Decomposition and Production of Dissolved Organic Matter by Aquatic Bacteria

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Dedication

This dissertation is dedicated to my wife Mollie and my son Huxley. Mollie, your patience and unwavering commitment to our family is what made this all possible. Huxley, your curiosity and love of all that is novel in the world remind me why I'm a scientist. I hope that you grow up to experience all of the gifts our world has to offer.

Preface

Aquatic ecosystems transport large amounts of organic matter from the landscape to the oceans. Along this pathway, heterotrophic bacteria rapidly cycle these compounds by acting as both degraders and producers of organic compounds. Understanding the ultimate fate of the organic matter and predicting how increased organic matter exports from terrestrial ecosystems will impact its delivery to the ocean, requires a better understanding of the factors that influence organic matter degradation and production in freshwater systems. While many scientists have approached this problem by focusing on microbial modifications of carbon (C), much less attention has been paid to other major elements found in organic molecules (namely nitrogen (N) and phosphorus (P)). A more integrated approach that incorporates microbial processing of both C and major macronutrients such as N and P is needed to describe the biogeochemical transformations of organic matter in freshwaters.

In this dissertation I examine the degradation and production of dissolved organic matter by heterotrophic bacteria, specifically focusing on dissolved organic phosphorus (DOP). In chapter 1, I quantify the degradation rates and overall bioavailability of DOP across 27 unique aquatic systems and explore important environmental and chemical regulators of these rates. Data from these systems show that DOP degradation rates are spatially variable, but are typically as high or higher than rates of degradation for C. Also, the chemical composition of organic matter was an important predictor of DOP bioavailability with DOP bioavailability being lowest when DOP was scarce relative to C. This relationship means that DOP is degraded by bacteria in systems that are more likely to experience P limitation, suggesting that DOP may be an important source of P to bacteria in these systems. Chapter 1 concludes by documenting the importance of incorporating estimates of organic matter bioavailability into estimates of resource imbalance experienced by aquatic bacteria. Accounting for the bioavailability of organic matter generally reduces the estimates of nutrient imbalance experienced by aquatic bacteria compared to estimates using bulk nutrient concentrations. This reduction in imbalance would result in more efficient C cycling by aquatic bacteria, which has important implications for understanding the composition of organic matter exported downstream and ultimately to the ocean.

Chapter 2 goes on to explore the production of organic matter by heterotrophic bacteria. It is well documented in marine systems that bacteria can produce an immensely diverse set of organic molecules, even when they are only given a single carbon source to start from. However, the factors that control this production and the extent to which bacteria also produce DOP remains unclear. Previous work has shown that bacteria in freshwaters have different stoichiometric strategies for dealing with nutrient imbalance, with some strains of bacteria capable of changing the chemical composition of their cells to more closely match that of their resources. This flexibility in biomass nutrient composition has important implications for the recycling rates of multiple nutrients and therefore likely impacts the production of organic molecules by bacteria as well. Using previously isolated bacterial strains that have had their biomass flexibility quantified, I test the impact of these different stoichiometric strategies on the composition of the organic matter the strains produce. In this chapter, I show that bacteria produce measurable amounts of dissolved organic phosphorus, even under strongly phosphorus limited conditions. Overall, bacteria converted ~0.01%-10% of the phosphate in the original media to dissolved organic phosphorus, with the highest conversion efficiencies under carbon limited growth conditions. Interestingly, the conversion efficiency was higher under extreme phosphorus limitation than moderate phosphorus limitation. This pattern was driven primarily by relatively high conversion efficiencies by bacteria with flexible biomass stoichiometry in the most phosphorus limited conditions demonstrating the importance of physiological responses to nutrient imbalance. This chapter also explores the impact of bacterial biomass flexibility on the optical properties of the organic matter produced by bacteria. I show that biomass flexibility is significantly and positively related to the specific ultraviolet absorbance at a wavelength of 254 nm, a measure of the aromaticity of the organic matter, when grown under extreme phosphorus limitation. This suggested that bacteria with more flexible biomass stoichiometry produce more complex carbon molecules under strong phosphorus limitation than less flexible strains do. While more work is needed to fully understand how the physiological growth strategies of different microbial taxa impact the production of DOM, this chapter provides some important first insights into this question.

In the final chapter, I transition away from research on aquatic ecology into what I consider to be another fundamental aspect of being a scientist: training the next generation of scientific thinkers. Over the last decade, there has been a clear call to shift the instructional methods used for teaching undergraduate biology courses. We now know that active learning approaches to teaching science lead to better science outcomes for students. Furthermore, engaging undergraduate science students in undergraduate research experiences has been shown to have a number of important benefits for students such as increased student engagement, interest in science careers, and understanding of the scientific process. To offer the benefits of research experiences to a broader set of students, many institutions have started offering Course-based Undergraduate Research Experiences (CUREs) in laboratory classes for students. It is common for these laboratory sections to be primarily facilitated by undergraduate or graduate teaching assistants (TAs) rather than full-time faculty members. For these TAs to efficiently achieve the goals of these CUREs they must understand both (a) the philosophical underpinnings of discovery-based inquiry, and (b) strategies for facilitating inquiry, based on evidence-based practices, in the teaching laboratory. However, TAs are rarely trained in pedagogy, which likely limits their abilities to effectively facilitate inquiry in the laboratory. Chapter 3 is a case study documenting the results of a theoretically grounded professional development pilot program. This pilot program revealed that novice TAs are initially concerned primarily about the logistical aspects of teaching: classroom management, content preparation, grading assignments, etc. These concerns limit their readiness for engaging with the more complex pedagogical concepts of evidence-based instruction or inclusive teaching. This means that TA professional development needs to be designed to parallel the dynamic nature of TA concerns and that programing focused on advanced teaching techniques is only effective after TAs have established a sense of comfort and confidence in their own teaching.

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Chapter 1: Dissolved Organic Matter Degradation by Heterotrophic Bacteria

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Summary

Freshwater aquatic systems are biogeochemical hotspots, with heterotrophic bacteria rapidly cycling the compounds that pass through them. P is a key nutrient that controls primary production in many freshwater ecosystems and is important for understanding eutrophication in lakes. Previous work has often focused on the dynamics of inorganic phosphorus and its impact on primary production, however, the role of nutrients bound in more complex organic forms (such as dissolved organic phosphorus, DOP) in supporting primary production and harmful algal blooms has been neglected. Here, we quantify the bioavailability of dissolved organic carbon (DOC) and DOP in 27 aquatic systems across the Upper Midwest United States. Using exponential decay models, long-term nutrient degradation assays revealed that decay constants for DOP ranged from- 0.001 per day to -0.12 per day with a median value of -0.01 per day. These rates were geographically variable and were as high or higher than DOC decay constants, which ranged from -0.003 per day to -.024 per day with a median value of -0.01 per day. Additionally, total bioavailability of DOP ranged from 0% to 100% with a median value of 78% of the DOP pool, demonstrating that DOP bioavailability was highly variable across systems. In contrast, bioavailable DOC was more tightly constrained with values ranging from 4.37% to 53.81% of the total DOC pool with a median value of 24.95%. DOP bioavailability was strongly correlated with the DOC:DOP of the organic matter pool, suggesting that bioavailable DOP is drawn down in systems that are more likely to be P limited. Finally, we show that including estimates of DOC and DOP bioavailability reduces estimates of elemental imbalance experienced by aquatic bacteria.

Introduction

Freshwater systems are incredibly active biogeochemical hot spots, particularly with regards to the processing of organic matter (Cole et al. 2007; Tranvik et al. 2009). Heterotrophic bacteria are major biogeochemical players in aquatic systems (Cotner and Biddanda 2002) and understanding how these microbes interact with organic matter is fundamental to predicting the flow of energy and nutrients through freshwaters. Dissolved organic matter (DOM) is a major resource pool for aquatic bacteria and has been the focus of numerous studies over the last 20 years. The vast majority of this work has focused on understanding how microbial modifications of DOM influence global carbon cycle processes, but it is important to remember that DOM is not solely composed of carbon. Microbial interactions with DOM are also likely important for understanding other biogeochemical cycles in freshwater such as the phosphorus (P) cycle, but our understanding of the role DOM plays in these other nutrients cycles remains limited (Maranger et al. 2018).

Humans have had profound impacts on the global P cycle by increasing the annual flux of P through ecosystems by a factor of 4-8 (Falkowski 2000; Schlesinger and Bernhardt 2013). This has important biogeochemical implications and has resulted in the eutrophication of freshwater systems worldwide leading to degraded water quality on a global scale. Fundamentally, eutrophication is a biogeochemical imbalance, where excess nutrients, often P in freshwater (Schindler et al. 2008), result in excessive accumulation of carbon (C) in the form of increased algal biomass. This continued anthropogenic modification of freshwater nutrient and organic matter pools, together with observations of shifts in planktonic community composition and organic nutrient pools (Teubner et al. 2003) suggest that the bioavailability of organic nutrient pools could an important factor affecting auto-heterotrophic coupling as well as harmful algal blooms.

Previous work has often explored the role of a single inorganic nutrient controlling primary production, but our rapidly evolving understanding suggests more complex scenarios are likely involved. Specifically, there is a growing appreciation for the role of nutrients bound in complex organic forms (such as dissolved organic phosphorus, DOP) in acting as important resources for aquatic organisms (Cotner and Wetzel 1992; Jackson

and Williams 1985; Björkman and Karl 2003; Nausch and Nausch 2007; Soares et al. 2017). Therefore, it is imperative to understand the bioavailability of DOP in natural systems in order to better predict its capacity to serve as a resource in the absence of (or supplementary to) inorganic phosphorus. Furthermore, understanding the composition and bioavailability of nutrients and organic matter, not just the total quantities in a system has been shown to have important impacts on the formation and toxicity of harmful algal blooms (Anderson et al. 2002; Donald et al. 2011) providing another compelling reason to further study DOP bioavailability in freshwater systems.

Organic P is the dominant form of P in most freshwater systems with DOP typically comprising 25%-50% of the total P pool (Wetzel 2001). Many studies have clearly demonstrated that at least some forms of DOP can serve as a P source for primary and secondary producers (Cotner and Wetzel 1992; Björkman and Karl 2003; Nausch and Nausch 2007; Li and Brett 2013) and DOP is therefore likely an important source of P when inorganic P is limited. While there have been some studies on DOP bioavailability specific to freshwater systems (Sonzogni et al. 1982; Boström et al. 1988; Cotner and Wetzel 1992; Li and Brett 2013), more studies have examined this topic in marine systems (Björkman and Karl 1994; Ruttenberg and Dyhrman 2005; Dyhrman and Ruttenberg 2006; Nausch and Nausch 2006, 2007). Nonetheless, in both marine and freshwaters, a portion of the DOP pool is readily available for assimilation into planktonic organisms. The relative bioavailability of specific DOP compounds can range from almost 0% to over 90% (Li and Brett 2013). While these studies have clearly suggested that DOP may be an important source of P for aquatic microorganisms, few studies have quantified bioavailable DOP in freshwater systems or examined how organic matter stoichiometry may affect the bioavailability of DOP compounds. In this paper, we present results from DOM bioavailability assays from 27 aquatic systems in the Upper Midwest of the USA. Our goal was to quantify the bioavailability of DOC and DOP from a diverse set of aquatic ecosystems and explore the potential environmental drivers of DOM bioavailability.

Materials and Methods

Study Sites and Sample Collection

During the summer season (June-September) water was collected from 27 fresh-water systems (24 lakes and 3 streams) in Minnesota and South Dakota (Appendix A Figure 1) from the upper mixed layer (0-2 m of depth) using a Van Dorn water sampler. The 27 systems covered three different Level III ecoregions as defined by the United States Environmental Protection Agency: The Black Hills in Western South Dakota (Middle Rockies), Itasca State Park in North Minnesota (Northern Lakes and Forests), and the Twin Cities greater metropolitan area (North Central Hardwood Forests). The Black Hills and Itasca State Park systems are relatively pristine systems with watersheds primarily dominated by coniferous forest whereas the Twin Cities greater metropolitan area is highly human impacted with watersheds dominated by hardwoods with urban land use or small-scale agriculture. General characteristics of each lake system can be found in **Table 1**.

Samples were collected in acid-washed HDPE amber bottles after pre-rinsing them with ~100 ml of sample water. Samples were stored on ice until they could be returned to the lab (always less than 4 hours), where they were stored at 4ºC until processed (<72 hours). For processing, ~100 ml of water was filtered through pre-combusted 0.7 µm nominal pore-size glass-fiber filters (Whatman, GF/F) and collected in pre-combusted borosilicate vials. Twenty ml of this sample was acidified with 10% HCL and used to measure total dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) on a Shimadzu TOC-L auto-analyzer with a TNM-L module (CSH/CSN model, Shimadzu Corp). Another 20 ml of filtrate was reserved for absorbance scans (wavelengths from 200-800 nm) using a Cary 50 spectrophotometer, which was used to calculate the specific UV absorbance at wavelength of 254 nm (SUVA). The remaining 60 ml of sample was used to measure total dissolved phosphorus (TDP), and soluble reactive phosphorus (SRP) using a molybdenum blue reaction with and without acid-persulfate digestion (Murphy and Riley 1962). DOP was calculated as the difference between TDP and SRP. Additionally, the GF/F filters were collected for fluorometric quantification of chlorophyll-a after being extracted in 90% acetone (Standard Methods 2005).

DOM Degradation Assays

DOM degradation assays were performed as long-term dark bottle incubations. Within 72 hours of collecting each sample, 900 ml of lake water was filter-sterilized using a 0.22 μm pore-size filter (EMD Millipore Steritop Filters) and collected in a pre-combusted 1 L amber glass bottle. Each bottle was inoculated with 100 ml of water from the same lake that had been filtered through a 1.6 μm pore-size glass fiber filter (Whatman, GF/A). While this approach did not standardize for the absolute inoculum size (i.e., number of bacterial cells) it did provided a consistent relative inoculum source for each assay of 10% by volume, similar to previous work (Wiegner et al. 2006; Lønborg et al. 2009b; Vonk et al. 2015). Furthermore, the relative size of the inoculum has been shown to have little to no effect on the overall degradation of DOC (Vonk et al. 2015). These bottles were incubated in the dark at 20ºC for a minimum of 230 days. Periodically throughout the incubation period (approximately monthly for the first 6 months and less often subsequently), 100 ml samples were removed from the incubations. These samples were filtered using a pre-combusted GF/F filters and DOC, TDN, DOP, SRP, and absorbance scans were measured as described above.

Data Analysis

To calculate DOM degradation rates, data were fitted to 3 unique models (linear, 2 component exponential, and 3 component exponential) and the model fit was compared using Akaike information criterion (AIC) scores. Because model fits were variable for different portions of the DOM pool, relative bioavailability of DOC and DOP was calculated by dividing the maximum nutrient loss (initial concentration minus the minimum measured concentration during the incubation period) by the starting concentration to obtain the percentage of the total pool that was degraded. This approach allowed for the direct comparison of relative lability between the DOP and DOC pools. The relationships between bioavailability and environmental parameters (such as SUVA, nutrients, etc.) were examined using simple linear regression. All analysis was performed using JMP® version 13 (SAS Institute Inc., Cary, NC, 1989-2007).

Results

Bulk Nutrient Analysis

The 27 systems studied covered a trophic gradient with chlorophyll-a values ranging from 0.25 µg/L to 57.19 µg/L and total dissolved phosphorus concentrations ranging from 0.07 µM to 2.31 µM (Table 1). Mean chlorophyll levels were much higher in the Twin Cities region compared to the two less human-impacted regions and chlorophyll values were also more variable in the Twin Cities compared to the other two regions. SUVA values were calculated for each system by dividing the specific UV absorbance of wavelength of 254 nm by the DOC concentration of the system. SUVA can be used as an index of the terrestrial contribution to the DOM pool, with higher SUVA values indicating more terrestrial influence. We sampled systems that exhibited a range of SUVA values (from 0.86 to 3.69) to cover a gradient of terrestrial influence on the DOM pool. Across the three regions sampled, chlorophyll and the relative contribution of DOC to the total dissolved carbon pool (DOC:TDC) showed strong differences, but DOP concentration and the relative contribution of DOP to TDP did not (Figure 1). Also, our study sites showed no regional differences in TDP or SRP concentrations, despite the fact that others have shown strong differences in total P concentrations across these ecoregions (Heiskary et al. 1987). Furthermore, DOP concentration was not significantly correlated with any of the measured lake characteristics (pH, alkalinity, chlorophyll, or SUVA), but was weakly positively correlated with DOC concentration and TDN concentration (Figure 2). Additionally, the Twin Cities dissolved carbon pool had a much higher fractional total organic carbon signature (with a median value of approximately 40% DOC) compared to the Black Hills and Itasca where DOC contributed less (11% and 18.5% respectively) to the dissolved carbon pool (Figure 1).

Degradation Rates of DOC and DOP

DOC degradation was best fit by an exponential decay model with a non-zero asymptote (equation 1, model resulted in an R2 value of 0.998 across all lakes; Appendix A Figure 2). Two lakes, Canyon Lake and Roubaix (both from the Black Hills region), resulted in model fits that had positive k values, despite both having lost DOC over the course of the incubation so they were excluded from analysis of DOC decay rates. In the

remaining 25 lakes, k values ranged from -0.003 to -0.024 per day with a median value of -0.009 and quartiles of -0.006 and -0.013 or median turnover time of 111 days (**Figure 3**). DOC degradation rates were not significantly correlated to measured elemental pools (DOC, TDN, TDP, SRP) or lake characteristics (pH, chlorophyll, SUVA) and there were no significant difference in DOC decay rates across region.

$$DOC_t = BDOC^{kt} + DOC_R$$

Eq. 1: Three parameter exponential decay model that was the best fit for long term DOC incubations were DOC_t is the concentration of DOC at time t, BDOC is the total pool of bioavailable DOC, k is the degradation rate, t is the time of incubation in days, and DOC_R is the size of the recalcitrant DOC pool.

In contrast, TDN dynamics were best fit by a simple linear model, but Fish Lake and Cedar Bog Lake (Twin Cities region) were the only two lakes that had slopes significantly different from zero. Both of these lakes showed significantly positive linear slopes, indicating an accumulation of TDN over the course of the long-term incubations. Given that degradation models had either non-significant degradation or positive accumulation, it was not possible to calculate TDN degradation rates or turnover times from this dataset. It is also important to note that TDN contains both organic and inorganic N, so it wouldn't be expected to follow the same patterns as DOC and DOP.

