

Pond nutrient storage across permanence and temporal gradients

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Full Time Independent Research/REU

Summer 2017

Abstract:

Our goal was to understand how range shifts in detritivorous larval caddisflies could affect nutrient storage along a pond permanence gradient in the Mexican Cut Preserve (Gunnison County, Colorado). As part of a larger project quantifying other ecological impacts and interactions arising from the caddisfly range shift, this study focused on measuring nutrient distributions along the permanence gradient. First, through an observational study, we measured levels of nitrogen and phosphorus in the water column and took samples of benthic organic matter (BOM) and sedge for analysis at a later date. By sampling these nutrient compartments with respect to time and pond permanence, we will be better able to understand the magnitude and timing of nutrient storage in oligotrophic subalpine ponds. Second, we designed an experiment to quantify the effects of caddisflies on pond nutrient distribution. We created an in-situ cage experiment to compare the effects of two Limnephilid caddisfly species: *L. externus* and *L. picturatus*. This allowed us to understand the nutrient cycling that caddisflies are responsible for within these ponds. This experiment also serves to contextualize our nutrient observations with respect to larval caddisfly activity and the ongoing research on caddisfly distributional shifts and nutrient excretion.

Introduction:

Climate change can be observed at RMBL through species range shifts as species move higher in elevation and latitude. In Gothic, the habitable zones for species have been rising in elevation at a rapid rate of 6-11m per decade. The warming climate is also shifting the permanence of glacial remnant kettle ponds in the Gothic Townsite, Gunnison National Forest, and the Mexican Cut Preserve. The hydroperiod of these ponds determines predator presence, which along with routine drying shapes the invertebrate community. As climate warming drives ponds to become more temporary, species which depend on pond hydroperiod are forced to follow these shifts. For example, larval caddisflies adapted to specific features of these hydroperiods, usually trading between the predator tolerance that comes with living in permanent ponds and the desiccation tolerance present in temporary pond species (Wissinger et al 2003). This makes them sensitive to these pond permanence shifts. Caddisfly larvae have been observed in new species combinations and densities in the Mexican Cut ponds. (Wissinger, Taylor, Grieg, 2015). Previous work has quantified the effects of these shifts on detritus processing and suggests linkages with other critical ecosystem functions (Klemmer et al. 2012). We predicted that these shifts would affect the nutrient cycling across the pond compartments from fixated nutrients in detritus to community available forms within other compartments.

The kettle ponds have two nutrient processing pathways. The “green” pathway cycles nutrients through living plants and algae, then primary and secondary consumers, while the “brown” pathway returns nutrients locked in dead biomass to the environment through detritivore consumption, then excretion and egression (Wissinger, Taylor, Grieg, 2015). Because caddisflies are the dominant detritivores in the Mexican Cut pond ecosystems and because they are undergoing species range shifts across elevation and permanence gradients, they made

excellent candidates for study. Historical data allows us to compare changes in ponds over time (Wissinger unpublished data), which allows us to predict the longer-term effects of climate change on species range shifts in the context of population, community, and ecosystem processes.

We predict the shifting distribution patterns across the permanence gradient are disrupting the niches that have been developed by the guild of detritivorous caddisflies (Wissinger et al. 2003). The tradeoffs between predator vulnerability and desiccation intolerance should cause complexities as the species shift along the gradient. This study aimed to quantify the shifts and their effect on ecosystem function with the added intent of contextualizing the data with respect to climate change predictions and projecting the consequences onto the known pond response to climate change.

This study is part of a four-step study, beginning with small-scale excretion measurements on larval caddisflies (Balik et al. *in prep*), then building models with this data. The models will then be tested in mesocosm-scale cattle tank experiments which will expand the model to the fourth step – in-situ experiments in kettle ponds. This REU Project investigated two questions: 1) How are nitrogen and phosphorus distributed within pond compartments and along the permanence gradient? 2) Does caddisfly excretion generate hotspots of nutrients in natural ponds? We addressed these questions using observational and experimental approaches, respectively.

