Priming Effects and Dissolved Organic Carbon Cycling in Aquatic Systems

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By

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Abstract:

Carbon cycling in freshwater is a large flux linking land to the sea and atmosphere, and better understanding some of the biogeochemical processes that occur in rivers is essential in our knowledge of nutrient cycling in aquatic systems, and the impact of CO_2 output of rivers as a whole. Much dissolved organic carbon occurs naturally and moves into rivers. A significant portion of this carbon is recalcitrant, and the processing of how it breaks down is not yet well understood. One hypothesis is the positive priming effect. Biologically available, or labile, carbon is believed by many to produce positive priming effects on the microbial breakdown of other, hard-to-break-down carbon forms, creating a greater CO_2 output via microbial respiration. We find that a better understanding of this aquatic priming effect is necessary, and seek to measure and observe this priming effect using traceable, labeled, recalcitrant ^{13}C. We tested the effects of sugar and nutrients on the breakdown of ^{13}C labeled recalcitrant carbon using experimental bioassays. We simplified the study of priming by directly testing the mineralization rate of aged, recalcitrant carbon. Across the treatments with sugar and nutrients, we observed evidence of positive priming effects. Increased CO_2 concentrations among the sugar and nutrient treatments from time zero indicate that the microbes are mineralizing carbon and respiring more than in the treatment without the added nutrients and carbon. Furthermore, an observed increase in the production of ^{13}C CO_2 indicates that much of this CO_2 came from the breakdown of our recalcitrant carbon: recalcitrant, labeled ^{13}C leachate. Using this recalcitrant labeled ^{13}C gives us a way

to more easily observe and calculate the respiration of the microbes breaking down the recalcitrant carbon. Because of this direct measurement, we observe clear evidence of the priming effect in an aquatic system. A better understanding of this process in aquatic systems is crucial in bettering our understanding of carbon cycling and CO_2 fluxes in our river systems.

Introduction:

Fresh water and riparian carbon sources are a crucial part of the overall global carbon cycle; it is estimated that over 5.7 petagrams of organic carbon is transported by river systems annually (Wehrli 2013; Ward et al., 2016). Of that, about three quarters of it is emitted into the atmosphere as carbon dioxide on a global scale (Ward et al., 2016). Aquatic microbes are responsible for decaying and transforming organic carbon in the water, which is a crucial part of the global carbon cycle, as it is transformed into CO_2 and either released or stored in the ocean (Cole et al., 2007; Battin et al., 2008; Ashberry et al., 2021).

Dissolved organic carbon (DOC) is the most important flow of organic carbon in streams and is a primary source of available carbon for heterotrophic microbes (Battin et al., 2008). Microbial breakdown is one of the primary pathways for conversion of organic carbon to CO_2 in aquatic environments (Ward et al., 2016). Understanding the breakdown of this dissolved organic carbon is needed to better estimate the (total) carbon being released and cycling through rivers.

Dissolved organic carbon is a heterogeneous pool with highly variable reactivity. Labile carbon is carbon with high bioavailability, meaning that it is more reactive, and has a structure that allows for microbes to be able to break it down easily, without expending excessive energy. Recalcitrant carbon on the other hand, is carbon that is extremely hard to break down, and may take excessive energy or time to be able to access. One reason for this is because of the molecular structure of much dissolved organic matter. Oftentimes, they are recalcitrant as they come in complex molecular forms such as tannins, lignins, and phenolic compounds. One of the possible reasons that rivers are able to convert so much carbon flux into the atmosphere is because of the priming of organic carbon breakdown (Battin et. al., 2015). In aquatic ecosystems, algae and microorganisms react in a way that some believe might facilitate or “prime” the breakdown of recalcitrant, or hard to break down, organic matter (Guenet et al., 2010; Ashberry et al., 2021). In other words, the algae release labile organic carbon that is easy for the microorganisms to decompose and use as energy, and “prime” the microbial breakdown of the recalcitrant organic matter more than they would without the labile matter released by the algae. The priming effect has been defined and well-documented for a long time in terrestrial environments (Ward, et al., 2016; Hotchkiss et al., 2014). However, study of priming effects in aquatic environments has come to a crossroads in its findings, with many studies’ findings in disagreement (Danger et al., 2013; Gontikaki et al., 2013; Guenet et al., 2014; Hotchkiss et al., 2013; Bengtsson et al., 2014; Kuehn et al., 2014; Attermeyer et al., 2015; Catalán et al., 2015).

One way to think about labile, recalcitrant carbon and the priming effect in this scenario is to imagine the recalcitrant carbon as a cracker. A child may not want to eat the cracker, because it is plain, there is no immediate draw. However, if we were to put frosting (labile carbon) onto the cracker, then the child will likely tolerate eating the cracker for the frosting. This is an observation of positive priming. We prime the cracker with sugar, which is what we are doing by adding glucose (sugar) to the aquatic system for the microbes to get energy from to further break down the recalcitrant carbon (cracker) at a faster rate.

