# The Effects of Common Stream Pollutants on Natural and Agricultural Streams' Metabolism and Nutrient Uptake

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**Abstract:**

Streams are highly influenced by the environment around them and have the capabilities to transport pollutants downstream or to terrestrial environments. The fluidity of streams makes them critically important to understand, especially as United States streams become increasingly more polluted with contaminants of emerging concern (CECs) and nutrients (e.g. nitrate). Many studies have looked at the ways in which streams are physically affected by either one of these types of pollutants, but few have looked at their combined effect on the functional capabilities of streams. Here, I studied how natural streams (i.e. limited exposure to pollutants) and agricultural ditches (i.e. likely have been exposed to pollutants) responded to sharp increases in caffeine and diphenhydramine. I measured metabolism and nutrient uptake rates. There were no differences for gross primary production, cellular respiration, or net primary production among treatment types (two-way ANOVA, GPP $F_{3,3}$=0.089, p>0.1; CR $F_{3,3}$=0.172, p>0.1; NPP $F_{3,3}$=0.689, p>0.1). For nutrient uptake rate, the agricultural ditches had higher nutrient uptake rates than the natural streams (two-way ANOVA, $F_{1,2}$ =12.59, p<0.001). This implies that the agricultural ditches are more accustomed to pollutants and therefore better able to process large amounts of nitrate under the pressure of new contaminants. The test also revealed the reference treatment to have the lowest uptake rate, the control treatment had the intermediate uptake rate, and the treatment with all contaminants had the highest uptake rate (two-way ANOVA, $F_{2,2}$ = 9.988, p<0.001). This revealed the CECs have the capability to increase nitrogen uptake rates. CECs are widely understudied, and the full breadth of their effects on other stream functions are yet to be known, but there is potential for caffeine and diphenhydramine to be used in remediation of streams loaded with excess nitrate levels if proven to have no serious adverse effects on other portions of the stream.

**Introduction:**

Streams are highly variable ecosystems temporally, spatially, functionally, chemically, and biologically (Hall, 1972; Minshall, 1978; Vannote et al., 1980; Izagirre et al., 2008). These fluvial systems are critically important to the wellbeing of other, seemingly separate, ecosystems, such as the terrestrial environments around them and depositional areas at the base of their watersheds (Vannote et al., 1980; Naiman et al., 1988; Bernhardt et al., 2003; NRC, 2000). For example, streams have influence on groundwater quality, which can harm the health of people otherwise isolated from the streams, and act as the transportation mechanism for nutrient pollution that causes harmful algal blooms in such as those in the Gulf of Mexico causing economic losses in the multibillion dollar fishing industry of that region (Vitousek et al., 1997; Alexander et al., 2000; Rabalais et al., 2002; Jekel et al., 2013)

Oftentimes, the health or wellbeing of a stream is determined by its physical features (e.g. substrate type, amount of large woody debris, insect composition, etc.), but it is less common and typically more telling to examine a stream's functional capabilities (Petersen, 1992; Mulholland et al., 2001). Understanding both a stream's structure and function is critical to getting the full picture of how the stream behaves and interacts with its surrounding environment (Bunn et al., 1999; Gesser and Chauvet, 2002).

Headwater streams are arguably the most functionally important part of watersheds, as their low volume-to-surface-area ratio provides large amounts of habitat for microbial communities to grow and thrive (Vannote et al., 1980). The main drivers of stream processes are biofilms, a collection of heterotrophic and autotrophic microorganisms living in an extracellular polymeric substance (Tank et al. 2006, Azim et al. 2005). These biofilms act similarly to filters, removing pollutants before they can head downstream (Alexander et al. 2000, Peterson et al.

2001). Classical studies have looked at the biofilm's microbial processes as they occur in natural, urban, and agricultural streams, but few have looked at crosses between them (Minshall, 1978; Lock et al., 1984; Johnson et al., 1997; Paul and Meyer, 2001; Kaushal et al., 2015).