Setup and Methods:

Observational Study: Nutrient distribution across ponds and compartments: Our observational study was conducted across two gradients, a temporal gradient as the season progressed, and a permanence gradient that spanned temporary to permanent ponds. Although we plan to continue sampling into autumn, the data presented here spans four weeks. Beginning on June 28 and 29, we sampled twelve ponds across the permanence gradient with four of each hydroperiod (Figure 6). Continuing for each pond on July 7, July 11 and 14, and July 34, we collected water chemistry samples of soluble reactive phosphorus (SRP), total dissolved phosphorus (TDP), total phosphorus (TP), ammonium, nitrate, total dissolved nitrogen (TDN), and total nitrogen (TN) for analysis at the lab. We also collected samples of benthic organic matter (BOM) from three locations at each pond, always including the southern bank, and in general near flags placed to measure the receding water line. A 21cm diameter stovepipe was planted in the pond bottom, and the area within the stovepipe was agitated manually. The mixture of benthos and pond water was collected with a 50 mL centrifuge tube. To collect sedge samples, we used a 0.25m² quadrat divided into twenty-five 0.1m² sections, placed at a random location within a uniform patch of sedge and took samples from three of the squares within using randomly assigned ordered pairs to select the squares. These were different for each pond and assigned before beginning collection each day.

The water chemistry samples were brought back to the lab and refrigerated until the time they were processed. The ammonium was analyzed immediately using a standard fluorometric method (Taylor et al. 2007) and the SRP was analyzed immediately with the molybdate blue method (Strickland and Parsons 1968). Meanwhile, TDN, TN, TDP and TP were first digested

using potassium persulfate additions heated with a pressure cooker. The digested phosphorus samples were then analyzed using the molybdate blue method and the digested nitrogen samples were using a second derivative spectroscopy method (Crompton et al. 1992). After analysis, nitrate samples were retained and frozen for measuring dissolved organic carbon (DOC) at a later date.

Before further analysis, 1 mL of each homogenized (shaken) BOM sample was extracted and filtered for chlorophyll analysis. The filters were frozen to lyse cells. They then were submerged in ethanol buffer for 4-6 hours and analyzed following a standard non-acidification method on a Trilogy Fluorometer (Welshmeyer 1994). The sedge and BOM samples were tinned and dried in an oven in order to preserve them. After weighing, the BOM was ground with a mortar and pestle and separated into 20mL scint vials to be ashed at RMBL for P, and the rest was kept for C:N analysis at the University of California at Davis. The sedge was bagged after weighing and kept for grinding using a ball mill at NC State.

The water chemistry was analyzed using two-way ANOVA to test for interaction between the permanence and time gradients, and then analyzed for treatment effect, using numbers from two-way ANOVA or one-way ANOVA depending on the significance of the interaction. The means were also calculated, and trends were analyzed using box plots presenting visual data. These tests will later be applied to the sedge and benthos samples.

Experimental Study: Preliminary in-situ measurements on caddisfly influences on surrounding chemistry: In Late July we used wire mesh cages (1m³) to contain 20 caddisflies of 2 species and a 200mL volume of sedge. Five cages contained *L. externus*, five contained *L. picturatus*, and five contained only the sedge as control. In the second week of August, water chemistry and BOM will be sampled at 0.5m and 1m away from the cages. The results from this experiment will inform the design of future experiments for testing the magnitude of caddisfly driven nutrient recycling.

Results:

Preliminary water chemistry data was analyzed using JMP and R. Analysis began using bar graphs displaying means and standard errors of each nutrient with respect to pond permanence and the sample week. These revealed visible trends across both gradients (Figure 1). Box and whisker plots were used to analyze means and variance across each time and permanence separately (Figures 2 and 3). There were increases in time in TP, TDP, TN, and TDN. Trends across time in ammonium and SRP were not evident. There did not seem to be an individual effect per treatment either. We saw much variation within each pond permanence type but did see trends across pond permanence for N and P analysis. We observed the highest average levels of P in semi-permanent ponds, while temporary ponds contained the most N.