Priming was originally defined in soil science as the quick cycling and breakdown of organic matter occurring as a result of adding very reactive organic matter. (Kuzyakov et al, 2000). Aquatic priming, as Ward et al., 2016 defined it, is using reactive matter to help amplify the effectiveness of breaking down less reactive organic matter. Essentially, priming (both positive and negative effects) often occur for a variety of factors. Positive priming may occur because more microbes are present as they consume more of the labile carbon; microbial biomass increases due to consumption of reactive substrates. Alternatively, microbes may gain more energy from breaking down labile carbon which is used for building enzymes that can more easily break down the recalcitrant carbon, energy gained from breaking down more reactive substrates leading to more microbial production of extracellular enzymes that can break down less reactive organic matter (Ward et al., 2016).

As for negative priming effects, an alternative hypothesis suggests that the addition of labile carbon could decrease the rate of breakdown of recalcitrant carbon through negative priming, possibly because the microbes selectively choose to consume the more labile/reactive carbon reactive substrates (Guenet et al., 2010; Ward et al., 2016).

Observations of the priming effect have been studied to a great extent in terrestrial environments, but much less is known about aquatic priming and the role it may play in use or the release of carbon (Ward, et al., 2016; Hotchkiss et al., 2014). Previous studies have examined priming in the breakdown of DOC and respiration in freshwater ecosystems. Within ecological settings, there have been very mixed results. Some studies saw no priming effects at all (Bengtsson et al., 2014; Attermeyer et al., 2015; Catalán et al., 2015), while some found negative priming effects (Gontikaki et al., 2013), and positive priming effects were also observed in the field (Danger et al., 2013; Guenet et al, 2014; Kuehn et al., 2014).

In contrast to ecological field studies of priming, in laboratory settings the evidence of priming can be much clearer without the ecological complexity interfering : Bianchi et al., found in a 2015 study that priming can enhance terrestrially derived DOC breakdown rates by as much as 75 times. Very few of these studies that have focused on aquatic priming effects in DOC breakdown have used labeled ^{13}C that is recalcitrant (Bengtsson et al., 2018). Using this recalcitrant labeled ^{13}C gives us a way to more easily observe and calculate the respiration of the microbes breaking down the recalcitrant carbon.

In this field sample-to-lab experiment, we examine the potential of priming effects in a lake water system, focusing on the breakdown of recalcitrant carbon through microbe respiration in the water. We do this via incubation experiments in the lab analyzing the differences in priming effects with treatments. Through bioassays, we examine the priming effects of both added glucose and added nutrients. The labeled ^{13}C comes from a plant-based leachate that is recalcitrant. This leachate was injected into closed-system incubation bottles with varying treatments.

3. Methods:

3.1. Experimental Setup

In our experiment, we are seeking to identify any potential aquatic priming effects of microbes through simplifying the study of priming by directly testing the mineralization rate of aged recalcitrant carbon. Treatments were primed with carbon and nutrients, and microbes' respiration was compared to that of a treatment without the carbon or nutrients. The ^{13}C leachate as a way to help measure respiration and the breakdown of the recalcitrant carbon, and used ^{13}C-labeled plant leachate to measure the biological breakdown of recalcitrant carbon in response to priming. Our plant leachate was formed from pure ^{13}C grass ground and brewed that was subsequently left to sit for two years, where we believe that most of the labile carbon broke down, leaving behind mostly recalcitrant ^{13}C labeled carbon.

We created four separate treatments for the experiment: lakewater, carbon, nutrients, and lastly carbon with nutrients; we used four bottles for each treatment. For the first treatment, we simply used the lake water and leachate. In the second treatment, we added glucose for a concentration of 1 mg C/L to four of the bottles for our labile carbon treatment, and four more bottles for our carbon, nitrogen and phosphorous treatment from a stock solution we created. For nitrogen, used N-NH4 (from NH_4Cl) at a concentration of 400 μmol N/L, and 50 μmol/L P-PO4 (KH_2HPO_4) for phosphorous; the second treatment was the "nutrients" treatment that included both nitrogen and phosphorus. The third treatment was nutrients and carbon (glucose) together.

We performed bioassays using this leachate. For the bioassays, water samples were collected from Flathead Lake, in Yellow Bay at coordinates: (47.87521, -114.03235). Our water samples were then filtered through a 45μL mesh in order to remove all of the zooplankton, but large enough for microbes to still be in the water. We diluted our 200 mg/L ^{13}C plant leachate by adding 0.5ml to 7L of water. We then filtered lakewater and subsequently poured the leachate into 28 individual 300 mL biological oxygen demand (BOD) bottles. After taking a time zero sample using the lake water treatment, we then stored the rest of the bottles in a closed cooler (for minimizing light exposure) at room temperature (20℃). We took samples of the just lake water and leachate treatment 0 (time zero), 22.5, 73.5, 126.5, 162, and 216 hours after the start of incubation to track the respiration of the microbes breaking down the recalcitrant carbon. We sampled

the other treatments (with carbon and nutrients) twice, at 165 hours and 217 hours after the start of incubation.