TDP, SRP, and DOP incubations revealed turnover times of approximately 150 days. After this period, DOP concentrations tended to increase in the incubations suggesting internal generation of DOP (see supplemental Figure 3). Given that there were no external sources of P to these incubations, this increasing concentration of dissolved P likely resulted from the degradation of particulate P that had accumulated over the early portion of the incubation. To examine the degradation of DOP over the course of the incubation, we excluded all data points after 150 days of incubation and excluded points when the calculated DOP concentration was below zero (this only occurred in 6 of the 230 total measurements). A 2-parameter exponential fit model best described the DOP data (the

same model as equation 1, omitting the recalcitrant pool) resulting in a model with an overall r2 value of 0.77.

DOP degradation rates (k) across the 27 lakes largely fell between 0 and -0.025 per day (22 of the 27 lakes). Three lakes had positive modeled k values with two of these systems in the Twin Cities metro area (Fish and Staring North) and one was in the Black Hills (Pactola, see supplementary data file). Because the estimated k values were positive despite the fact that concentrations of DOP decreased in the incubations, these lakes were excluded from other analysis of DOP degradation rates. Additionally, two lakes form the Itasca region (Boot and Elk) had extreme negative k values of -0.096 and -0.123 respectively (Figure 3). The median value for all 27 systems was -0.010 corresponding to a median turnover time of 100 days. DOP degradation rates were not significantly correlated to measured elemental pools (DOC, TDN, TDP, SRP) or lake characteristics (pH, chlorophyll, SUVA).

To compare the relative rate of DOP turnover to DOC turnover, we calculated a kDOC:kDOP value for the 22 systems that had negative k values for both DOC and DOP. Overall, turnover rates of the two nutrients were remarkably similar with a median kDOC:kDOP of 0.98, upper quartile of 1.83, and lower quartile of 0.50. However, there were several lakes with more extreme values with the most extreme system having a DOP turnover rate nearly 20 times faster than DOC (Elk Lake in Itasca State Park). The Itasca region did have significantly lower kDOC:kDOP values than the other two regions (Figure 4, Chi Square Median test, p= 0.0297). The Itasca region also had a typical kDOC:kDOP value less than 1 (Wilcoxon signed-rank, p=0.014), indicating that for this region DOP degradation constants were significantly higher than DOC degradation constants.

Estimates of DOC and DOP Bioavailability

It took approximately 9 months for degradation models to give reasonable predictions for the total size of the recalcitrant DOC pool (i.e., the 3 parameter fit models outperformed the 2 parameter fit models). Prior to 9 months, 2 parameter fit models outperformed the 3 parameter models so a clear asymptote was not indefinable. In contrast, for DOP, the 2-parameter fit model was always a better fit than 3 parameter fit model, so a

modeled estimate of the recalcitrant DOP concentration was not possible. Therefore, in order to estimate and compare the relative sizes of the bioavailable pools of DOC and DOP, we calculated these values using the lowest measured value of DOC and DOP over the course of the incubation. By subtracting this lowest value from the starting concentration, we estimated the amount of DOC or DOP that had been degraded during the incubation period and used this as an estimator of the size of the BDOC and BDOP pools. BDOC values in these lakes ranged from ~19 μM to ~397 μM with a median value of 118 μM, and relative BDOC values ranged from 4.4% to 53.8% of the total DOC pool with a median value of 25.0% with no regional differences in BDOC. In contrast, a much larger portion of the DOP pool tended to be bioavailable. Eight systems had BDOP values over 95% of the total DOP pool and the median value for all the lakes was 78%. Relative BDOP was also more variable than BDOC and had an interquartile range of 40.8% to 97.5% compared to 21.0% to 30.6% for BDOC (Figure 5). Absolute values for BDOP concentrations ranged from 0.01 μM to 0.82 μM and three-quarters of the samples had BDOP concentrations below 0.26 μM. As with BDOC, there were no significant regional difference in BDOP.

In these systems, relative %BDOP was positively correlated to the initial concentration of DOP in the system, suggesting that systems with a larger DOP pools not only had more BDOP, but also had a larger fraction of the DOP pool that was bioavailable (Figure 6, $p=0.0043$). Additionally, relative BDOP was negatively correlated to the initial DOC:DOP ratio, indicating that DOP was less bioavailable when it was scarce relative to DOC (Figure 7, $p=0.0002$). However, relative BDOP was not significantly correlated to other individual element pools (DOC, TDN, DIC) nor was it significantly correlated to any of the lake characteristics measured (pH, alkalinity, chlorophyll or SUVA). Temperature data was only available for the 10 lakes in the Itasca region, but within this subset of the data temperature, was not a significant predictor of BDOP concentration or relative BDOP percentage. Absolute concentrations of BDOP also showed a strong negative correlation with the DOC:DOP ratio, suggesting that BDOP was being drawn down at high DOC:DOP ratios (Figure 8), but absolute BDOP was not significantly correlated to other elemental or lake characteristic measurements.

Relative %BDOC was not significantly correlated with any of the measured elemental parameters (initial DOC, TDN, TDP, SRP, DOP or DOC:TDN, DOC:TDP, DOC:SRP, or DOC:DOP). Interestingly, relative %BDOC was also not significantly correlated to SUVA but the absolute size of the BDOC pool did show a significant positive correlation with SUVA (Figure 9; $p= 0.0217$). This positive trend with SUVA is likely partially explained by the fact that high SUVA systems tend to be high in DOC (and the absolute amount of BDOC was strongly positively correlated to DOC concentration), but in combination with the fact that relative BDOC did not change with SUVA, this suggests that even systems dominated by more aromatic organic matter contain large amounts of bioavailable DOC. Furthermore, absolute BDOC concentration was strongly positively correlated to TDN, TDP, and chlorophyll (Figure 10, $p<0.0001$) consistent with the accumulation of labile DOC under high nutrient conditions and high productivity.

Stoichiometry of Bioavailable Nutrients

Overall, ratios of bioavailable C and P were much lower than the bulk chemistry pools. DOC:TDP ratios ranged from 319-7122:1 with a median of 1595:1 while DOC:DOP ratios ranged from 679-15,360:1 with a median of 2449:1 in the systems we examined. In comparison, BDOC:BTDP ranged from 133-8848:1 (this high point was an outlier with the next highest value being 2943) and median value of 746:1. BDOC:BDOP ranged from 144-9719:1 with a median value of 843:1. Previous work showed that assemblages of aquatic heterotrophic bacteria in lakes have mean biomass C:P ratios around 102:1 (Cotner et al. 2010), while individual strains can have highly variable biomass composition with values well over 1000:1 (Godwin and Cotner 2015a). Therefore, the stoichiometry of the bioavailable nutrients measured in this study more closely match typical bacterial biomass stoichiometry than measures of bulk nutrient chemistry. Bioavailable nutrient stoichiometry was also positively correlated with bulk nutrient stoichiometry (Figure 11, $p <0.0001$ and Figure 6, $p<0.0001$).

To better understand the relative changes in the DOC and DOP pools over the incubation periods, we also examined how DOC:DOP ratios changed over the first 150 days of the incubation. We choose the first 150 days because this was the active degrada-

tion period for DOP (see above) and we were most interested in how stoichiometry changed during DOM degradation. We fit simple regression functions at each lake to determine if there was an increase or a decrease in DOC:DOP throughout the incubations. Out of our 27 systems studied, 20 of them had positive slope parameters, indicating that DOC:DOP generally increased during incubation. However, the relationship between DOC:DOP and incubation time was generally weak and only 1 lake (Boot) show a statistically significant relationship.

Discussion

The data on degradation and bioavailability of DOC and DOP provide insights into three important areas. First, across all lakes, median BDOC and BDOP turnover times were approximately equal for both BDOC and BDOP (~100 days), but in Itasca State Park lakes, BDOP turnover was significantly faster than BDOC (in some cases by as much as 20 times). This spatial variability highlights the need for more empirical measurements of DOP degradation rates from a variety of systems to better understand potential spatial patterns. In our study, degradation rates of DOC and DOP could not be explained by the other elemental pool sizes or environmental characteristics, further emphasizing the need for more work in this area. Second, we show that the portion of the DOP pool that is bioavailable is extremely variable across systems, but often exceeds 50% and is strongly related to the DOC:DOP ratio of the system. BDOP was drawn down in systems with high DOC:DOP ratios (where P is more likely limiting) and therefore it is likely that in many systems, DOP represents a high-quality P resource to supplement inorganic P availability. Thirdly, the bioavailability of the DOM pool suggests that the nutrient stoichiometry of available resources in aquatic systems may be more similar to the biomass demands of aquatic microbes than previously thought. This has important implications for understanding the experienced nutrient imbalance by heterotrophic bacteria and in turn, understanding how bacteria couple multiple elemental cycles in aquatic systems.

Degradation Rates of DOC and DOP

Degradation rates of DOC in freshwater systems have been the topic a numerous papers and a recently published meta-analysis showed that DOC decay rates can vary by several orders of magnitude across different systems (Catalán et al. 2016a). Their dataset included 33 bioassay measurements from lakes in a similar climatic region to our study and these 33 lakes had a median k_{DOC} value of 0.0021, the same order of magnitude as our median value of 0.0077. Estimates of DOP degradation rates are scarcer in the literature and are dominated by estimates from marine systems. One study in the Baltic Sea estimated DOP turnover times to be between 3 to 4 days, about twice as fast as the shortest turnover time in our data set (Nausch and Nausch 2006). In contrast, DOP turnover times from the North and South Atlantic Ocean subtropical gyres were 5.5 months and 10.5 years respectively (Mather et al. 2008). Another study from Station ALOHA, in the North Pacific Subtropical Gyre found that DOP turnover time increased with depth and ranged from 12 to 268 days at a single sampling site (Björkman and Karl 2003). One major difference between these systems that could explain the differences in turnover times is productivity, with the Baltic being highly productive compared to Station ALOHA. However, DOP degradation rates were not significantly correlated to chlorophyll levels in our study, so within our systems, productivity was not a good predictor of DOP turnover. The variability of literature measurements, along with the variability in DOP turnover times in this study, highlight the need for more direct measurements of DOP degradation across a variety of systems to better constrain typical DOP degradation dynamics and to better understand the factors controlling them.

Here, it is also important to consider the difference in model fits between the DOC and DOP degradation curves. DOC degradation was incredibly consistent across systems, allowing for the degradation models to very accurately estimate the recalcitrant portion of the DOC pool and estimate of degradation rate (Appendix A Figure 2). However, in the case of DOP there was more variation in degradation pattern across system and the proximately of many of the DOP measurements to a non-zero asymptote made it difficult to use the degradation model to accurately estimate a refractory DOP pool (Appendix A Figure 3). Nonetheless, we elected to use a two-parameter decay model to esti-

mate DOP degradation rates because the model provided a reasonable overall fit (R^2-0.77, Appendix A, Figure 3) and gave rates that could be directly compared to the DOC estimates. This approach does by definition infer that a refractory DOP pool is not present (i.e. 100% bioavailability), and tends to homogenize the overall DOP degradation patterns. A closer view of the DOP degradation plots (Appendix A Figure 3) demonstrated at least three different general patterns for DOP degradation: 1) DOP is rapidly degraded to a zero intercept, 2) DOP is slowly degraded to a zero intercept, or 3) DOP degrades slowly to a non-zero intercept but a zero intercept is inferred by the fit model. This third case would suggest that there is in fact a refractory DOP pool in these systems and this observation further supports our use of using measured concentrations differences rather than DOP degradation models to calculated the size of the bioavailable DOP pool in our systems.

Bioavailability of DOC and DOP

Our measurements of the relative bioavailability of DOC are well within the range measured in other aquatic systems (Sondergaard and Middelboe 1995; Stets and Cotner 2008; Catalán et al. 2015; Helton et al. 2015; Frey et al. 2016). All but one of the systems we measured had BDOC values less than 50% of the total DOC pool, further supporting the idea that the bulk portion of DOC in freshwater is recalcitrant. However, these relative BDOC measures are quite high compared to marine systems, suggesting that exports to freshwater represent a younger, more labile carbon source than those found in marine systems. Furthermore, absolute BDOC concentrations were positively related to TDN, TDP, and chlorophyll concentrations suggesting that nutrient availability is an important control on the accumulation of BDOC. This finding contrasts with previous work that showed no significant correlation between BDOC and nutrient conditions (Stets and Cotner 2008); however, it should be noted that Stets and Cotner also measured positive correlations between BDOC and both TDP and chlorophyll concentrations but the relationships were not statistically significant in the twelve lakes they studied. Nonetheless, our data also suggest that stoichiometry may be an additional constraint to BDOC accumulation. The fact that the slope in Fig. 11B was more than 1 suggested that BDOC was accumulating disproportionately when DOC:DOP ratios were highest.

It was interesting to note that while the amount of BDOC was positively correlated to SUVA, the relative lability was not (Figure 9). SUVA has been shown to be highly correlated with the aromaticity of the DOM pool (Weishaar et al. 2003), so this pattern suggests that increasing aromaticity of the DOM pool does not significantly decrease its bioavailability. Furthermore, SUVA is considered a useful proxy for terrigenous organic matter with higher SUVA systems receiving large terrigenous inputs. SUVA was not strongly correlated to relative or absolute concentrations of BDOP, so we suggest that terrigenous inputs in our study systems represent a labile source of organic carbon but not organic phosphorus.

As with DOP degradation kinetics, estimates of the relative pool size of BDOP are sparse. However, the values reported in the literature are in good agreement with the values we measured here. In a Baltic Sea study, the DOP pool was 75% bioavailable (Stepanauskas et al. 2002) and a similar value (33.2%-60%) was reported for 3 stations in the central Baltic as part of a different study (Nausch and Nausch 2007). A more recent analysis suggested that ~40% of the DOP in four boreal lakes was bioavailable (Soares et al. 2017). Our study of 27 unique systems supports the idea of very labile BDOP with a median value of ~78%, but also highlights the large amount of variability in BDOP across systems. It should be mentioned, however, that our incubations lasted much longer than these other studies (150 days compared to ~7 days), which should have resulted in higher estimates of BDOP as our incubations would capture both rapidly degrading and slowly degrading DOP compounds. The fact that many of our incubations continued to show DOP losses up until 150 days into the incubations (Appendix A Figure 3) demonstrates the need for longer term incubations to fully describe the BDOP pool. On the other hand, our first sampling period occurred after ~30 days of incubation, which limited our ability to describe the degradation rates of the fastest degrading DOP pool. Given the rates of degradation documented in the literature and also the fact than many of our incubations showed major losses of DOP within the first 30 days, a stratified sampling method with high frequency measurements over the first few weeks and then less frequent measurements over several months may provide the best overall picture of DOP degradation.

Our findings also suggest that organic matter stoichiometry is an important control on the bioavailability of DOP. We found that the DOC:DOP ratio was a significant predictor of both absolute and relative BDOP (Figure 7 and Figure 8), with higher DOC:DOP ratios correlated to lower BDOP concentrations and percentages and a higher BDOC:BDOP ratio relative to the DOC:DOP pool (Fig.11). These patterns suggest that relative size of the BDOP pool decreased when the DOP was small relative to DOC. Presumably when the DOC pool size was large relative to DOP, microbes were more likely to be P-limited and consumed any bioavailable P. In lakes with lower DOC:DOP ratios, the organic matter pool would more closely resemble the biomass requirements of aquatic bacteria and/or the microbes are more likely to be limited by organic C rather than P.

Stoichiometry of Bioavailable Nutrients

Ecological stoichiometry provides a guide for predicting the cycling of multiple nutrients by examining the elemental balance between organisms and their resources. However, a fundamental problem associated with understanding these imbalances is our capacity to know what the resource availability is that organisms actually experience. Our ability to accurately describe the resource imbalance experienced by bacterial communities in situ is hindered by our lack of simultaneous measurements of the bioavailability of multiple elements (Berggren et al. 2014; Soares et al. 2017). Here, we observed that the experienced BDOC:BDOP resource ratios of aquatic bacterial communities were typically less than measured DOC:DOP pools (Figure 11). Therefore, bulk chemical measurements likely overestimate the size of the labile DOC pool or underestimate the size of the DOP pool. The fact that our measurements of BDOP indicated that large fractions of the DOP pool could be bioavailable while there clearly was a non-labile pool of DOC suggests that DOC measurements overestimate the BDOC pool, which many other studies have observed. Nonetheless, previous measurements of imbalance using bulk chemistry data likely overestimate the actual imbalance experienced by these communities particularly in more carbon-limited systems. Furthermore, our results suggest that it is at lower C:P ratios, i.e., more eutrophic systems, where chemical measurements of DOC:DOP are likely to overestimate the bioavailable pool of DOC. Although there is more BDOC being produced in these systems, the microbial biomass is more likely to be

limited by the availability of organic carbon, resulting in more drawdown and an increased proportion of the DOC pool being recalcitrant. This has important implications for understanding how bacteria couple C and P cycles in freshwater because the experienced imbalance between consumer and resources governs the differential recycling of those nutrients. If heterotrophic bacteria in aquatic systems experience a more balanced resource pool in terms of the C:P ratio than previously thought, this should result in more efficient C cycling as compared to predictions based on bulk chemistry ratios.

Conclusions

The 27 aquatic systems examined in this study demonstrate that DOP bioavailability was quite variable across systems but was strongly predicted by the DOC:DOP ratio of the system. The bioavailability of DOC was more tightly constrained due to an increased proportion of recalcitrant material relative to DOP and it was not predicted by organic pool stoichiometry but rather was strongly related to nutrient conditions (both TDN and TDP concentration). Exponential decay models fit the loss of DOC tightly, but were not as strong of a fit for DOP. Given that DOP turnover times were calculated using an exponential model with no asymptote (and therefore assuming 100% bioavailable DOP in all samples) our estimates for turnover times are likely skewed high, particularly for systems with sizeable recalcitrant DOP pools. Despite these potential limitations, our data suggest that DOP turnover time was significantly faster than DOC turnover in the Itasca region (in one case, ~20 times faster), but DOC and DOP had similar rates of turnover in the other two regions. This suggests that overall DOP in freshwater systems is turning over as quickly or more quickly than DOC. More measurements are needed in order to properly assess this spatial variability and determine if there are any broader geographic patterns in the relative turnover rates of DOC and DOP in freshwaters, particularly because variability in DOC and DOP degradations rates could not be explained by inorganic nutrient pool sizes or lake characteristics measured in this study.

Furthermore, we have shown that organic matter stoichiometry is an important control on the accumulation of bioavailable DOP in aquatic systems. Relative bioavailability of DOP was positively related to the concentration of DOP in the sample and negatively correlated to the initial DOC:DOP ratio, suggesting that DOP accumulates in sys-

tems that are less P-limited. In contrast, the initial organic matter stoichiometry was not predictive of relative or absolute BDOC. Instead, BDOC accumulation was associated with high nutrients (TDN and TDP) and high production (chlorophyll). Absolute BDOC was also strongly correlated to SUVA values, providing evidence that terrestrial subsidies represent a labile source of DOC in the systems studied. Finally, incorporating measures of nutrient bioavailability decreased the predicted nutrient imbalance experienced by heterotrophic bacteria in aquatic systems, which has important implications for understanding the coupling of C and P biogeochemical cycles.