The issues facing agricultural streams are largely those of nutrient pollution (Tank et al. 2006). Nutrients are also a problem for urban streams (Carpenter et al. 1998), which nearly ubiquitously deal with the issues caused by pharmaceutical, personal care products, and pesticide pollutants as well (Bunch and Bernot, 2010). The biofilms in headwater streams have the ability to process excess nutrients before they travel downstream and cause eutrophication issues, but recent studies have found links between pharmaceutical pollution and decreased biofilm growth (Sanderson et al., 2009; Rosi-Marshall et al., 2012; Lawrence et al., 2012; Rosi-Marshall et al. 2013).

There are many experiments that look at the ways in which urban streams differ from natural streams and the effects that urban pollutants have on already impacted streams, but there is limited research on what a sudden increase in pollutants does to a stream (Bunch and Bernot 2010, Lawrence et al. 2012, Jekel et al. 2013, Li et al. 2015). In this experiment, I tested how the microorganisms in streams functionally deal with a sharp change in the chemical environment around them by introducing contaminants of emerging concern (CECs) and nutrients while measuring their metabolism and nutrient uptake rates. I predicted that the contaminants would negatively affect the respiration, primary production, and nutrient uptake rates. I compared the responses of natural streams to those of agricultural ditches to see if the agricultural ditches were less susceptible to the negative effects of the contaminants. Since agricultural ditches typically are more polluted, I predicted the agricultural streams to be less affected by the introduction of CECs than the natural streams.

**Methods:**

*Site Locations:*

The natural streams and agricultural ditches were chosen so as to receive samples from streams that are in different stream systems if possible (not simply upstream or downstream) as well as different watersheds where possible (Figure 1). This project was conducted on the Flathead Reservation in Lake County and Sanders County, Montana. For this project, I chose three natural streams, where the water was largely untouched by the agricultural environment of the region, and three agricultural ditches where the water was generated mostly from run off from nearby farmland or cattle grazing area (Figure 2). The agricultural ditches were surrounded by farms and roads, while the natural streams were either on protected land or in hiking areas. In general, the natural streams were more shaded than the agricultural ditches since they were often forested. In addition, the natural streams maintained their own floodplains and were not dredged or channelized. The agricultural ditches were managed, follow straight stream paths, and had minimal riparian vegetation covering the stream bed.

*Pollutants:*

The CECs I chose to study in this experiment were caffeine and diphenhydramine. These two chemicals are commonly found in streams across the United States (Kolpine et al., 2002; Daughton, 2014; Du et al., 2014) and have been shown to have adverse effects on streams' functional capabilities (Kolpine et al., 2002; Berninger et al, 2010). The concentrations of pollutants that I used were those that could be found naturally in polluted streams—for caffeine, 1 µg $L^{-1}$ (Kolpine et al., 2002) and for diphenhydramine 6 µg $L^{-1}$ (Daughton, 2014).

*Stream Metabolism:*

For the metabolism studies, I had three treatments (caffeine, diphenhydramine, and both contaminants) as well as a control with no contaminants added. I created mesocosms using glass jars of approximately 500 mL, filling them with water from the stream and a small amount of the upper layer of substrate. Mesocosm samples were taken from five different regions of the stream which served as replications. I used five light and five dark replicates for each treatment type and control in each stream (Figure 3), having the light jars to reveal the photosynthetic microbial processes and the dark to represent the respiration.

This in-stream metabolism was estimated using methods adapted from Odum (1956) and other papers (Bott, 2006; Izagirre et al., 2008). I found gross primary production (GPP), cellular respiration (CR), and net primary production (NPP) by measuring the change in dissolved oxygen (DO) levels in the closed mesocosms that were incubated in the stream. This *in situ* method was possible assuming that the changes in gas concentrations from the atmosphere and groundwater was negligible. I used the following formula where C is the dissolved gas concentration, P is photosynthesis, R is respiration, and E is the influences from the ecosystem (Bott, 2006).

$$C_{\text{dissolved } O_2} = P - R \pm E$$

The mesocosms were left to incubate in the stream for 2-3.5 hours and the DO measurement (mg $L^{-1}$) was taken at the beginning and end of the incubation time using a handheld oxygen, conductivity, salinity, and temperature system (YSI Model 85, YSI Incorporated, Yellow Springs, OH, USA). I found the GPP or CR (mg $O^2$ $m\text{-}^2$ $h^{-1}$) using the following formulae and finding the surface area of the substrate used in the mesocosms.

