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The source of excess nutrients to Pine Draw, Turnbull National Wildlife Refuge

Henry Price

Eastern Washington University

THE SOURCE OF EXCESS NUTRIENTS TO PINE DRAW, TURNBULL NATIONAL WILDLIFE REFUGE

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THESIS OF Henry Price APPROVED BY

___________________________________ **Date:** ______________

Camille McNeely, Graduate Study Committee

___________________________________ **Date:** ______________

Krisztian Magori, Graduate Study Committee

___________________________________ **Date:** ______________

Stacey Warren, Graduate Study Committee

MASTER'S THESIS

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i. Abstract

Nitrogen and phosphorus are the most common limiting nutrients for biological activity in freshwater ecosystems. Applying inorganic nitrogen fertilizer has increased crop productivity but caused excess nitrogen inputs to the hydrosphere. Nitrate contamination is a worldwide environmental problem. The fate of nitrogen in ecosystems is variable based on land type and hydrogeological interactions. Excess nitrogen can be retained in soils, sequestered in stream organisms, denitrified or transported downstream. The goals of this study were to monitor nitrogen concentrations in Pine Draw, Turnbull National Wildlife Refuge (TNWR), Washington, U.S.A., and to determine the source of nitrogen loading. Pine Draw is unique because it's located in the channeled scablands and has minimal anthropogenic impacts but has experienced excess nutrients for at least 20 years. Symptoms of nutrient loading observed on TNWR are an overabundance of primary producers, decreased biological diversity, extensive algal blooms, low dissolved oxygen, episodic anoxia, loss of vascular plant life and fish kills. I sampled nine surface water, three groundwater inputs on Pine Draw and three surface water sites on Philleo drainage monthly from October 2016 to October 2017. I documented nitrate+nitrite (NO_3-N), ammonium (NH^{4+}-N) and phosphate (PO_4^{3-}) concentrations as well as specific conductance, conductivity, dissolved oxygen, temperature, pH and discharge in both watersheds for the duration of the sampling period. We used stable isotope ratios of oxygen and nitrogen in nitrate to determine that the source of nitrogen to Philleo drainage, groundwater and Pine Draw was a combination of ammonium fertilizer and soil nitrate varying seasonally based on water source.

ii. List of Tables

iii. List of Figures

1. Introduction

Nitrogen

Nitrogen is one of two common limiting resources in freshwater and marine ecosystems and is needed for protein synthesis [Conley et al. 2009]. Most of the nitrogen resides in the atmosphere as N_2 gas that is not usable as source by most organisms [Vitousek et al. 1997]. Nitrogen fixation is the process of converting nitrogen gas (N_2) to biologically available ammonia (NH_3). The natural process of nitrogen fixation occurs mostly through nitrogen-fixing bacteria acting either independently or in symbiosis with a plant. Ammonia that is not quickly used by organisms to support growth is often quickly nitrified. Nitrification is the oxidation of ammonia to nitrite then nitrite to nitrate by bacteria and archaea in multiple steps which require oxygen. Denitrifying bacteria reduce nitrate via nitrite, nitric oxide and nitrous oxide to N_2 gas with organic carbon often donating electrons in anaerobic environments. Denitrification is the most significant process through which nitrogen becomes biologically inert and is removed from ecosystems [Vitousek et al. 1997].

In the early 1900's the Haber-Bosch process was developed to emulate nitrogen fixation and convert nitrogen gas to ammonia for fertilizers to support the increasing demand for food; through the Haber-Bosch process we have continued to increase the application of synthetic fertilizers over the last 40 years [Sebilo et al. 2013]. The excess nitrogen that is not used by crops is transported to the atmosphere, soils, groundwater and downstream aquatic ecosystems causing eutrophication of streams and estuaries [Stoliker et al., Granger & Wankel 2016]. Eutrophication refers to excess nutrient enrichment leading to high rates of respiration and depletion of dissolved oxygen. The amount of attention to nitrogen contamination has increased in the last two decades [He & Lu 2016]. Up to 60% of nitrogen from synthetic fertilizer is taken up by crops [Sebilo et al. 2013], but the rate can be substantially lower. For example, in China nitrogen uptake efficiency is about 28% for rice and wheat and 26% for corn [Miao et al. 2011]. Excess nitrogen is considered the third most important environmental problem globally, behind biodiversity loss and climate change [Yevenes et al. 2016]. In order to manage for nitrogen contamination it is necessary to identify the flow paths and sources to aquatic systems [Granger & Wankel 2016].

Nitrogen can significantly affect terrestrial ecosystems as well as aquatic ones. Riparian species that are adapted to low nitrogen levels are often outcompeted by native or invasive plants that can use the higher levels of nitrogen more efficiently [Pardo et al. 2011]. Symbionts such as mycorrhizal fungi, and nitrogen-fixing bacteria live on or in host plant roots and exchange nutrients, such as nitrogen, for carbon compounds [Pardo et al. 2011]. In a stream lacking anthropogenic impacts nitrogen fixers would likely have a large competitive advantage, suggesting excess nitrogen would decrease fitness [Vitousek & Howarth 1991].

Phosphorus

Phosphorus is necessary for many organisms for a variety of physiological processes and creation of structures such as phospholipids, DNA and RNA [Conley et al. 2009, Childers et al. 2011]. The phosphorus cycle is sedimentary. Natural phosphorus sources are weathering and erosion of minerals [Bennett et al. 2001]. Phosphorus is made anthropogenically available through mining. The atmospheric cycle consists of dust transport because phosphorus doesn't have a stable gaseous form [Mahowald et al. 2008]. Phosphorus runs through a series of systems from terrestrial to freshwater then to the marine environment. The fate of phosphorus is to be buried in sediments, mostly in the ocean with less in lakes and wetlands [Bennett et al. 2001].

Anthropogenic impacts on the phosphorus cycle involve mining and transporting mineral phosphorus for various uses including agricultural fertilizer and detergents [Childers et al. 2011]. Most excess phosphorus from humans is due to inefficient use of phosphate resources at some point in transport, production or application [Childers et al. 2011]. Around 20 million tons of phosphorus is mined a year, with 9 million tons a year is flowing into our oceans [Childers et al. 2011]. When inputs of phosphorus exceed the outputs, phosphorus accumulates in terrestrial and aquatic systems. This accumulation can lead to long-term impacts that will be harder to manage as the total phosphorus levels increase.

Estuaries and Coastal Waters

Eutrophication of estuaries is one of the best understood consequences of excess nitrogen loading. Estuarine nutrient limitation often changes seasonally, but inputs of nitrogen are most likely to cause eutrophication. Rates of nitrogen fixation are low in estuaries and coastal seas due to detrimental effects of salinity on nitrogen fixers [Conley et al. 2009]. Nitrogen is usually the limiting nutrient during summer due to an increase in phosphorus recycling in the water column and an increase in rates of phosphorus desorption from clay and other particles [Conley et al. 2009]. Estuary eutrophication results in anoxia, hypoxia, loss of biodiversity, including a reduction in seagrasses [Conley et al. 2009], excess algal growth and toxic blooms of dinoflagellates [Musgrove et al. 2016]. Eutrophication of estuaries can be exacerbated by only controlling for one limiting nutrient. If phosphorus is managed in upstream watersheds while nitrogen is ignored, the stream is likely to be nitrogen saturated, allowing for more nitrogen to flow downstream into estuaries and coastal seas [Conley et al 2009]. If both phosphorus and nitrogen aren't managed eutrophication will occur at points downstream based on which resource is limiting during nutrient pulses.

Nitrate cycling in streams and the role of nitrate

Within a watershed, significant sources of nitrogen to freshwater ecosystems include atmospheric deposition, local nitrogen fixation, and inputs from the terrestrial environment including fertilizer and leaching of soil organic matter, manure, and sewage [Ding et al 2014, Lu et al. 2015, Vitousek et al. 1997]. The impact nitrogen will have is directly dependent on the microbial communities present at the groundwater-surface water interface and biogeochemical processes within the system [Stoliker et al. 2016]. In most aquatic habitats, nitrate is the predominant form of reactive nitrogen because it is very soluble and easily leached from soils [Yevenes et al. 2016]. Ammonium is less abundant because it is usually adsorbed to charged clay particles, converted to nitrate via nitrification or consumed by microbes or algae [Yevenes et al. 2016]. Solutes, including nitrogen and phosphorus, in streams will transition from biotic to abiotic repeatedly while being transported downstream in a process known as nutrient spiraling (Figure 1). The spiraling length is the distance traveled while the nutrients cycles once between inorganic and organic forms [Thomas et al. 2003, Hauer & Lamberti 2006].

The concentration of nitrate in streams is controlled by the inputs of total nitrogen to the stream, but also to rates of uptake, nitrification, and denitrification. Nitrate turnover rates in the hyporheic zone are controlled by the availability of labile biologically available carbon, streambed residence times, and oxygen concentrations in the hyporheic zone. To convert a mole of ammonium to nitrate, two moles of oxygen are needed [Yevenes et al. 2016], which suggests higher oxygen consumption in areas with higher nitrification rates. Nitrification occurred more frequently with residence time-controlled increases in turnover rates until dissolved oxygen concentrations were low enough. Long residence times in low dissolved oxygen environments favored denitrification [Krause et al. 2013]. The rates of anammox (anaerobic ammonium oxidation), denitrification and nitrification could be linked to rates of organic N-mineralization (conversion of organic nitrogen to inorganic nitrogen), which would increase N-mobility and transport downstream [Stoliker et al. 2016]. Nitrifiers compete with heterotrophic bacteria for ammonium, and nitrification rates are positively correlated with nitrate concentrations in streams [Bernhardt et al. 2002]. Denitrification rates can be high enough to remove 48% of the nitrate in a freshwater system, but rates are hard to measure because a high percentage of denitrification occurs in small spatially distributed sites [Yevenes et al. 2016].

Isotopes in nitrate as tracers

Nutrient isotope ratios are a useful tool in understanding the sources of contamination in surface and groundwater. Nitrogen from various origins and processes has predictable isotope ratios [Yevenes et al. 2016]. We can track the sources of nitrogen through the nitrogen and oxygen isotope ratios in the nitrate molecule [Musgrove et al. 2016]. Isotope ratios may vary based on rates of denitrification because bacteria preferentially consume ^{14}N over ^{15}N in nitrate during this process [Kendall 2004]. Recent literature suggests that biogeochemical reactions occurring in groundwater may alter these ratios, which could cause an error in estimation of the relative contributions of nitrate sources. This effect can be mitigated by using multiple tracers such as nutrient concentrations, chlorine and bromide ratios, manganese and iron concentrations, boron isotopes, or oxygen isotope ratios [Pasten-Zapata et al. 2014]. The ratios of ^{15}N to ^{14}N in a sample may be compared to those of potential sources. Air is used as a standard because the average composition is very stable at 0.366‰ [Kendall 2004]. Other sources of nitrogen influence the ratio in predictable patterns, e.g. fertilizers produced from atmospheric nitrogen can change the isotope ratio by ± 3‰, while animal manure have ratios in the range of +10 - +25‰ [Kendall 2004].

Nitrate sources can vary seasonally [Yevenes et al. 2016]. For example, nitrate pollution is a severe problem in aquatic systems in Taihu Lake Basin, China. A dual nitrogen and oxygen isotope approach was applied to identify diffuse nitrate inputs in a stream in an agricultural field at the basin in 2013. Results showed that reduced nitrification in the soil was the main nitrate source throughout the year. Manure and sewage contributed more nitrate during the dry season (22.4%) than during the rainy season (17.8%). Atmospheric deposition generated its highest amounts of nitrate in May (18.4%), June (29.8%), and July (24.5%) [Ding et. Al 2014].

Groundwater

Nitrate contamination of groundwater is a widespread problem globally [Wu & Sun 2016]. Transport and transformations of nitrogen in groundwater differ from surface water in several ways. Groundwater systems have much slower flow velocities and longer residence times compared to surface water. Groundwater also can show a longer residence time seasonally; in summer residence time can be longer than winter [Yevenes et al. 2016]. Usually groundwater flow paths will converge near shorelines where they intersect surface water, but the pathways can vary spatially and temporally based on water depth, aquifer thickness, sediment spatial heterogeneity, seasonal changes in hydrologic gradient, wind and waves and presence of littoral zone [Stoliker et al. 2016]. Nitrate concentrations in groundwater can be higher and more stable than surface waters. For example, in the Abujiao River basin, China, surface water nitrate levels were substantially changed by precipitation and land use changes while groundwater nitrate levels did not fluctuate [Lu et al. 2015].