Figure and Tables

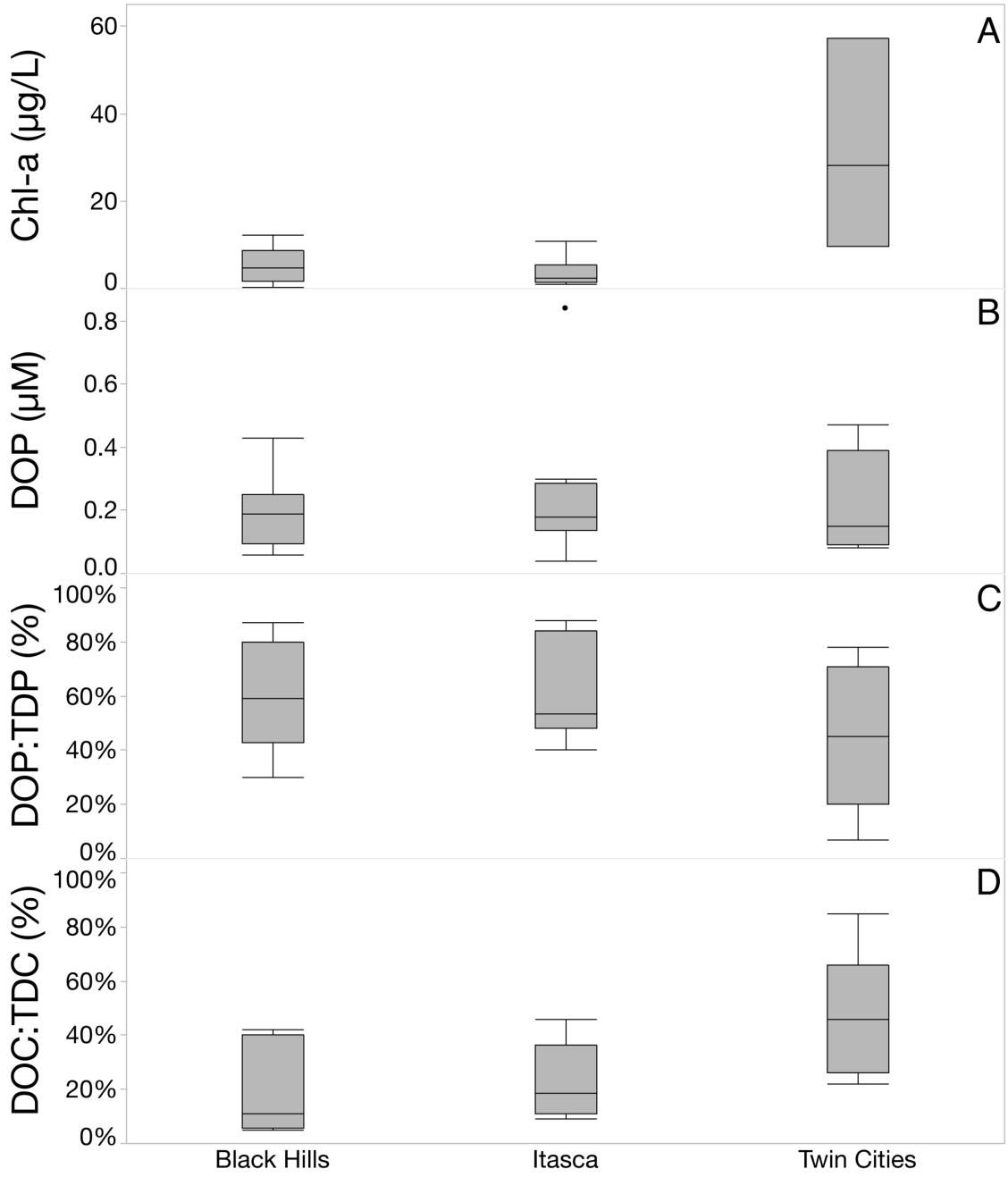

Figure 1-1: Regional variability in measured lake characteristics.

Panel A shows that chlorophyll concentrations were highest and most variable in the Twin Cities (urban) region. Panel B shows no significant differences in total DOP concentration across the three study regions and panel C shows no significant differences in

the relative contribution of DOP to the total dissolved P pool, however the Twin Cities does show the largest range of relative DOP contribution. Panel D shows the contribution of DOC to the total dissolved carbon pool with the Twin Cities showing a much higher contribution of DOC compared to the other two regions, in other words, inorganic carbon dominates the dissolved carbon pool in the Black Hills and Itasca regions.

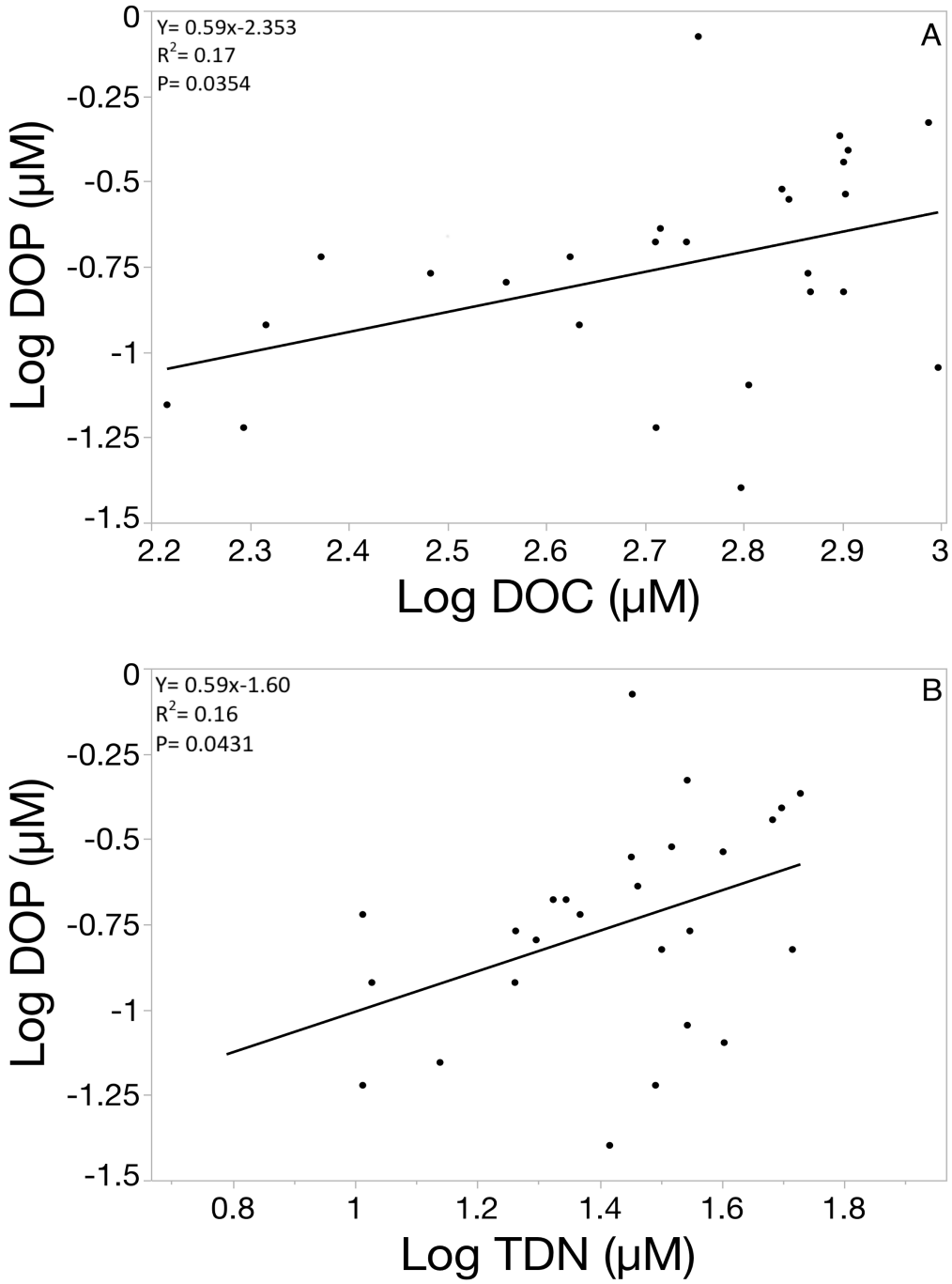

Figure 1-2: Scatterplots showing the relationship between DOP and DOC (A) and between DOP and TDN (B).

All values are log transformed. DOP shows a significant positive correlation to both DOC and TDN, but the relationship is weak in both cases with R^2 values below 0.2 for both regressions.

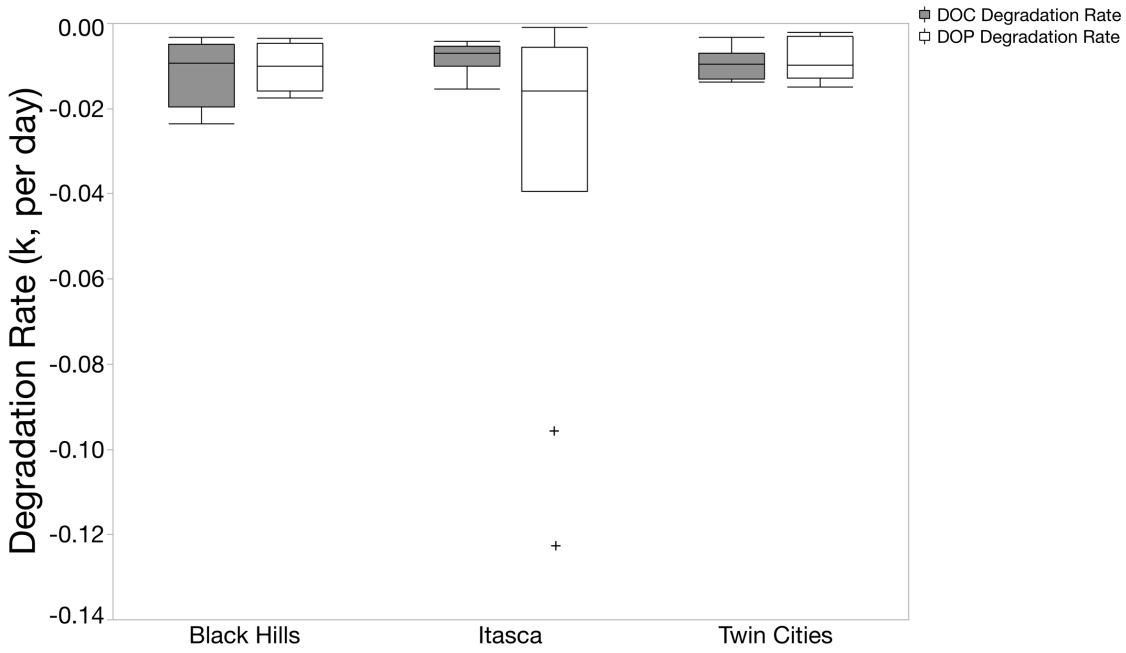

Figure 1-3: Box and whisker plots of DOC and DOP degradation constants (k) from exponential decay models.

Only lakes that had negative k values are included in this figure. In the Black Hills and Twin Cities regions, k values are very similar for DOC and DOP. However, DOP k values are more negative (meaning faster degradation) than DOC k values in the Itasca region. For figure including positive k values, see supplemental materials figure 1.

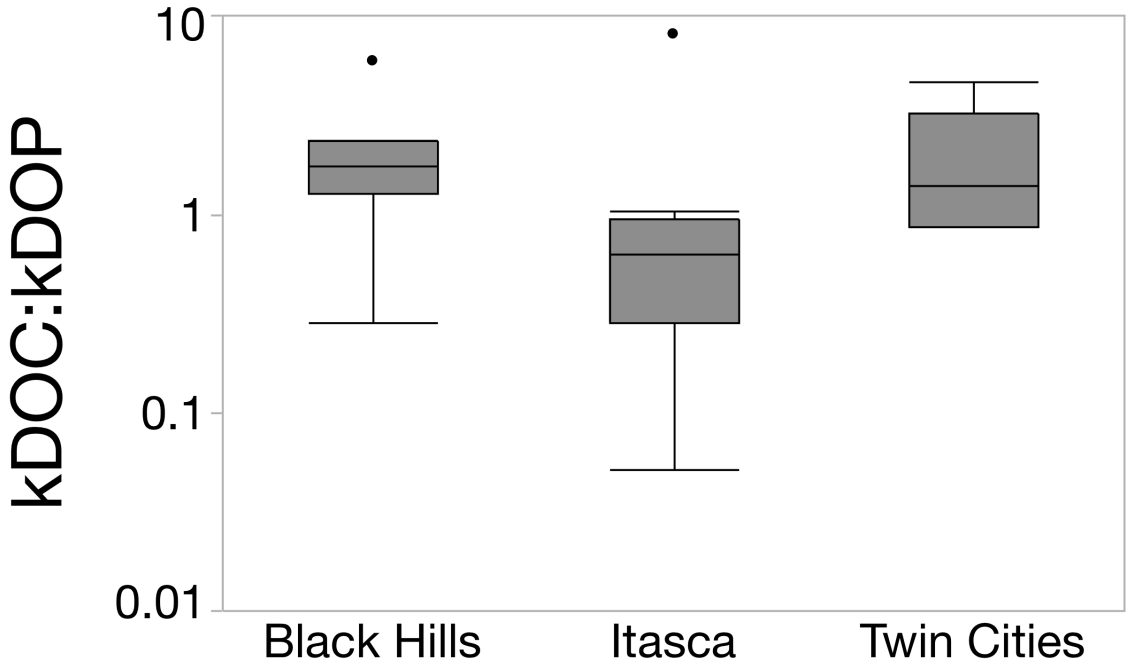

Figure 1-4: Box and whisker plot showing the variability in kDOC:kDOP values across the three study regions.

The Itasca region had a significantly lower value than the other two regions (Chi Square Median test, p= 0.0297) meaning DOP turnover was faster relative to DOC turnover in the Itasca region compared to the Black Hills and Twin Cities. The majority of samples form the Itasca region also had values less than 1, indicating that DOP was typically turning over faster than DOC in this region.

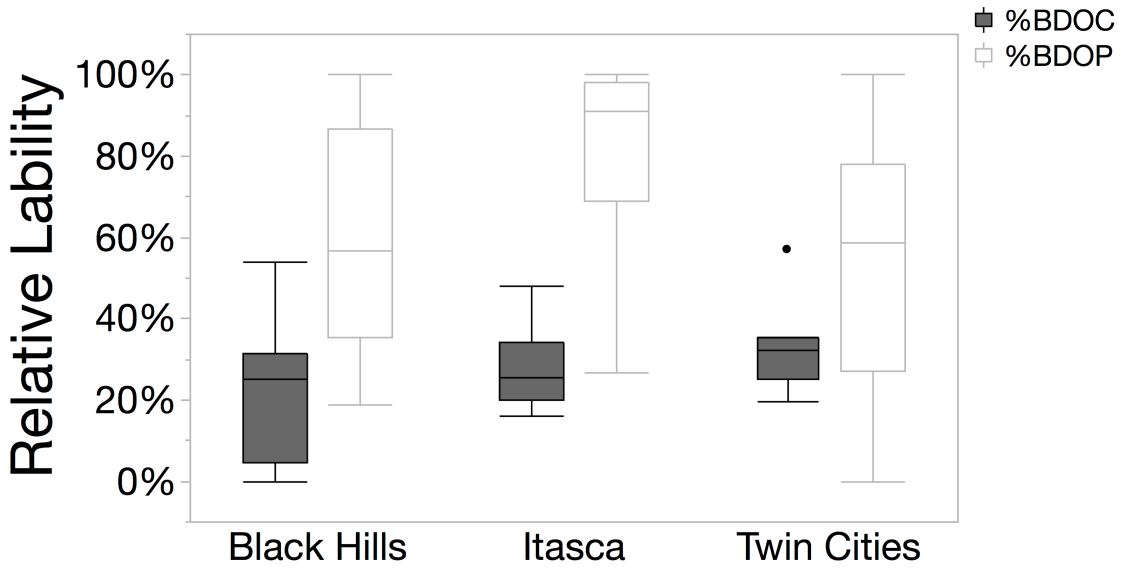

Figure 1-5: Box and whisker plots showing the relative lability of DOC and DOP in the three regions studied.

Relative %BDOP was higher than relative %BDOC in all three regions (pairwise t-tests, $p<0.01$). Relative %BDOP also showed a much larger range of values compared to relative %BDOC.

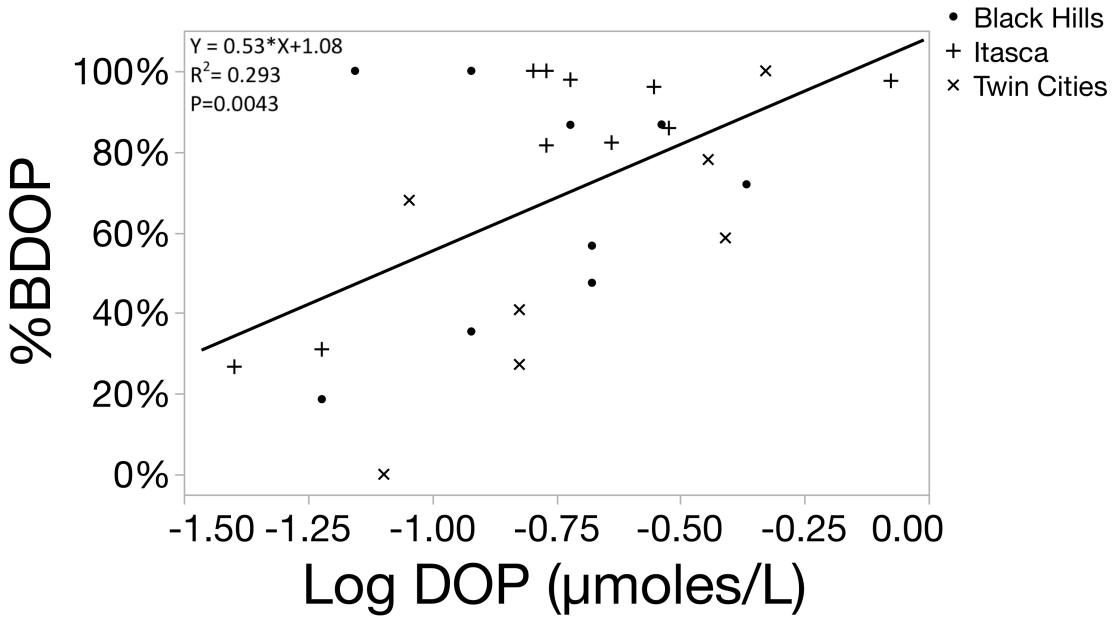

Figure 1-6. Scatterplot showing the linear regression between the %BDOP and the original concentration of DOP in the sample (log transformed) for 26 lakes.