In taking samples, two bottles of the chosen treatment (first was the lake water and leachate treatment) are taken out of the cooler. We used four syringes to each draw 70 mL of the sample from the bottles (2 syringes for each bottle). A sample of a pooled 60 mL sample between the two bottles was taken to be saved for alkalinity measurements to later calculate the dissolved inorganic carbon. 40 mL of water is then drawn from each bottle and put through a 0.45 μm filter that is attached to the syringe as it is released into a 40mL vial. Then a third replicate is taken, with 20 mL from each bottle, so that there were 3 40mL vials of sample in total. 80 μg of 85% phosphoric acid is then added to each vial to prevent any further changes in the dissolved inorganic carbon. The alkalinity and DOC samples are then stored in a ~4℃ refrigerator.

For the syringe samples, we subsequently added 70 mL of zero air (no CO_2) to each syringe. They were then equilibrated by mixing the water and air through shaking the syringes for three minutes. The leftover liquid was then discarded, keeping the newly-equilibrated 70 mL of gas in the syringe. To measure the concentration of CO_2 and the $\delta^{13}C$ value of the CO_2 in the headspace of the syringe, we used the Picarro (G2201-i Isotopic Analyzer), where 20 mL of air was pushed out of the system, and then the last 50 mL of sample is processed into the Picarro.