ANOVA does not reveal significance in any of these qualitative observations. Using two-way ANOVA we determined there was significant interaction between permanence and time for TN ($p < 0.05$) Means tables from this analysis are displayed in Tables 1-6. The time gradient had a significant effect on TN as well ($p < 0.001$) but there was no significant effect from permanence. Plotting the interaction showed variation in patterns of responses for both ammonium and

nitrogen (Figures 4 and 5). One-way ANOVA revealed few significant effects in the other variables across either gradient, but trends for each across permanence and time were evident. TDN and TDP each showed significant increases in concentration over time ($p < 0.02$ and $p < 0.001$ respectively). There were no other significant results.

Discussion:

Given these findings we cannot determine a concrete causal link between the permanence and the levels of nutrients, but the trends were visible enough that it merits more research. For example, there are 40+ additional ponds at Mexican Cut that could be sampled in future studies. One trend we observed was that semi-permanent ponds are the most varied in their nutrient storage. This is expected because they are the most varied in hydroperiod and size. We also observed trends along the permanence gradient featuring the highest amount of water column nutrients in temporary ponds. Although this seems contradictory to our hypothesis that permanent ponds would contain the most nutrients, it will not be definitive without the sedge and BOM data on these nutrients. While this may be due to higher overall nutrient levels in more temporary ponds, we believe the water column is the most vulnerable to losses during drying of these ponds. We believe that BOM and sedge will fixate nutrients for most of these ponds.

The precipitation was also extreme relative to previous years (Barr 2015). There was far more rainfall and consequently, semi-permanent and temporary ponds sustained water far past their usual drying times. This likely extended the amount of time that organisms were able to populate these ponds and could have contributed to different nutrient dynamics. Additionally, the results in the TDN and TN samples indicate problems with processing the samples. Ammonium levels should be lower than TDN and TN samples, and higher numbers indicate wrong values for these samples, which could mean that time doesn't have as significant of an effect on TN and TDN as our results indicate.

In summary, we can conclude that while there is more work to be done, there are likely impacts of permanence and time in the season on each of these nutrients. It will be interesting to investigate the impact of a particularly rainy or dry season on pond nutrients in the coming years as well, as these extremes may have implications for the life cycles and abundance of many species. In the future, a replicate study could be conducted with higher power on the pond nutrients to determine if there is a statistically significant difference in nutrient storage across temporal and permanence gradients.

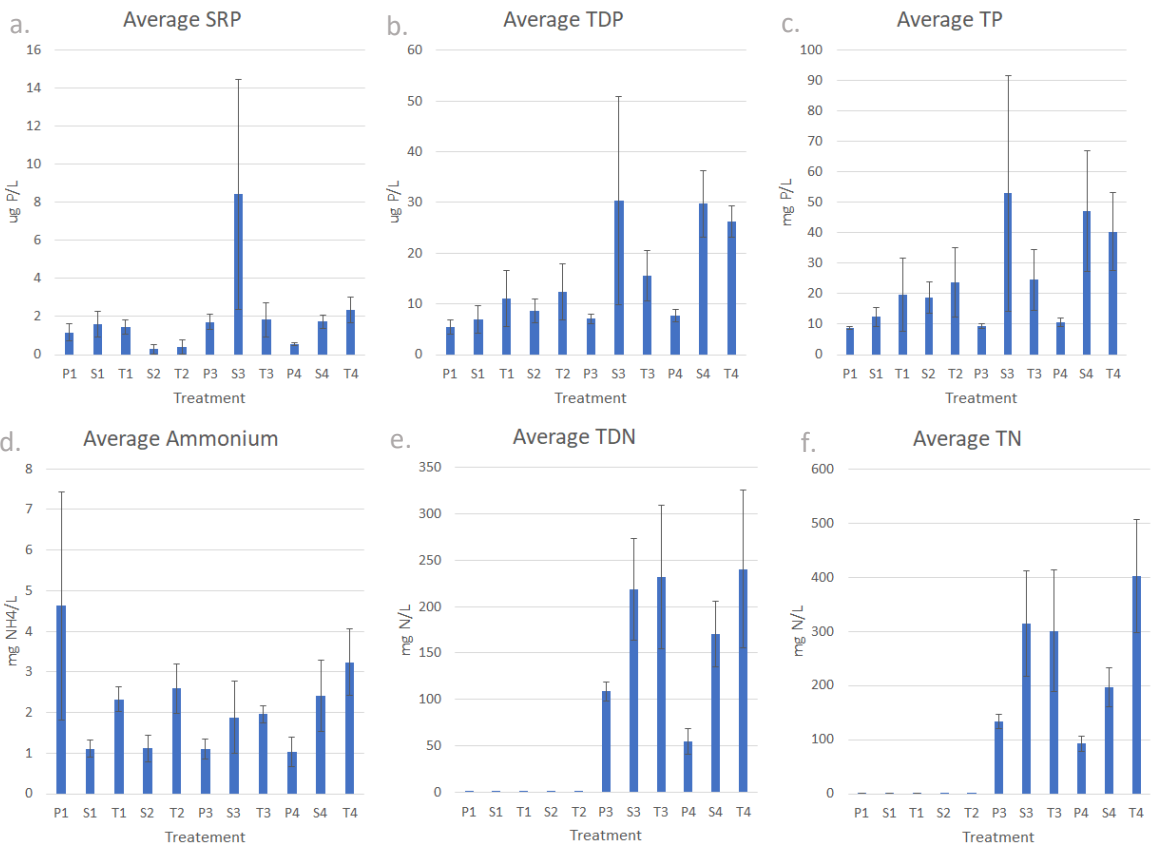
Acknowledgements:

Thank you to Brad Taylor and Jared Balik for working closely with me and guiding my research and for their patience as I learned my way around the lab, and the Mexican Cut, and the research expectations and practices at RMBL, and for making it possible for me to participate in this program, and to Jared's help in editing each presentation and paper I worked on at RMBL. Additional thanks to Amanda DeVecchia, Hamish Greig, and Scott Wissinger for their work in beginning this research and support along the way. Thanks to Derek West for teaching me about

his research, taking me to his experiments, and for his patient answers to any questions I had. More thanks for Elin Binck, Jackie Fitzgerald, and Benjamin Swift for all of the work they did with sample processing and for good conversation during long work days, and thanks to Susan Campbell, Leah Franzluebbbers, Abrianna Sadler, Michelle Woods, for welcoming me upon my arrival into the activities of their lab, Susan especially for the work we shared and for her contributions of GPS data and other experiments and feedback throughout this study and to Isaac Shepard for answering any questions and for his patience in the midst of shenanigans. Additional thanks to Jessica Kast for companionship during long days in the lab and for helping me figure out experiments, and for mutual accountability agreements. I also would like to thank Ian Billick and Jennie Reithel for running a fantastic field station, Emily Mooney for conducting a rigorous, educational, and fun REU program, Shannon Sprott for being amazing in the GIS office, to the National Science Foundation for funding this research and my time at RMBL, to the NC State Environmental Sciences and Biology departments, and to Greg Wilson especially for encouraging me to apply to and then accept the REU program, and to every person I've met this summer who has made this such an enriching, educational, and overall fulfilling and exciting time.

Charts and Tables:

Figure 1: Treatment Bar Graphs



Each letter represents the Hydroperiod, P for Permanent, S for Semi-Permanent, and T for Temporary.

Each number represents the sample week. Week 1 was June 28 and 29, Week 2 was July 7, Week 3 was July 11 and 14, and Week 4 was July 24.

Figure 1: Nutrient levels across hydroperiod and time for each treatment. In order, a.) SRP, b.) TDP, c.) TP, d.) Ammonium, e.) TDN, f.) TN. Bar heights represent means, and the error bars contain standard error calculated by JMP, added and subtracted in both directions.