$$GPP = DO_{\text{final}} - DO_{\text{initial}}$$

$$CR = DO_{initial} – DO_{final}$$

In order to determine the NPP, I used the following formula.

$$NPP = GPP – CR$$

*Nutrient Uptake:*

I found the nutrient uptake of the streams by creating mesocosms similar to those described above, filling them with a small amount of substrate and water from each stream, and using the same jars. This portion of my study had three treatment types, a reference, a control, and a contaminated group (Figure 3). The reference mesocosms only contained whatever nutrients were already present in the stream—no additional nutrients were added to the mesocosm. These mesocosms therefore represented the way the stream acts without a huge flux of nutrient additions. The control mesocosms had only the additional nitrate added, which represents the way the stream would act if it was to be suddenly flooded with nutrients, as would be the case during fertilizing season on nearby farms. The treatment mesocosms were filled with both the nitrate and the CECs, representing the way a stream would react to a sudden influx of both nutrients and CECs. For the control and treatments, I added nitrate solution to make the concentration of the water approximately 126 mg $L^{-1}$. There were three replicates per treatment type per stream (Figure 3). I measured the nitrate levels in the mesocosms approximately every two hours for six hours on the day the sample was taken in order to produce a nitrate uptake rate for each stream and treatment type. Nitrate was measured using the cadmium reduction method (NitraVer Nitrate Reagent Powder Pillow, Hach, Loveland, CO, USA) and reading on a spectrophotometer (Spectrophotometer V-1200, VWR International LLC, Radnor, PA, USA).

*Statistics*:

For metabolism, I ran three two-way ANOVAs with treatment (control vs caffeine vs diphenhydramine vs both) and stream type (agricultural ditch vs natural stream) as the independent factors and GPP, CR, and NPP as the dependent factors. For nutrient uptake, I ran a two-way ANOVA with treatment (reference vs control vs treatment) and stream type (agricultural ditch vs natural stream) as the independent factors and nutrient uptake rate as the dependent factor. In the metabolism portion of the study, there are 5 replicates per treatment; in the nutrient uptake portion, there are 3 replicates (Figure 3). I used Shapiro-Wilks normality tests. If the two-way ANOVA was significant, a post hoc of Tukey HSD test was run. I accepted p-values less than 0.1, which is typical for most stream ecology papers.

**Results:**

*Stream Metabolism:*

There were no differences in the treatment type for the stream metabolism in terms of GPP ($F_{3,3}$=0.089, p>0.1), CR ($F_{3,3}$=0.172, p>0.1), or NPP ($F_{3,3}$=0.689, p>0.1, Figure 4). In addition, the two-way ANOVA revealed there was no difference in the stream type for GPP ($F_{1,3}$=0.455, p>0.1), CR ($F_{1,3}$=8.854, p>0.1), or NPP ($F_{1,3}$=2.268, p>0.1, Figure 4). To normalize the data, I used a logarithmic transformation. The variation was greater for the agricultural ditches than the natural streams. Analysis was done on the streams individual responses to the different treatment types as well, which also resulted in no true differences in the treatment types for all three metabolism metrics (Figure 5).

*Nutrient Uptake:*

A two-way ANOVA was used to examine the differences in nutrient uptake. Both the treatment type and the stream type had an effect on the rate of nutrient uptake within the streams (Figure 6). A logarithmic transformation was completed in order to normalize the data. The agricultural ditches (mean =-9.051 ±1.255 mg $L^{-1}$ $hr^{-1}$) had higher nutrient uptake rates than the natural streams (mean = -6.144 ±2.536 mg $L^{-1}$ $hr^{-1}$, $F_{1,2}$ =12.59, p<0.001). The test also revealed the reference treatment to have the lowest uptake rate (mean = -3.759 ± 1.022 mg $L^{-1}$ $hr^{-1}$), the control treatment had the intermediate uptake rate (mean = -6.400 ± 1.393 mg $L^{-1}$ $hr^{-1}$), and the treatment with all contaminants had the highest uptake rate (mean = -12.634 ± 3.649 mg $L^{-1}$ $hr^{-1}$, $F_{2,2}$ = 9.988, p<0.001).