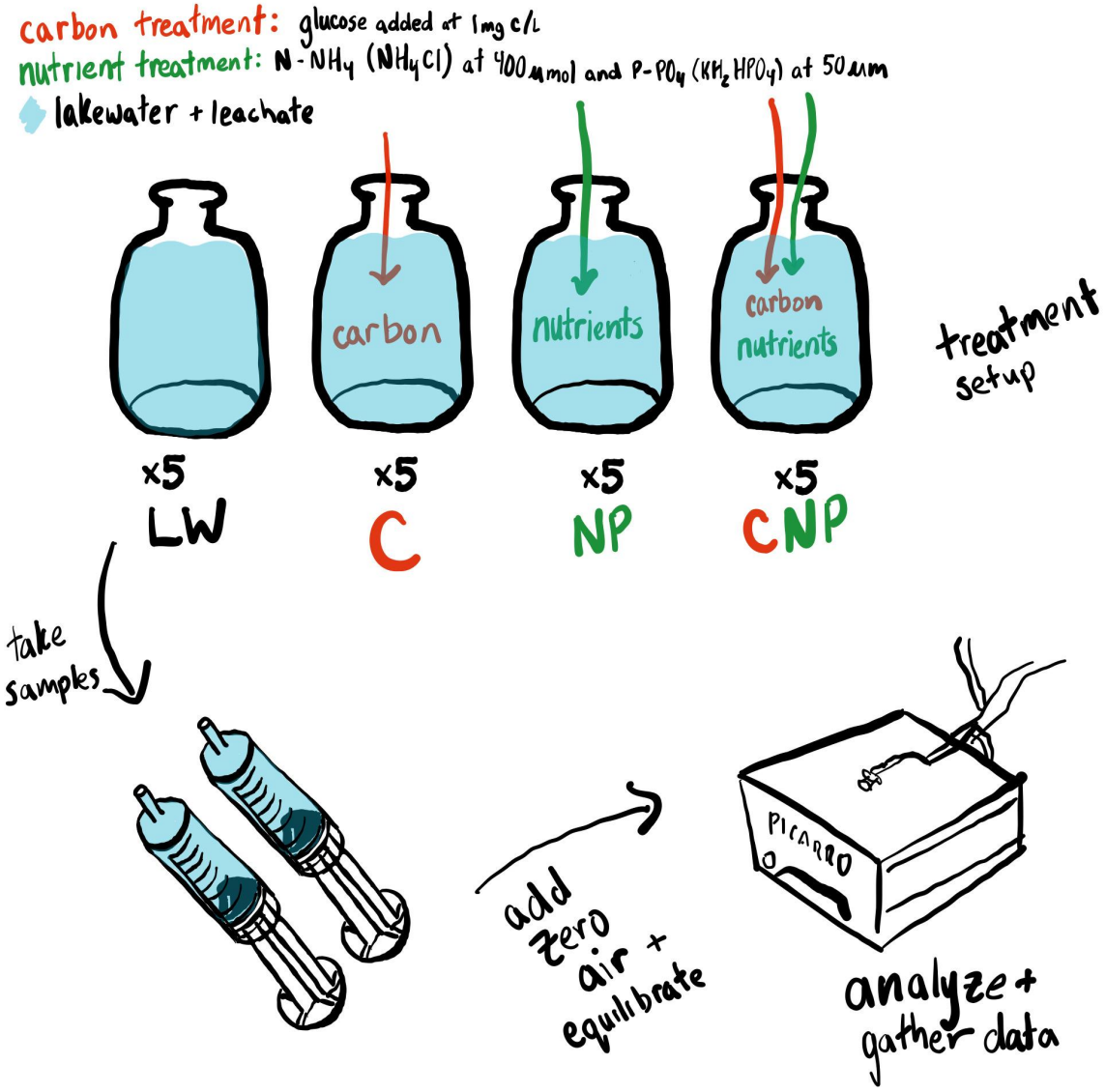

Fig. 1: We started with five replicates of all four treatments. These include lakewater and leachate (LW), lakewater leachate with glucose added (C), lakewater leachate with nitrogen and phosphorus (NP), and lakewater leachate with both glucose and nitrogen and phosphorus (CNP). After incubating, samples were taken with syringes and equilibrated before being processed into the isotopic analyzer, where we got our raw data.

3.2 Data Analysis

To estimate the respiration that occurred in each of our treatments, we calculated the excess dissolved inorganic carbon based on the difference in CO2 concentrations at time 6 and time 0. To do this, we first calculated the dissolved CO2 concentration in the water samples before equilibration based on our measured CO_2 concentrations in the headspace using Henry's Law. Through our samples, we found the alkalinity by titration, across all the treatments. We did not find any systematic difference in alkalinity across these treatments, and as a result used one single value for alkalinity in our calculations. The following equations were the basis of our calculations to find alkalinity and DIC:

$$\frac{[H^+][HCO_3^-]}{[CO_2]} = k_1$$

$$\frac{[H^+][HCO_3^{2-}]}{[HCO_3^-]} = k_2$$

$$DIC = CO_2 + HCO_3^- + CO_3^{2-}$$

$$Alk = HCO_3^- + 2CO_3^{2-}$$

The first two of these equations are the bicarbonate and carbonate equilibrium equations respectfully, where k_1 and k_2 are temperature-dependent equilibrium constants. The third equation represents the sum of all of the carbon in the system. The final equation is representative of the sum of the charge in the system. In our experiment, we directly measured CO_2 and H^+. The constants k_1

and k_2 are known based on temperature, leaving us to solve for the unknowns between these four equations to calculate DIC and alkalinity.

We then converted the fraction of ^{13}C based on atomic fraction and $\delta^{13}C$ value. To calculate excess ^{13}C dissolved inorganic carbon, we first calculated an average value of ^{13}C and ^{12}C DIC in the samples at the initial starting time (time zero). We then calculated the change in ^{13}C DIC over time by subtracting the concentration at time zero from the concentration at time six.We calculated change over time by dividing this difference by the incubation time, delta t.

$$\Delta^{13}C\ DIC\ = \frac{(^{13}C[DIC]t_6 - {}^{13}C[DIC]t_1)}{\Delta t}$$

Using the excess ^{13}C CO_2 and the collected CO_2 data, we were able to create plots depicting the decomposition rate in the lake water treatment, and the differences in microbial respiration overall, and of specifically the leachate, and see a significant difference between the samples at the initial and last time intervals.

4. Results:

Clear evidence of increased microbial respiration can be seen with the samples primed with glucose (C), as well as nutrients and the treatment of both glucose and nutrients together. Concentrations of both CO_2 and $\delta^{13}CO_2$ increased

among treatments primed with glucose and nutrients added in comparison to those with no added glucose or nutrients.

Dissolved inorganic carbon concentrations were drastically different across treatments. DIC concentrations were initially at approximately 18.2 µmol/L. Over time, the lakewater and leachate DIC steadily increased until it the final time sample, where it became slightly higher to about 18.4 µmol/L. Meanwhile, the DIC of the carbon (C) and nutrient (NP) treatments were both at an average around 18.85 µmol/L, and the CNP treatment was higher, averaging around a value of 19.5 µmol/L. Adding glucose and nutrients stimulated respiration of the plant leachate. The change in DIC was approximately 0.0167µmol*L^{-1} per day. In comparison, the change in DIC for the C and NP treatments were 0.0108 and 0.0919 µmol*L^{-1} per day, and the CNP treatment was 0.0173 µmol/L per day. A fraction of this change in DIC per day is attributed to the ^{13}C plant leachate.

We took samples every day in the lakewater (LW) treatment to assess if the curve was linear. Based on this lakewater sample treatment, we determined that the rate of decay was constant throughout our experiment.

The ^{13}C concentrations increased in treatments with glucose, nutrients and glucose and nutrients together to an even larger extent in comparison to the lake water treatment (see figure 4). The lake water treatment average was 0.013 µmol/L per day. The largest increase in ^{13}C respiration came from the carbon and nutrient treatment, which increased by an average of 0.12 µmol/L per day. The

carbon treatment average increase was 0.059 μmol/L per day. The nutrients treatment increased by 0.048 μmol/L ^{13}C respiration per day.