One lake had to be excluded because the initial SRP concentration was below the method detection limit, so a DOP concentration could not be calculated. A positive relationship shows that as DOP concentration increased among systems, the relative lability of DOP increased.

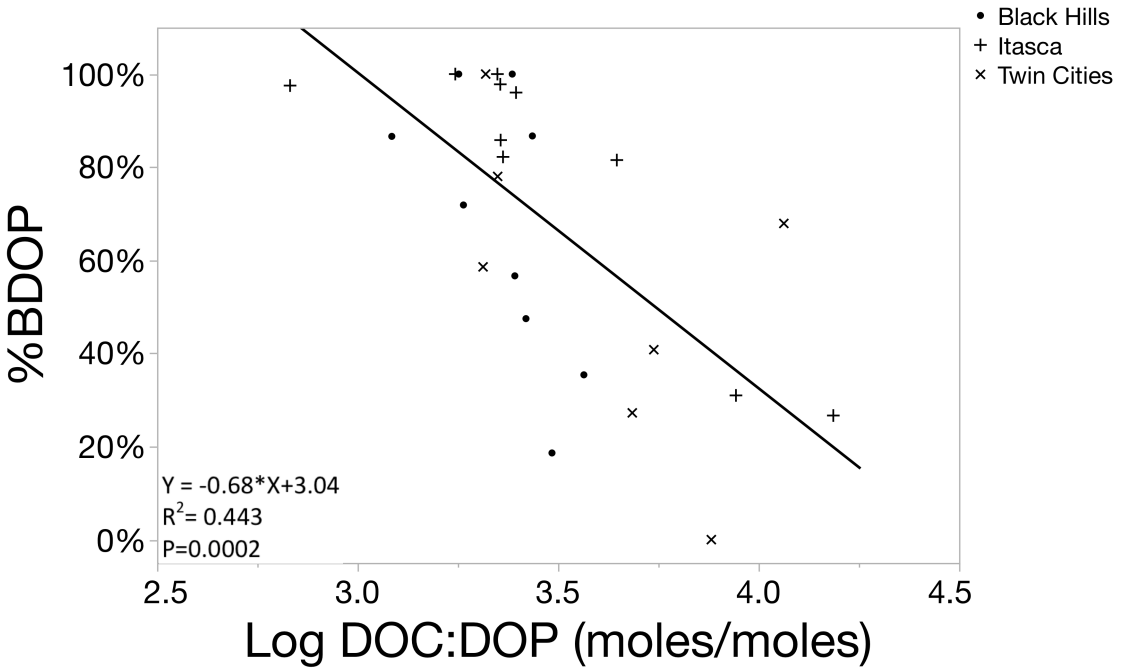

Figure 1-7. Scatterplot showing the linear regression function comparing the percentage of BDOP to the DOC:DOP ratio of the initial sample (log transformed). The significant negative relationship demonstrates that the relative bioavailability of the DOP pool decreases as DOP becomes scarce relative to DOC.

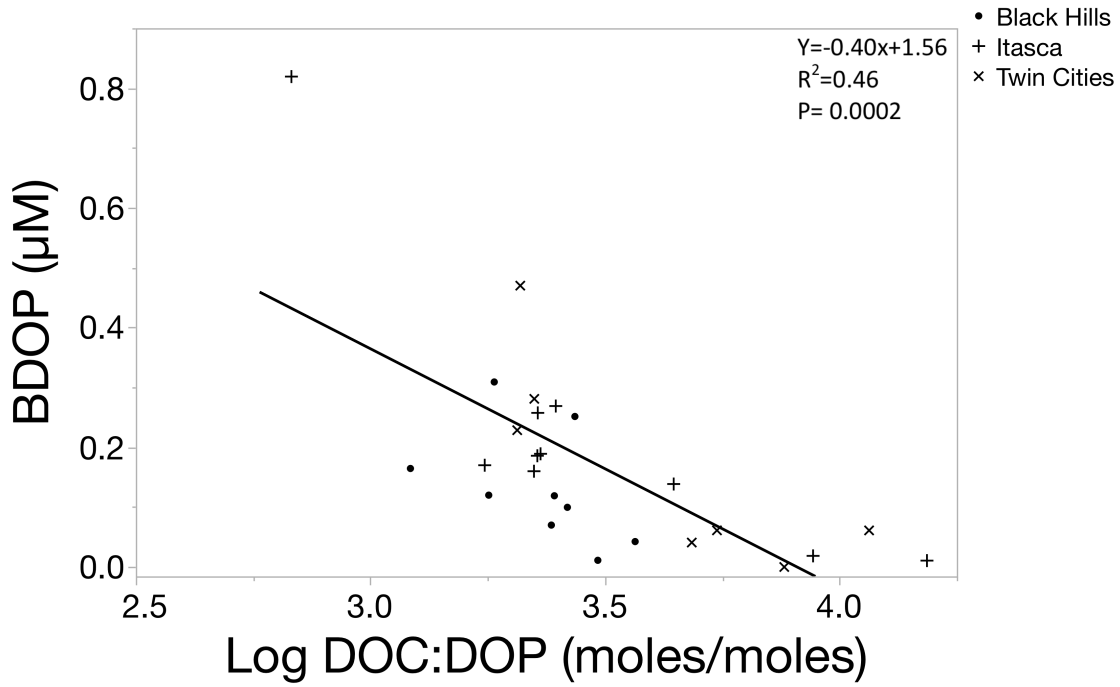

Figure 1-8: Scatterplot showing the linear regression function comparing the concentration of BDOP to the DOC:DOP ratio of the initial sample (log transformed). The significant negative relationship demonstrates BDOP is drawn down as DOP becomes scarce relative to DOC.

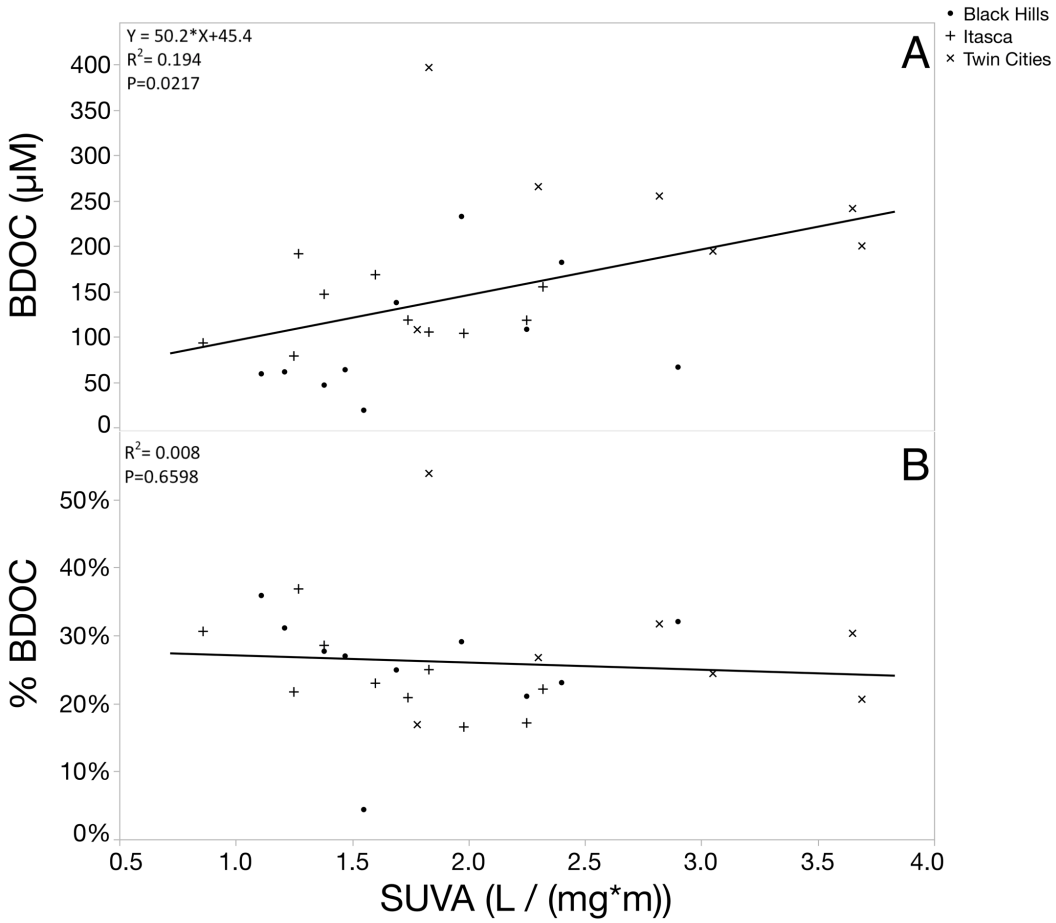

Figure 1-9. Scatterplots showing the relationship between BDOC and SUVA. **(A)** Shows the absolute size of the BDOC pool and **(B)** shows the relative size of the BDOC pool. The absolute amount of BDOC showed a significant positive association with SUVA whereas the relative BDOC percentage was not significantly related to SUVA. This could be at least partially driven by the fact that high SUVA systems tend to have larger total DOC pools, but it also suggests that systems dominated by more aromatic carbon compounds (high SUVA) still have large pools of BDOC.

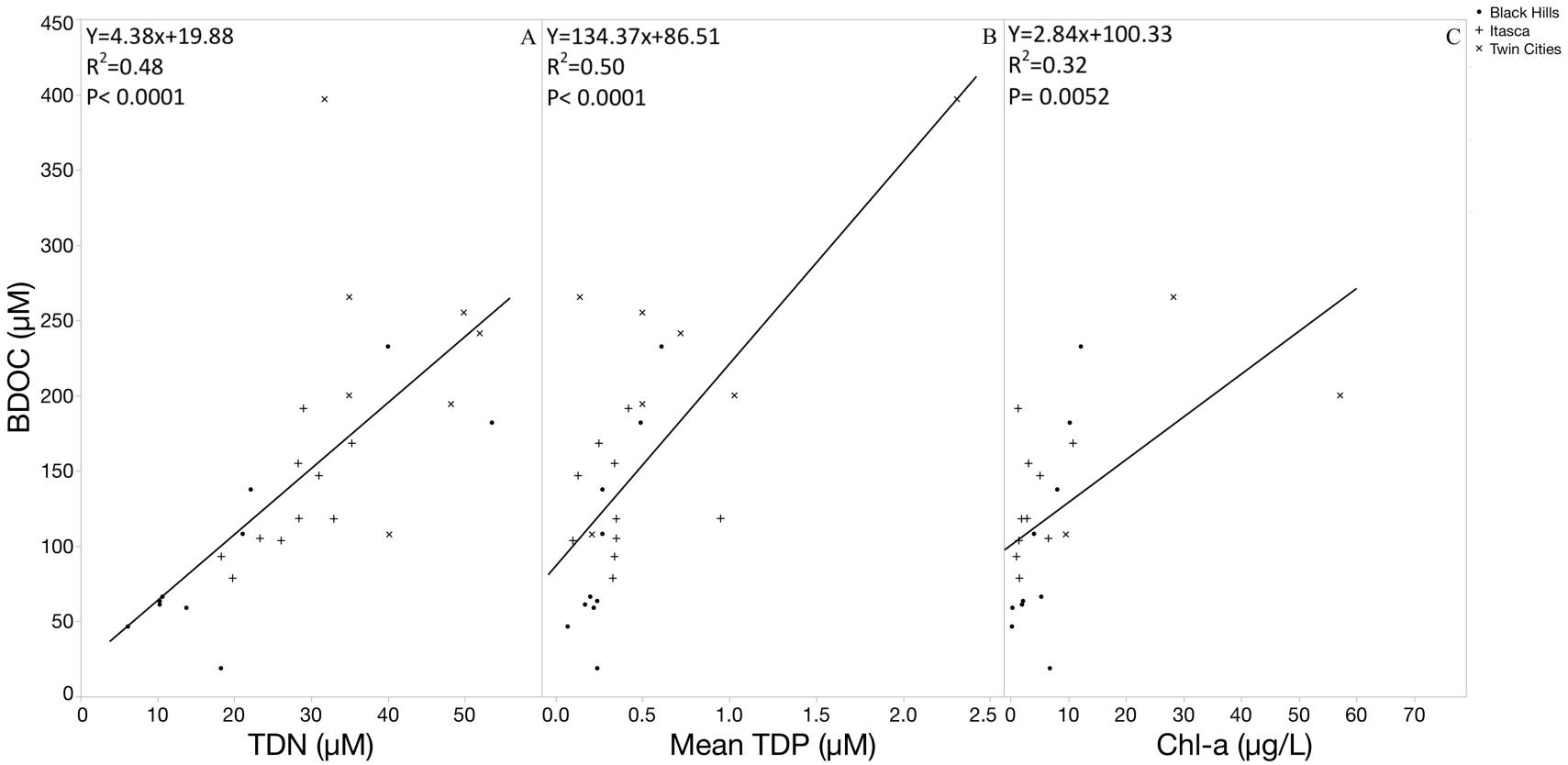

Figure 1-10: Linear regression functions showing the relationship between BDOC concentration and trophic status indicators. Strong positive relationships between BDOC and TDN **(A)** and TDP **(B)** suggest BDOC accumulation in high nutrient conditions. BDOC is also positively correlated with productivity, measured as chlorophyll-a concentration **(C)**.

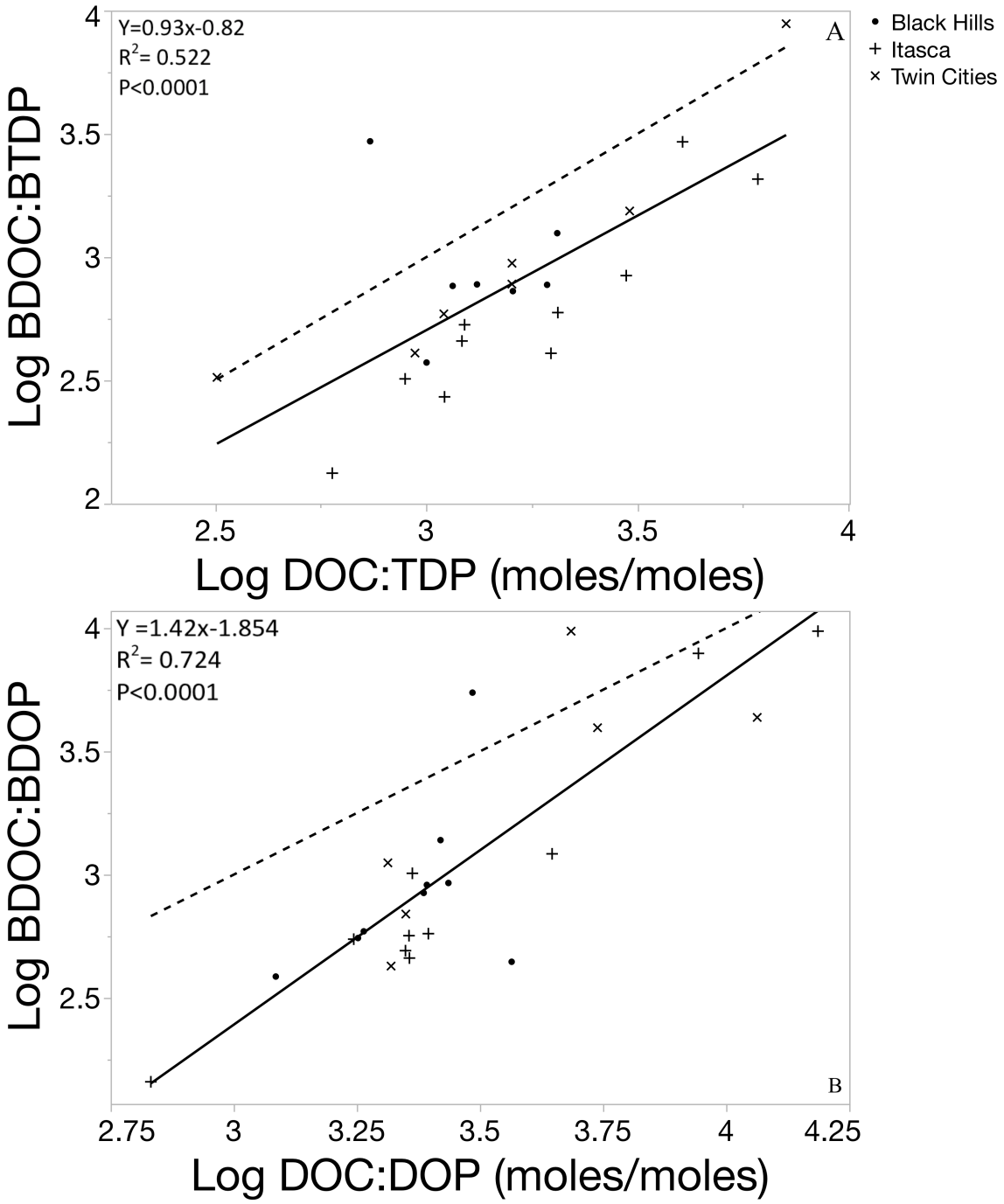

Figure 1-11. Comparison of bioavailable nutrient stoichiometry to bulk nutrient stoichiometry

(A) Linear regression fit (solid line) comparing the log transformed values for BDOC:BTDP ratio to the bulk DOC:TDP ratio for 24 lakes, 3 samples had to be removed from the data set because estimated BTDP was 0. Dotted line represents a 1:1 for comparison. The regression line falls below the 1:1 reference indicating that the C:P ratio of

the bioavailable pool is lower than the measured C:P of the system. In other words, bulk resource ratios measured using whole water DOC:TDP would overestimate the experienced resource ratio of the microbial community (BDOC:BTDP). **(B)** Linear regression fit (solid line) comparing the log transformed values for BDOC:BDOP ratio to the bulk DOC:DOP ratio for 24 lakes, 3 samples had to be removed from the data set because estimated BTDP was 0. Dotted line represents a 1:1 for comparison. The regression line falls below the 1:1 reference indicating that the C:P ratio of the bioavailable pool is lower than the measured C:P of the system. Additionally, the slope of the regression fit is greater than 1 meaning that BDOC:BDOP ratios more closely match bulk DOC:DOP ratios when they are higher (in systems that are DOP poor relative to DOC).