**Discussion:**

*Stream Metabolism:*

Results from the stream metabolism portion of this study showed no differences in the metabolism rates of natural streams and agricultural ditches. In addition, the introduction of CECs caffeine and diphenhydramine had no effect on the metabolism rates. This finding differs from my original hypothesis and previous studies, which showed these CECs having adverse effects on biofilm respiration (Rosi-Marshall et al., 2013). GPP, CR, and NPP were all highly variable among and within the streams and ditches (Figure 4A, B, and C). The variation that occurred in the streams prevented any real differentiation being detected (Figure 5). This variability likely resulted from the combination of two different factors.

Firstly, some streams in this study proved to be heterotrophic (i.e. higher respiration rates than photosynthesis rates) while others were autotrophic. For example, the National Bison

Range stream was very open to sunlight which allowed it to be more autotrophic, but the other two natural streams (Magpie Creek and Swartz Creek) were covered by forest canopy and therefore were more heterotrophic (Figure 5). Similarly for the agricultural ditches, the Foust Farm ditch had recently been dredged and therefore did not have a very extensive autotrophic community living in the stream bed. This was represented in the GPP values, which were all heterotrophic, despite the fact that the stream bed was open and available to light (Figure 5). This variation obscured any potential affects the introduction of CECs had on the streams' metabolism.

Secondly, there were issues in the actual methods of the measurement of metabolism. This was made most clear when looking specifically at how each individual stream responded to different treatment types, which also had no statistical differences in treatment types and extremely high variation. Metabolism is extremely difficult to measure in streams and even the most expensive and time-consuming methods have their problems (Izagirre et al. 2008). If this study were to be completed again, a more extensive open-stream method using propane and weirs would be ideal (Mulholland et al., 2001; Bott et al., 2006; Izagirre et al., 2008).

*Nutrient Uptake:*

A two-way ANOVA revealed differences in the nutrient uptake rates of the streams based on treatment type as well as stream type (Figure 6). The agricultural ditches had higher rates of nutrient uptake than the natural streams. I predicted this would be the pattern seen since the microorganisms living in the agricultural ditches are more accustomed to receiving large amounts of nutrients and therefore are more likely to be able to quickly deal with a large flux in nitrate additions. Unexpectedly, the mesocosms treated with the CECs responded to the introduced contaminants by processing the nitrate input faster than the other two treatment types

(Figure 6). The control treatment type, which included a nitrate addition but no CECs, had the intermediate uptake rate; the reference, which represented the way the stream behaves without any introductions, had the lowest uptake rate (Figure 6). This implies that streams with these CECs present are better able to handle any nitrate pollution that may be introduced. Since the reference and control treatments for the types of streams were statistically the same, it can be said that the change in nutrient uptake rate was due to the CEC inputs (Figure 6).

Further studies are needed in order to understand the full mechanism behind caffeine and diphenhydramine's influence on the nutrient uptake. Limitations in time and resources prevented a full examination of the individual effects, but it would be interesting to separate the CECs from one another and determine if one of the contaminants has more of an effect than the other or if it is the combination of the two which caused my results. In addition, further studies should look at the potential effects other stimulants or antihistamines have on nutrient uptake rates.

*Implications:*

While there are many potentially harmful effects of CECs yet to be fully understood, caffeine and diphenhydramine prove to not have adverse effects on nitrate uptake rates of streams, both in agricultural ditches and in more natural streams (Berninger et al., 2011; Jekel et al., 2013; Daughton, 2014). In the short term, streams polluted with caffeine and diphenhydramine actually are better able to process nitrogen pollution. Essentially, the pollution of CECs cancels out the pollution of the nutrient. This could prove useful for better predicting the aftermath of spills or accidents from waste water treatment plants or from unexpected septic tank leaks.

In the future, some may attempt to use these CECs as a remediation practice to remove excess nitrogen from streams. While this may be beneficial in critical situations where the nitrogen is harming groundwater supplies or human health, I do not believe it would be the best practice in terms of preventing harmful algal blooms. CECs are widely understudied and the full breadth of their negative effects are yet to be known. These contaminants have potential to harm vegetation, microorganisms, riparian regions, and many other critical pieces of streams, but studies have not been fully completed yet. Better understanding of the way streams react to these CECs has the potential to greatly benefit stream ecosystems as well as human health.