With enough time, nitrate contamination of groundwater may respond to reduced nitrogen input. In central Vermont, elevated nitrate concentrations ranging from 12 to 34 mg/l nitrate were discovered in groundwater from domestic wells near a large dairy farm [Kim et al. 2016]. In this medisedimentary bedrock aquifer, the groundwater flow is controlled by fractures, bedding/foliations and basins and ridges in the bedrock surface [Kim et al. 2016]. The nitrate sources were a ravine filled with manure and the surrounding fields. Once the nitrogen contamination from the ravine was gone, the nitrate levels in groundwater dropped from 34 mg/l to less than 10mg/l in ten years [Kim et al. 2016].

Groundwater dynamics vary based on land type [Pasten-Zapata et al. 2014]. Sediments in groundwater flow regions can affect the fate of nitrogen by removing it or altering mobility. In Ireland two fractured aquifers with contrasting geomorphology were compared using nitrogen and oxygen isotopic ratios in nitrate (NO_{3^-}) to determine the sources of nitrate contamination [Orr et al. 2016]. The moderately fractured, diffusely karstified limestone favored nitrification. In contrast, in the low-transmissivity, highly-lithified sandstone and pyrite-bearing shale aquifer showed lower rates of nitrification and lower nitrate concentrations [Orr et al. 2016]. The variability of nitrate concentrations in this area is due to the influence of bedrock hydrogeology on nutrient mobility [Orr et al. 2016]. Bedrock type is a key factor as it affects residence time, aquifer depth and flow rate [Orr et al. 2016]. This is important in showing us that even similar aquifers can vary substantially in nitrogen behavior based on geomorphology and each aquifer needs to be examined closely.

Groundwater nitrate contamination may also vary with depth. In the United States in the San Joaquin Valley, California shallow wells were more likely to be contaminated than deeper wells [Lockhart et al. 2014]. Nitrate levels in shallow groundwater wells are generally higher than streams [Yevenes et al. 2016] Nitrate levels near the surface water-groundwater interface are high but decrease rapidly with soil depth suggesting significant nitrate consumption [Yevenes et al. 2016].

2. Study Site

Pine draw runs through the Channeled Scablands on the Turnbull National Wildlife Refuge (TNWR) south of Cheney, Washington, USA (Figure 2). The channeled scablands are characterized by pothole wetlands and large wetland sloughs between grasslands, shrub steppe and ponderosa pine forest. TNWR has more than 150 wetlands totaling more than 3000 acres. These wetlands provide habitat for 16 species of waterfowl, 5 amphibians and 113 total species [Davidson et al. 2006]. One threatened plant species - the water howelia, *Howellia aquatilis*, is present in these wetlands as well [Davidson et al. 2006].

Excessive nitrogen, phosphorus and sediment are likely the primary pollutants affecting water quality on the TNWR [Davidson et al. 2006]. Symptoms of nutrient loading on TNWR include an overabundance of primary producers, decreased biological diversity, low dissolved oxygen, episodic anoxia, loss of vascular plant life, fish kills and potentially toxic algal blooms [Davidson et al. 2006]. There were fish mortality events on TNWR that were attributed to oxygen depletion from respiring and decaying algae [Davidson et al. 2006]. During the fish mortality event on TNWR in 1987, refuge staff detected low dissolved oxygen and high ammonia-nitrogen concentrations in the aquatic system [Davidson et al. 2006].

Pine Draw is a tributary to the Palouse River, covering 10.8% of the drainage area [Carroll 2007]. The Palouse River is on the 303d list for impaired waterways, which means it must be managed under the Clean Water Act [Carroll 2007], suggesting contaminant management on Pine Draw is important. For thirty years Pine Draw has experienced extensive algae mats which directly influence submerged aquatic vegetation including the threatened water howelia, waterfowl and amphibian populations [Davidson et al. 2006]. The algal mats compete with plant species for light resulting in a decrease in biomass and seed production. The submerged aquatic plants act as substrate for aquatic invertebrates, which are an important source of protein and fat for pre-fledgling waterfowl, and as food for waterfowl and other wetland species [Davidson et al. 2006]. Nutrient loading has been identified as one of the contaminants contributing to the decline in amphibian populations [Davidson et al. 2006].

The presence of the springs (GW1, GW2 & Spring) feeding Pine Draw have an influence on the concentrations and loading of certain nutrients originating from their location and continuing downstream [Davidson et al. 2006]. They found total phosphorus, soluble reactive phosphorus, nitrite-nitrate and ammonia concentrations all increased between PD3 and PD4 (Figure 3) [Davidson et al. 2006].

The suspected recharge source for the groundwater flowing into Pine Draw is the Stubblefield Lake drainage which receives significant agricultural runoff from dryland wheat farming [Davidson et al. 2006]. Stubblefield is fed by an agricultural stream, Philleo drainage. Surface water entering TNWR from Philleo drainage has high nutrient concentrations [Davidson et al. 2006]. This is expected as the off-refuge portion of Philleo is heavily anthropogenically impacted [Davidson et al. 2006]. Surface water concentrations collected from Philleo drainage by refuge staff found had four times the nitrate concentrations (mean=5.95 mg/L) as the other sites sampled, including Pine Draw [Davidson et al. 2006]. Stubblefield Lake has no outflow, which suggests it is losing the high levels of nutrients to either groundwater or the atmosphere.

Objectives

1. Record seasonal nitrogen concentrations in groundwater and surface water in Pine Draw and Philleo drainages for the 2016-2017 water year.
2. Determine the source of nitrogen to Pine Draw by mapping changes in nitrogen concentrations and analyzing stable isotope ratios of nitrogen and oxygen in nitrate.

Hypotheses

1. Groundwater is a seasonal contributor of excess nitrogen to Pine Draw downstream sites.
2. Nitrate concentrations in the study area will follow the trend:

Philleo Drainage>Groundwater>Pine Draw Downstream> Pine Draw Upstream

3. The isotopic ratios of the nitrate in Pine Draw downstream and groundwater will be similar to those of Philleo drainage.
4. The isotopic ratios of nitrate in Pine Draw and Philleo drainage will be similar to nitrogen from fertilizers.

3. Methods

I sampled 3 surface water sites on Philleo drainage, 9 surface water sites on Pine Draw and 3 groundwater sites for a total of 15 sampling sites. The groundwater sites included 3 springs that feed into Pine Draw. The selected sampling sites are above and below wetlands and known groundwater inputs. I attempted to sample every site monthly during the study period but were unable to get complete data due to seasonality of streams or adverse weather conditions. No sites were sampled in December 2016 due to bad road conditions near the study site. Data from late September – early October are incomplete due to sampling error. No sampling was done in April 2017 due to sampling dates falling on the 29th of March and the 1st of May.

In the field I recorded temperature, dissolved oxygen, pH and conductivity using a YSI 556 Multimeter. After returning to the lab I filtered water samples into acid washed

HDPE bottles for analysis of the dissolved ammonium, nitrate, and phosphate. [OIA 2009 a, b, c], Samples were filtered through 0.7 μm glass fiber filters. Field blanks were nano-pure water obtained from the EWU geochemistry lab, carried into the field in acid-washed 500 ml HDPE bottles, and treated identically to collected water samples. All samples were frozen until analysis. Three of the filtered samples from each site were individually analyzed on the Alpkem 3 Flow Analyzer for phosphate (PO_4^{3-}), nitrate (NO_3^-), and ammonium (NH_4^+) [OIA 2009a, b, c]. Nitrate was analyzed using nitrate plus nitrite nitrogen [OIA2009 b], ammonium using in-line total kjeldahl nitrogen and ammonia by gas diffusion [OIA2009 a] and phosphate using the orthophosphate method [OIA2009 c] For determination of stable isotopes of oxygen and nitrogen in nitrate, I collected two water samples at each of 10 sites once in each season (February, May, August, November). These samples were filtered with 0.1 μm membrane filters, frozen, and sent the University of California, Davis Stable Isotope Facility for analysis via bacterial denitrification assay (http://stableisotopefacility.ucdavis.edu/no3.html). Isotope ratios at the facility are obtained using a ThermoFinnigan GasBench + PreCon trace gas concentration system interfaced to a ThermoScientific Delta V Plus isotope-ratio mass spectrometer. Initially, 2 replicates were analyzed for each sample. Due to low variability, only 1 out of every 5 subsequent samples was replicated.

Statistical Analyses

All statistical analyses were done using RStudio statistical software. Nitrate+nitrite, ammonium and phosphate concentrations were log transformed for statistical analyses to obtain a more normal distribution for ANOVAs. ANOVAs were used to compare log transformed values of ammonium, nitrate and phosphate concentrations to water type and month. Sites were classified into the water type categories: philleo drainage sites (PH), groundwater springs (GW), pine draw sites downstream of groundwater influence (PDD) and pine draw sites upstream of groundwater influence (PDU). PH consisted of PH1, PH2 and PH3, PDU consisted of PD2 and PD3, GW consisted of GW1, GW2 and Spring and PDD consisted of PD4, Dock, PD5, PD7, Cheever Lake, PD8 Bend and PD8 Transect (Figure 2). A subset of sites was used to represent the variation in data by month based on relation to groundwater inputs which included PH1, PD3, PD5, GW1, GW2 and PD8TR. Total loads were calculated by multiplying the discharge data by ammonium, phosphate and nitrate concentrations from the same site and date.

Principal Components Analysis (PCA) plots were done using an incomplete dataset. Variables used were ammonium, phosphate and nitrate concentrations as well as temperature, specific conductance, conductivity, pH and dissolved oxygen concentrations. Sites need to have complete data to be added to the PCA. This required us to drop all sites with incomplete data which caused only 115 of the 147 original data points to be usable.

4. Results

Nitrate Isotopes

Isotope values varied based on water source and seasonally. Philleo drainage, the expected source of nitrogen, had isotope ratios resembling ammonium fertilizer in fall through spring, but saw more influence of soil nitrate in summer (Figure 4). Groundwater sites included two adjacent springs, GW1 and GW2 and the more spatially isolated

Spring which is further downstream. GW1 and GW2 showed ammonium fertilizer as a source in winter which changed the source to soil nitrate in the spring months, while the Spring site showed ammonium fertilizer for the duration of the sampling period. Pine Draw sites downstream of groundwater inputs and Philleo Drainage showed isotope ratios resembling ammonium fertilizer in the fall and winter, but shifting more towards soil nitrate in the spring and summer. Pine draw sites upstream of groundwater influence show soil nitrate as a contributing source, though summer data was unavailable due to a lack of flowing surface water in the summer months. Pine Draw upstream sites showed signs of ammonium fertilizer influence in the winter months while runoff was low, suggesting a minor influence of groundwater.

Nitrate + Nitrite

Monthly Data

Nitrate+nitrite concentrations were affected by season (P=.0004). Pine Draw sites closer to groundwater influence varied more seasonally and had similar peaks to groundwater sites which fell in January-February and November-October. There was a consistent drop in nitrate levels between March-May in GW1, GW2, PD5, PD8 Transect and PD3, but not in PH1 (Figure 5).

Variation by Site & Water Source

Nitrate concentrations varied by water type and season (Table 1). Philleo drainage had high levels of nitrate that didn't vary seasonally (Figure 6). Philleo drainage nitrate+nitrite levels were significantly different than all other concentrations (Tukey post-hoc test: PH-PDD: P=0.0018, PH-PDU: P=<.0001) except groundwater (P=0.436).

Groundwater mean nitrate concentrations were significantly different than Pine draw downstream (P=0.044) and Pine draw upstream (P=<.001). Pine draw downstream mean nitrate+nitrite concentrations were significantly different than Pine draw upstream (P=.0016).

Nitrate+nitrite Total Loads

Nitrate+nitrite total loads were highest in the snowmelt months of February-May (Figure 7). Philleo Drainage had the highest concentrations of nitrate+nitrite but due to the low discharge had rather small total loads. Pine Draw had the highest loads in April-May.

Ammonium

Monthly Data

Ammonium concentrations were significantly different among water types (Table 2). Groundwater mean ammonium concentrations tended to be lower than either Philleo Drainage or Pine Draw (Figure 8). PDD and PDU were not significantly different (P=0.57), but PDD and GW showed significant difference (P=0.00016). Ammonium showed highest values in spring and fall.

