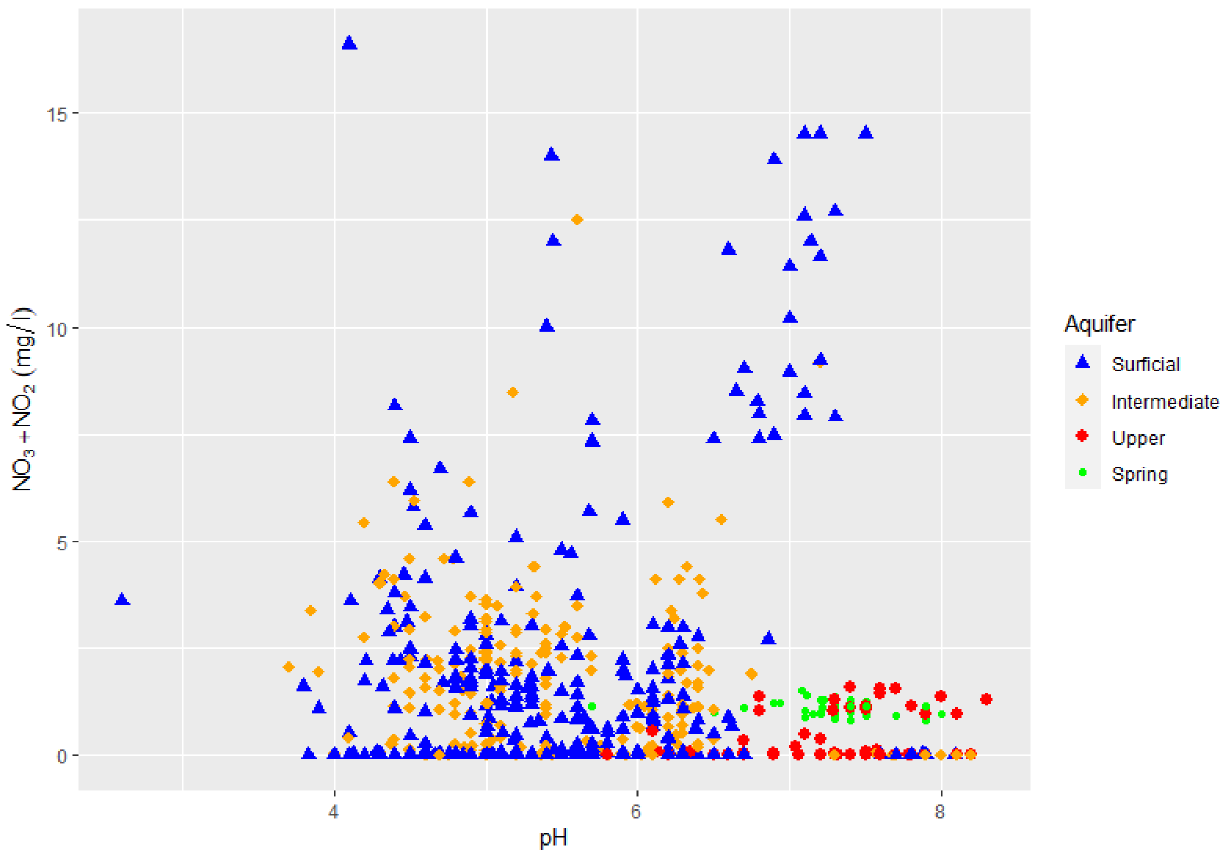


Figure 5. Specific Conductance ("conductivity") versus nitrate.

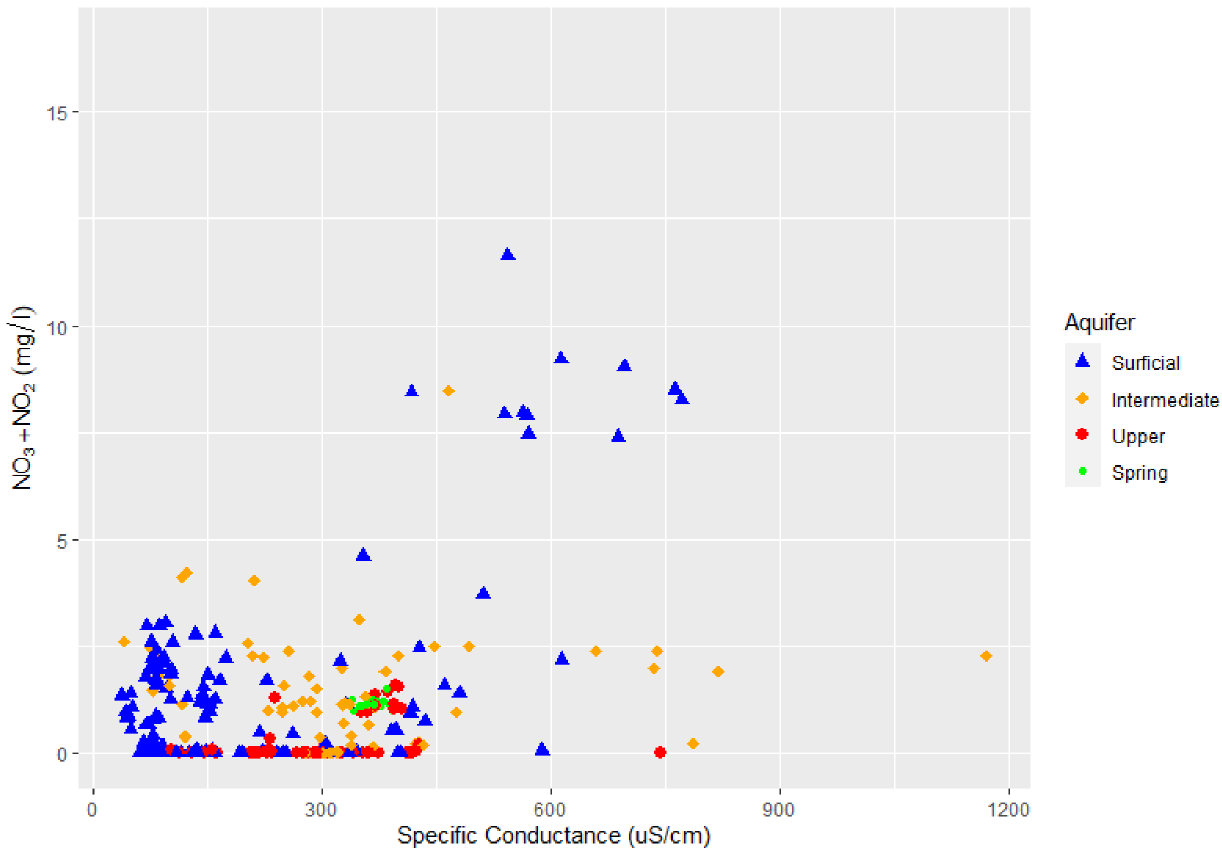


Figure 6. Total nitrogen versus nitrate.