Figure 2: Time Box Plots

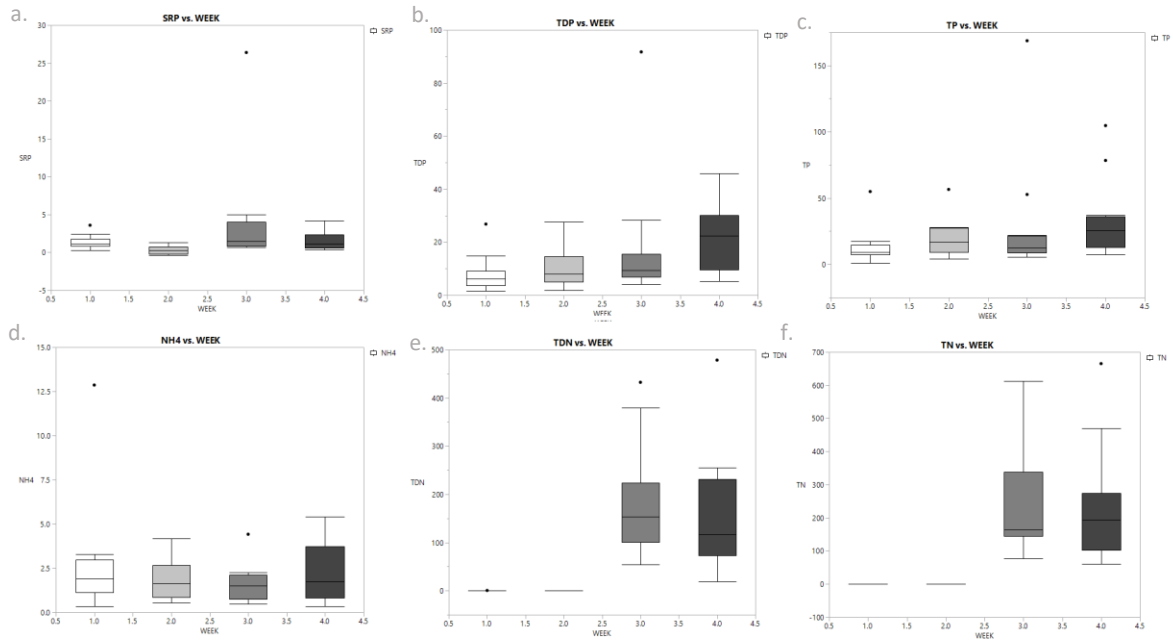


Figure 2: Box and whisker plots created with JMP indicating trends across time. Taller boxes indicate higher variance, and higher boxes indicate higher levels of nutrients. Trends are visible across most plots but few are significant. Nutrients are as follows: a.) SRP, b.) TDP, c.) TP, d.) Ammonium, e.) TDN, f.) TN