Variation by Site and Water Source

Ammonium concentrations were highest in surface water sites, with lowest concentrations being in groundwater sites. Surface water sites had slight peaks in January-March that GW sites did not share (Figure 9).

Ammonium Total Loads

Ammonium total loads were highest in the high flow periods (Figure 10). Ammonium concentrations seemed to correlate with periods of high rates of decomposition in the spring and fall months.

Phosphorus

Seasonal Variation

Philleo Drainage monthly phosphate concentrations were more variable than Pine Draw and groundwater concentrations showing spikes in the fall and spring, which correlate with fertilizer applications (Figure 11). Phosphate concentrations on Pine Draw varied with distance downstream but were higher in the spring during the high flow events. Phosphate concentrations had a larger range in PDD than GW sites.

Variation by Site & Water Source

Phosphorus mean values were highest in Philleo Drainage which were not significantly different than groundwater concentrations (Table 3). PDU and PDD showed significant difference from groundwater (P=<0.0001 and P=<0.0001 respectively) but not between each other (P=.36). Phosphate concentrations showed a decreasing trend in phosphate concentrations from PH →GW → PDD → PDU (Figure 12)

Total Loads

Total phosphate loads are highest in spring (Figure 13) which correlates with the highest discharge months. Phosphate values were not high in the fall, which was expected due to increased discharge with increased precipitation. This could be due to the variability of phosphorus inputs from agricultural fertilizer. If inputs of fertilizer are in the fall, but not spring we would see a lag of phosphorus inputs to Pine Draw based on the residence time of the groundwater system.

Other Water Quality Parameters

Dissolved oxygen low values in surface water occur in March-May and October-November with the highest values occurring in January-February (Figure 14). Low temperatures in surface waters occur in September-May while highest temperatures occur in June-July (Figure 15). The lack of dissolved oxygen in the March-May and September-November while water temperatures are still cooler suggests consumption of oxygen because we would expect to see higher dissolved oxygen concentrations throughout the entire low temperature period.

Temperature varies negatively with pH (Figure 16), and our dissolved nutrient concentrations. Temperature doesn't have as much of a negative relationship with dissolved oxygen as expected, suggesting a different variable influencing dissolved oxygen concentrations. Pine Draw downstream and groundwater vary similarly in relation to water quality parameters. Philleo drainage tends to vary more with with temperature and pH while staying more consistent on nitrate and phosphate concentrations.

5. Discussion

The Source of Nitrogen

The three primary sources of nitrate identified by stable isotope analysis were soil nitrate, manure & septic systems and nitrate originating from ammonium fertilizer. Philleo drainage, groundwater springs and Pine Draw sites downstream of groundwater inputs had nitrogen and oxygen isotope ratios consistent with ammonium fertilizer during at least some seasons. Soil nitrate and denitrification effects were strongest in summer.

The source of the excess nitrogen to Pine Draw was through groundwater springs. PDD nutrient concentrations were highest just downstream of groundwater inputs at sites PD4, PD5 and PD8 Bend and decreased with distance downstream. Upstream of groundwater influence, the source of nitrogen was consistently soil nitrate, while downstream the source was soil nitrate with seasonal contributions of ammonium fertilizer. I observed a concentration cascade of nitrogen originating from ammonium fertilizer with the highest values in PH, closest to the source, relatively moderate values in GW and lower values in PDD. PDU showed no influence from this concentration cascade, suggesting that nitrogen in downstream Pine Draw did not originate from upstream within the watershed.

Groundwater springs have been contributing excess nitrate+nitrite and phosphate to Pine Draw for at least 20 years. Not much ammonium flows into Pine Draw through these groundwater systems, but the eutrophication caused by the excess nutrients could contribute to ammonium production. Eutrophication consumes oxygen but also leads to increased biomass and more decomposition contributing more ammonium to the system.

More dissolved oxygen is consumed because of nitrification using oxygen to process the excess ammonium created [Yevenes et al. 2016]. These processes likely both contribute to hypoxic conditions in Pine Draw [Davidson & Rule 2006].

Isotopes

The low $\delta^{15}N$ values in Philleo in fall through spring seasons correlate with typical fertilization pattern of winter wheat, with fertilizer being applied in the fall and the peak of ammonium fertilizer influence being in the winter months [Westerman et al. 1994]. Philleo Drainage $\delta^{15}N$ values being low in spring suggests spring runoff continues to remove the fall application of fertilizer, which decreases in the summer months when nitrogen remains in the soils due to a lack of runoff.

Pine Draw upstream correlation with groundwater is influenced by the ratio of the volume groundwater to surface water (GW:SW) in the system seasonally. In the spring and Fall PPD and GW $\delta^{15}N$ values are less similar, but more similar in the summer and winter when GW:SW ratio increases. The source of nitrate to PDU was soil nitrate throughout the sampling period, indicating the excess nitrogen isn't being transported from upstream, but is only present in the sites downstream of groundwater inputs.

Groundwater Inputs of Nitrogen

Groundwater systems are the source of nitrogen to Pine Draw. Seasonally we see the source of nitrogen upstream of the GW influence to stay soil nitrate, while downstream of groundwater inputs we see a substantial shift towards ammonium fertilizer. The excess nitrogen must be coming from a nearby source through groundwater systems due to the lack of ammonium fertilizer upstream of these groundwater inputs and the lack of use of ammonium fertilizer on TNWR.

Patterns of nutrient concentrations and water quality parameters differed between the more spatially separated groundwater sources (GW1 and GW2 vs. Spring), suggesting the possibly of different sources, flow paths, or changes in residence time. The lack of ammonium fertilizer impact in the fall could be due to a relatively short residence time in these aquifers leading to all the nitrate originating from ammonium fertilizer to be transported downstream quickly. The residence time could also be exceeding one year, we could be seeing a lag where we were sampling nitrogen which originated in previous years. A change in flow pattern can also alter the fate of nitrogen and oxygen in GW [Tecklenburg et al. 2013].

Limitations of Study

Groundwater springs were clearly flowing from an aquifer at our GW sites. While this was useful, I was unable to sample groundwater at depth to quantify the amount of nitrogen in the aquifer. Nitrogen and oxygen can be altered substantially based on depth, which limited sampling to potentially altered concentrations or isotopic ratios.

I had a lack of information on the practices of farmers on the upstream portions of Philleo Drainage. While I was able to research the common winter wheat practice, we were unable to confirm that only Fall applications of fertilizer were being used. If an additional application is occurring in spring, our summer conclusions will need to be revisited.

Some of my sites were only available seasonally. Pine Draw and GW sites were difficult to reach in the winter months due to surface freezing and significant snowfall. Upstream Pine Draw and Philleo Drainage sites were only available in high flow periods and dried during the hottest summer months. The lack of GW sampling method also limited this data due to the unavailability of sampling hyporheic flow.

Importance & Implications

Eutrophication is a worldwide problem, causing large hypoxic zones in estuaries and coastal waters [Conley et al. 2009]. The importance of eutrophication ranges from small streams [Davidson & Rule 2006] to coastal waters and estuaries [Conley et al. 2009]. The fate of these nutrients is usually being transported downstream to our oceans [Reddy et al. 1999]. Upon reaching marine waters phosphorus will increase in solubility making it more biologically available, causing nitrogen to be a common limiting resource.

Knowing the flow paths of nitrogen is important in managing hypoxia from small streams to our marine environments. While we manage individual watersheds for surface water concentrations, looking at hyporheic flow in nutrient transport can be the missing piece to the puzzle. Unimpacted streams, such as Pine Draw, can be influenced significantly influenced by groundwater flow pathways. The potential of a contaminant to impact adjacent systems is not limited to one stream, multiple groundwater flow pathways could be branching off one source.

Management in the future needs to not only look at surface waters downstream of contaminant inputs, but also adjacent watersheds that could be connected via underground flow pathways. We would also need to look at the volume and spatial distribution of aquifers to better assess their transport capabilities.

6. Tables

Table 1. ANOVA between dependent variable: nitrate, and independent variable water type divided into seasons.

	DF	Sum of Sq	Mean Sq	F Value	P
Water Type	3	148.48	49.49	28.787	8.71E-14
Season	3	33.8	11.27	3.22	0.000409
Water Type:Season	9	22.95	2.55	1.483	0.163182
Residuals	109	187.4	1.72		

Table 2. ANOVA between dependent variable: ammonium, and independent variable water type divided into seasons.

	DF	Sum of Sq	Mean Sq	F Value	P
Water Type	3	0.0678	0.022597	5.574	0.00141
Water Type:Season	9	0.0478	0.005311	1.31	0.24124
Season	3	0.562	0.0183734	4.621	0.00454
Residuals	100	0.4054	0.004054		

Table 3. ANOVA between dependent variable: SRP, and independent variable water type divided into seasons

	DF	Sum of Sq	Mean Sq	F Value	P
Water Type	3	0.14538	0.04846	16.821	6.16E-09
Season	3	0.02034	0.00678	2.353	0.0766
Water Type:Season	9	0.01009	0.00112	0.389	0.9379
Residuals	101	0.29098	0.00288		

7. Figures

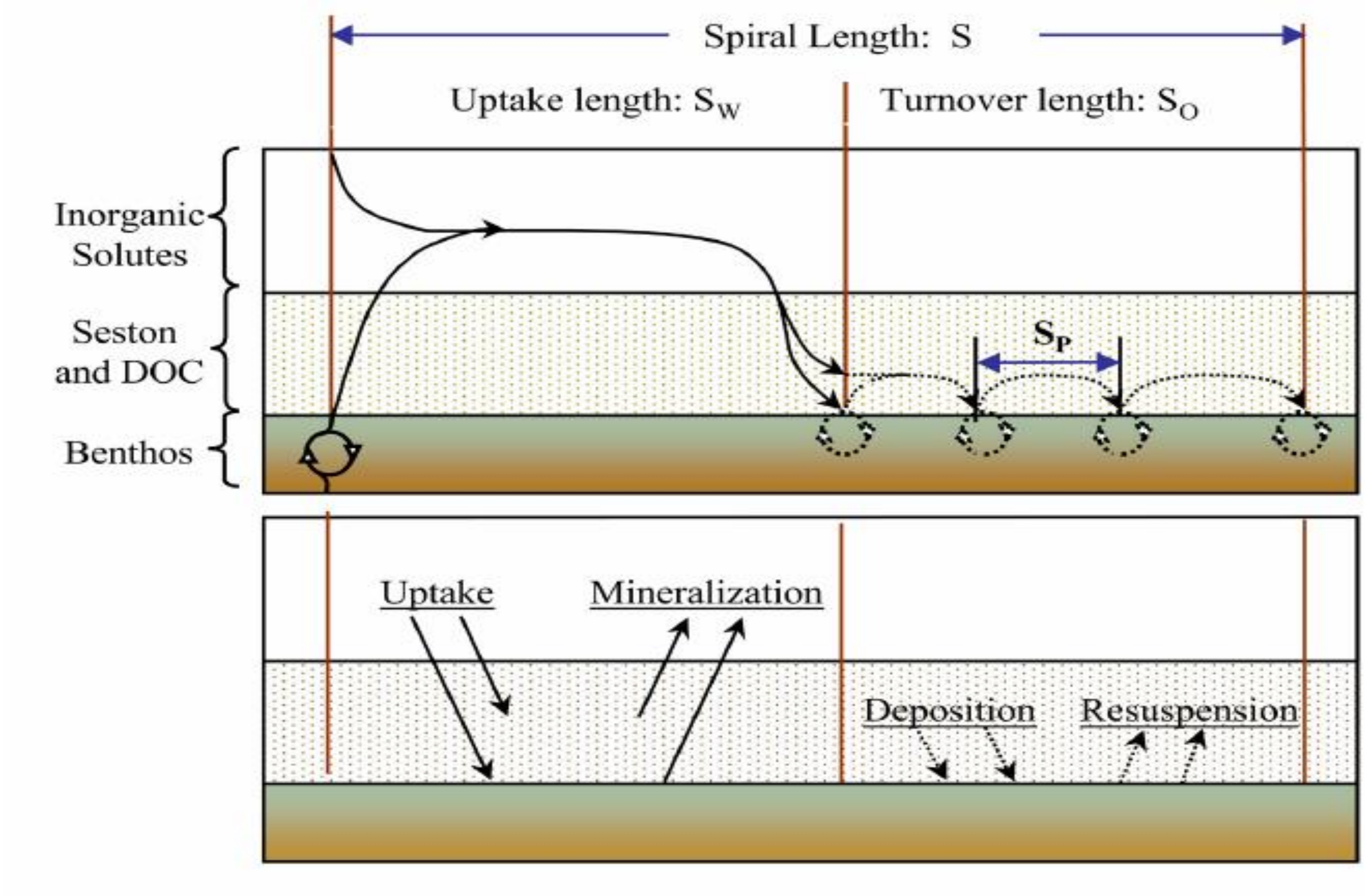

Figure 1. Nutrient spiraling in a stream. Solid arrows represent inorganic transport, dashed represent organic transport. Sw=Uptake Length, So=Turnover Length and Sp represent particle transport distance. (Thomas et al. 2007)