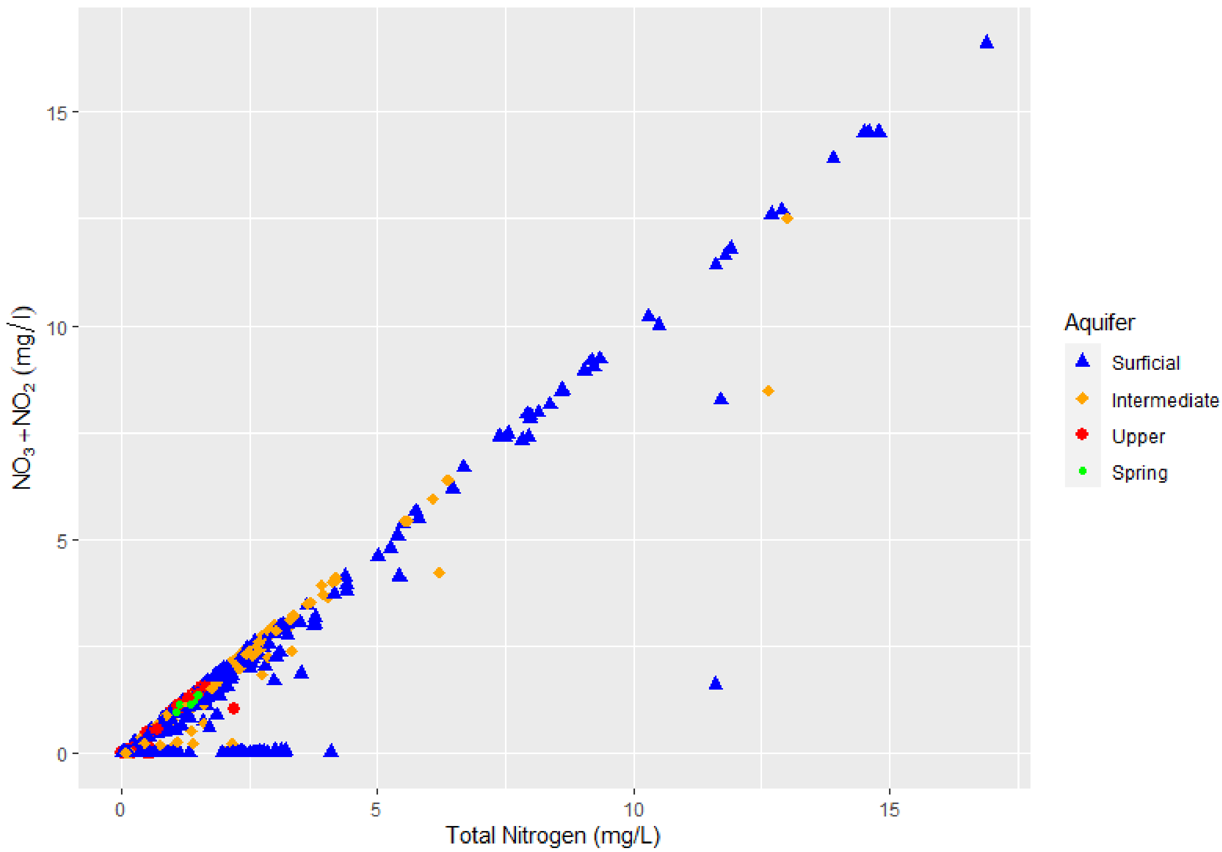


Figure 7. Total phosphorus versus nitrate.

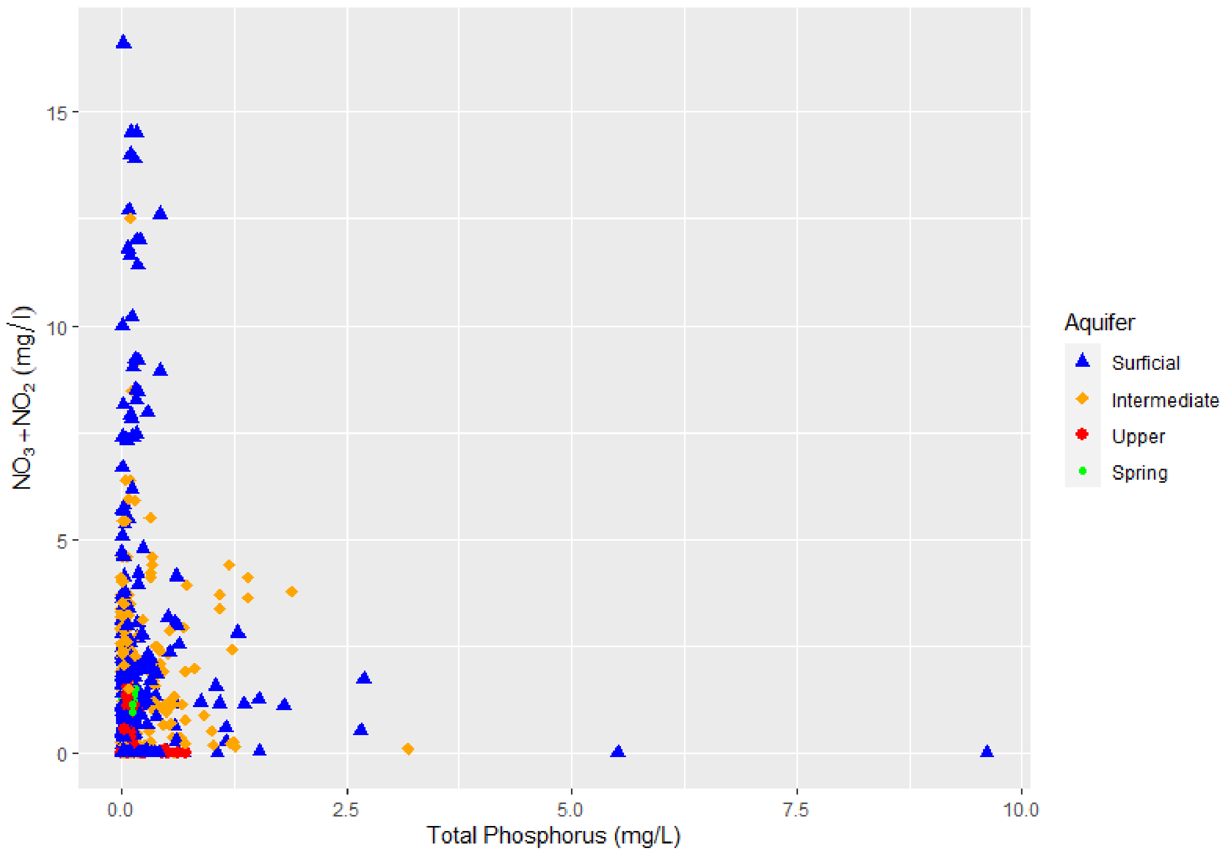


Figure 8. TSS versus nitrate.