Overall respiration increased with nutrients, carbon, and the carbon with nutrients treatments (fig. 2). Glucose and nutrients together had a bigger impact, with glucose and nutrients separately increasing respiration comparably.

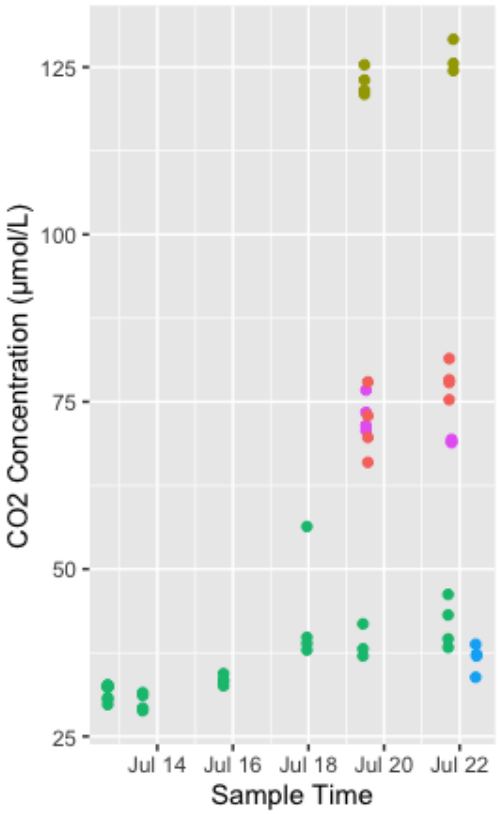

Fig. 2: This plot depicts the change in CO_2 concentration values for each treatment and over time.

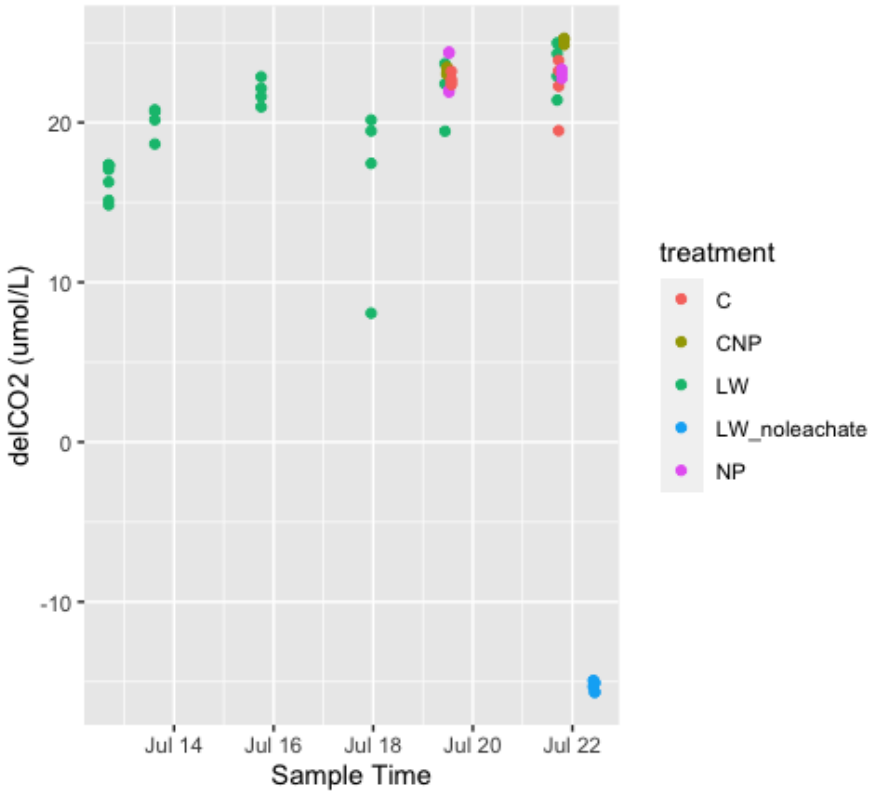

Fig. 3: This plot shows δCO_2 values for each treatment at each sampling time.