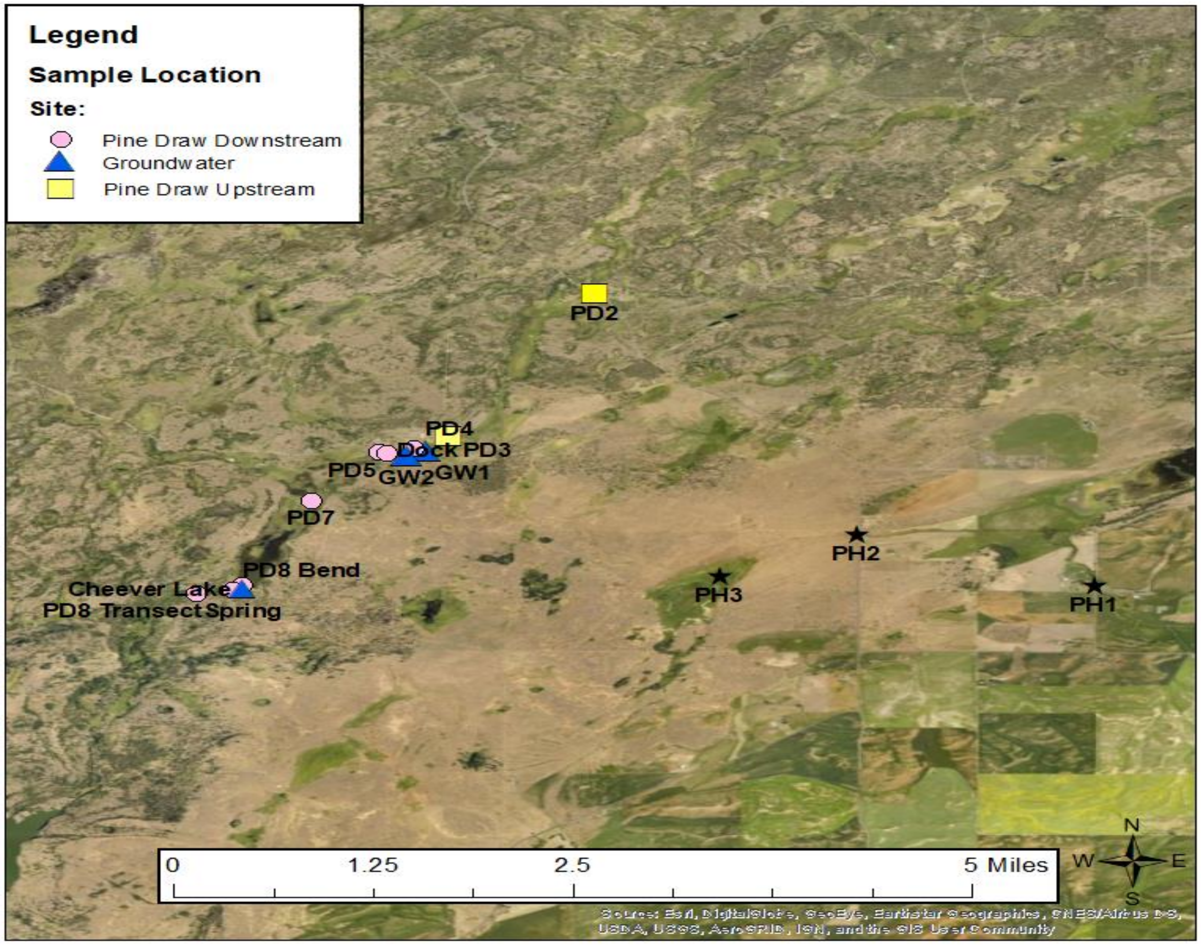

Figure 2. Sampling Sites on Pine Draw, Philleo Drainage and groundwater springs on Turnbull National Wildlife Refuge, WA. USA. Sites are divided into Pine Draw Downstream (Pink Circles), Pine Draw Upstream (Yellow Squares), Groundwater (Blue Triangles) and Philleo Drainage (Black Stars). Sources: Esri DigitalGlobe, GeoEye, Earthstar Geographics, CNES Air us DS, USDA, USGS, AeroGrid, IGN and the GIS user community

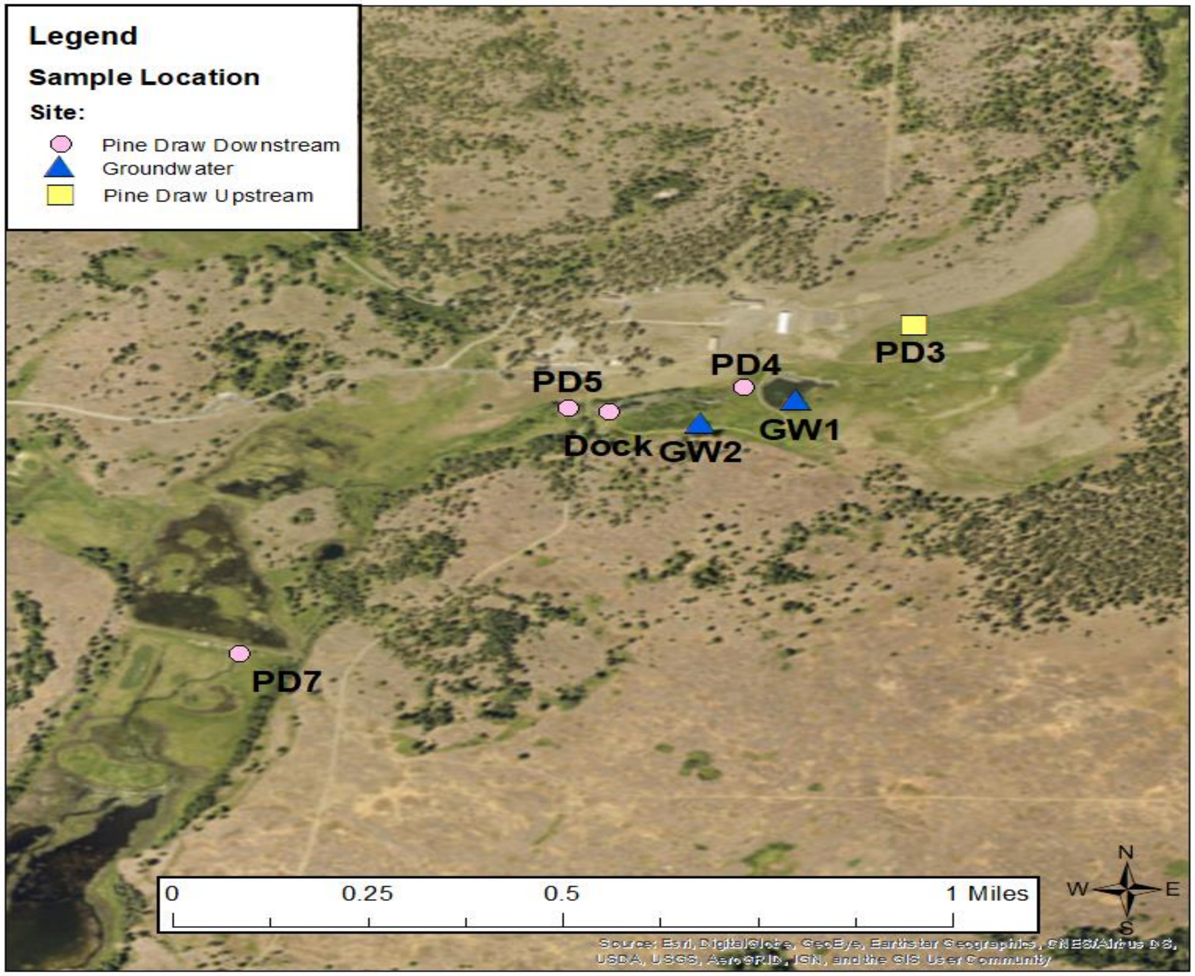

Figure 3. Sampling Sites on Pine Draw, Philleo Drainage and groundwater springs on Turnbull National Wildlife Refuge, WA. USA. Sites are divided into Pine Draw Downstream (Pink Circles), Pine Draw Upstream (Yellow Squares) and Groundwater (Blue Triangles). Sources: Esri DigitalGlobe, GeoEye, Earthstar Geographics, CNES Air us DS, USDA, USGS, AeroGrid, IGN and the GIS user community

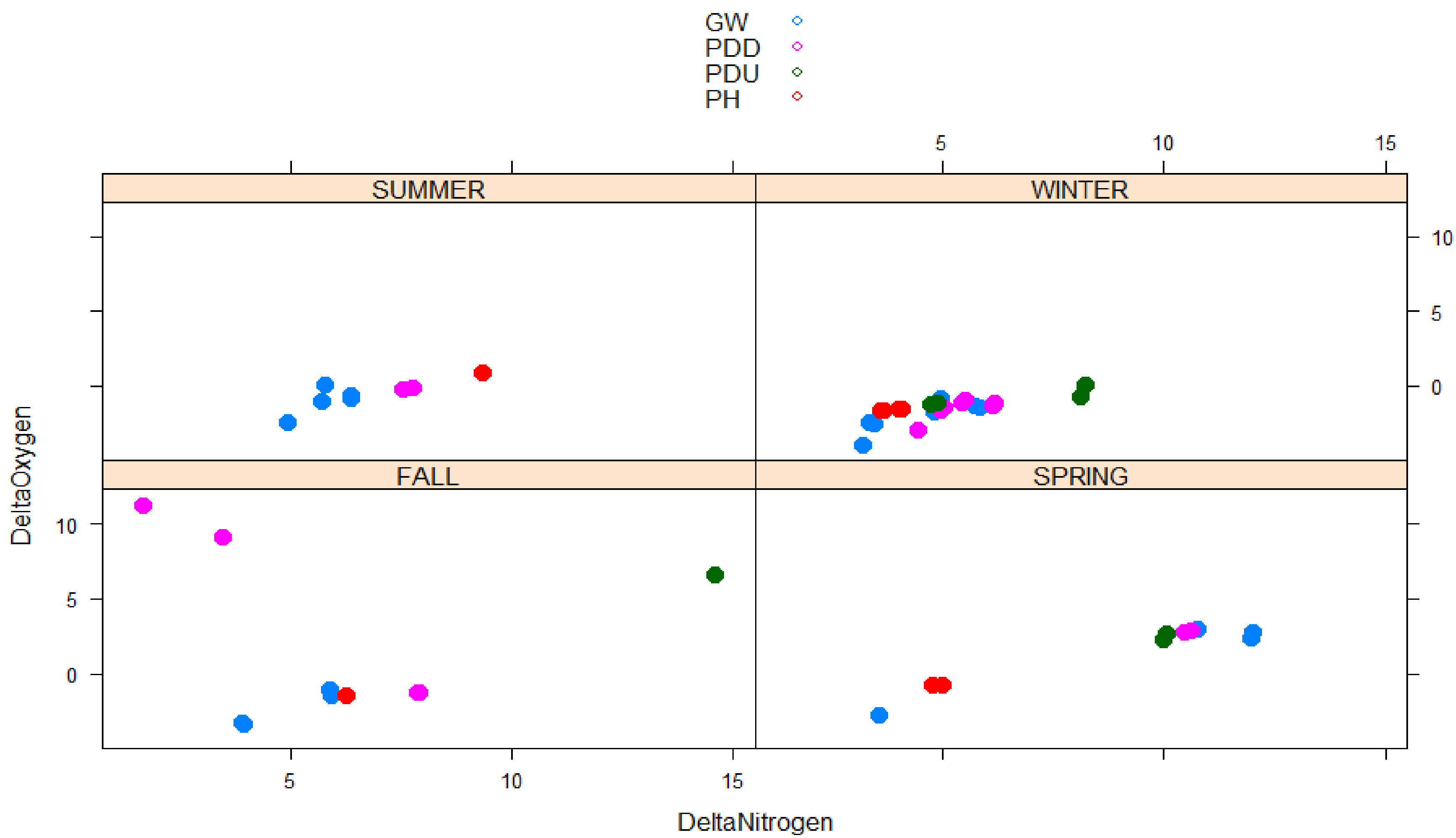

Figure 4. Stable isotope ratios for nitrogen and oxygen in the nitrate molecule from four seasons on Pine Draw, Philleo Drainage and groundwater springs on Turnbull National Wildlife Refuge, Washington, USA. DeltaNitrogen= $\delta^{15}N$. DeltaOxygen= $\delta^{18}O$.