Table 1-1: Table showing general characteristics of each of the 27 sampling sites

Name	Region	DIC (µM)	DOC (µM)	TDN (µM	TDP (µM)	SRP (µM)	DOP (µM)	Chl-a (µg/L)	SUVA (L / (mg*m))	pH	Alkalinity (µeq/L)
Bismark	Black Hills	1097	790	53.5	0.49	0.06	0.43	10.29	2.40	7.5	1157
Canyon Lake	Black Hills	3051	165	13.8	0.22	0.16	0.07	0.35	1.11	8.3	3042
Center	Black Hills	779	514	21.1	0.27	0.06	0.21	4.09	2.25	7.7	830
Dark Canyon	Black Hills	2912	168	6.2	0.07	<0.038	>0.32	0.25	1.38	8.6	2939
Deerfield	Black Hills	3780	236	10.3	0.24	0.04	0.19	2.18	1.47	8.6	3799
Pactola	Black Hills	2914	197	10.3	0.17	0.11	0.06	2.00	1.21	8.4	2964
Roubaix	Black Hills	2623	207	10.7	0.20	0.08	0.12	5.34	2.90	8.5	2615
Sheriden	Black Hills	2366	430	18.3	0.24	0.12	0.12	6.83	1.55	8.5	2398
Stockade	Black Hills	1952	800	40.0	0.61	0.31	0.29	12.21	1.97	8.5	2024
Sylvan	Black Hills	803	552	22.1	0.27	0.06	0.21	8.11	1.69	8.5	857
Arco	Itasca	920	515	31.0	0.13	0.07	0.06	5.14	1.38	7.5	830
Boot	Itasca	3086	363	19.8	0.33	0.17	0.16	1.55	1.25	8.4	2848
Deming	Itasca	1206	733	35.3	0.25	0.08	0.17	10.85	1.60	8.0	1145
E. Twin	Itasca	3083	701	28.3	0.34	0.06	0.28	3.13	2.32	7.9	2991
Elk	Itasca	2526	568	28.4	0.95	0.11	0.84	2.86	1.74	8.5	2913
Itasca	Itasca	3372	421	23.3	0.35	0.16	0.19	6.58	1.83	8.5	3101
Josephine	Itasca	604	520	29.0	0.42	0.20	0.23	1.34	1.27	7.8	555
Long	Itasca	3005	304	18.3	0.34	0.17	0.17	1.04	0.86	8.5	3059
Mary	Itasca	2847	627	26.1	0.10	0.06	0.04	1.47	1.98	8.4	2860
Ozawindib	Itasca	1603	690	33.0	0.35	0.05	0.30	1.92	2.25	8.2	1631
Beckman	Twin Cities	180	993	35.0	0.14	0.05	0.09	28.27	2.30	5.4	29
Cedar Bog	Twin Cities	1030	971	34.9	1.03	0.57	0.47	57.19	3.69	6.9	1039
Fish	Twin Cities	374	639	40.2	0.21	0.13	0.08	-	1.78	9.2	552
Como	Twin Cities	754	738	31.8	2.31	2.16	0.15	9.61	1.83	9.1	770
Staring Creek N	Twin Cities	2806	797	51.9	0.72	0.58	0.15	-	3.65	8.0	2203
Staring Creek S	Twin Cities	2303	797	48.2	0.50	0.14	0.36	-	3.05	7.9	2292
Staring Lake	Twin Cities	2180	805	49.9	0.50	0.11	0.39	10.29	2.82	7.8	2718

Chapter 2: Dissolved Organic Matter Production by Heterotrophic Bacteria

Summary

Heterotrophic bacteria are key biogeochemical regulators in freshwater systems. Through both the decomposition and production of organic matter, bacteria link multiple biogeochemical cycles together. While there has been a multitude of work on understanding the role of microbes in the aquatic carbon cycle, improving our understanding of these important linkages will require more information about how organic matter transformations impact other nutrients, such as phosphorus. In this study, a culture-based laboratory experiment was used to examine the production of dissolved organic matter by heterotrophic bacteria under varied nutrient conditions. In addition to quantifying the production of dissolved organic carbon, we also measured the production of dissolved organic phosphorus and characterized the microbially-produced organic matter using optical properties. Results from these experiments show that measurable amounts of dissolved organic carbon and dissolved organic phosphorus were produced by heterotophic bacteria under nutrient conditions ranging from carbon-limitation to strong phosphorus-limitation. Additionally, optical characterization revealed that organic matter produced by bacteria grown in high phosphorus conditions is highly aromatic with similar optical properties to terrestrially derived organic matter. Overall, these findings suggest that heterotrophic bacteria can be important producers of organic matter in freshwaters and that continued trends of increased nutrient concentrations (eutrophication) may fundamentally change the composition of microbially produced organic matter in freshwater systems.

Introduction

Heterotrophic bacteria are important regulators of multiple biogeochemical processes in aquatic ecosystems including the cycling of carbon (C) and phosphorus (P) (Cotner and Biddanda 2002; Cotner et al. 2010; Schlesinger et al. 2011; Jeyasingh et al. 2017). However, our understanding of how these key elemental cycles are linked in aquatic systems remains limited (Maranger et al. 2018b). It has long been acknowledged that inland waters are biogeochemically active “pipes” connecting terrestrial systems with

the oceans, but this active pipe concept has traditionally only been used to consider how inland waters process C (Cole et al. 2007; Tranvik et al. 2009; Aufdenkampe et al. 2011). Recently, there has been a call to better understand how the freshwater pipe concept could be applied to macronutrient cycling in inland waters (Maranger et al. 2018b), and developing our understanding of how freshwaters serve as active pipes for multiple elements requires an understanding of both the production and decompositions of organic matter.

Dissolved organic matter (DOM) is a major biogeochemically active carbon pool in freshwater systems (Tranvik 1988; Stets and Cotner 2008; Tranvik et al. 2009; Catalán et al. 2016b). To date, the bulk of the research conducted on DOM has focused on the production and decomposition of dissolved organic carbon (DOC). In marine systems, microbial production of DOC can result in a pool of slow-degrading (or recalcitrant) carbon that can be exported to and buried in the deep oceans (Jiao et al. 2010, 2011; Lechtenfeld et al. 2015). This microbial carbon pump is now widely accepted as an important mechanism of storing carbon in the ocean. Microbial carbon production has also been presented as the dominant pathway for recalcitrant organic matter production in soils (Liang and Balser 2011; Cotrufo et al. 2015) resulting in soil DOM that has been heavily modified by microbial metabolism. While this pathway has been less explored in freshwater systems, it also appears to be an important control on DOM composition in freshwaters (Kawasaki and Benner 2006; Guillemette and del Giorgio 2012). Despite this known importance for global C cycling, the implications of microbial production of DOM on other nutrient cycles (such as DOP production) are not well known.

One major pathway for microbial DOM production is through the excretion of bacterial metabolites (Lechtenfeld et al. 2015). The environmental conditions, such as availability of nutrients, experienced by bacteria can greatly affect the production of specific types of metabolites. For example, bacterial production of phosphatase is strongly related to nutrient conditions (Cotner and Wetzel 1991). Recent work has shown that bacteria have several strategies for dealing with nutritional imbalance, including changing their biomass composition to more closely match the chemical composition of their resources (Mooshammer et al. 2014; Godwin and Cotner 2015b; Danger et al. 2016; Godwin et al. 2017). Biomass composition flexibility likely has important consequences

for the composition of organic matter that is produced by heterotrophic bacteria, but how these different stoichiometric compositions impact organic matter transformations by heterotrophic bacteria remains unknown. In this chapter, we explore the production of DOM by heterotrophic bacteria and determine how differing stoichiometric strategies impact the chemical composition of DOM produced. This was accomplished by growing bacterial strains that exhibited a range of biomass flexibility under various conditions of nutrient limitation and assessing the composition of organic matter that was produced.

Materials and Methods

Bacterial Culturing Media

WC Medium was prepared according the recipe in Guillard and Lorenzen (1972) with ultrapure water (Milli-Q System). Media was mixed in glassware that had been soaked in 10% hydrochloric acid for a minimum of 1 hour and rinsed with ultrapure water to remove any trace phosphorus contamination. All chemical stocks used to make the media were ACS grade or equivalent. Glucose was added as the sole organic carbon substrate with a final concentration of 6.66 mM carbon. Nitrogen was supplied as sodium nitrate at a concentration of 1 mM resulting in a media C:N molar ratio (6.6:1) equal to the Redfield ratio (Redfield 1958). Micronutrients, vitamins, and trace metals were supplied consistent with the recipe (Guillard and Lorenzen 1972). To manipulate the C:P of the media, phosphorus was added as potassium phosphate at three different levels: 0.067 mM P, 0.014 mM P, and 0.0067 mM P, resulting in media C:P of 100:1, 500:1, and 1000:1, respectively.

Strain Selection

A large field campaign was conducted in 2013 where water samples taken from lakes across the state of Minnesota were used to culture and isolate heterotrophic bacteria following the procedures outlined by Godwin and Cotner (2015a). Through these efforts, a culture repository of over 1000 unique bacterial strains isolated from freshwater system was established. To quantify the variability in stoichiometric flexibility within this library, a sub-sample of ~135 strains were grown in continuous culture at 25% of their maximum growth rate at two media C:P levels (100:1 and 10,000:1) (see Godwin and Cotner 2018). Biomass flexibility for these ~135 strains was calculated as the relative

percentage increase in biomass C:P when grown in high C:P conditions compared to the biomass C:P in the low C:P media using equation 1 below. Archival stocks of each strain were stored at -80ºC in glycerol for future use.

$$\frac{(Biomass\ C{:}P\ at\ 10{,}000{:}1 - Biomass\ C{:}P\ at\ 100{:}1)}{Biomass\ C{:}P\ at\ 100{:}1} \times 100$$

Equation 1: Relative change in biomass stoichiometry expressed as a percent change from the biomass stoichiometry when grown under media conditions with a C:P of 100:1.

To select strains for this study, the ~120 strains described above were sorted by C:P biomass flexibility and split into quartiles. The 1st quartile (represented the lowest C:P flexibility values) were classified as inflexible strains and the 4th quartile were classified as flexible strains. From these quartiles, we attempted to recover strains from the -80ºC freezer, resulting in 9 strains being easily recovered from the deep freezer (5 inflexible strains and 4 flexible strains). These 9 strains were the then used for the present study.

Culturing Bacteria

Once bacteria had been successfully recovered from the -80ºC, a pair of starter cultures were generated for each strain by inoculating each strain into 2 ml of WC media with a C:P of 100:1. Resazurin was added as a respiratory indicator at a concentration of 20 μM to monitor the growth of bacteria in these starting cultures. Once resazurin indicated growth (pink cultures), these 2 mL starter cultures were used to inoculate duplicate 250 mL cultures of each strain by diluting the 2 mL starter with ~248 ml of fresh WC media (without resazurin) with a C:P of 100:1. These cultures were incubated at room temperature (~22 ºC) on a tabletop shaker set at 150 rpm. Growth in 250 mL cultures was monitored using optical density readings and cultures were harvested when peak biomass was achieved. This process was then repeated to generate cultures in WC media with a C:P of 500:1 and 1000:1 with one deviation. Because it was assumed that these high C:P cultures would contain less concentrated biomass (and therefore need more volume filtered to measure the biomass), a final culture volume of 500 mL rather than 250 mL was used.

Collecting Microbially Produced DOM

Cells from cultures were collected onto pre-combusted, pre-weighed Whatman GF/F filters (0.7 μm) to measure microbial biomass. Filters were then oven dried at 60ºC for at least 24 hours and re-weighed. Microbial biomass was calculated by subtracting the pre-weight of the filter from the post-weight after oven drying overnight at 60 ºC . Remaining media was filter sterilized using a 0.22 μm polyethersulfone (PES) bottle top filter and the residual media was collected in muffled amber glassware and stored at 4 ºC until analyzed (samples were analyzed within 2 weeks of filtration).

Characterizing Microbial DOM Production

To characterize the chemical composition of the residual media, we measured dissolved nutrients and the specific-UV absorbance at wavelength 254 nm (SUVA254). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were measured using a Shimadzu TOC-L auto-analyzer with a TNM-L module (CSH/CSN model, Shimadzu Corp). To measure total dissolved phosphorus (TDP) and soluble reactive phosphorus (SRP), we used a molybdenum blue reaction with and without acid-persulfate digestion (Murphy and Riley 1962). To conservatively estimate DOP, we measured TDP and SRP in triplicate and subtracted the upper 95% confidence interval of the SRP measurement from the lower 95% confidence interval for TDP for each sample. Absorbance scans (wavelengths from 200-800 nm) were performed using a Cary 50 spectrophotometer, which was used to calculate SUVA254 by dividing the absorbance at wavelength 254 nm by the total DOC concentration (produced DOC plus any residual glucose). To account for the amount of glucose that was not consumed during the incubation period, residual media glucose was measured using Amplex™ Red glucose/glucose oxidase assays (Invitrogen, catalog number A22189) according to the manufacturer's protocol. Microbially produced DOC was then calculated by subtracted the residual glucose measurement form the total DOC concentration.

Data Analysis

We used a generalized factorial ANOVA model with interaction effects to examine how stoichiometric flexibility and media composition impacted DOC and DOP production. When significant predictors were found, we performed post-hoc Tukey HSD

tests to determine which levels of the predictor variables were significantly different form one another. Additionally, we used a general linear regression model to explore relationships between a quantitative measure of biomass flexibility (expressed as the percent change in biomass C:P when grown under media C:P conditions of 100:1 and 10,000:1) and the optical properties of the organic matter produced. All statistical analysis was performed in R version 3.5.1 (https://www.R-project.org).

Results

Quantifying Microbially Produced DOM

To quantify the production of microbially-produced DOM, we measured DOC and DOP concentrations in cell-free media after microbial growth had plateaued. We also measured the amount of glucose remaining in the residual media to account for any of the starting carbon source that had not been consumed (Figure 1). We calculated the total amount of DOC produced by subtracting the amount of residual glucose from the total amount of DOC in the residual media. As expected, residual glucose was lowest when strains were grown at a C:P of 100:1, with less than 5% of the DOC in the residual media being glucose (Figure 1). This efficient drawdown of glucose strongly supports the idea that organic C was limiting microbial growth in this treatment. In comparison, the residual DOC from strains grown under more P-limited conditions typically contained 10% to 20% of the DOC as glucose (Figure 1).

DOC production was highest for strains growing at an intermediate media C:P (500:1), with values ranging from 713 μM to 5193 μM and a median value of 3301 μM. In comparison, DOC production for strains grown at a C:P of 100:1 ranged from 369 μM to 2931μM with a median value of 503 μM and 656 μM to 4512 μM with a median value of 1288 μM for strains grown at a C:P of 1000:1 (Figure 2). Across the three treatments, this represented DOC production ranged from ~6% to ~82% of the original glucose pool. A full factorial ANOVA (with nutrient stoichiometry, biomass flexibility, and an interaction effect as factors) was used to examine the effect of media stoichiometry and stoichiometric flexibility (separately and interactively) on DOC production. The whole model revealed that only media stoichiometry was a significant predictor of DOC production ($p = 0.003$), and a Tukey HSD post-hoc test confirmed that DOC production at 500:1 was

significantly higher than DOC production at 100:1 (although neither was significantly different from the 1000:1 group). Despite the fact that stoichiometric flexibility was not a significant predictor of mean DOC production, median values for flexible strains were lower than inflexible strains under more P-limited conditions (C:P of 500:1 and 1000:1), a pattern that warrants more thorough future investigation.

Phosphate levels in the residual media were highly impacted by the media C:P, with over 90% of the media SRP being removed in the 500:1 and 1000:1 treatments compared to ~40% to 50% removal rates when the media C:P was 100:1 (Figure 3). This again supports the idea that bacteria were experiencing C-limitation at the lowest media C:P and transitioned to P-limitation at the two higher C:P values. DOP production was strongly influenced by media condition. DOP production was 1-2 orders of magnitude larger under C-limited conditions compared to P-limited conditions (Figure 4) but was detectable in all growth conditions (although not for all strains). Of the 26 total samples that were collected, 6 had DOP levels below detection (3 strains grown at 100:1 that were all inflexible and 3 strains grown at 500:1, two flexible and 1 inflexible). One inflexible strain only produced measurable DOP under the most phosphorus limited condition, but all other strains had measurable DOP production for at least two media levels. For the strains that produced measurable DOP, values ranged from 0.01 μM to nearly 10 μM (Figure 4), which represented ~0.006% to 12.5% of the original phosphate pool (Figure 5). As with DOC, a full factorial ANOVA was used to examine the effect of media stoichiometry and stoichiometric flexibility (separately and interactively) on DOP production. In terms of absolute DOP production, media stoichiometry was a significant predictor in the whole model ($p=0.02$), but stoichiometric flexibility was not and there was no significant interaction effect. A Tukey HSD test confirmed that absolute DOP production was higher under C-limited conditions than under P-limited conditions, but there was no significant difference between the two P-limited conditions. However, when we account for the difference in initial phosphate concentration of the original media by expressing DOP as a percentage of the media SRP, neither media stoichiometry nor stoichiometric flexibility were significant predictors of relative DOP production. This seems to be primarily driven by both extremely high variability in relative production within media

types (with values varying over an order of magnitude within each media type) and a relatively small sample size.

Optical Characterization of Microbially Produced DOM

$SUVA_{254}$ was used as an indicator of organic matter quality. All samples showed increased $SUVA_{254}$ values (in comparison to the $SUVA_{254}$ of the starting media) in the residual media, consistent with microbial production of aromatic carbon compounds (Figure 6). Mean $SUVA_{254}$ values were significantly impacted by the media C:P (Factorial ANOVA, $p< 0.0001$), but biomass flexibility did not have a significant effect on the mean $SUVA_{254}$ of produced organic matter. However, flexible stains did show much larger variation in $SUVA_{254}$ values compared to inflexible strains when grown at a media C:P of 100:1. Furthermore, strains grown at 100:1 C:P showed significantly higher $SUVA_{254}$ values than strains grown at higher C:P (Tukey HSD, $p <0.0001$). In contrast, under more P-poor conditions, $SUVA_{254}$ values were much lower (typically less than 1) and much less variable. While there was no interaction effect between biomass flexibility and media stoichiometry in the full factorial ANOVA model, the mean residual $SUVA_{254}$ value for flexible strains grown under the most P-limited conditions was over 3 times larger than the inflexible strains (0.88 compared to 0.29). To more fully explore this, we used a simple linear regression model to examine if the quantitative metric of biomass flexibility was predictive of $SUVA_{254}$ values in the strains grown at a C:P of 1000:1. This analysis showed a significant positive relationship between biomass flexibility and $SUVA_{254}$ values ($p=0.026$, $R^2= 0.59$). In other words, when grown under the most P-limited conditions, more stoichiometrically flexible strains produced organic matter with higher $SUVA_{254}$ values. This pattern did not persist in the other media treatments, where there was no significant relationship between quantitative biomass flexibility and $SUVA_{254}$ of the produced organic matter.