Figure 3: Hydroperiod Boxplots

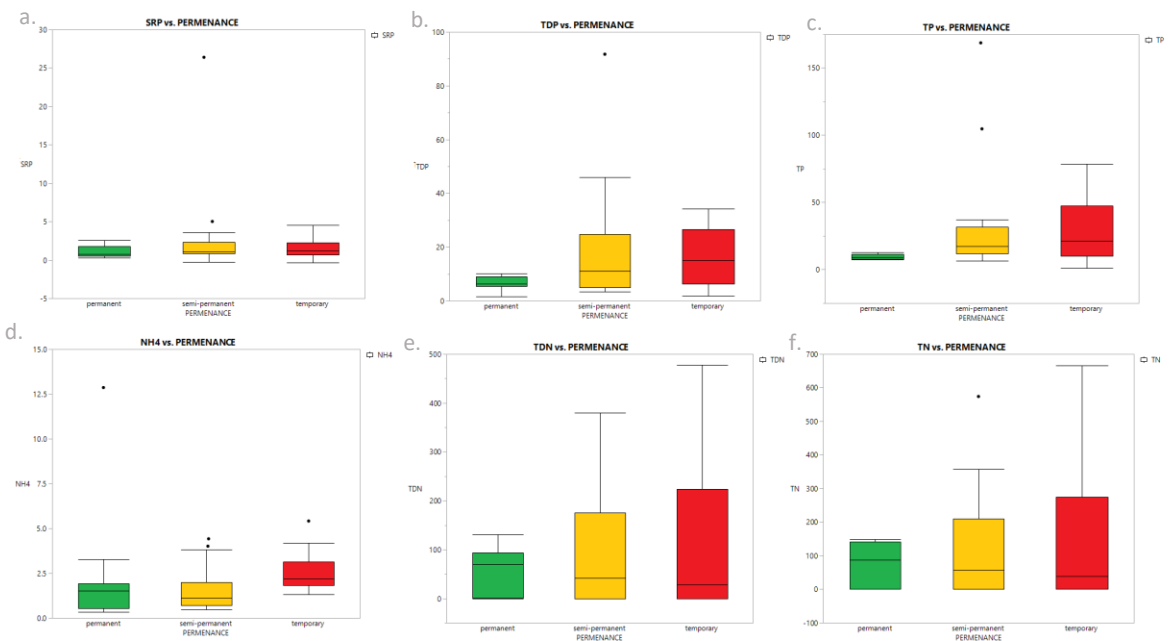


Figure 3: Box and whisker plots created with JMP indicating trends across permanence. Taller boxes indicate higher variance, and higher boxes indicate higher levels of nutrients. Trends are visible across most plots but few are significant. Nutrients are as follows: a.) SRP, b.) TDP, c.) TP, d.) Ammonium, e.) TDN, f.) TN

Figure 4: NH₄ Interaction Plots

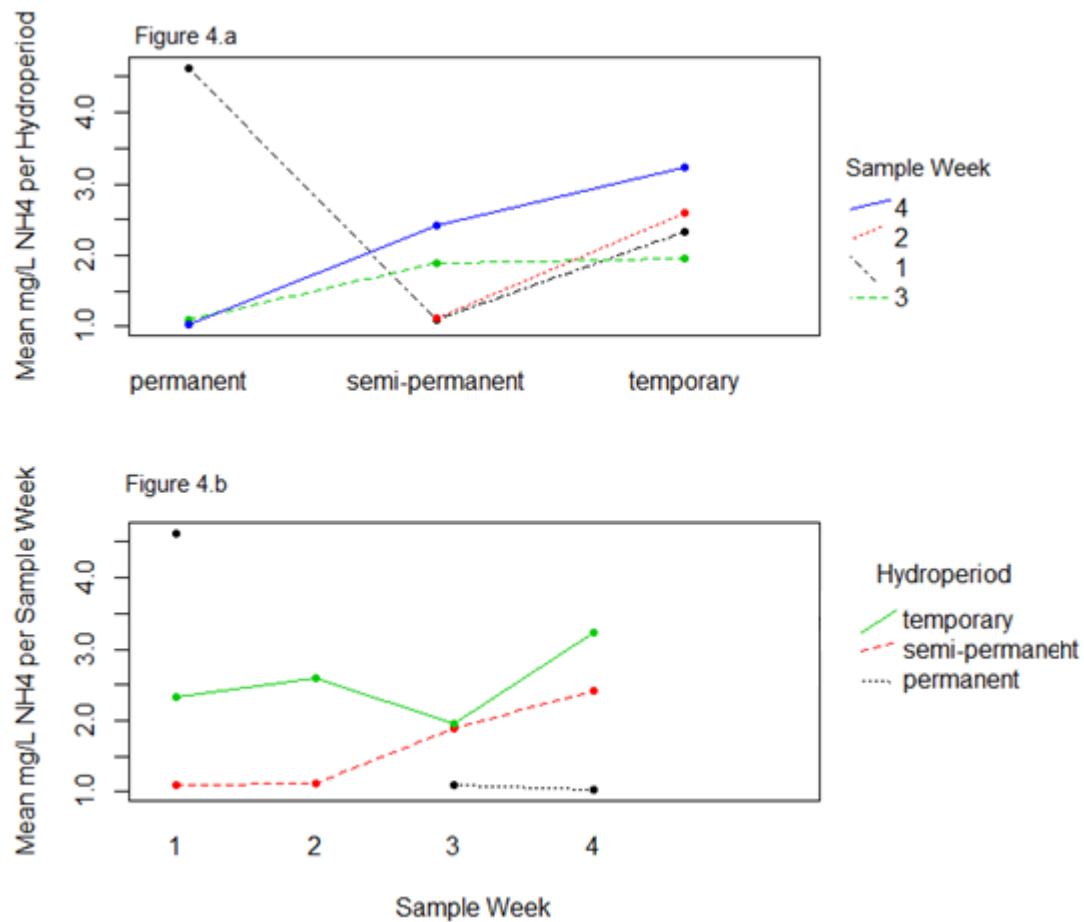


Figure 4: Interaction across sample week and permanence for ammonium made in R. Week 1 is June 28 and 29, week 2 is July 7, week 3 is July 11 and 14, and week 4 is July 24. a.) Traces are the week, b.) traces are the hydroperiod. Both are shown in order to contextualize interactions.