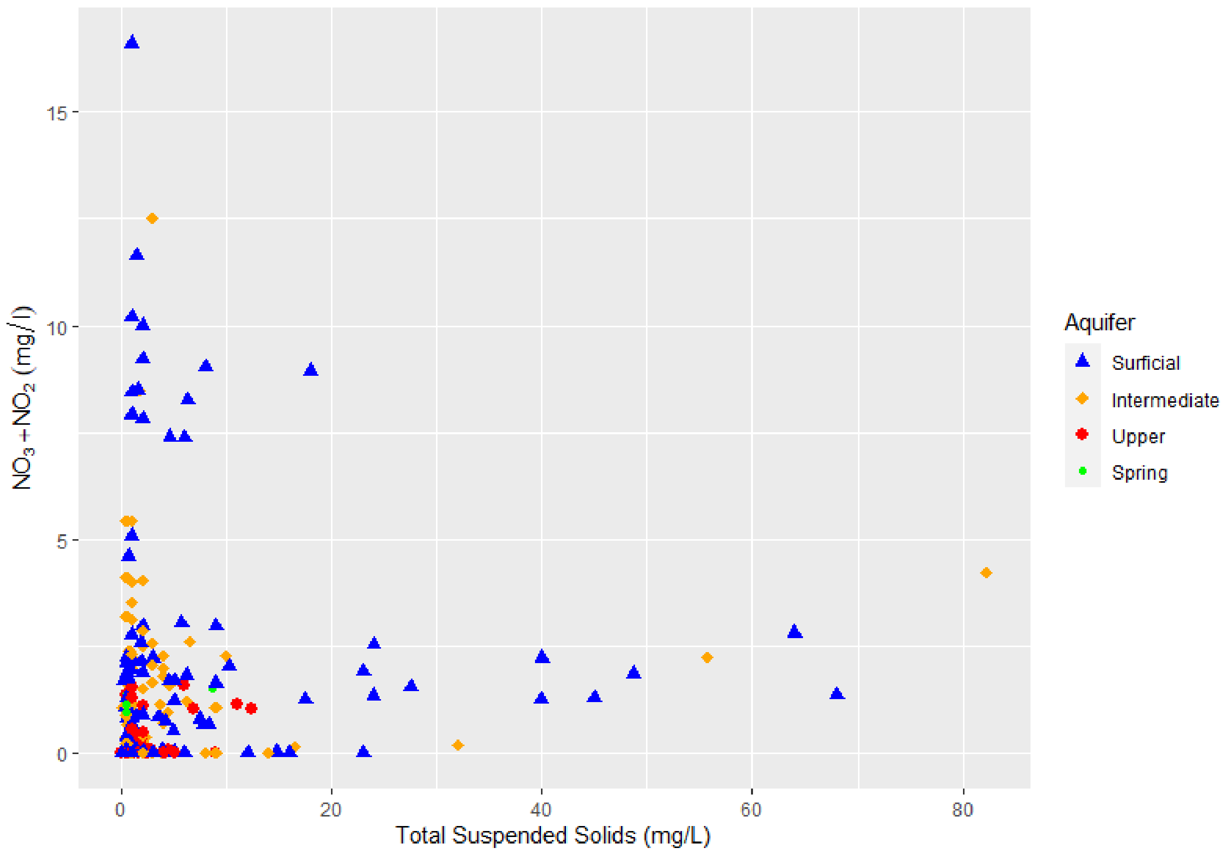
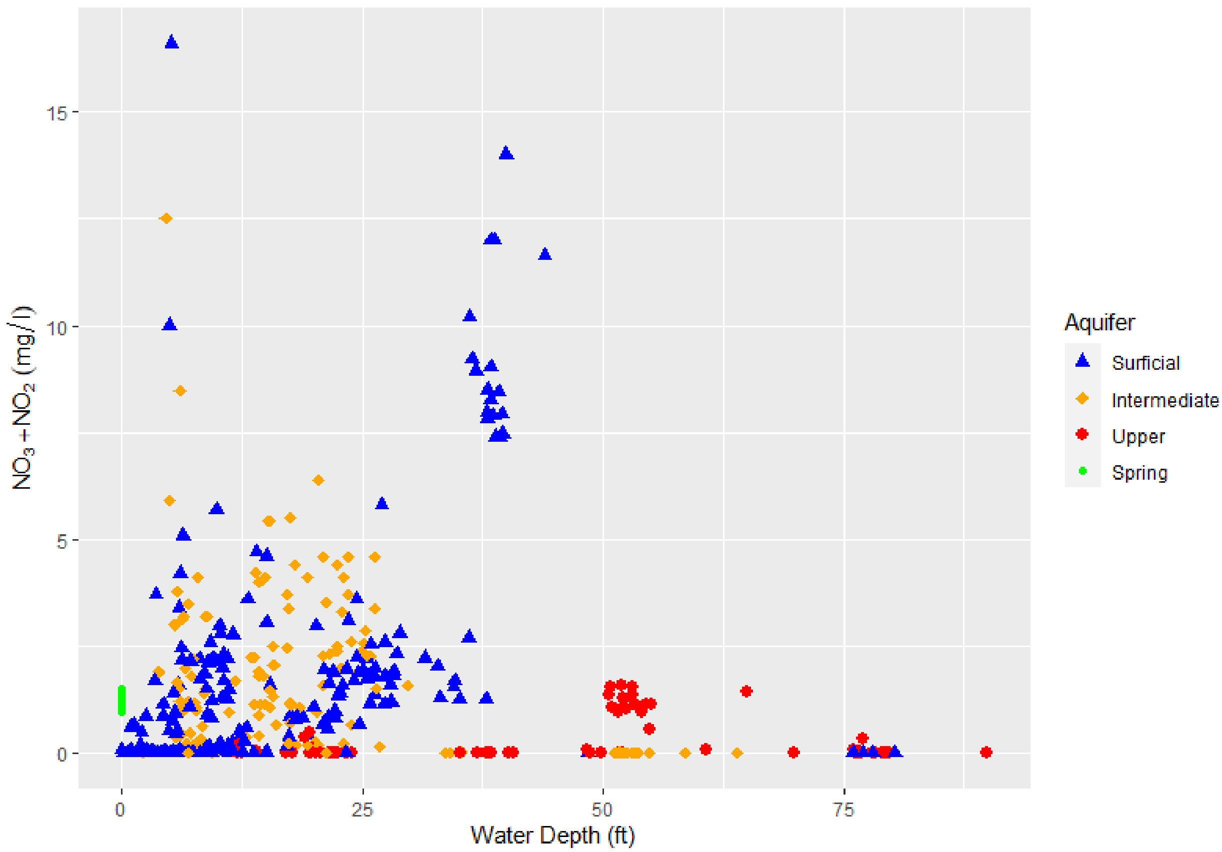


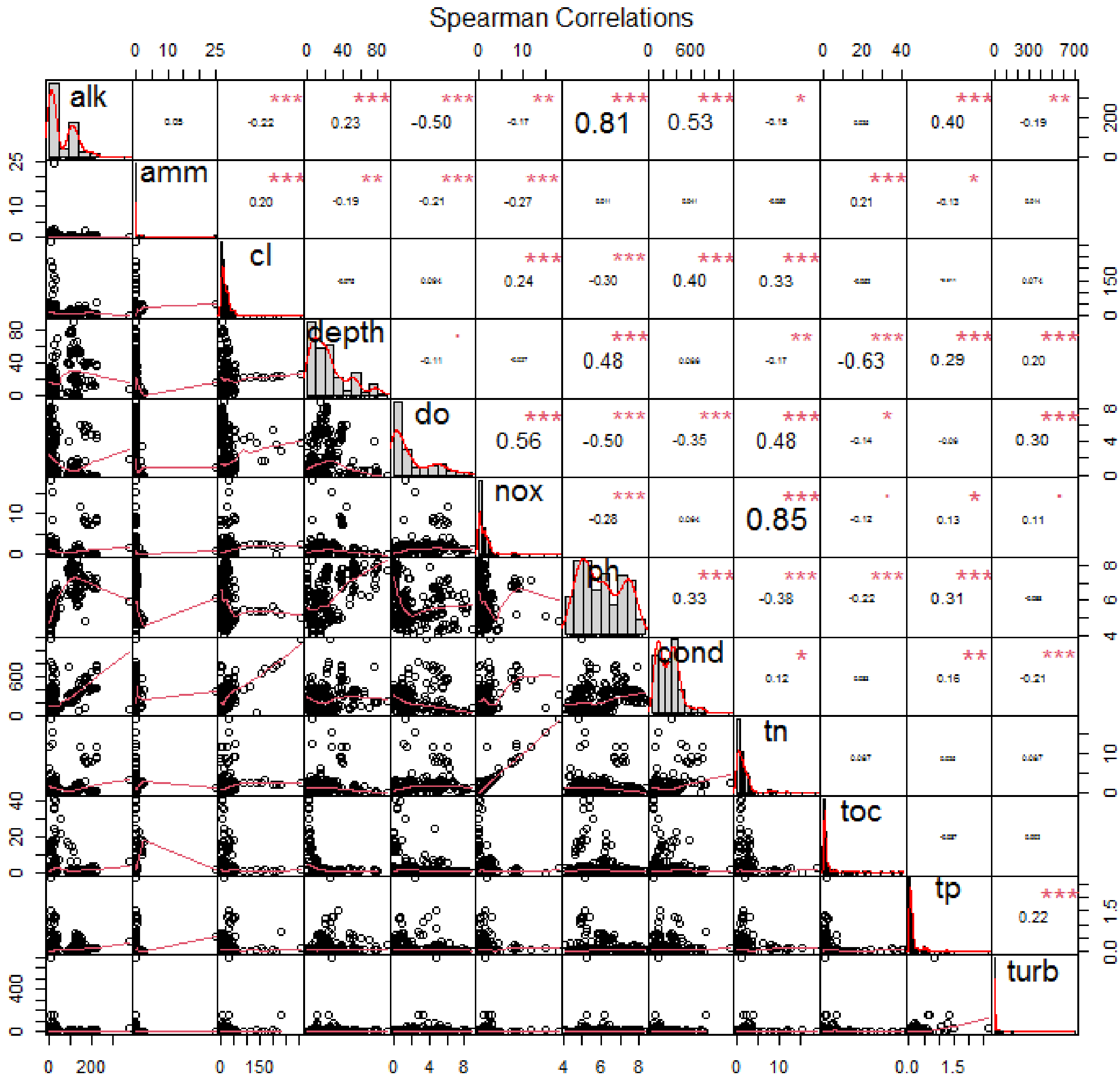
Figure 9. Water depth versus nitrate



APPENDIX 5

Correlation Matrices for Comparison among Water Quality Variables and Nitrate Concentrations among Wells

Figure 1. Spearman Correlations among water quality variables, based on 293 observations.



Notes:

- The distribution of each variable is shown on the diagonal.
- On the bottom of the diagonal is the bivariate scatter plots with a fitted line
- The top of the diagonal is: the value of the correlation plus the significance level as stars
- Each significance level is associated to a symbol: p-values(0, 0.001, 0.01, 0.05, 0.1, 1) = symbols("***", "**", "*", ".", " ")

Figure 2. Spearman correlation matrix for nitrate + nitrite concentrations in wells and the spring vent from November 2017 through June 2020. Size of circles represent strength of relationship, and the color represents a positive (blue) or a negative (red) relationship.