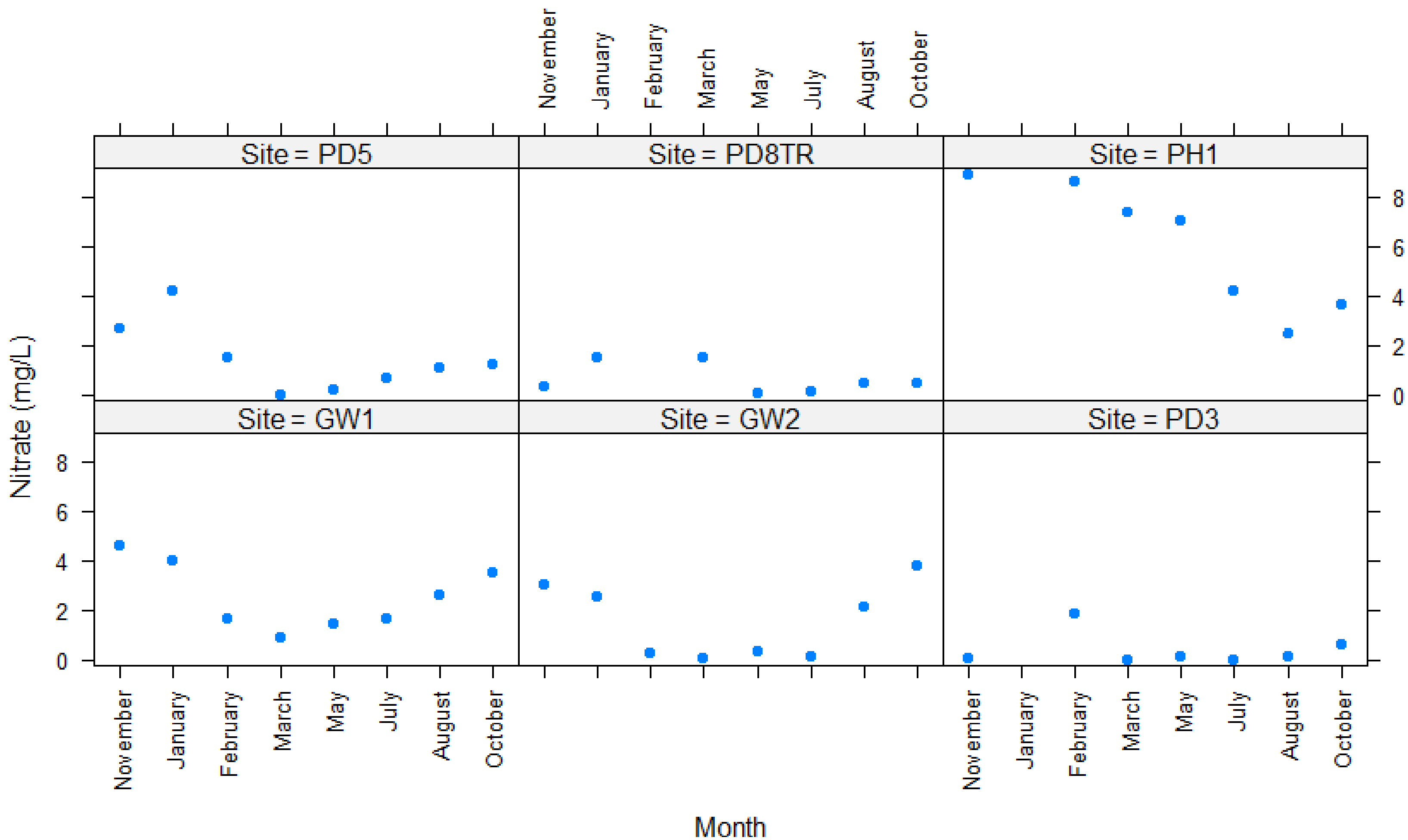

Figure 5. Nitrate concentrations monthly on key sites from Philleo Drainage (PH1), Pine Draw (PD5, PD3 & PD8TR) and groundwater springs (GW1 & GW2) located on the Turnbull National Wildlife Refuge, Washington, USA.

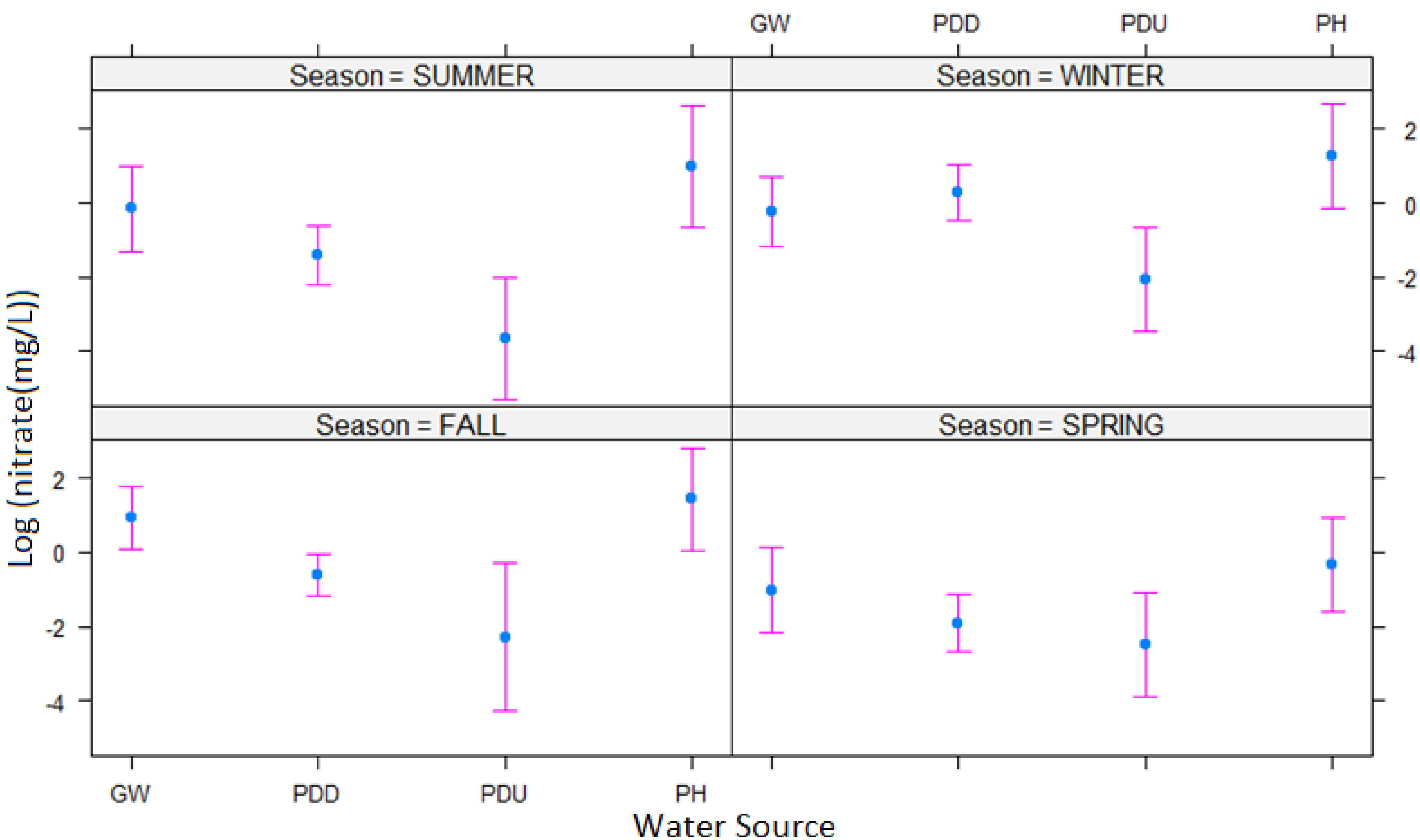

Figure 6. Seasonal nitrate concentrations (log transformed) in the water types Pine Draw Downstream (PDD), Pine Draw Upstream (PDU), Groundwater (GW) and Philleo Drainage (PH).

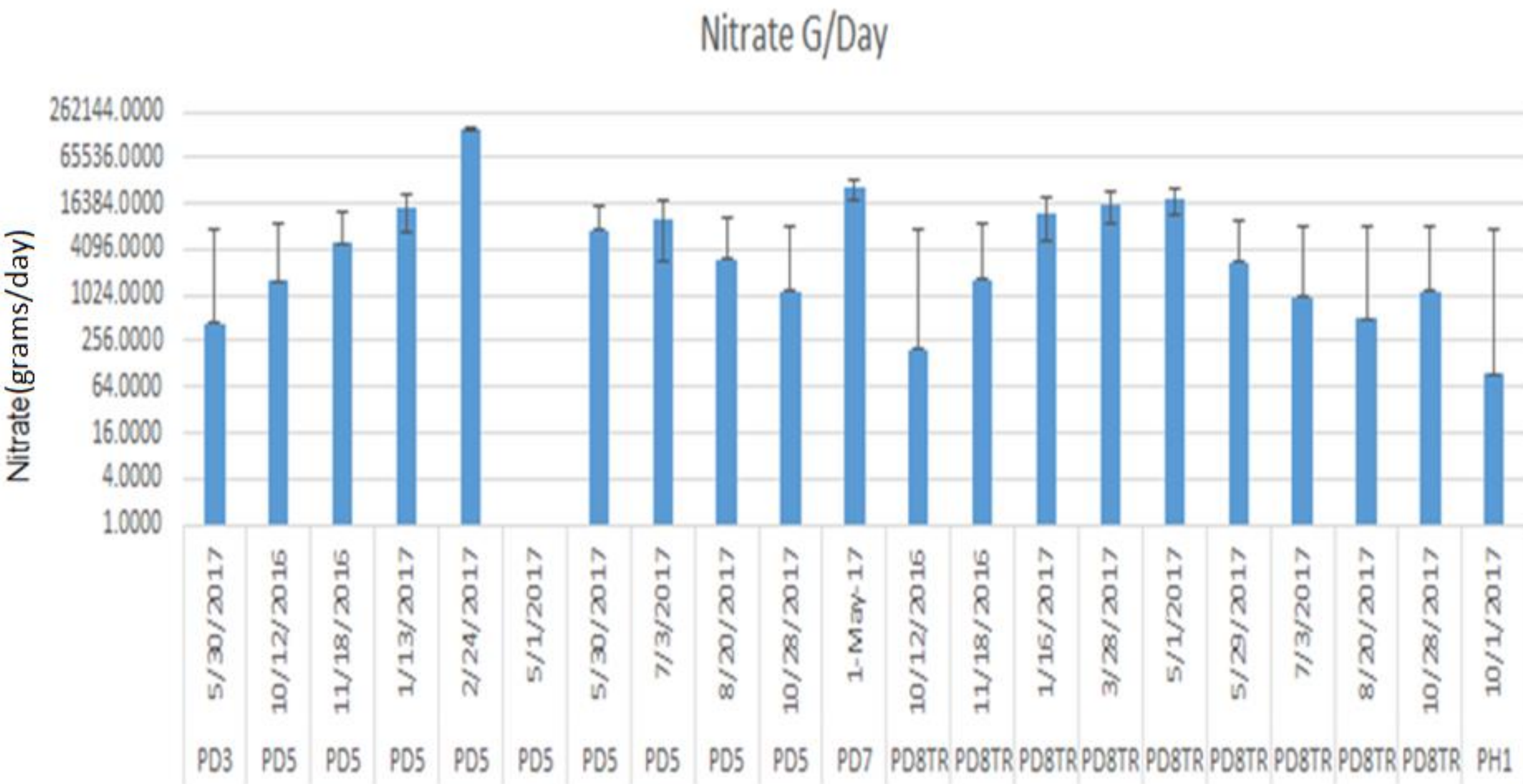

Figure 7. Nitrogen total loads calculated from discharge and nitrogen concentrations in stream. Lack of negative error bars suggests error bars extend into negative values.