Stoichiometry of Microbially Produced DOM

To examine how biomass flexibility and media conditions impacted the relative processing of C and P, we examined the stoichiometric ratios of the residual media. Overall, the nutrient composition of the residual media was significantly impacted by the media stoichiometry (Figure 8, Factorial ANOVA $p =0.0023$). Under P-limiting condi-

tions, the nearly complete removal of available phosphate (Figure 3) resulted in C:P (calculated as DOC:TDP) values 2 or 3 orders of magnitude higher than residual media from C-limited incubations (Figure 8). Conversely, strains grown in C-limiting conditions had C:P values ranging from ~10-60 with a median value less than 20. In other words, the relatively low removal efficiency of phosphate under C-limited conditions (~50%, Figure 3) resulted in P-rich residual media. To isolate the effect of our treatments on the stoichiometry of the organic matter being produced by the bacteria, we also calculated DOC:DOP ratios. Bacteria growing under C-limiting conditions produced organic matter that was relatively P-rich (lower DOC:DOP) compared to cultures experiencing P-limitation, but the differences were not statistically significant in the full factorial ANOVA model, despite median values for DOC:DOP being an order of magnitude lower under C-limitation than under P-limitation (Figure 9). This could in part be driven by the extraordinarily large variability in the intermediate media C:P, which varied over two orders of magnitude for flexible strains. This variability compounded with the limited sample size at each treatment level results in fairly conservative conclusions from the ANOVA model. When examined in isolation from the other treatments, the difference in DOC:DOP was statistically significant between flexible and inflexible strains (t-test, p=0.0419). Additional replication would be needed to fully resolve this discrepancy.

Biomass production by Bacteria under Different Growth Conditions

In addition to measuring organic matter production, we quantified bacterial biomass accumulation in our cultures as a measure of microbial growth potential. Microbial biomass was significantly impacted by media type (Factorial ANOVA, p=0.02) and was highest at low media C:P (Tukey, p=0.02). Bacterial biomass accumulation was also significantly impacted by stoichiometric flexibility, with flexible strains having higher biomass accumulation than inflexible strains (Tukey, p=0.02), but there was not a significant interaction effect between flexibility and media stoichiometry.

Discussion

In this study, we explored the production of dissolved organic matter by heterotrophic bacteria under different nutrient conditions and examined how flexibility in biomass nutrient composition impacts the quality and quantity of microbially produced or-

ganic matter. Here, we discuss the implications of three key findings based on this work. First, we demonstrate measurable amounts of DOC and DOP production by heterotopic bacteria under nutrient conditions ranging from C-limitation to strong P-limitation, but limitation status showed a strong influence on the production of DOC vs DOP (Figure 2, Figure 5). Second, optical characterization of microbially produced organic matter revealed that DOM produced by bacteria grown under C-limited conditions is highly aromatic with $SUVA_{254}$ values as high as 3 $L*mg\text{-}C^{-1}*m^{-1}$, a value comparable to organic matter extracted from peatland soils (Hansen et al. 2016). This finding suggests that under C-limited conditions, microbial metabolism can produce DOM with similar optical properties to terrestrially derived organic matter, indicating that limitation status of the microbial community processing the organic matter may be a more important driver of $SUVA_{254}$ than the original source of the material. Lastly, stoichiometric flexibility of bacteria had variable effects on DOM production, but the effects were generally most pronounced under the strongest limitation conditions (both lowest and highest media C:P values). In general, we observed more variable DOM production in the flexible strains at low C:P and more variation in the inflexible strains at high C:P. This likely reflects the fact that the flexible strains are good competitors for P (our designation of flexibility was based on P) and the inflexible strains are better adapted as competitors for C.

While more work is needed to fully understand how the physiological growth strategies of different microbial taxa impact the production of DOM, this work provides some important insights into this question. For example, biomass flexibility was positively correlated to $SUVA_{254}$ values when strains were grown under the most P-limited condition (Figure 7) but not under lower C:P media treatments. This indicates that strains with flexible biomass composition were able to produce more aromatic carbon compounds (i.e., more similar to the organic matter produced under C limitation in this study) under strong P-limitation than inflexible strains. Overall, these findings have important implications for understanding the role of heterotrophic bacteria as significant producers of DOM in aquatic systems and lend insights in how we might expect this role to change under different nutrient conditions.

DOC and DOP Production

Under C-limited conditions, typically ~5%-10% of the media C was converted to DOC, whereas ~10%-70% of the original media C was typically converted to DOC under P-limited conditions (Figure 2). Biomass accumulation was also higher at low C:P compared to high C:P (Figure 10), suggesting that strains growing under C limitation preferentially allocated available carbon to biomass rather than DOC. While we did not directly measure carbon respired in this study, this observed tradeoff in C allocation parallels previous work indicating that bacterial growth efficiency decreases as media C:P increases (Godwin et al. 2017). To explore this more fully, we examined the relative allocation of carbon into each potential pool (biomass, respiration, DOC, and residual glucose) by flexible and inflexible strains under high and low media C:P (Table 1). We did not have direct measurements of biomass C or respiratory C, so we estimated these parameters assuming C was 50% of dry biomass and then calculated respiratory C using a mass balance approach. This basic accounting showed that allocation of C to DOC was an important pathway under strong P limitation for both flexible and inflexible strains and even potentially exceeded respiratory C for inflexible strains (Table 1).

Relative DOP production showed a similar pattern in that DOP production was lowest when P was limiting (as DOC production was lowest when C was limiting), but overall DOP production as a percentage of original media P was much lower than it was for C. For example, relative DOP production peaked under C-limited conditions with ~12% of the original media P being converted into DOP (compared to peak conversion rates of over 70% for DOC). Additionally, under mild P-limitation (media C:P of 500:1), DOP conversion fell to ~0.006%-2% much lower than the DOC values (~5%-10%) under C-limitation. Interestingly, DOP production efficiency went back up under the most extreme P-limitation, but still had relatively modest values with 7 of the 9 samples converting less than 2% of the original media P to DOP (Figure 5). Taken together, these values suggest that the composition of microbially produced DOM is strongly impacted by the C:P of the resources available to heterotrophic bacteria. Furthermore, there seems to be a trade-off between allocating carbon to biomass vs allocating it to DOP compounds. For example, inflexible strains in this study had lower biomass accumulation at both high and low media C:P (Figure 10) relative to the intermediate C:P and produced higher amounts

of DOP in these conditions (Figure 4) indicating the tradeoff in C-allocation. This suggests that for inflexible strains, maintaining a uniform biomass composition (by excreting excess nutrients in DOM) is more important than allocating additional C to biomass.

Microbial production of DOM has been well studied in marine systems but has received comparatively less attention in freshwaters. Additionally, the bulk of the work in marine systems has been carbon centric (Ogawa et al. 2001; Koch et al. 2014; Lechtenfeld et al. 2015), although there are several key studies that have explored DOP production as well (Orrett and Karl 1987; Thingstad and Rassoulzadegan 1995; Lønborg et al. 2009a; Tsuda et al. 2014a). Generally, these studies have relied on *in-situ* experiments designed to estimate ecologically relevant rates of DOP production, but this design has made it challenging to isolate the relative production of autotrophic and heterotrophic organisms. Nonetheless, these studies provide important context for this work.

Our observed conversion rates of 5-10% of media glucose into DOC over the course of the incubation period for cultures with a media C:P of 100:1 are in line with previous work, which has shown DOC conversion values ranged from 5%-15% during long term incubations in artificial seawater with a media C:P of 106:1 (Ogawa et al. 2001; Koch et al. 2014; Lechtenfeld et al. 2015). Additionally, our values under P-limited conditions align well with DOC production estimates (0.2-0.9%) of a single *Pseudovibrio* sp. grown in pure culture under phosphate limitation (Romano et al. 2014). This similarity in DOC production efficiency between various marine microbial communities and the individual freshwater strains that we tested here suggested a high degree of similarity in the potential of freshwater bacteria to be significant producers of DOC as has been acknowledged in marine systems (Kawasaki and Benner 2006; Jiao et al. 2011; Lechtenfeld et al. 2015).

Measurements of DOP production in controlled incubation experiments are sparse in the literature, but we found one study that strongly paralleled this work in a marine setting (Lønborg et al. 2009a). This study used artificial seawater with media C:P values ranging from 32-311 and found a DOP production efficiency of 17%, which is very comparable to the 12% we measured in our cultures grown at a C:P of 100:1. Several other studies have attempted to measure DOP production *in situ* in marine systems (Orrett and Karl 1987; Thingstad and Rassoulzadegan 1995; Tsuda et al. 2014b). These studies in-

corporated both phytoplankton and heterotrophic bacteria in their microbial pools making it hard to make a direct comparison to our work, but at least one of these studies measured DOP production as ~5% of the SRP drawdown during an open ocean diatom bloom with most of the production being attributed to the autotrophic diatoms (Tsuda et al. 2014b). The data presented here suggested that freshwater bacteria can be at least as important as producers of DOP as this marine autotrophs, which have been acknowledged as pivotal players in the marine P cycle (Ruttenberg and Dyhrman 2005; Karl and Björkman 2007).

In addition to overall amounts of production, we also explored how nutrient conditions impacted the stoichiometry of produced organic matter. Not surprisingly, DOC:DOP values increased under P-limited conditions, suggesting that DOM produced under P-limitation is relatively P-poor compared to DOM produced under C-limitation (Figure 9). Importantly, microbially DOM produced under all nutrient conditions was P-poor relative to the classic Redfield ratio of 106:1 as even DOM in the lowest media C:P treatment had a median DOC:DOP on the order of 10,000:1. Recent work has shown that lakes in the upper Midwest United States typically have DOC:DOP values ranging from ~700-10,000 (Thompson and Cotner 2018), indicating that in natural systems this microbially produced DOM is likely a source of P-poor organic matter compared to the compositions of the standing stock DOM. Furthermore, that same study demonstrated that the bioavailability of DOP was negatively associated with the DOC:DOP ratio, which, paired with our findings here, suggests that DOP produced under P-limited conditions should be more resistant to further microbial processing and may exacerbate P limitation. On the other hand, cultural eutrophication in freshwaters is likely shifting the experienced resource ratios of microbial communities more towards C-limitation due to increased P loading and this trend should result in more P-rich organic matter production, which in turn could be exported to downstream systems as a bioavailable form of DOP.

Optical Characterization of DOM

Optical properties have long been used to characterize the composition of DOM and to infer the sources of production (McKnight et al. 2001; Stedmon et al. 2003; Hansen et al. 2016). One such optical property, $SUVA_{254}$ is strongly correlated to the ar-

omaticity of DOM (Weishaar et al. 2003) as well as molecular weight (Chowdhury 2013) and has been used as an indicator of terrestrially derived organic matter, with higher $SUVA_{254}$ values being associated with more terrestrial influence (Helms et al. 2008; Hansen et al. 2016). Therefore, $SUVA_{254}$ was used as an optical characterization of the DOM produced by bacteria under different limitation conditions.

For context, $SUVA_{254}$ values for freshwater systems typically range from 1-6 $L*mg\text{-}C^{-1}*m^{-1}$ (Hansen et al. 2016). In this study, the original media had a $SUVA_{254}$ value of 0.136 $L*mg\text{-}C^{-1}*m^{-1}$ and increased over the incubation period in all cultures. DOM associated with algal production and aquatic plant leachates is typically assumed to have $SUVA_{254}$ values less than 1, whereas aged terrestrial organic matter typically has a value of 3 or higher (Pellerin et al. 2010; Hansen et al. 2016). In this study, we show the microbial production of DOM from a single, non-aromatic carbon source could produce $SUVA_{254}$ values as high as 3, more similar to leachates from peatlands than from traditional autochthonous sources (Hansen et al. 2016). Importantly, we saw the highest values for $SUVA_{254}$ measured when bacteria were growing under C-limited conditions, consistent with the hypothesis that C-limitation should result in increased C-processing and leave behind more complex and less bioavailable C-substrates. This finding has important implications for the use of $SUVA_{254}$ as a predictor of DOM source in freshwater, particularly in eutrophic lakes. The data presented here show that DOM previously assumed to be of a strong terrestrial signature, could simply be processed DOM produced by heterotrophic bacteria under C-limiting conditions. Therefore, our work may suggest that $SUVA_{254}$ may be more indicative of nutrient limitation by microbes processing organic matter than it is of the original organic matter source. Recent work has demonstrated that the vast majority of soil organic matter is highly processed by microbial communities before being exported to aquatic systems (Cotrufo et al. 2015), so the limitation of those microbial communities may be a more salient predictor of the final composition of this organic matter than the original source. This suggests that the relatively high $SUVA_{254}$ values commonly associated with terrestrially derived organic matter may reflect persistent high C-demand in soil microbial communities (see Ekblad and Nordgren 2002; Demoling et al. 2007; Spohn and Kuzyakov 2013; Heuck et al. 2015) rather than specific sources of organic matter production.

Furthermore, the pattern of increased P-loading into freshwater systems would tend to drive the C:P of microbial resources down, which should promote the production of high $SUVA_{254}$ DOM by bacteria. These eutrophic systems have been shown to be more efficient at burying organic matter in their sediments, which has been attributed to the overall higher rates of production in these systems (Heathcote and Downing 2012). Our study suggests a potentially complementary and synergistic mechanism for the increased carbon burial rates in eutrophic systems. As eutrophication occurs, lakes become more P-rich, which in turn promotes high $SUVA_{254}$ DOM production by the heterotrophic community. DOM with high $SUVA_{254}$ has been shown to be largely resistant to bacterial degradation (Frey et al. 2016), which should increase the burial efficiency of this material as its protected from microbial remineralization. It should be noted, that while many studies have linked low DOM bioavailability to high aromaticity, several other studies have found contradicting evidence (see Kalbitz et al. 2003; Weishaar et al. 2003; McDowell et al. 2006; Hosen et al. 2014; Frey et al. 2016; Thompson and Cotner 2018) and it has also been shown that high $SUVA_{254}$ material is more susceptible to photodegradation , which could also increase bioavailability (Bertilsson and Tranvik 2000; Moran et al. 2000). Therefore, more explicit measurements of the bioavailability of microbially produced organic matter is needed to fully explore the mechanisms of enhanced carbon burial proposed here.

Connecting Stoichiometric Flexibility to DOM Production

Applying the framework of ecological stoichiometry has greatly increased our understanding of the coupled cycling of multiple nutrients in aquatic systems. Fundamental to applying this framework is understanding how organisms interact with resource pools that are chemically imbalanced relative to their biomass needs. Recent work has shown that aquatic bacteria can be highly flexible in manipulating their biomass composition to help alleviate experienced resource imbalance (Godwin and Cotner 2015a), which has important implications for predicting how these communities will respond to changing nutrient conditions. This newly identified stoichiometric plasticity could have important implications for the production of DOM by microbial communities, but this area is largely unexplored in the literature. Additionally, the impacts of changing resource

stoichiometry on DOM production by bacteria is not well constrained. Understanding the interactions between changing resource stoichiometry and physiological plasticity will provide important insights into how aquatic bacteria will biogeochemically couple multiple nutrients under changing nutrient conditions in the future.

In this study, we provide some insights that address this gap in knowledge. We observed that resource stoichiometry is a strong control the microbial production of DOM, both from a quantity (Figure 1, Figure 4, Figure 5) and quality (Figure 6, Figure 7) perspective. Overall, DOM of microbially-produced organic matter seemed to be driven by the limitation status of the bacteria, with relatively high DOC production occurring under P-limited conditions and high DOP production occurring under C-limited conditions. This pattern makes intuitive sense because one would predict that actively growing bacteria would efficiently utilize the nutrient limiting to growth and therefore would not export high levels of that nutrient from their cells. This pattern was also observed in the quality of DOM, measured here as $SUVA_{254}$. Under C-limited conditions $SUVA_{254}$ values were indicative of more aromatic DOM, consistent with repeated microbial processing of the DOM that efficiently removed simple, highly-degradable, carbon substrates and leaving behind the more complex aromatics (Figure 6).

Pinning down the effect of biomass flexibility and how this physiological strategy interacted with changing nutrient substrate proved challenging. There were large amounts of variation in DOM production within each of the two different strategies (flexible vs inflexible) and our modest number of strains examined made it difficult to establish the strength of patterns we observed. However, a few interesting patterns did emerge, one being that carbon centric metrics like mean DOC production and mean $SUVA_{254}$ were very similar for flexible and inflexible strains when grown under C-limiting conditions, but in both cases flexible strains exhibited more variability than inflexible strains. In contrast, there were more significant divergences in mean values under extreme P-limitation. For example, DOC production was lower in flexible strains than inflexible strains (Figure 2) and biomass accumulation was higher for flexible strains than inflexible strains (Figure 10), consistent with preferential allocation of C into biomass (as opposed to DOC or excess respiration) by flexible bacteria under P-limitation. In addition, biomass flexibility was significantly corelated with SUVA under strongly P-limited conditions (Figure 7),

but not under mild P-limitation or C-limitation. The positive association between biomass flexibility and $SUVA_{254}$ values of the DOM is again consistent with enhanced microbial processing of C by flexible bacteria under P-limitation. Interesting, biomass flexibility seemed to have potential effects on DOP production under both C-limitation and strong P-limitation. Both the DOP production and the stoichiometry of produced DOM suggested more efficient P use by flexible strains under C-limited conditions, resulting in lower relative DOP production (Figure 4, Figure 5) and higher DOC:DOP values (Figure 9). The same pattern was observed for DOP production under highly P-limited conditions (Figure 9), although the difference between flexible and inflexible strains was more muted in this treatment. In summary, biomass flexibility did seem to have some impact on the efficiencies of DOM production, with possible interactions with substrate stoichiometry. Given the small sample explored here and the sizeable variation we measured, these relationships need further exploration to more precisely describe the interactions between biomass flexibility and nutrient stoichiometry and understand the impact on microbial DOM production.

Previous work has shown that relative growth rate and resource stoichiometry interactively control biomass flexibility (Godwin et al. 2017), which suggests that temperature could be an important consideration for microbial DOM production in natural systems through its control of relative growth rate. Additionally, the batch culture approach used in our study allows bacteria to grow at maximum growth rate during the early phases of the incubation and variable relatively growth rates later in the incubation period. Given that biomass flexibility seems to maximized at low relative growth rates (Godwin et al. 2017), our experimental approach could have damped the effect of biomass flexibility on DOM production. Repeating this basic design in a continuous culture, where relative growth rate can be controlled, may provide a better estimate of the effect of biomass flexibility by more fully activating the physiological response to nutrient imbalance.