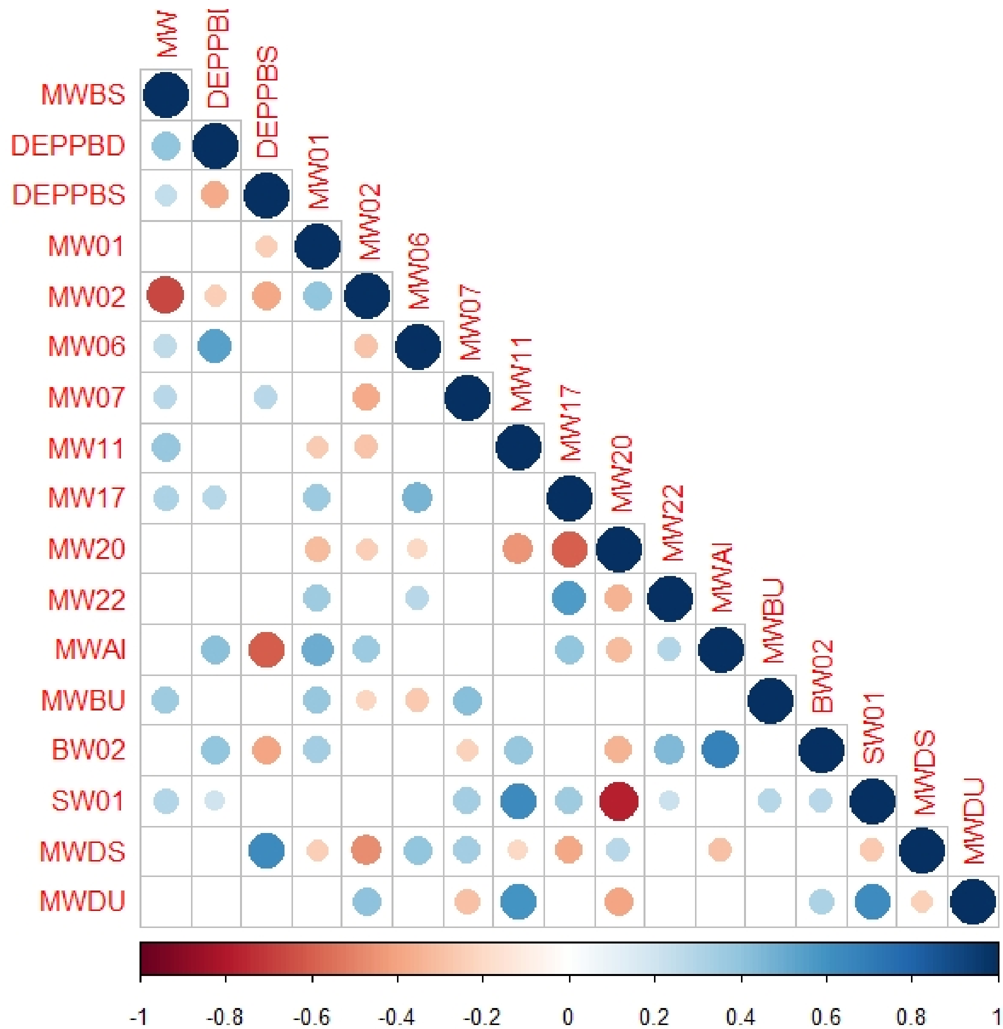
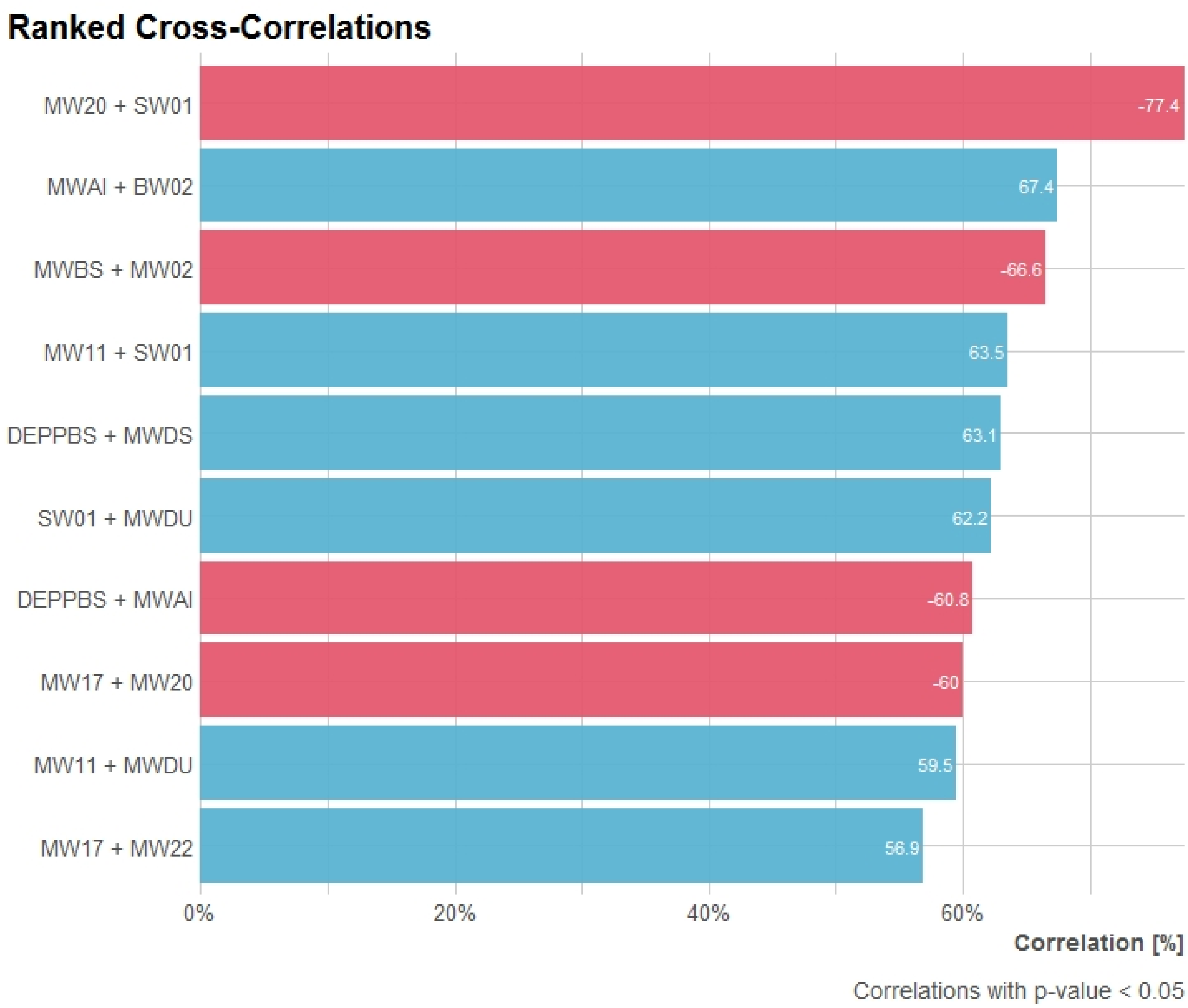


Figure 3. Spearman cross-correlations ranking the top ten significant (p<0.05) correlations among sample sites.



APPENDIX 6

Output from Principal Components Analyses of Water Quality for 18 Sample Sites

Note: amm = ammonia-N, cl = chloride, DO = dissolved oxygen, nox = nitrate + nitrite, ph = pH, cond = specific conductance, toc = total organic carbon, tp = total phosphorus, turb = turbidity, so4 = sulfate.