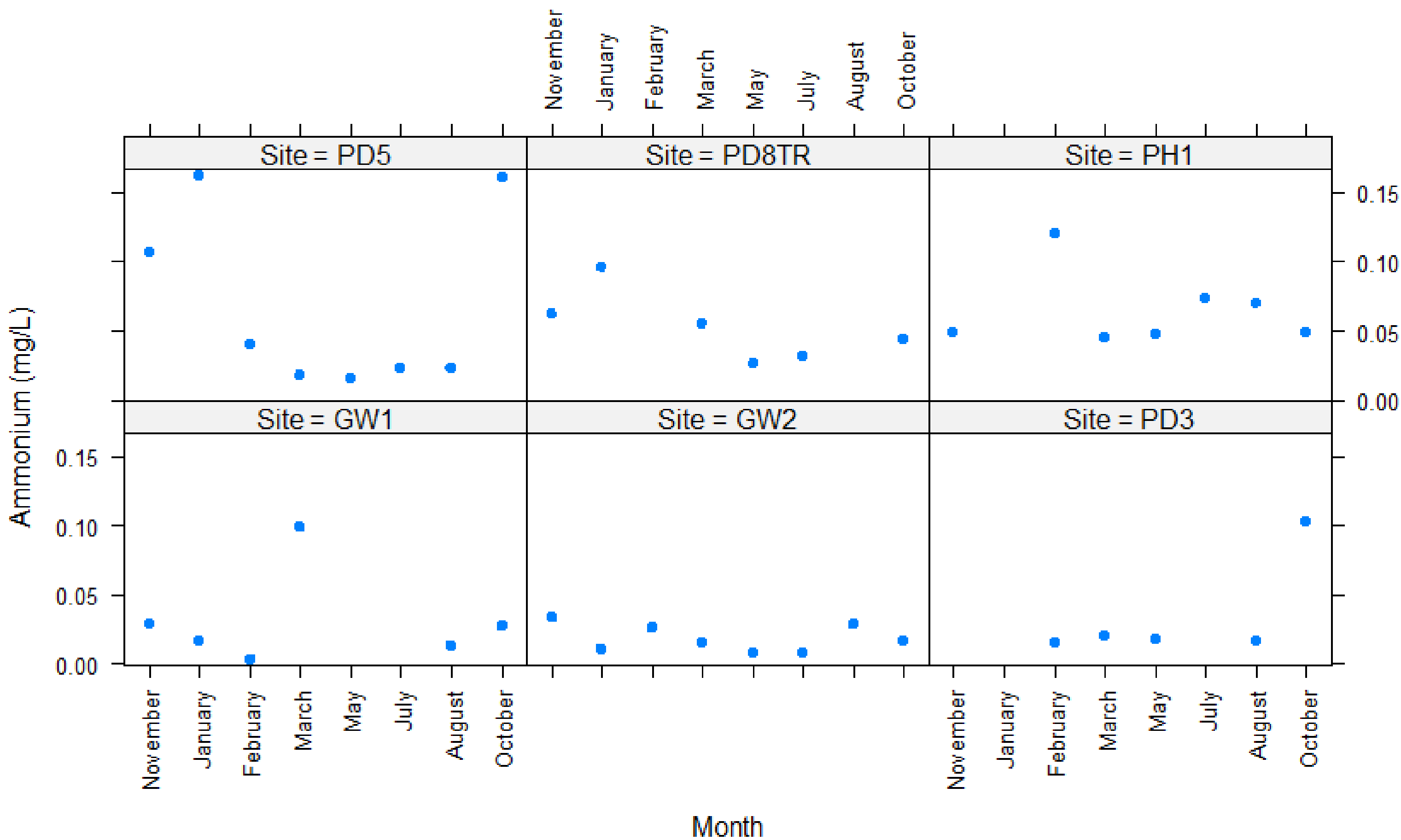

Figure 8. Ammonium concentrations monthly on key sites from Philleo Drainage (PH1), Pine Draw (PD5, PD3 & PD8TR) and groundwater springs (GW1 & GW2) located on Turnbull National Wildlife Refuge, WA, USA.

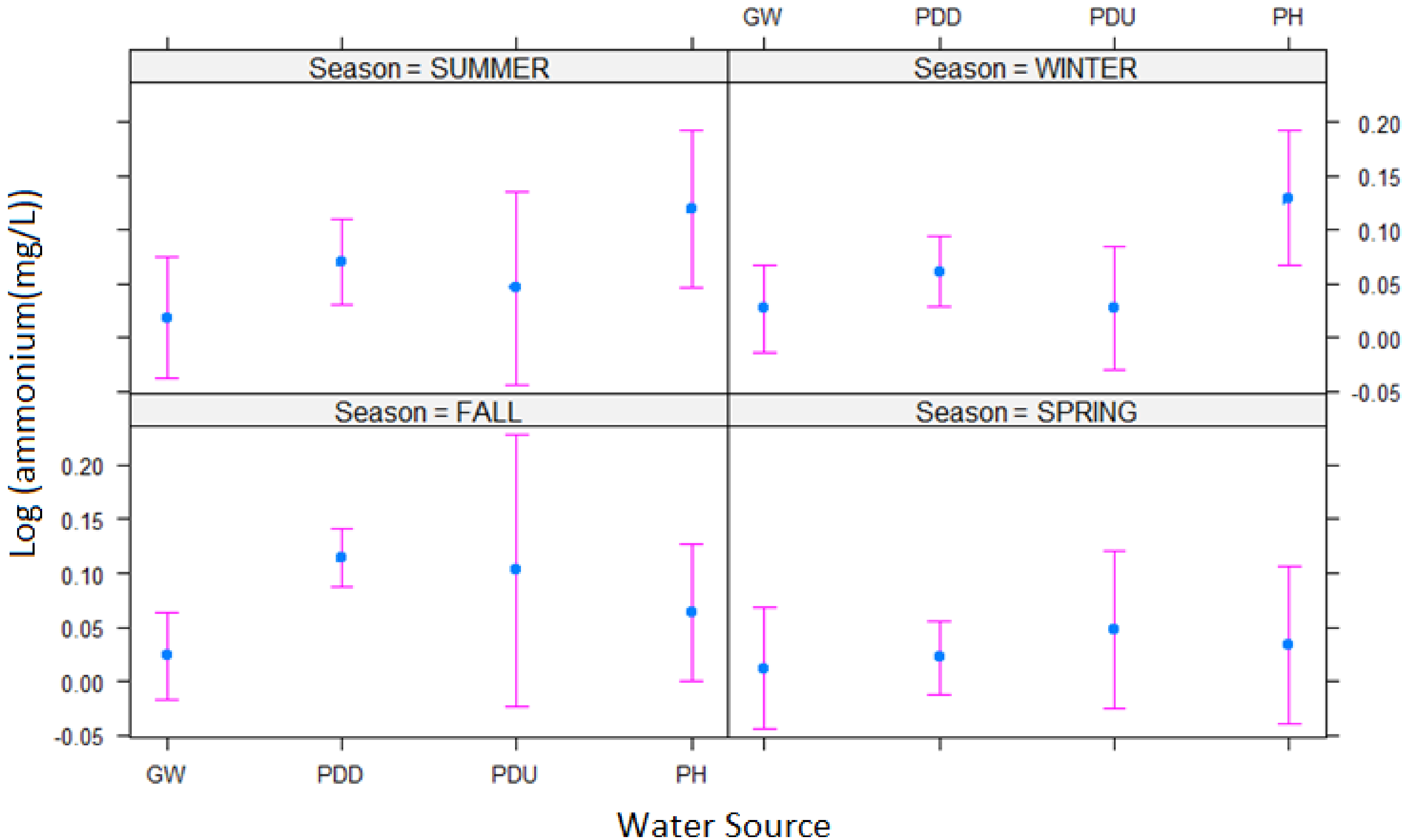

Figure 9. Seasonal ammonium concentrations (log transformed) in the water types Pine Draw Downstream (PDD), Pine Draw Upstream (PDU), Groundwater (GW) and Philleo Drainage (PH).

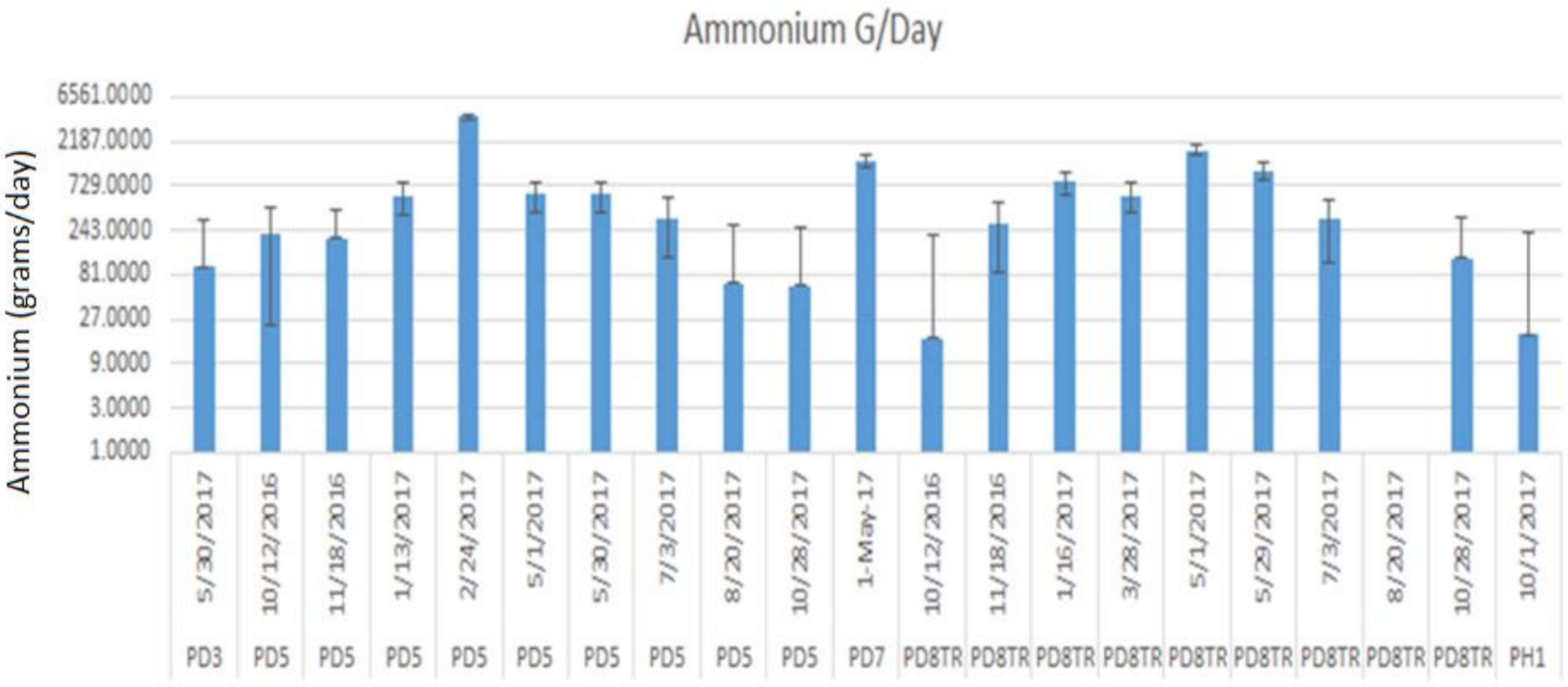

Figure 10. Ammonium total loads calculated from discharge an ammonium concentrations in stream. Lack of negative error bars indicate error bars extending into negative values.