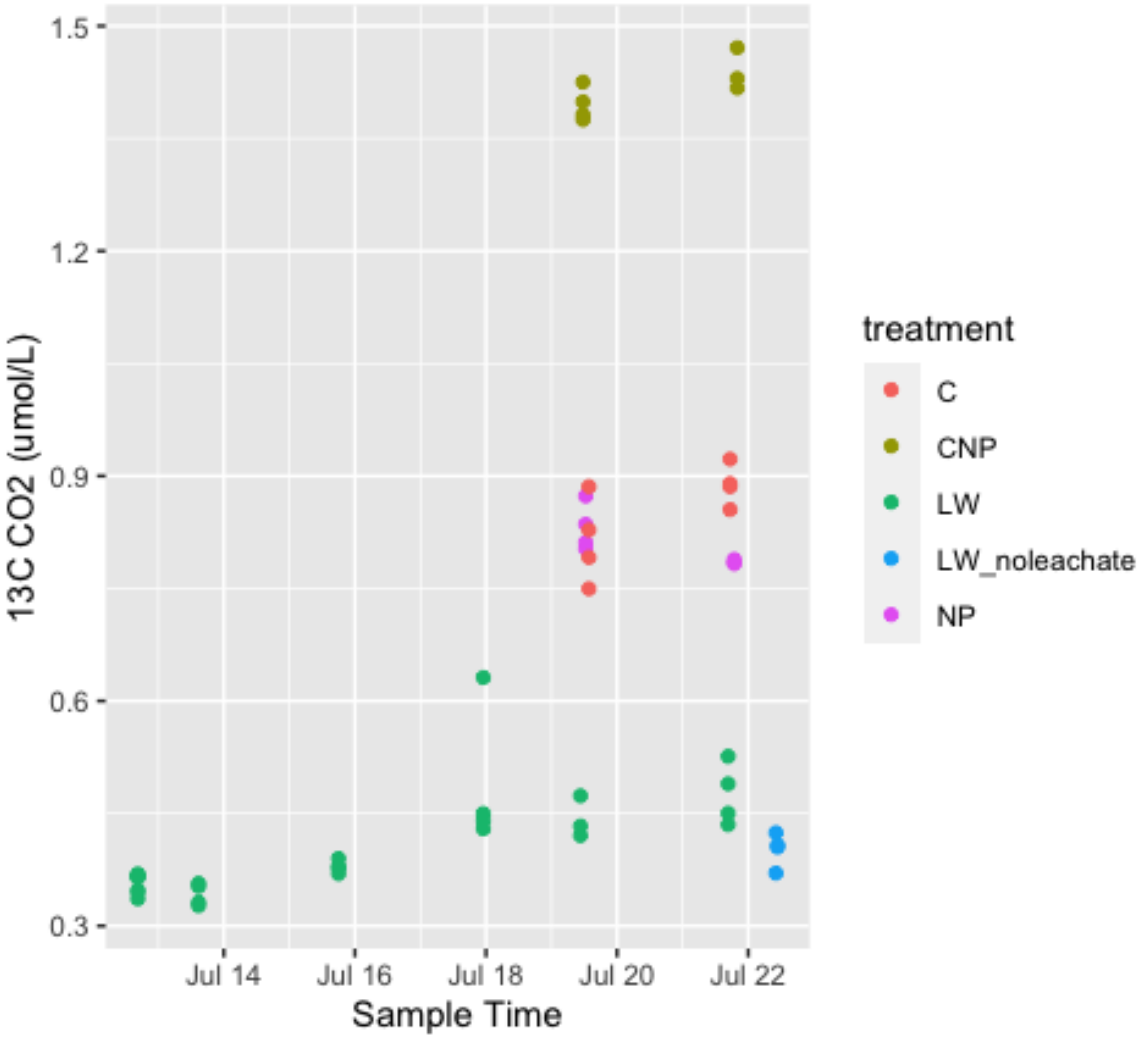

Fig. 4: This plot shows the $^{13}C\ CO_2$ respiration for each treatment at the time points samples were taken. Compare the initial time zero (Jul 12) to the time six interval (just before Jul 22)

The effect of positive priming from glucose and adding nutrients is evident within the accumulated excess ^{13}C CO_2 data. The fraction of the respiration that can be attributed to the breakdown of the leachate is the excess ^{13}C that we found. Excess ^{13}C CO_2 increased with the treatment that had sugar, versus those that did not (Fig 7). Respiration increased of the leachate with nutrients versus no nutrients as well.

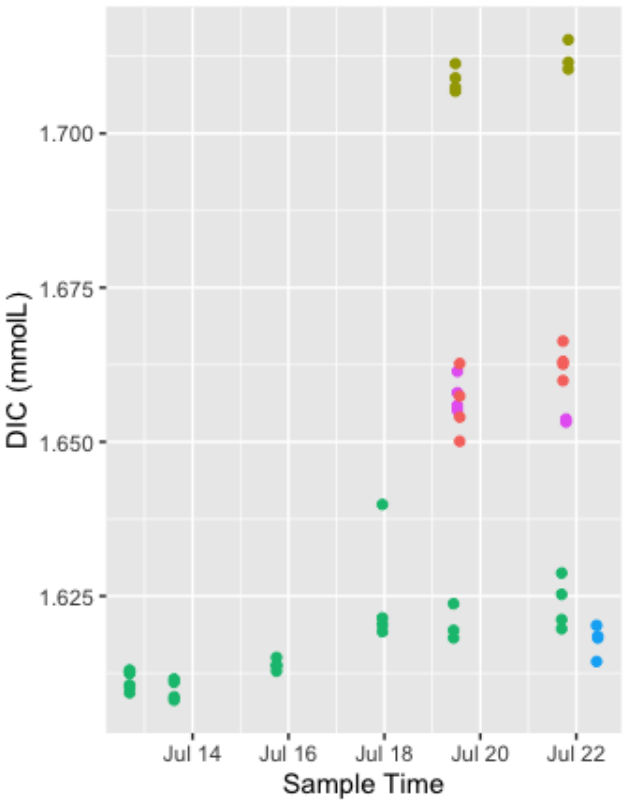

Fig. 5: This plot depicts the amount of total dissolved inorganic carbon (DIC) for each treatment at the sampled time points.