PCA

Principal Component Analysis

Data worksheet
Name: Data4
Data type: Environmental
Sample selection: All
Variable selection: All

Eigenvalues

PC	Eigenvalues	%Variation	Cum.%Variation
1	2.48	22.5	22.5
2	2.12	19.3	41.8
3	2.11	19.2	61.0
4	1.19	10.8	71.8
5	0.81	7.4	79.1

Eigenvectors
(Coefficients in the linear combinations of variables making up PC's)

Variable	PC1	PC2	PC3	PC4	PC5
amm	0.304	0.243	0.381	-0.307	0.178
cl	0.304	-0.268	0.136	0.018	-0.780
cond	-0.043	-0.375	0.488	0.237	0.056
depth	-0.410	-0.299	0.069	-0.295	-0.003
do	0.267	-0.206	-0.490	0.230	0.232
nox	0.264	-0.492	-0.041	0.323	0.364
ph	-0.539	-0.122	0.210	0.043	0.164
so4	0.359	-0.028	0.418	0.063	0.074
toc	0.153	0.491	0.073	0.113	0.177
tp	0.226	-0.272	0.092	-0.614	0.312
turb	0.103	-0.154	-0.345	-0.458	-0.096

Principal Component Scores

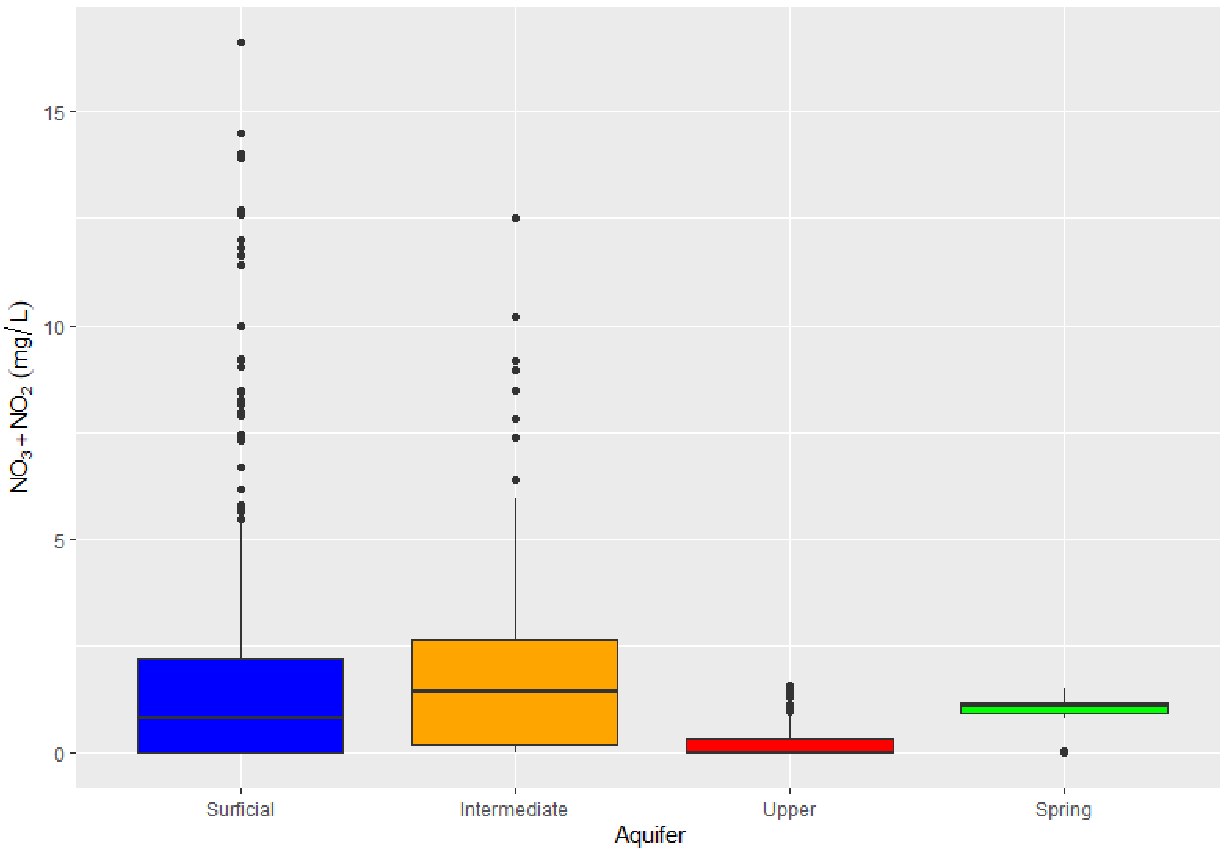
Sample	SCORE1	SCORE2	SCORE3	SCORE4	SCORE5
BW02	0.164	3.33	-0.268	0.806	0.582
DEPFLD	-1.7	-0.0751	1.41	0.257	-0.0468
DEPPBD	-1.62	0.468	0.579	0.371	0.026
DEPPBS	-0.102	0.354	-2.39	0.426	0.661

MW01	2.57	3.13	2.29	-0.24	0.128
MW02	-0.239	-0.726	0.751	0.131	0.077
MW04	0.999	-4.08	0.395	1.48	2.03
MW06	-0.319	1.17	-1.21	0.161	-0.149
MW07	0.461	0.36	-2.56	-0.163	0.725
MW11	1.53	-1.73	-0.214	0.546	-3.13
MW14	1.18	0.553	-0.789	-0.567	0.0785
MW15	2.56	-0.917	2.72	-2.61	0.915
MW17	0.999	0.178	0.491	0.703	-0.852
MW20	2.25	-0.459	1.04	1.67	-0.325
MW22	1.07	-0.0358	-2.14	1.11	0.359
MWAI	-2.28	-0.335	0.824	-0.136	-0.53
MWBS	1.03	-1.12	-2.45	-2.59	-0.612
MWBU	-1.98	-0.667	1.04	0.145	-0.143
MWCI	-1.35	-0.00553	-0.197	-1.6	0.177
MWDS	-0.107	0.389	-1.12	-0.0978	0.113
MWDU	-3.04	-0.41	0.204	-0.792	-0.0567
MWEU	-1.29	0.467	0.921	0.0497	0.0776
SW01	-0.785	0.151	0.678	0.944	-0.105

APPENDIX 7

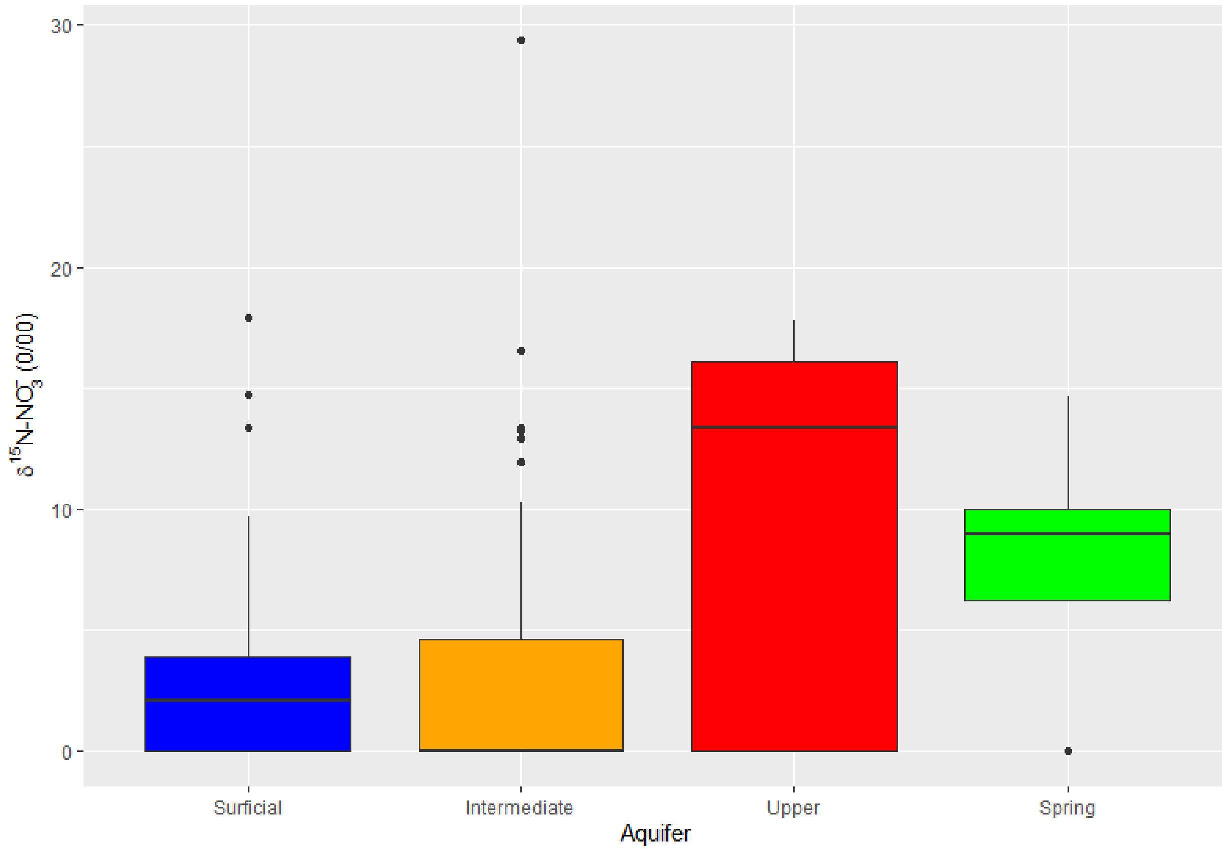
Comparison of Nitrate Concentrations and $\delta^{15}N$ Stable Isotope Ratios by Aquifer