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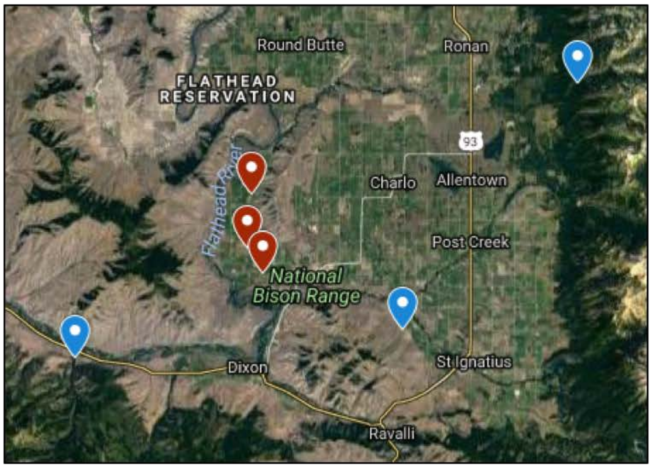
**Figures:**




| Name | Coordinates |
|---|---|
| Magpie Creek | 47°19'22.7"N, 114°28'51.4"W |
| Dan's Ditch | 47°25'46.6"N, 114°18'36.0"W |
| Otter's Ditch | 47°23'50.6"N, 114°18'54.0"W |
| Foust Farm | 47°22'40.2"N, 114°17'06.1"W |
| National Bison Range Stream | 47°21'53.8"N, 114°10'36.8"W |
| Swartz Creek | 47°30'14.2"N, 113°59'31.9"W |

Figure 1: Map of sites and table of coordinates. Blue sites are deemed natural and red are agricultural ditches.

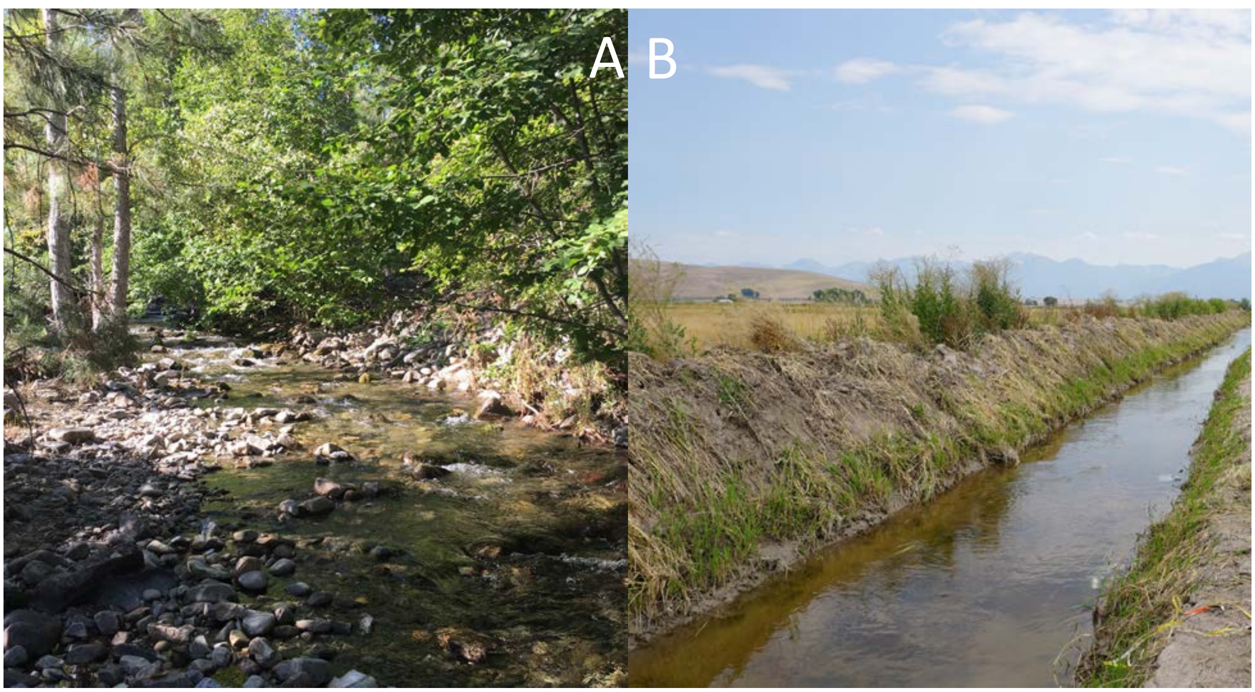



Figure 2: A typical natural stream (A) and agricultural ditch (B) in the region.