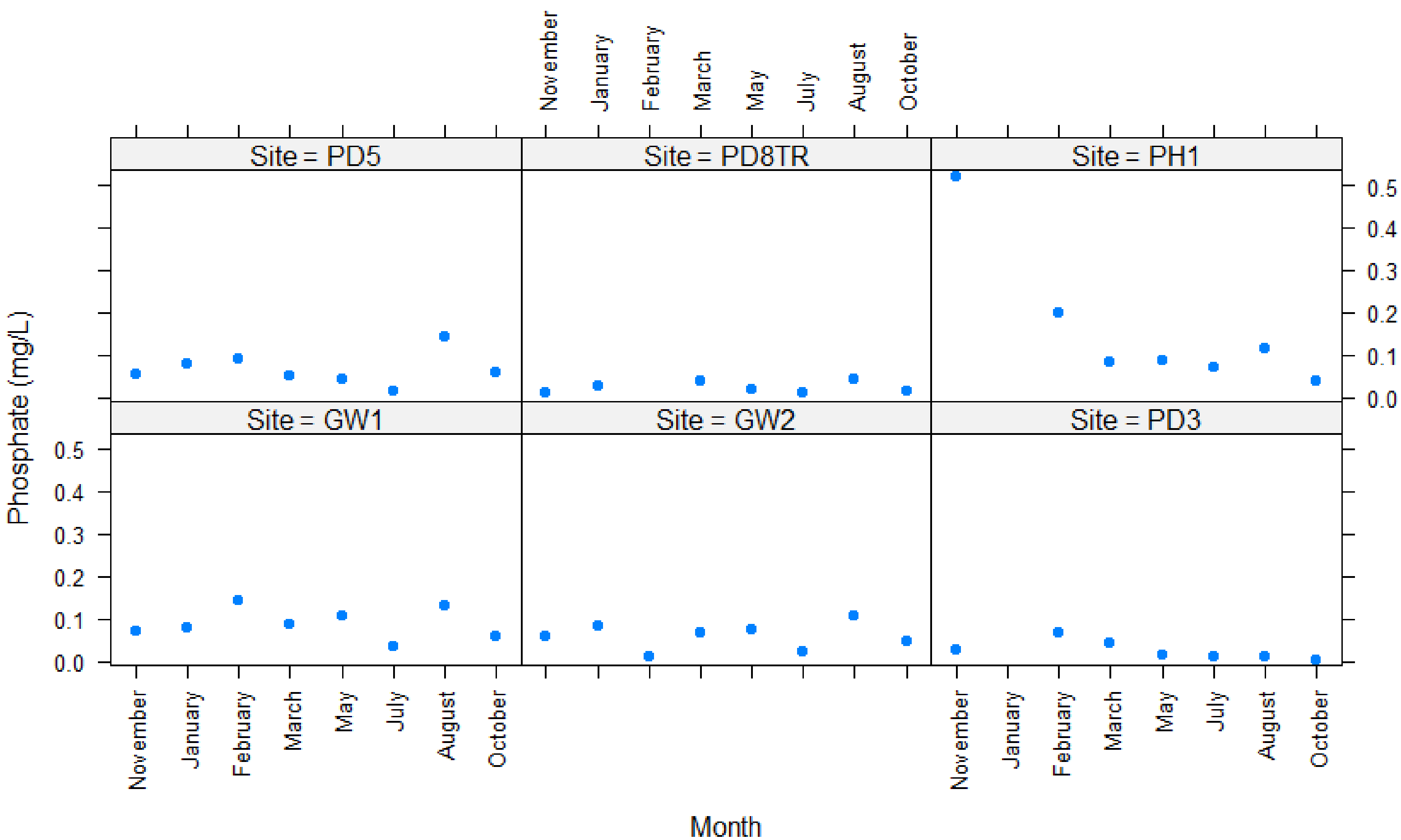

Figure 11. Soluble Reactive Phosphorus (SRP) concentrations monthly on key sites from Philleo Drainage (PH1), Pine Draw (PD5, PD3 & PD8TR) and groundwater springs (GW1 & GW2) located on Turnbull National Wildlife Refuge, WA, USA.

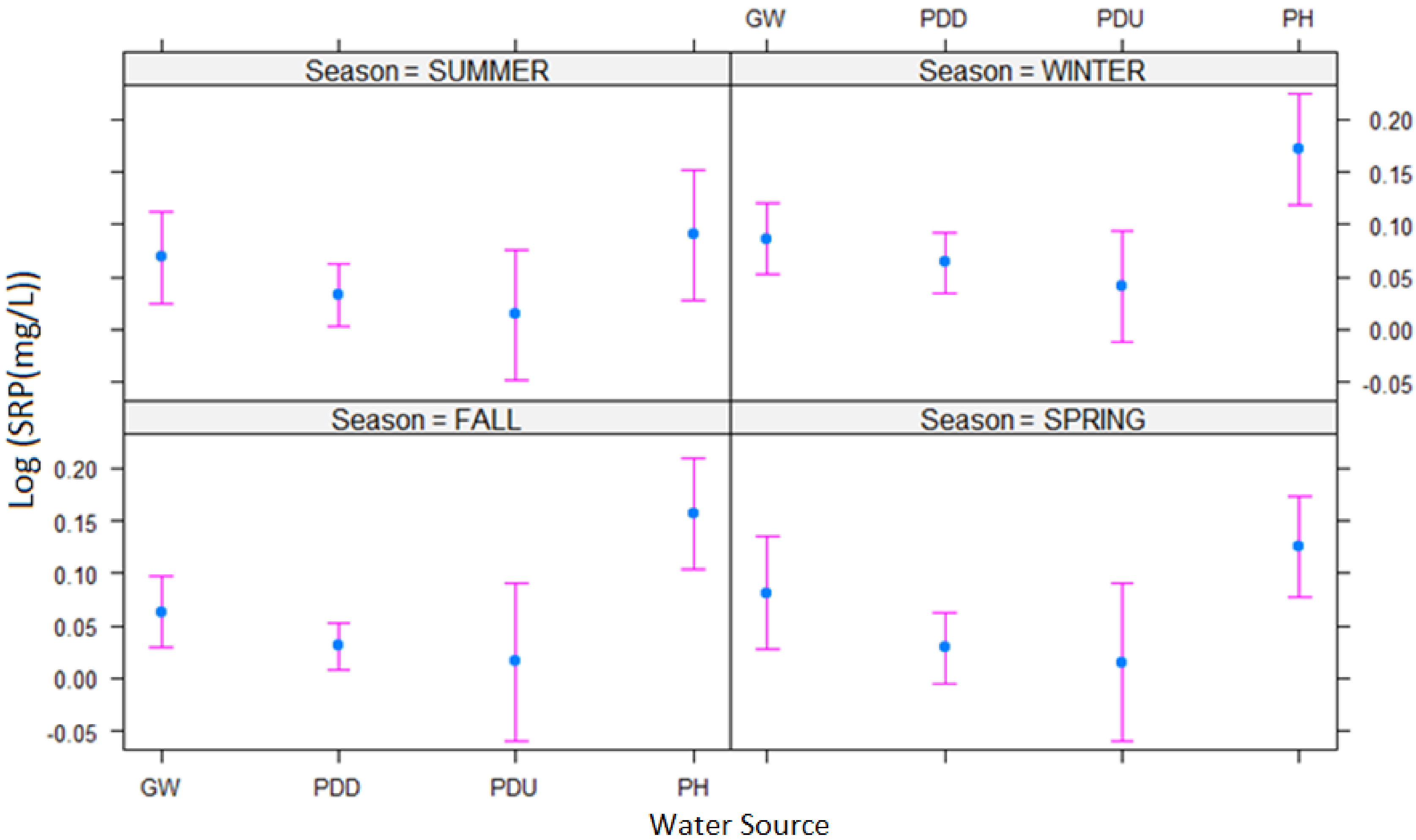

Figure 12. Seasonal soluble reactive phosphorus (SRP) concentrations (log transformed) in the water types Pine Draw Downstream (PDD), Pine Draw Upstream (PDU), Groundwater (GW) and Philleo Drainage (PH).

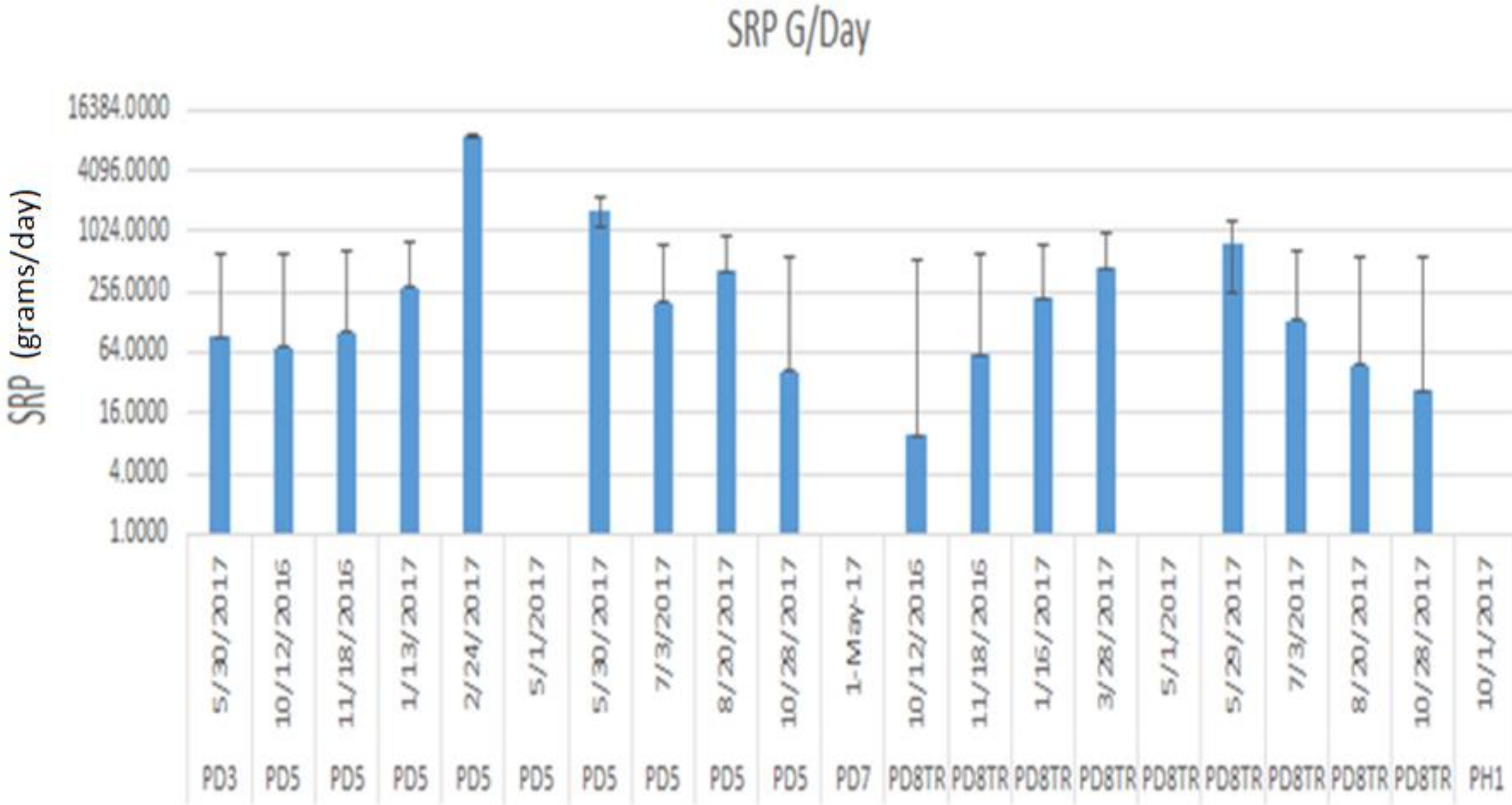

Figure 13. Soluble reactive phosphorus total loads calculated from discharge and phosphate concentrations in stream. Lack of negative error values indicate error bars extending into negative values.