Figure 1 - Nitrate + Nitrite Concentrations by Aquifer and Spring Vent



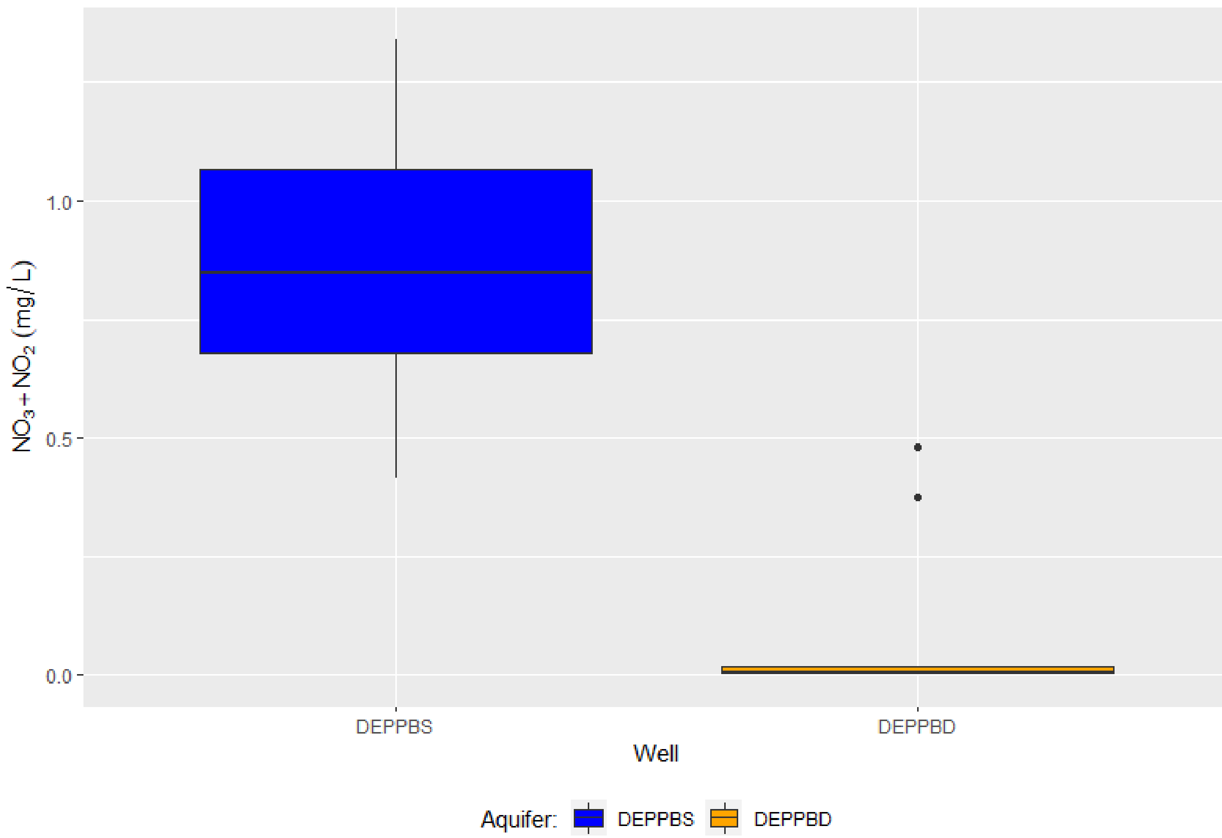
Note: Results include data from November 2017 through June 2020. Median values are represented by the horizontal line, and the 25th and 75th levels are the lower and upper part of the boxes. Whiskers represent 1.5x the interquartile range Dots are outliers.

Figure 2 - δ^{15}N Stable Isotope Ratios by Aquifer and Spring Vent



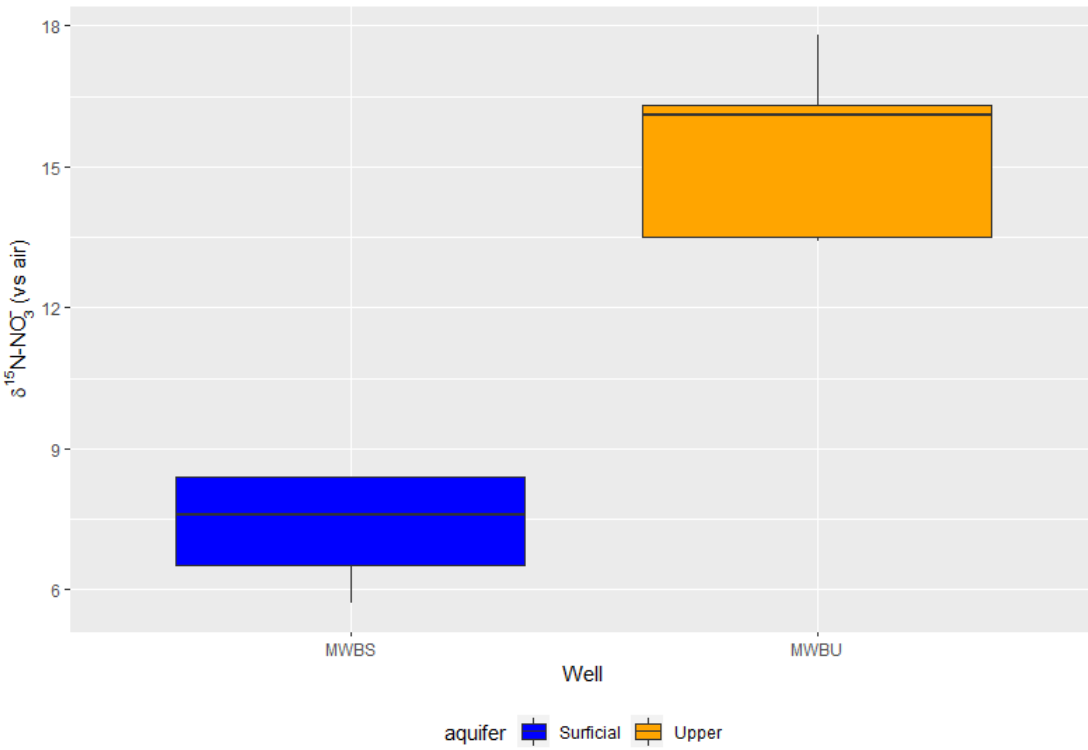
Note: Results include data from November 2017 through June 2020. Median values are represented by the horizontal line, and the 25th and 75th levels are the lower and upper part of the boxes. Whiskers represent 1.5x the interquartile range Dots are outliers.