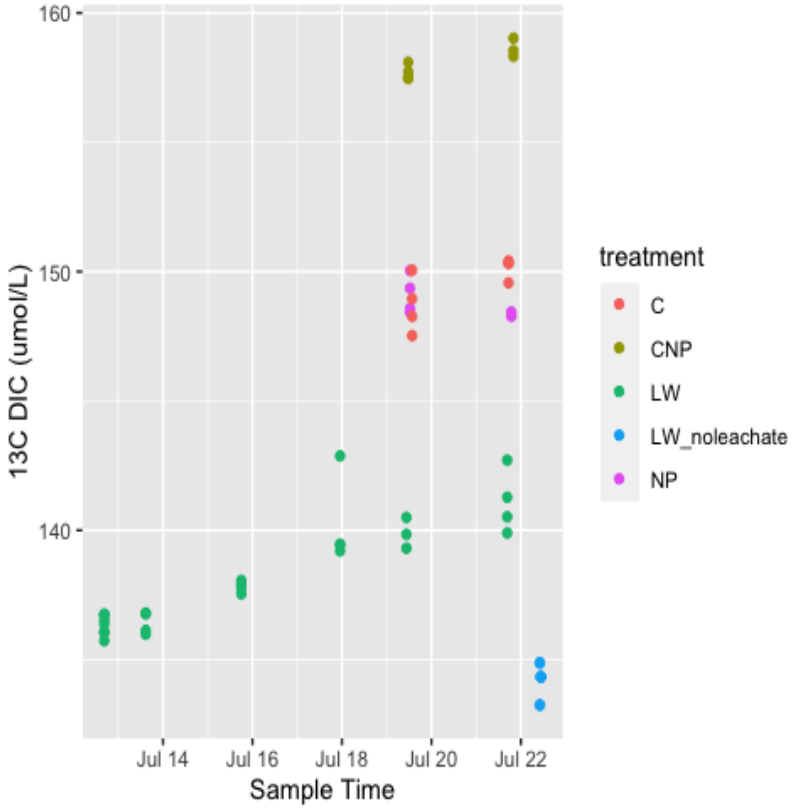

Fig. 6: This plot depicts the amount of ^{13}C dissolved inorganic carbon (DIC) for each treatment at the sampled time points.