The work presented here lends important insights into the role of aquatic bacteria as producers of organic matter in freshwater systems as well as identifies key interactions between microbial physiology and nutrient conditions that may impact DOM production by heterotopic bacteria. We demonstrate the potential for substantial DOM production by aquatic bacteria under variable nutrient limitation conditions, including the production of

highly aromatic compounds under high P conditions. Furthermore, this analysis suggests that interactions between biomass flexibility and nutrient condition have important controls on the efficiencies and nutritional composition of DOM production, particularly in relation to DOP. Finally, we demonstrate measurable amounts of DOP production by bacteria even under extremely P limiting conditions, identifying a potential mechanism for the accumulation of low levels of DOP in oligotrophic systems. Taken together, these findings improve our understanding of the fundamental linkages between aquatic bacteria and DOM cycling and allow us to better predict how these linkages may change under future nutrient conditions.

Figures and Tables

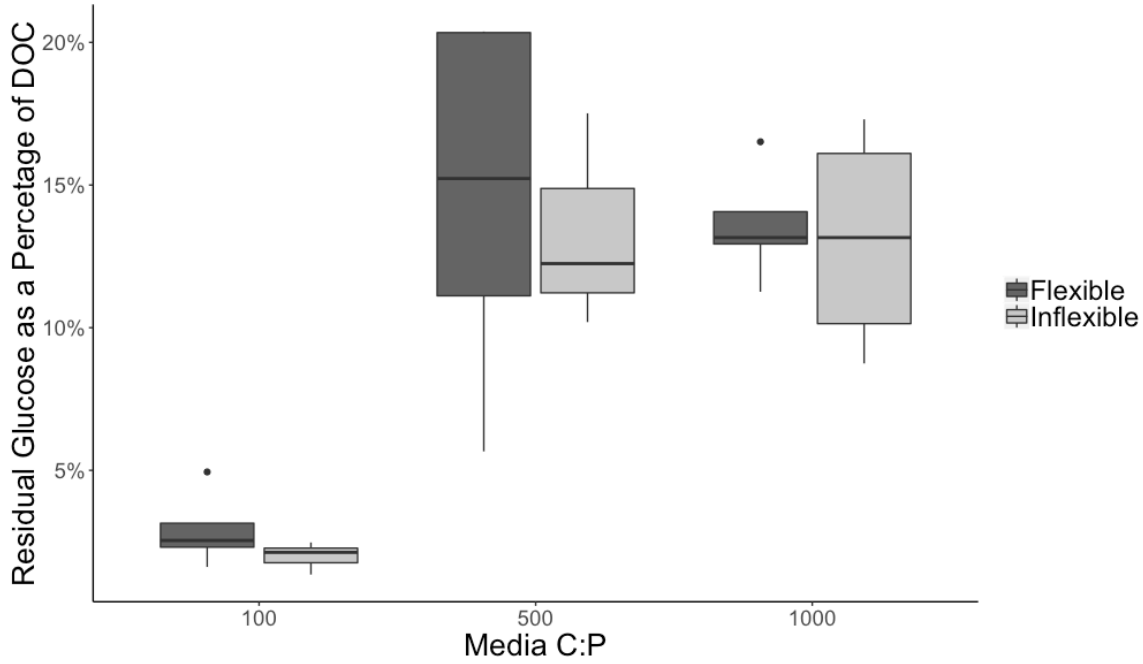

Figure 2-1: Residual Glucose concentration as a percentage of the residual DOC pool.

When grown at a media C:P, typically less than 5% of the residual carbon pool was in the form of glucose. In contrast, glucose made up a significantly higher fraction of the residual DOC pool (typically between 10-20%) under the higher media C:P treatments (Factorial ANOVA, $p<0.0001$). Nonetheless, even under the P-limited conditions, the majority of the residual DOC pool was microbial produced, not simply glucose that was not consumed during the incubation period.

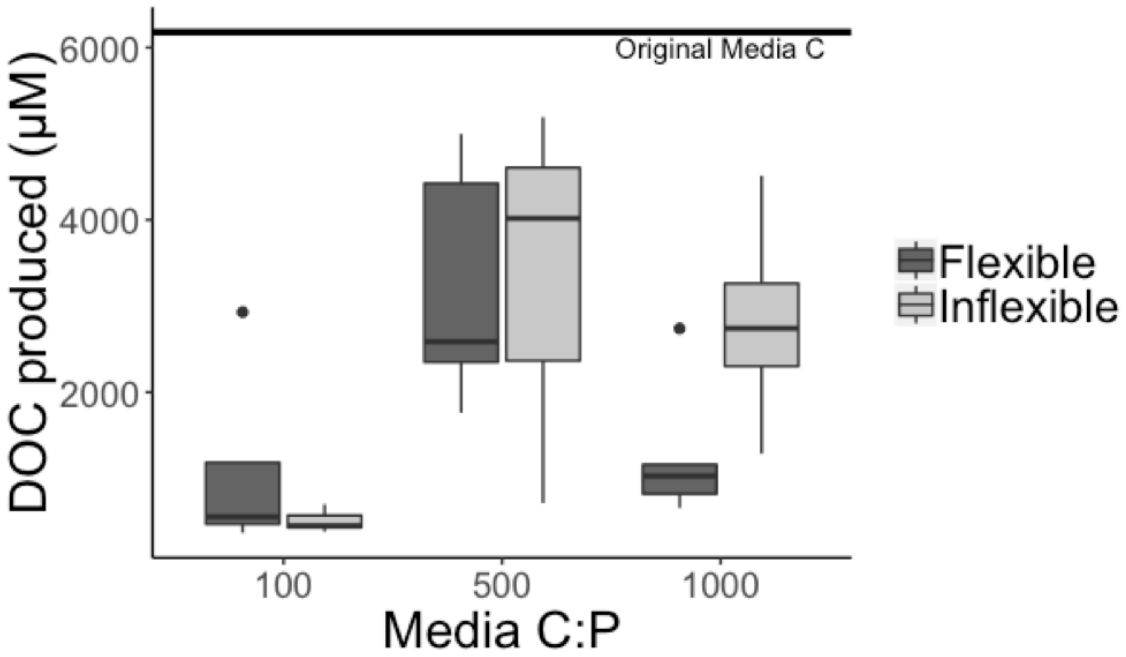

Figure 2-2: Concentration of the amount of DOC (µM) produced by flexible and inflexible strains grown at three unique media C:P ratios.

DOC produced was calculated by subtracting the amount of glucose in the residual media from the total measured DOC concentration in the residual media. Mean DOC production was not significantly different between flexible and inflexible strains within any of the media treatments. However, there was substantially more variability in DOC production by flexibly strains in the low C:P treatment, whereas in the high C:P treatment inflexible strains showed larger variation in DOC production. When grown at a media C:P of 100, residual DOC was generally less than 10% of the original media C concentration (with one exception) and was more variable among flexible strains than inflexible. In contrast, when strains were grown in media with a C:P of 500 the residual DOC concentrations were typically above 50% of the original C concentration and highly variable for both flexible and inflexible strains. When grown under extreme P limitation (C:P of 1000), flexible strains had lower residual DOC concentrations than inflexible strains (but not statistically significant) with median values of ~25% and ~ 50% of the original C respectively.

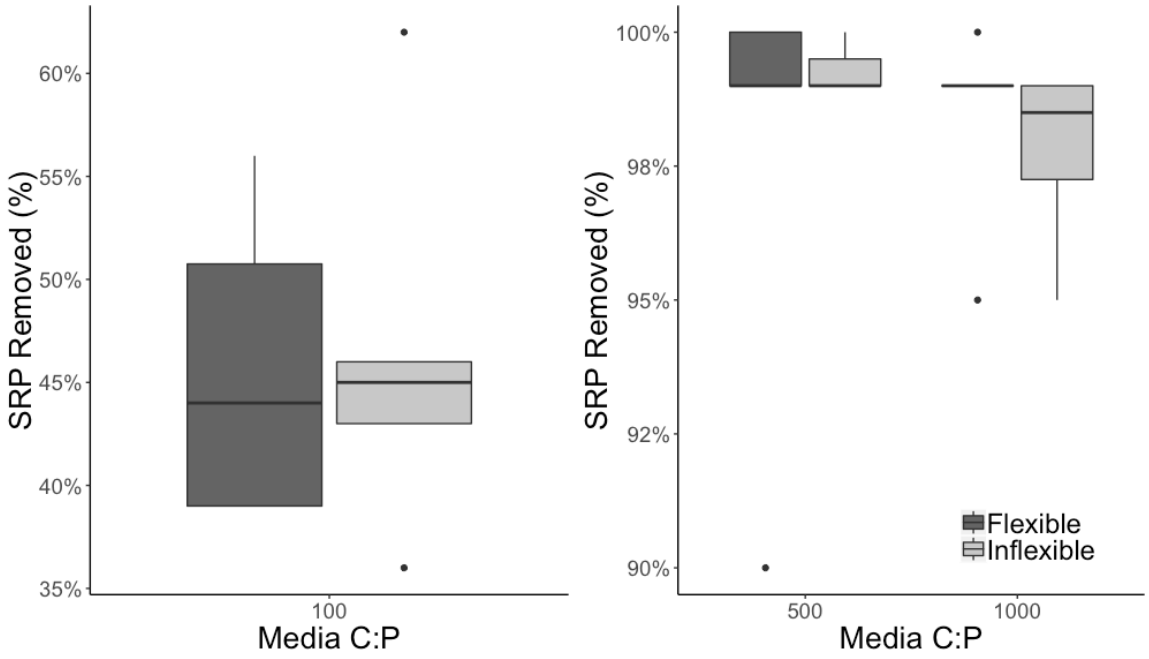

Figure 2-3: SRP removal efficiency by flexible and inflexible bacterial strains grown at three different media C:P ratios.

P removal efficiency was significantly lower at the lowest media C:P compared to the removal efficiency at the two higher media C:P levels (Factorial ANVOA with Tukey, $p<0.0001$). Nearly complete uptake of SRP was achieved when media C:P was 500 or higher, with all samples achieving at least 90% SRP removal. In contrast, typically 50% or less of the SRP was removed during the incubation period when strains were grown at a C:P of 100:1. Stoichiometric flexibility did not significantly affect the SRP removal efficiency at any media C:P level.

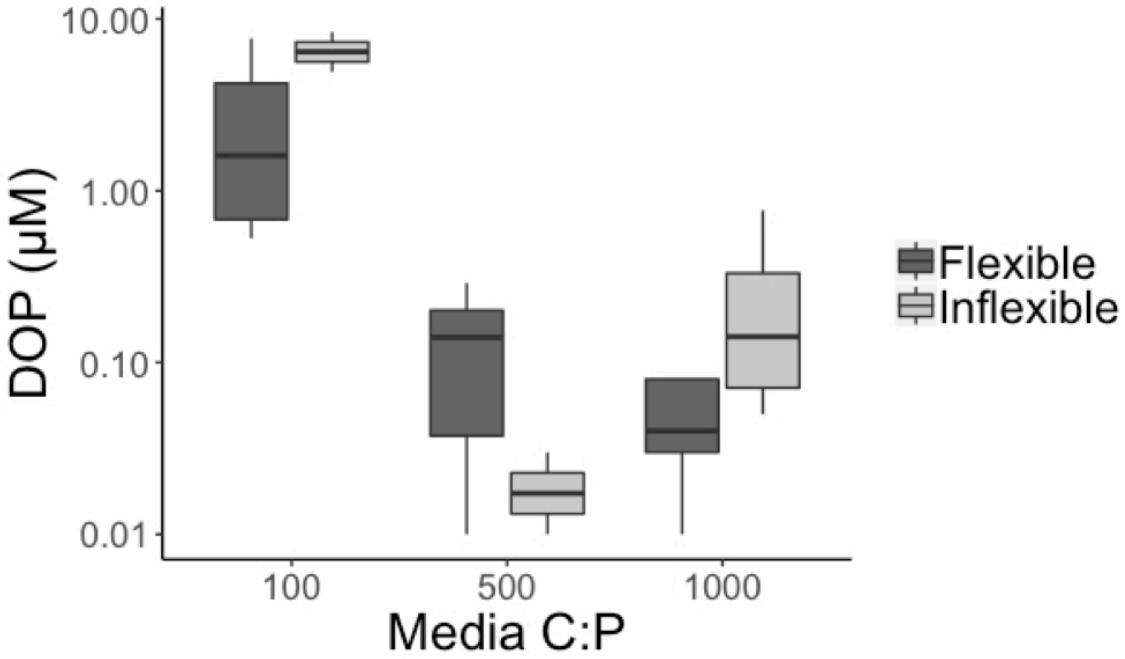

Figure 2-4: Minimum amount of DOP produced over the incubation period.

Minimum DOP production was calculated by subtracting the upper bound of the 95% confidence interval for SRP from the lower bound of the 95% confidence interval for TDP. Values are in micromolar and are plotted on a log scale. Inflexible strains generally produced more DOP than flexible strains (but this pattern was not statistically significant) when grown at the highest and lowest C:P values. Conversely, when grown at an intermediate C:P (500:1), flexible strains produced more DOP than inflexible strains (again this pattern was not statistically significant). Despite no statically significant effect of biomass flexibility on the mean values of DOP production at different treatment levels, there were clear trends in the variability associated with the different flexibilities. Variability in DOP production was highest under low media C:P conditions for flexible strains and decreased at high media C:P conditions, whereas inflexible strains showed the opposite pattern with variability in DOP production increasing as media C:P increased.

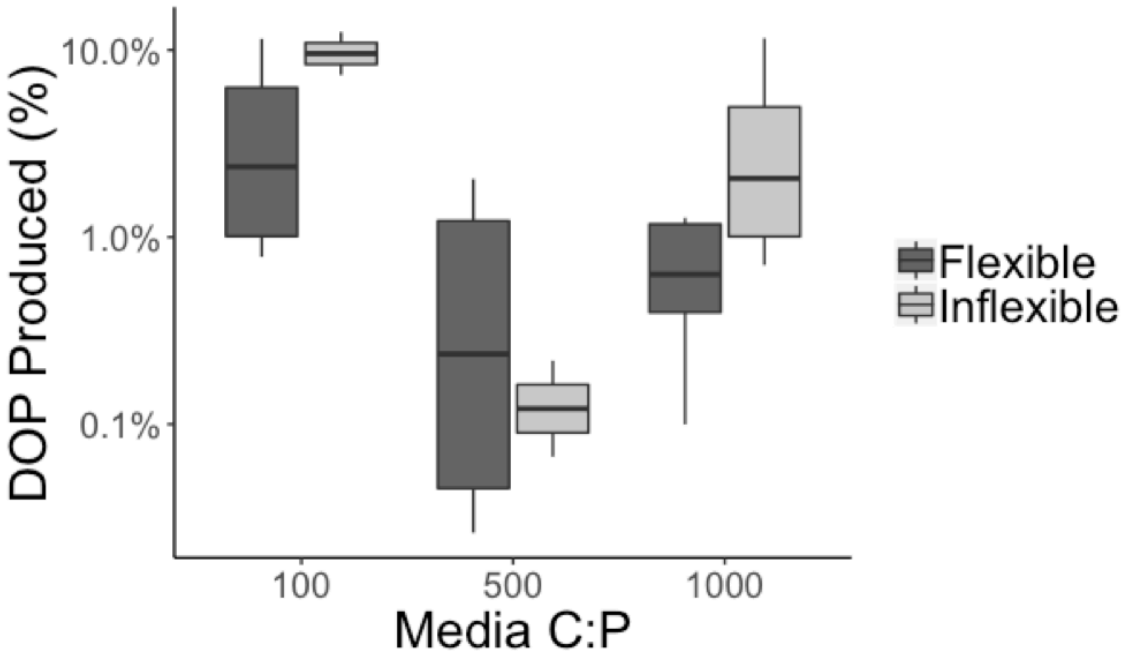

Figure 2-5: DOP production as a percentage of media phosphate concentration.

Relative DOP production is presented on a log scale for both flexible and inflexible strains across all media C:P levels. 5 samples that had DOP production below the method detection limit are removed from the figure. Variation in DOP production was high both within media treatments and across media treatments. Across all samples with measurable DOP, relative DOP production ranged from 0.03% of media phosphate to 12.5% of media phosphate and within media treatment variation covered at least one order of magnitude in all cases. Analysis by factorial ANOVA showed that neither media stoichiometry not stoichiometric flexibility were significant predictors of relative DOP production. However, it is worth noting that under both extreme C:P values (100:1 and 1000:1) median DOP production was higher for inflexible strains than for flexible strains, a pattern that warrants further exploration.

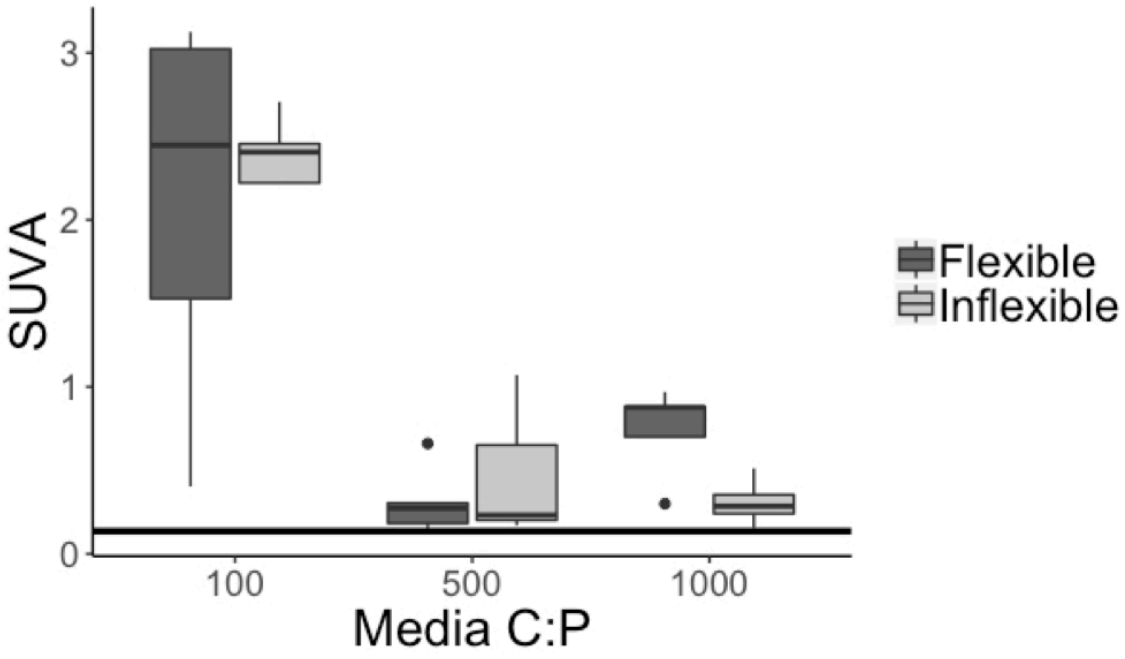

Figure 2-6: Specific-UV-Absorbance at wavelength 254 (SUVA, L * mg-1 * L-1) for flexible and inflexible strains grown at three different phosphorus levels.