Figure 3 - Comparison of Nitrate+Nitrite Concentrations in Paired Wells DEPPBD and DEPPBS



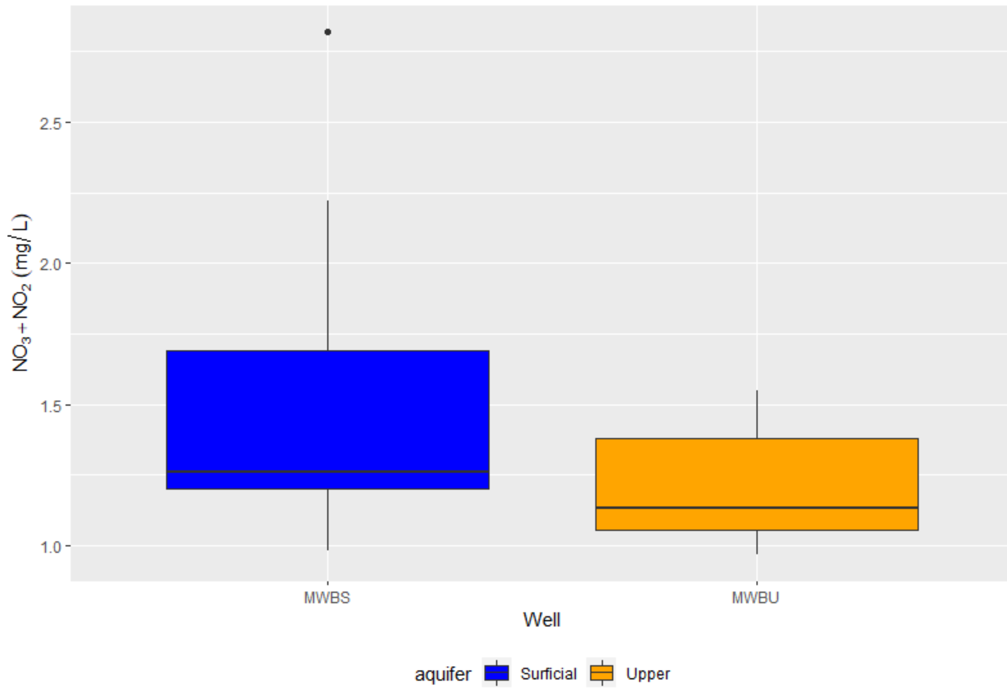
Note: Results include data from November 2017 through June 2020. Median values are represented by the horizontal line, and the 25th and 75th levels are the lower and upper part of the boxes. Whiskers represent 1.5x the interquartile range Dots are outliers.

Figure 4 - Comparison of δ[15]N Stable Isotope Ratios in Paired Wells DEPPBD and DEPPBS



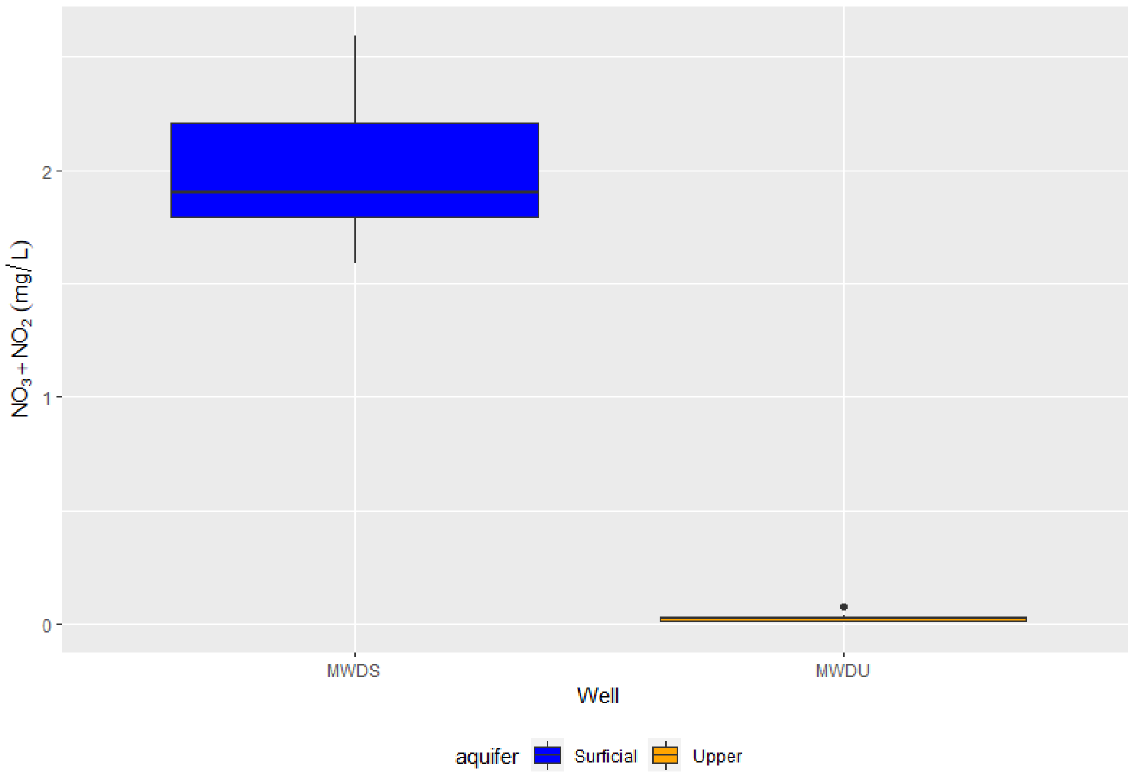
Note: Results include data from November 2017 through June 2020. Median values are represented by the horizontal line, and the 25th and 75th levels are the lower and upper part of the boxes. Whiskers represent 1.5x the interquartile range Dots are outliers.

Figure 5 - Comparison of Nitrate + Nitrite Concentrations in paired wells MWBS and MWBU



Note: Results include data from November 2017 through June 2020. Median values are represented by the horizontal line, and the 25th and 75th levels are the lower and upper part of the boxes. Whiskers represent 1.5x the interquartile range Dots are outliers.

Figure 6 - Comparison of Nitrate + Nitrite concentrations in Paired Wells MWDS and MWDU



Note: Results include data from November 2017 through June 2020. Median values are represented by the horizontal line, and the 25th and 75th levels are the lower and upper part of the boxes. Whiskers represent 1.5x the interquartile range Dots are outliers.

APPENDIX 8

Nitrate + Nitrite Concentrations and $\delta^{15}N$ Stable Isotope Ratios by Aquifer for Septic Tank Density and Acres of Residential Land Use Within 1-km Monitoring Well Buffers