Figure 5: TN Interaction Plots

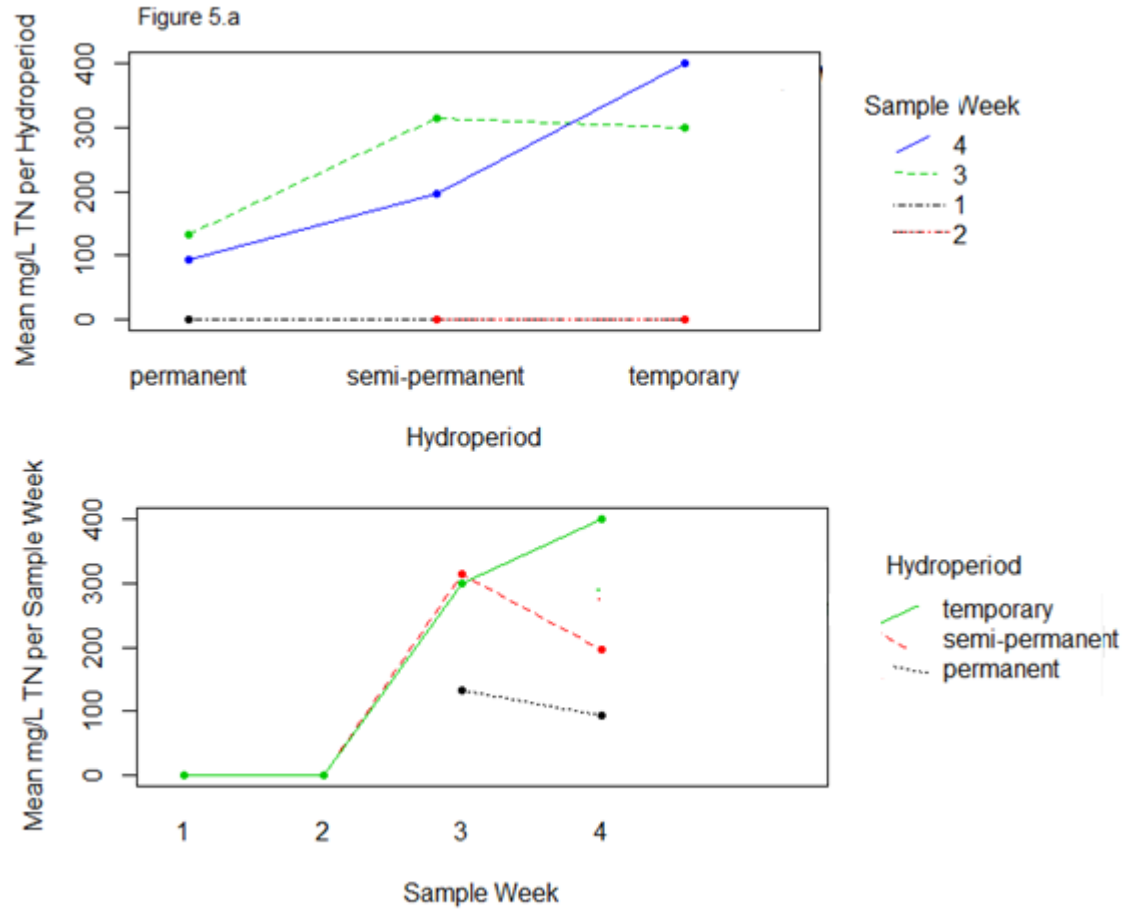


Figure 5: Interaction across sample week and permanence for TN made in R. Week 1 is June 28 and 29, week 2 is July 7, week 3 is July 11 and 14, and week 4 is July 24. a.) traces are the week, b.) traces are the hydroperiod. Both are shown in order to contextualize interactions.