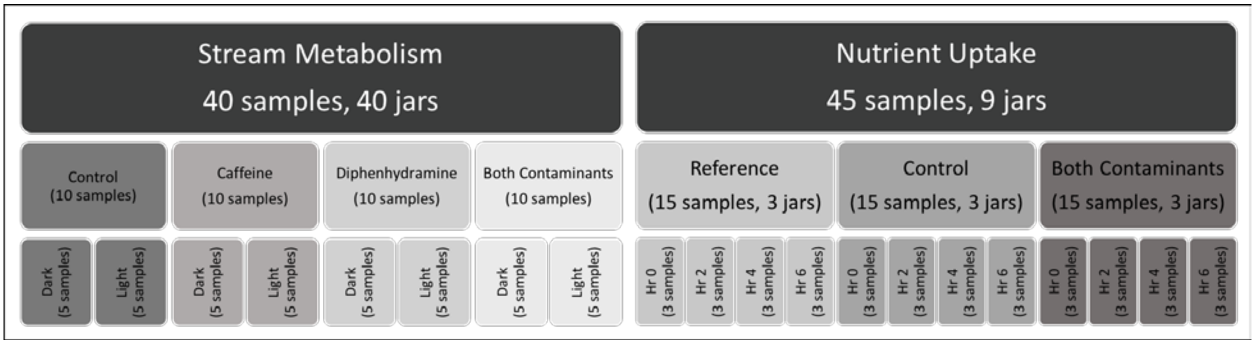



Figure 3: Diagram of sample sizes and treatment groups.


Figure 4: Metabolism of streams in terms of gross primary production (A), cellular respiration (B), and net primary production (C). Values are means with standard error bars (n=5 replicates). A two-way ANOVA revealed there was no difference in treatment type for GPP ($F_{3,3}$=0.089, p>0.1), CR ($F_{3,3}$=0.172, p>0.1), or NPP ($F_{3,3}$=0.689, p>0.1). In addition, the two-way ANOVA revealed there was no difference in the stream type for GPP ($F_{1,3}$=0.455, p>0.1), CR ($F_{1,3}$=8.854, p>0.1), or NPP ($F_{1,3}$=2.268, p>0.1).