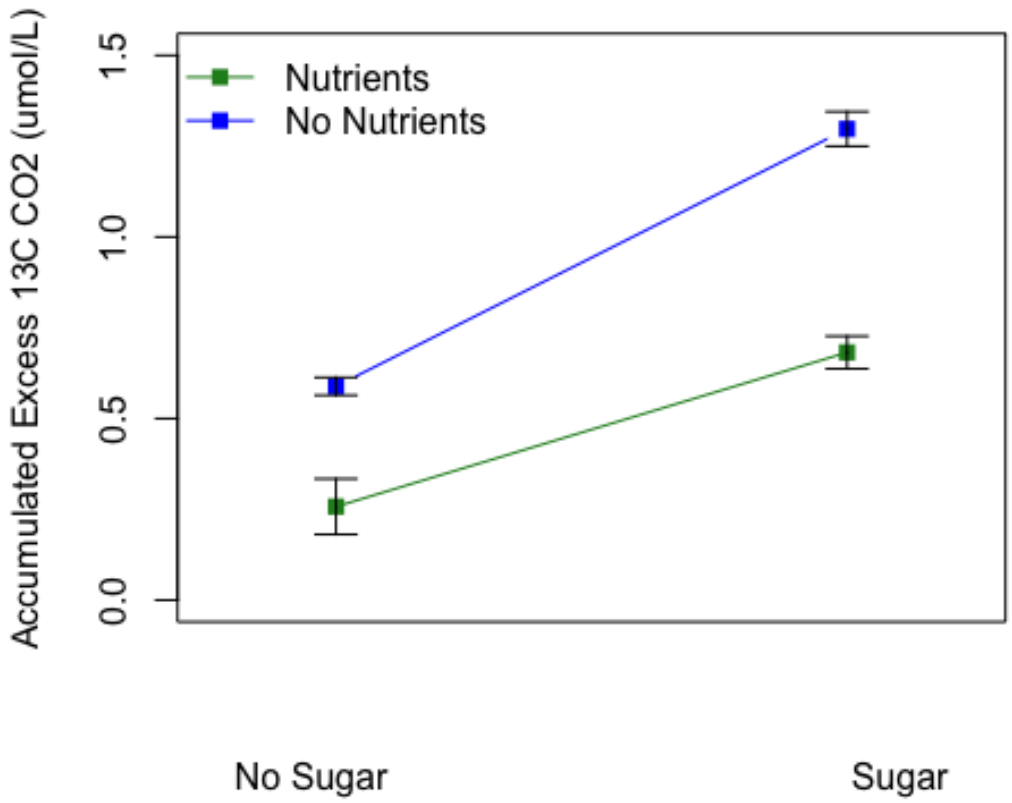

Fig. 7: Using the alkalinity and DIC calculations to find the excess $^{13}C\ CO_2$, this graph depicts the priming effect of glucose (sugar) versus no glucose, both with and without the presence of nitrogen and phosphorus (nitrogen and phosphorus in blue, no nutrients in green). Our results show a clear indication of priming with higher excess $^{13}C\ CO_2$ with both nitrogen and phosphorus and with sugar; based on our results, evidence of positive priming was observed in our experiment. Error bars show one standard deviation from the average of the five replicates.

Discussion:

CO_2 concentrations, ^{13}C respiration and accumulated excess ^{13}C DIC increased among treatments with glucose, as well as nutrients. Furthermore, CO_2 increased more in the treatment with glucose and nutrients. The excess ^{13}C DIC derived from respiration of the leachate, and evidence of the priming effect in these treatments (source here).

Increased CO_2 concentrations among the glucose and nutrient treatments from time zero indicate that the microbes are mineralizing carbon and respiring more than in the treatment without the added nutrients and carbon. Furthermore, we observed an increase in the production of ^{13}C CO_2 in the nutrient and glucose treatments as well. This indicates that much of this increased CO_2 came from the recalcitrant leachate.

Similar to other lab aquatic priming experiments, our results show increased CO_2 respiration and evidence of priming in treatments with added glucose as well as nutrients. Many other studies (Guenet et al., 2010; Bianchi et al., 2015) performed in lab settings provided evidence of positive priming effects, whereas other field-based experiments were more mixed with priming results (Danger et al., 2013; Guenet et al, 2014; Kuehn et al., 2014, Gontikaki et al., 2013).

However, other priming experiments used differing methods to calculate and infer observations of priming attributing it to breakdown of recalcitrant material, and few studies have used labeled, directly traceable recalcitrant carbon (Bianchi et al., 2015). In our experiment, we used a labeled ^{13}C recalcitrant

substrate to be able to directly observe the priming effect via excess ^{13}C DIC. The increases in CO_2 respiration, ^{13}C respiration, excess $^{13}CO_2$ DIC from the glucose, nutrients, and glucose plus nutrients treatments indicate clear observations of positive priming effect; there was further breakdown of the recalcitrant carbon with these treatments that were primed than the treatment with solely the lake water and leachate. In other words, the addition of glucose and nutrients increased the microbial capability to more efficiently decompose the recalcitrant (harder to break down) carbon.

The importance of being able to study aquatic priming effects and better understand carbon cycling is crucial to the carbon fluxes in river systems. Much of the terrestrially derived dissolved organic carbon that ends up in rivers is more recalcitrant, which is why it is important to study these interactions. Priming could explain why terrestrially-derived soil organic matter often builds up for decades, but when put into a river, it can decay quickly (Battin et al., 2008).

One of the main improvements that this experiment could use is its scale. To better understand the aquatic priming effect, further studies using labeled recalcitrant ^{13}C should be performed, on a larger spatial scale. As a first run using the ^{13}C leachate, there is promise for further studies to be done in this regard towards a better understanding of these aquatic priming effects. Another aspect of our experiment that is worth mentioning is the fact that it was all lab-based and lake water was used. By using lake water, we are merely using the microbes in the water as a proxy for microorganisms and respiration that would occur in a

river system. Gathering samples from rivers, such as rocks that include heterotrophic bacteria and microbes could be good to be able to better explore priming effects for a river ecosystem field setting.

This distinction of using microbes in the water versus studying in a true field experiment is important, because there are many other ecosystem factors in river systems to be taken into account, such as which types of carbon in river systems are best at leading to priming (algae driven by producing DOC, or driven by terrestrial-sourced labile carbon like leaves). Other important ecosystem knowledge that could be important to look into is seasonality of the priming effect. For example, if the priming is driven by algae, how does light availability in the different seasons impact the effect? If it is driven by other carbon sources such as leaves falling into rivers, then perhaps the priming effect would be higher in another season. While our study delves deeper into directly measuring priming effects, much more work is to be done in applying these concepts in studying natural aquatic ecosystems. There is great opportunity for next steps in further studies of priming effects in relation to aquatic ecosystem knowledge and the interactions of carbon cycling in the field, in a natural environment.

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