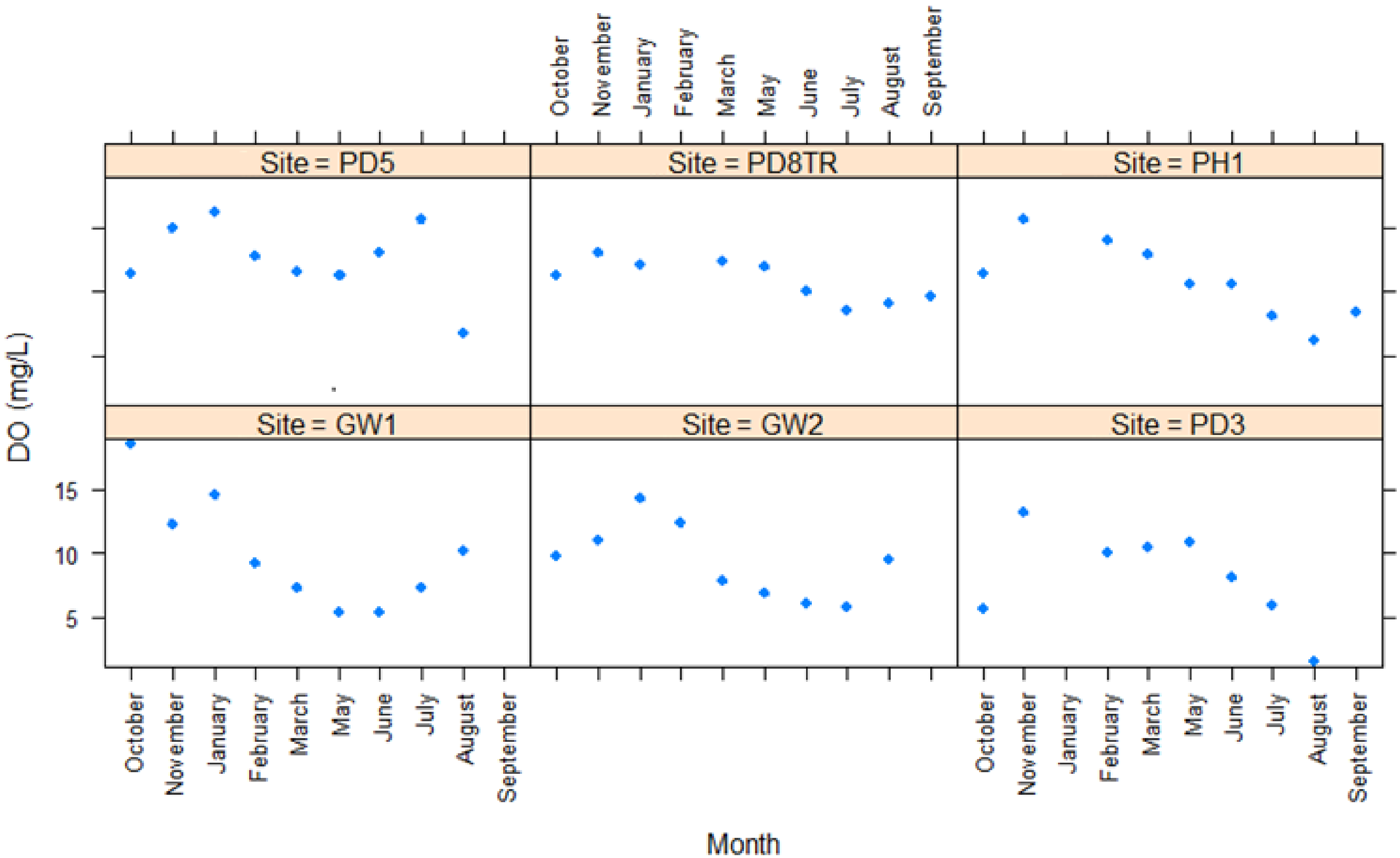

Figure 14. Dissolved Oxygen (DO) in concentrations (mg/L) monthly on key sites from Philleo Drainage (PH1), Pine Draw (PD5, PD3 & PD8TR) and groundwater springs (GW1 & GW2) located on Turnbull National Wildlife Refuge, WA, USA.

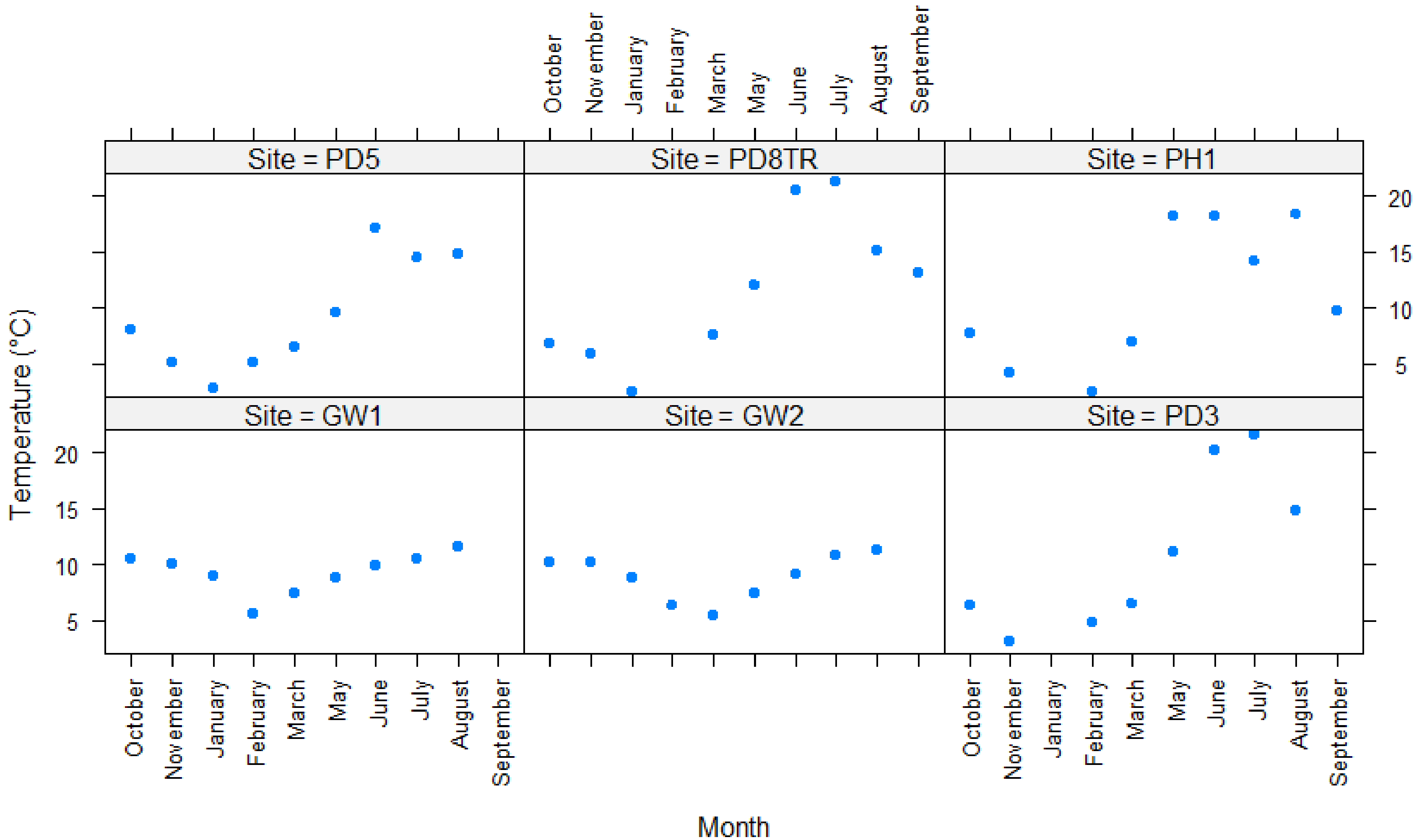

Figure 15. Temperature (°C) in different water types in four sampling periods on Pine Draw, Philleo Drainage and groundwater springs.

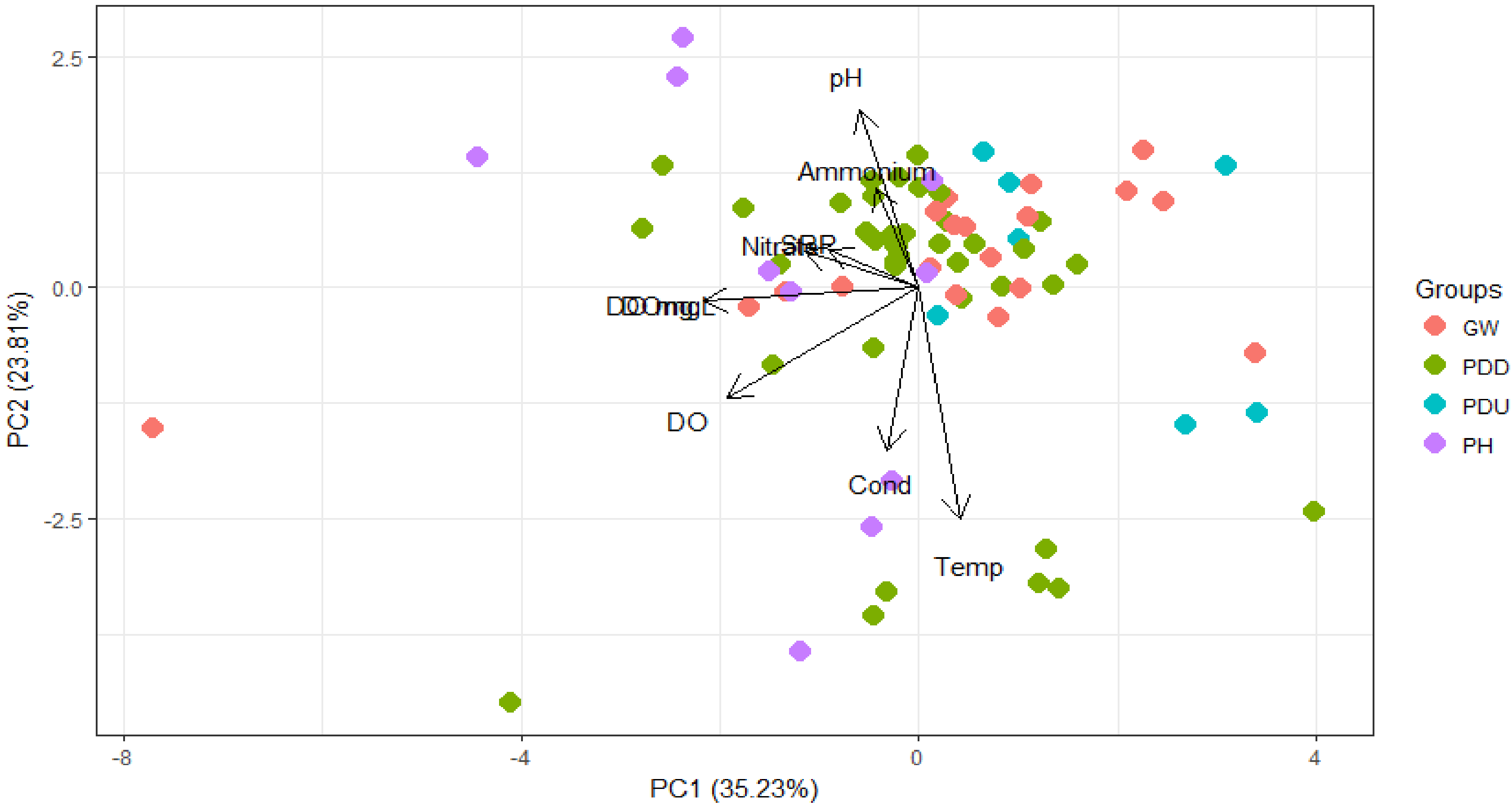

Figure 16. Biplot of Principal Compnent Analysis including Temp, Conductivity, Specific Conductance, Dissolved Oxygen Concentrations, Nutrient Concentrations and pH. Data from Pine Draw, Philleo Drainage and groundwater springs on Turnbull National Wildlife Refuge, WA, USA.

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VITA

Author: Henry L. Price

Place of Birth: Bellingham, Washington

Undergraduate Schools Attended: Wenatchee Valley College

Eastern Washington University

Degrees Awarded: Bachelor of Science, 2016, Eastern Washington University

Associate of Arts and Science, 2012, Wenatchee Valley College

Honors and Awards: Graduate Assistantship, Biology Department, 2017-2018, Eastern Washington University

Travel Grant, for presentation at Ecological Society of America conference, Portland, Oregon, 2017

Professional Experience: Biology Intern, L.B. Stone Properties, Spokane, Washington, 2017

Invertebrate Technician, Eastern Washington University, Cheney, Washington, 2017

Field Technician, Washington Department of Fish & Wildlife, Creston, Washington, 2016

Field Technician, The Great Basin Institute, Reno, Nevada, 2015

Field Assistant, Adams Conservation District, Ritzville, Washington, 2014