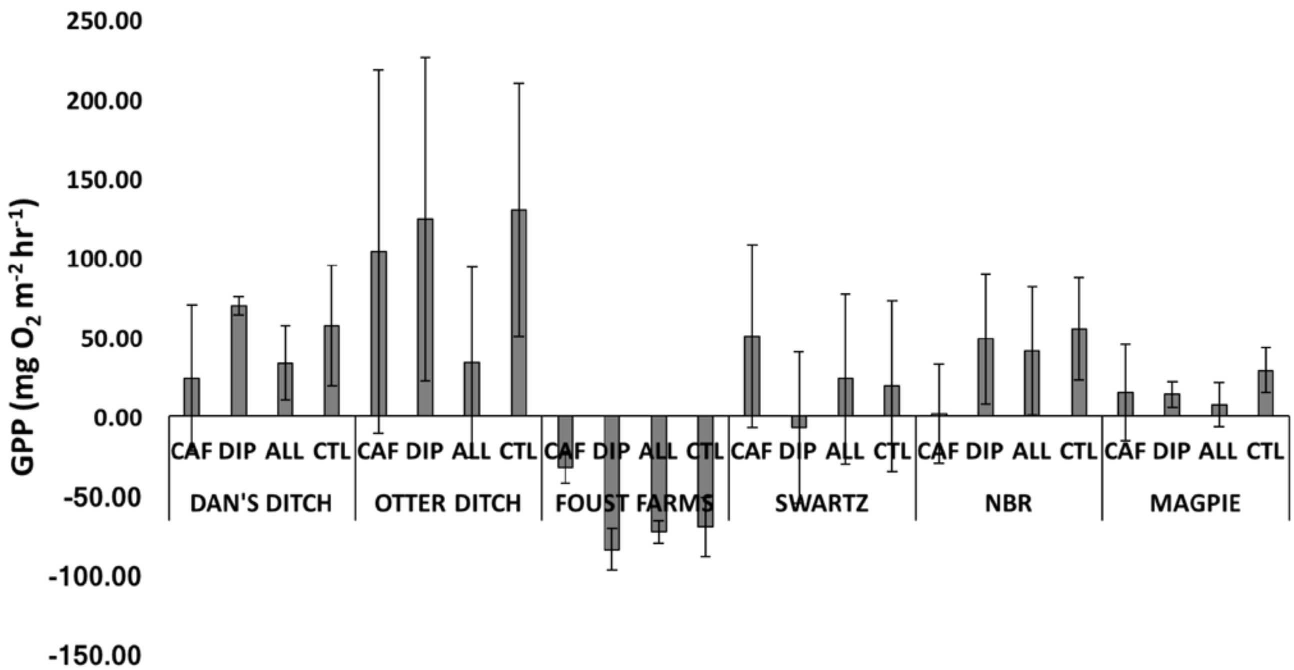



Figure 5: Individual streams' NPP responses to caffeine (CAF), diphenhydramine (DIP), and both contaminants (ALL), with the control (CTL) having no contaminants added. Values are means with standard error bars (n = 5 replicates).

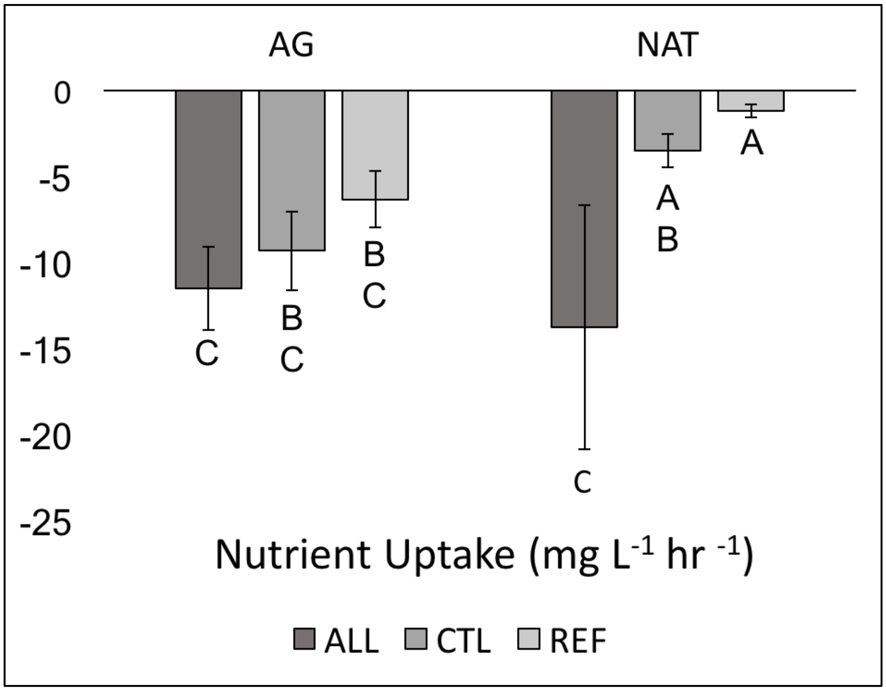



Figure 6: Nutrient uptake of streams. Values are means with standard errors (n=9 replicates). A two-way ANOVA revealed that agricultural ditches (mean =-9.051 ±1.255 mg $L^{-1}$ $hr^{-1}$) had higher nutrient uptake rates than the natural streams (mean = -6.144 ±2.536 mg $L^{-1}$ $hr^{-1}$, $F_{1,2}$ =12.59, $p<0.001$). The test also revealed the reference treatment to have the lowest uptake rate (mean = -3.759 ± 1.022 mg $L^{-1}$ $hr^{-1}$), the control treatment had the intermediate uptake rate (mean = -6.400 ± 1.393 mg $L^{-1}$ $hr^{-1}$), and the treatment with all contaminants had the highest uptake rate (mean = -12.634 ± 3.649 mg $L^{-1}$ $hr^{-1}$, $F_{2,2}$ = 9.988, $p<0.001$). Capital letters denote differences in means.