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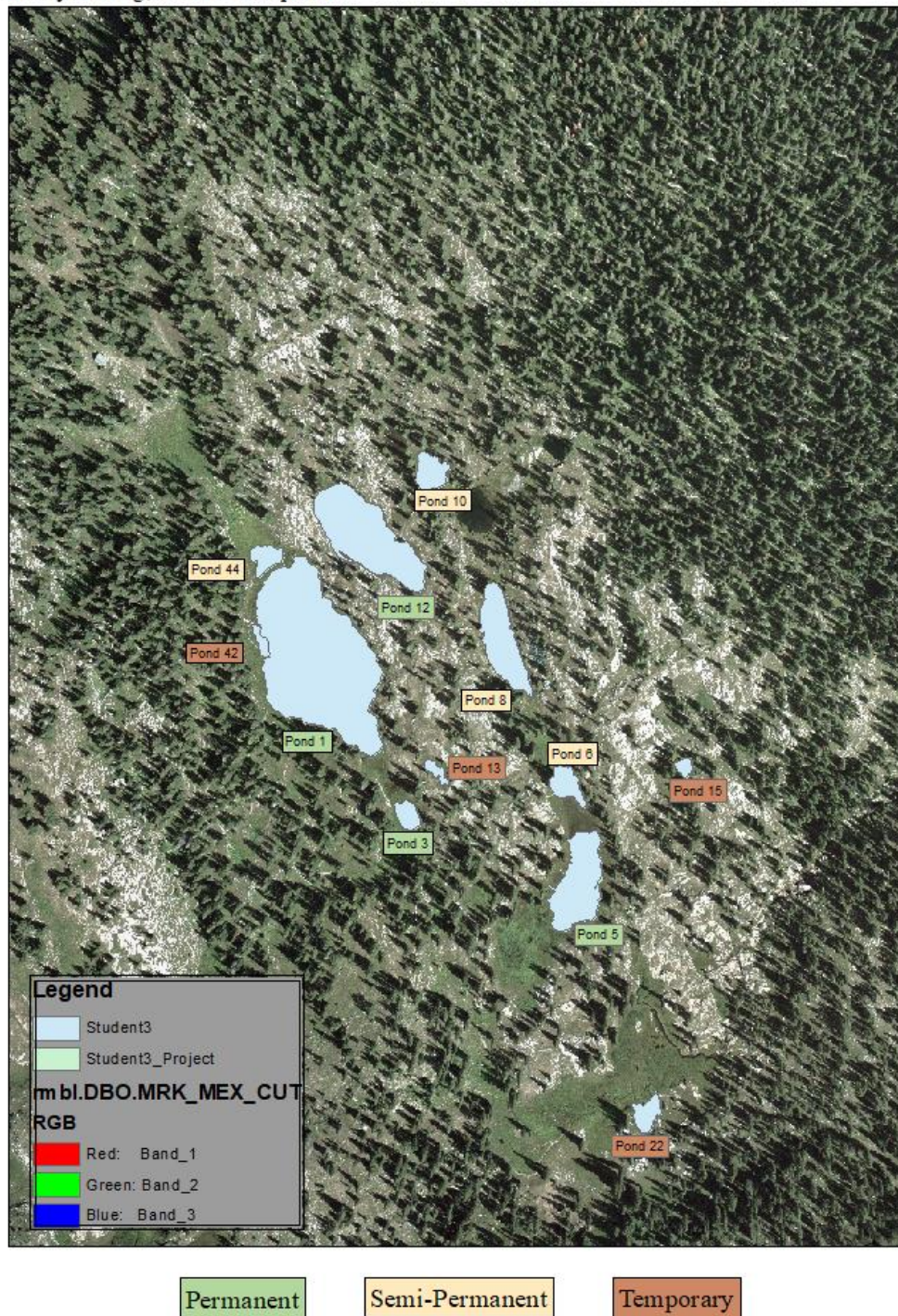


Figure 6: Ponds in the Mexican Cut, data taken by Susan Campbell, displayed by permanence.

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