Figure 1 - Septic Tank Density Versus $\delta^{15}N$ Stable Isotope Ratios

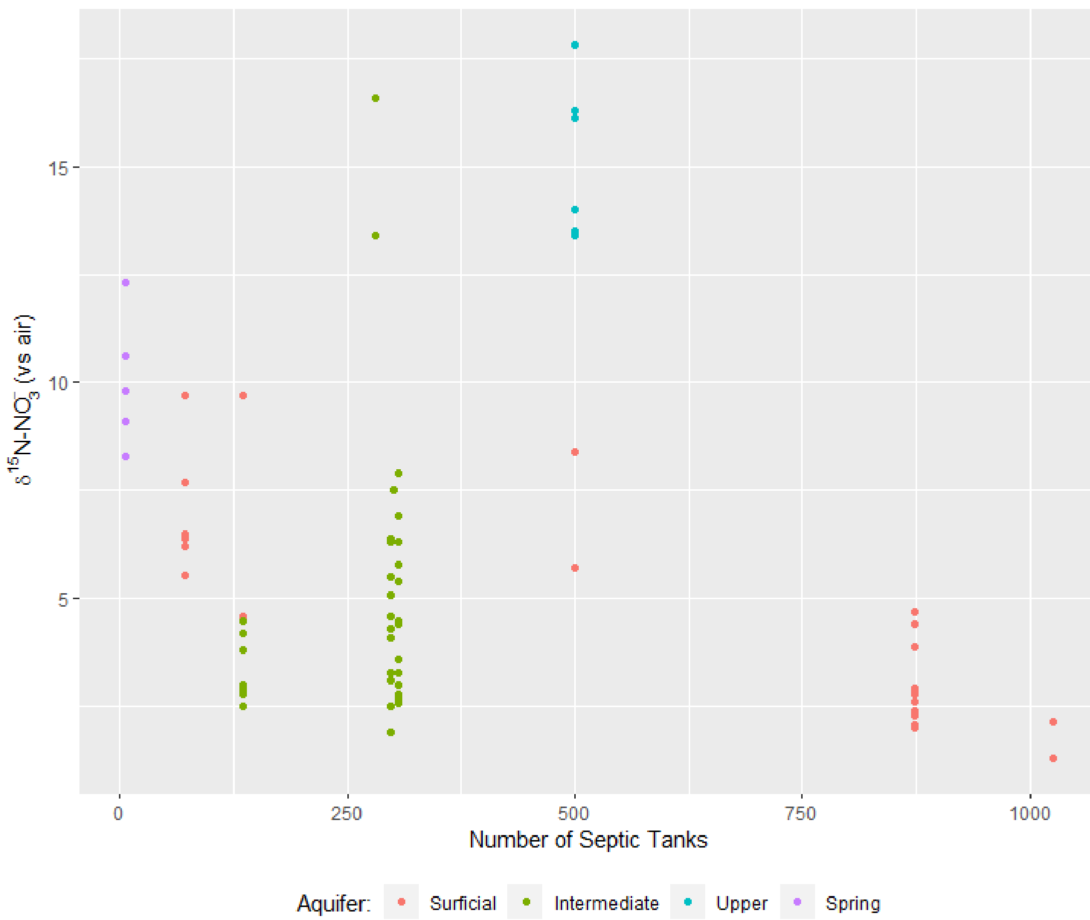


Figure 2 - Septic Tank Density Versus Nitrate + Nitrite Concentrations

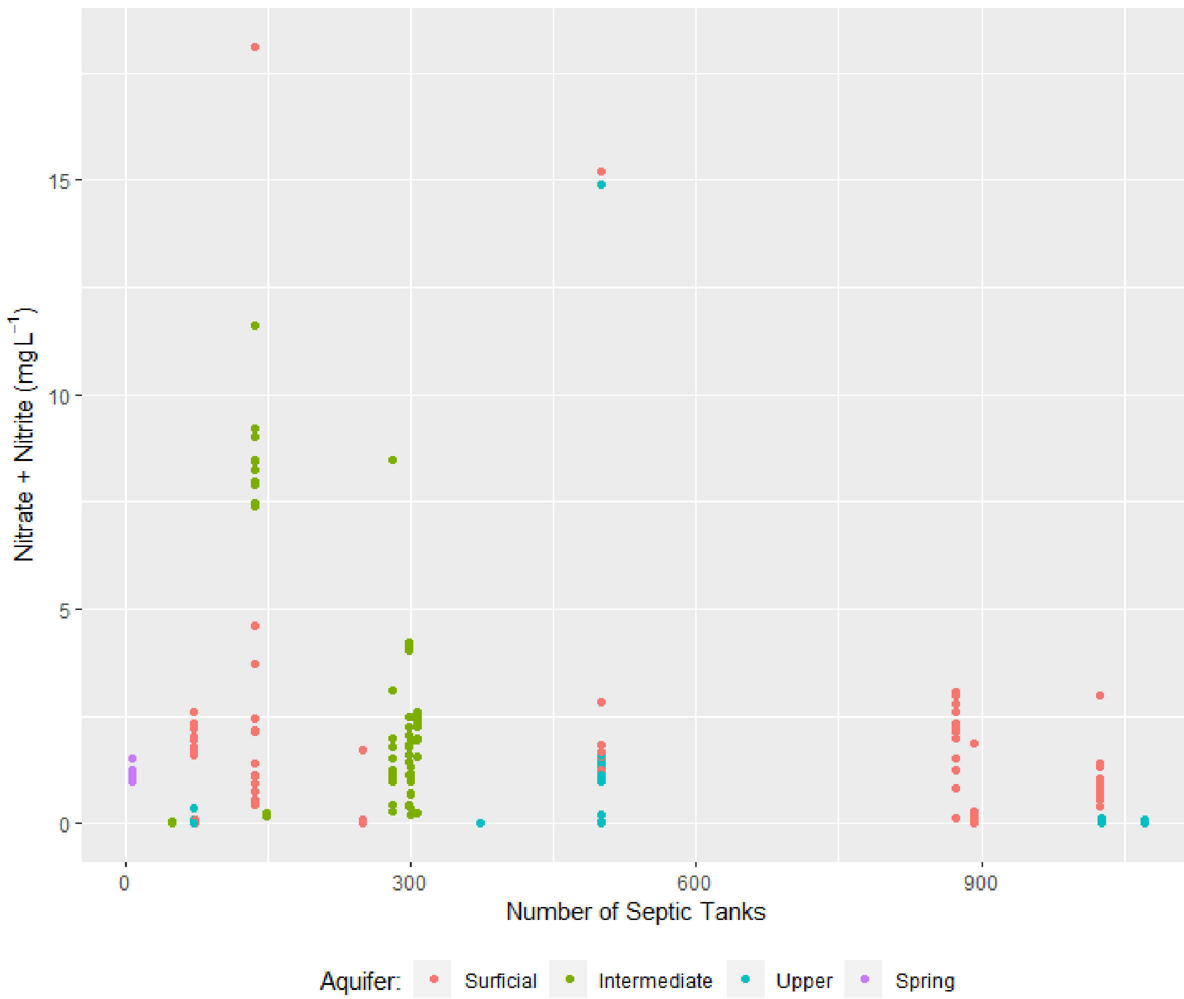


Figure 3 - Residential Land Use Area Versus δ^{15}N Stable Isotope Ratios

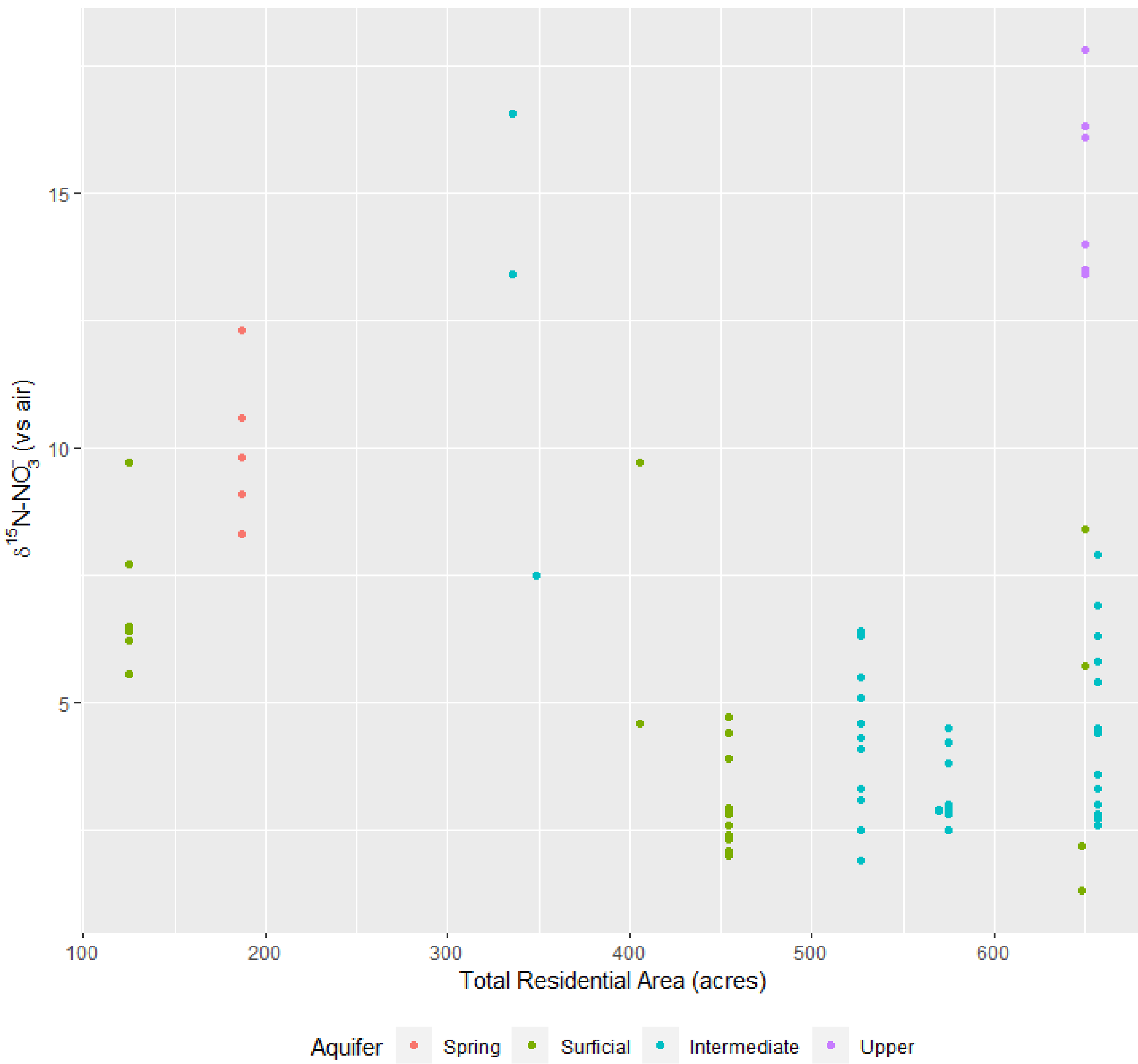


Figure 4 - Residential Land Use Area Versus Nitrate + Nitrite Concentrations