All cultures showed an increased SUVA value relative to the value for the original media (black line). Under the most sever P limitation, flexible strains showed elevated SUVA values in the residual media compared to the inflexible strains.

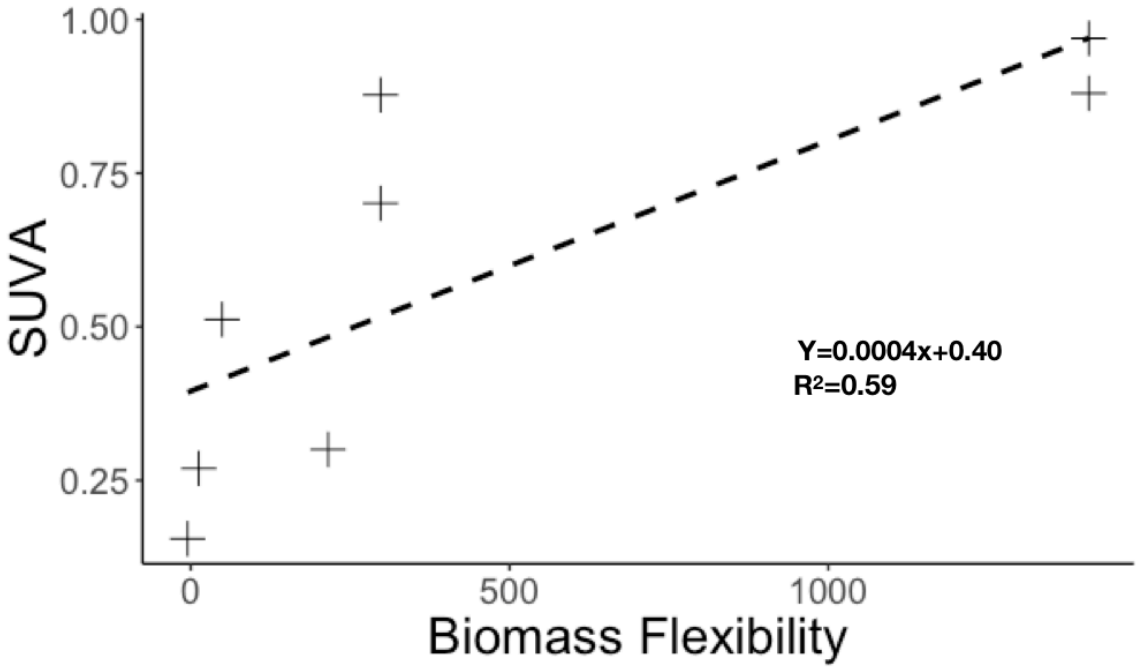

Figure 2-7: SUVA values as a function of biomass flexibility for strains grown in media with a C:P of 1000.

Biomass flexibility was calculated as the percent change in biomass C:P when grown under media C:P conditions of 100:1 and 10000:1. Under severely P-limiting conditions, SUVA of the residual media was strongly and significantly correlated to the measured biomass flexibility of the strain ($p=0.026$). Under the other two culture conditions, there was no relationship between SUVA and biomass flexibility, suggesting that this pattern may be driven by strong P-limitation.

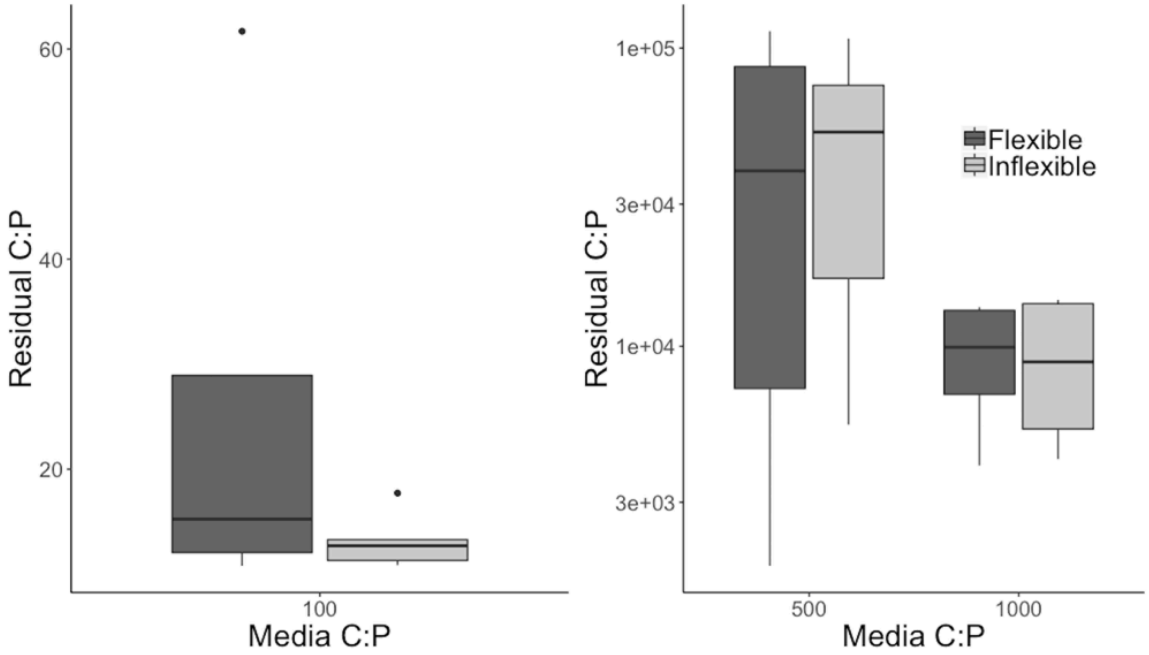

Figure 2-8: C:P values in residual media for inflexible and flexible strains across all three media C:P levels.

Residual C:P was calculated as DOC:TDP and showed significant differences across media C:P level (Factorial ANOVA, p= 0.0023). At a media C:P of 100:1, residual C:P values were strongly influenced by residual phosphate that was not consumed during the incubation period (see Figure 3) and had residual C:P values 2-3 orders of magnitude lower than the higher media C:P treatments, which had nearly complete phosphate removal during the incubation period. Biomass flexibility was not a significant predictor of residual C:P, with media values be very similar in flexible and inflexible strains within each media treatment.

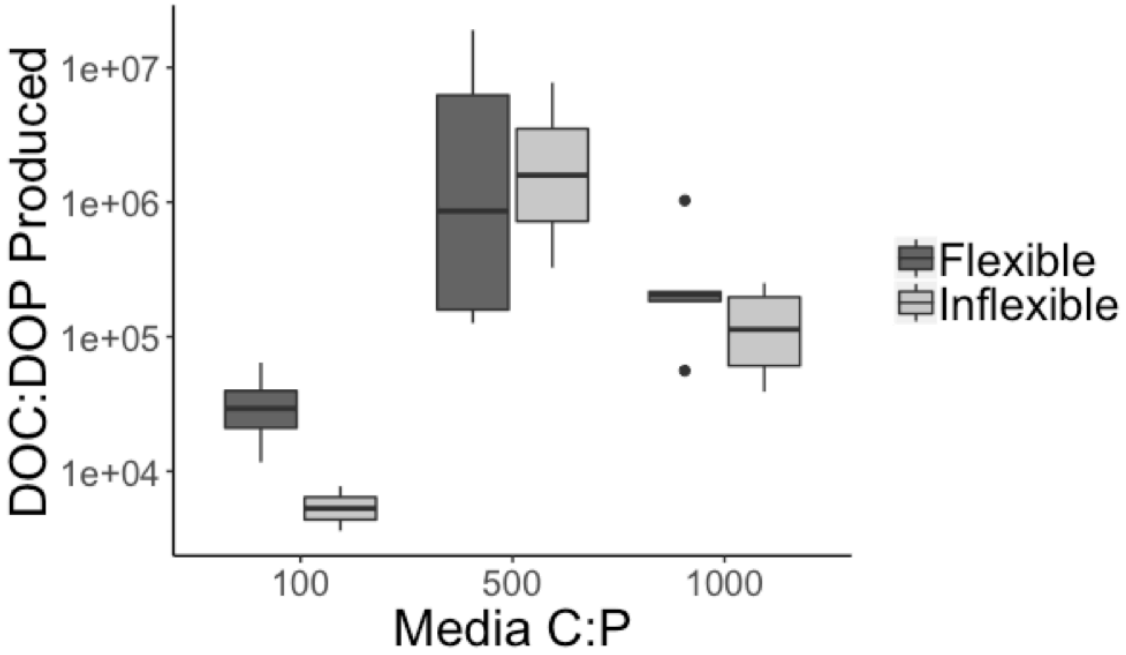

Figure 2-9: DOC:DOP values of residual organic matter across all three media C:P levels for both flexible and inflexible strains, plotted on a log scale.

Generally, microbial produced organic matter was more P-rich in C-limiting media conditions compared to P-limiting media conditions, but neither media treatment or biomass flexibility were statistically significant predictors of DOC:DOP in the residual media. Residual DOC:DOP showed high variation, with values spanning over 3 full orders of magnitude across the media treatments. Strains grown at a media C:P of 500:1 showed the most within treatment variation with 2 orders of magnitude covered. Additionally, strains under C-limiting conditions showed some separate in median DOC:DOP values, with flexible strains producing higher C:P organic matter compared to inflexible strains, but this difference was not statistically significant in our small dataset.

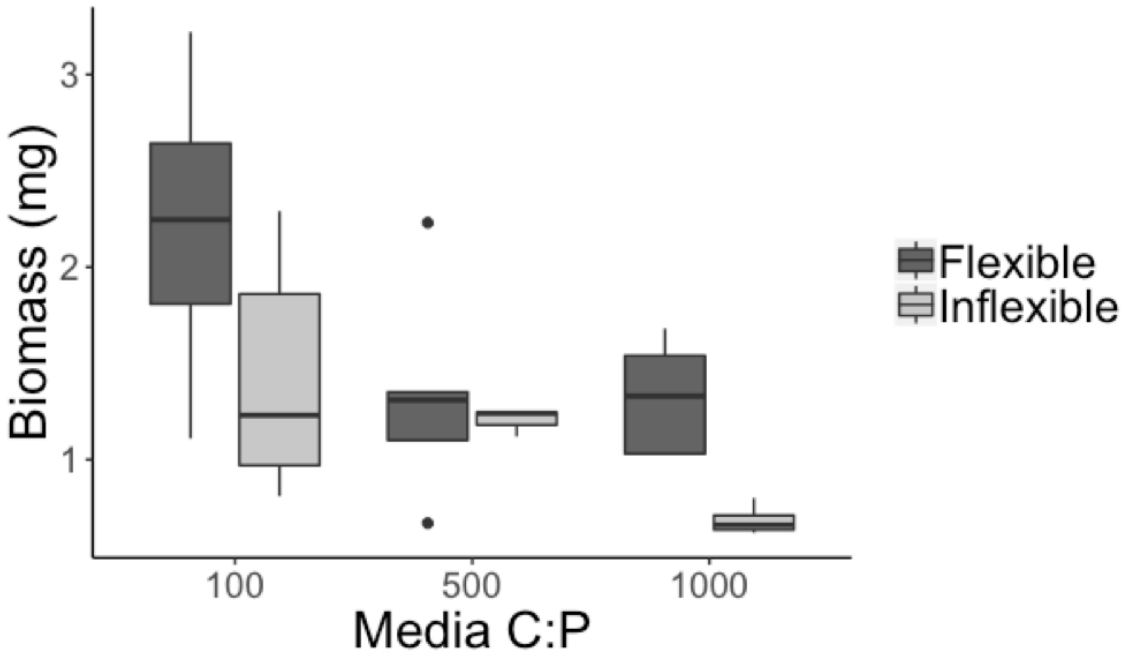

Figure 2-10: Biomass production by flexible and inflexibly strains across all three media C:P levels.

Biomass is shown as mg dry mass per 100 ml of cultural volume. A factorial ANOVA showed that both biomass flexibility and media stoichiometry were significant predictors of biomass production, although there was no significant interaction effect. Post-hoc analysis showed that inflexible strains had significantly less biomass production than flexible strains overall (Tukey, p= 0.02) and that biomass production was lower at a media C:P of 1000:1 than at a media C:P of 100:1 (Tukey, p= 0.02).

Table 2-1: Estimated allocation of resource C by bacteria

Table showing the estimated relative allocation of carbon by both flexible and inflexible strains under high and low media C:P. Biomass C was estimated as 50% of total dry mass, and CO_2 was calculated by mass balance. All estimates represent median values. The estimated growth efficiency was calculated by dividing the biomass C estimate by the total C drawdown (biomass+DOC+CO_2).

Strain Type	*Media C:P*	*Biomass C (μM)*	*DOC (μM)*	*Residual Glucose (μM)*	*CO_2 (μM)*	*Estimated Growth Efficiency (%)*
Flexible	100:1	1.9	0.6	0.01	4.29	28%
Inflexible	100:1	1.0	0.5	0.01	5.29	17%
Flex	1000:1	1.2	1.2	0.16	4.32	17%
Inflexible	1000:1	3.2	3.2	0.39	2.61	14%

Chapter 3: A Case Study Describing a Theoretically Ground Training Program for Undergraduate Teaching Assistants in the Life Sciences

*Substantial portions of this chapter are currently in review for publication at the International Journal for Designs in Learning

Summary

Research over the last decade has indicated that active learning and student-centered instruction lead to better learning outcomes in undergraduate biology courses than traditionally common methods, such as lecturing. This shift in pedagogical approach has been applied to both high-enrollment lecture-based courses as well as smaller enrollment laboratory courses. In laboratory courses, the primary instructor is often a graduate or undergraduate student teaching assistant. These novice instructors often lack the pedagogical knowledge and experience to effectively implement student-centered instructional practices such as inquiry. Therefore, to fully realize the benefits of inquiry-based laboratories for students, the instructors of these courses require support.

In this paper, we present a design case for a theoretically and contextually grounded professional development program aimed at providing pedagogical support for undergraduate teaching assistants in a college biology laboratory course. In its first iteration, four undergraduate teaching assistants participated in a 12-week program to develop their pedagogical knowledge and skills. Participants were assigned weekly readings, turned in periodic reflective writings, and met with an experienced teaching mentor (Author 1) on a monthly basis. As designers, we grounded our design in the current literature, but also built in flexibility to be responsive to participants needs throughout the experience. Participants found it challenging to reflect on pedagogical strategies early in their experience, but found the additional support provided by the program very useful as they developed. Finally, we discuss the participant feedback that is being incorporated into future designs of the professional development programming.

Introduction

Over the last decade, there has been a clear call to shift the instructional methods used for teaching undergraduate biology courses (Brewer & Smith, 2011; Olson & Riordan, 2012). We now know that active learning approaches to teaching science lead to better science outcomes for undergraduate students (Freeman et al., 2014). In light of this evidence, many institutions of higher education have begun shifting their undergraduate biology curriculum focus to a more student-centered approach. This pedagogical shift has been documented in traditionally lecture based courses (McClanahan & McClanahan, 2002; Sivan, Leung, Woon, & Kember, 2000; Walker, Cotner, Baepler, & Decker, 2008), as well as in laboratory courses where students spend their time participating in inquiry activities (Cotner & Hebert, 2016; Weaver, Russell, & Wink, 2008). Traditionally, laboratory courses (those courses where students spend a significant amount of time doing practical activities in a laboratory setting) are often taught as "cookbook style" laboratory experiences, where students follow procedural instructions from a laboratory manual to test a narrow and specified experimental question, but more recently many have shifted towards more open-ended experiences driven by student-led inquiry (Lord & Orkwiszekski, 2006).

Despite this shift in the structure of laboratory courses and increased opportunities for students to participate in open-inquiry, there has been little change in the pedagogical learning opportunities for those teaching the laboratory section. Therefore, programs that support the pedagogical development of laboratory instructors are needed to effectively transition laboratory courses away from an instructor-centered model and towards a student-driven inquiry model. Instructors in these inquiry-based laboratory courses require a skill set that is distinct from that of the instructor in more traditional, "cookbook style" laboratory courses (Gormally et al., 2016). For example, instructors of a "cookbook style" laboratory are primarily responsible for organizing laboratory logistics and troubleshooting student's questions on defined procedures. In contrast, instructors of inquiry-based labs primarily act as facilitators of learning and must develop skills that support students asking questions, analyzing data, and drawing conclusions.

Furthermore, many higher education institutions use graduate or undergraduate students in the role of teaching assistant (TA) to lead laboratory sections. Most TAs, who are the primary points of contact for many college laboratory sections, are not trained in pedagogy and may lack the skill sets necessary to effectively facilitate inquiry. As students themselves, their exposure to college level inquiry-based labs may be limited because the majority of the coursework offered to undergraduate and graduate students is content-driven and lacks fundamental aspects of inquiry. Additionally, current practice for preparing TAs is not conducive to promoting effective teaching strategies, as very few programs provide specific pedagogical support for TAs and instead often focus on ensuring TAs have mastered the content they are expected to deliver (Dotger 2010). Therefore, when undergraduate students are asked to serve as facilitators of inquiry in their role as TAs, they have very little experience or education to draw from.

While some studies have attempted to evaluate and describe effective preparation programs for graduate TAs (e.g., Barrus 1974; Clark & McLean 1979; Roehrig, Luft, Kurdziel, & Turner 2003; Rushin et al., 1997), very little information exists for undergraduate TA programs, particularly in laboratory settings (but see Romm, Gordon-Messer, & Kosinski-Collins 2010 and Gromally, Sullivan & Szeinbaum 2016). Undergraduate TAs can serve as a vital piece of the academic puzzle, providing a level of instructor contact that cannot be facilitated by a faculty member alone in large college courses. Therefore, pedagogical support programs for TAs have the potential to drastically improve the educational experience for many students. In this paper, we present the design case for a theoretically and contextually grounded undergraduate professional development program for laboratory TAs in introductory-biology courses at a large research university.

Context

Institutional Background

Over the past 5 years, the College of Biological Sciences (CBS) at the University of Minnesota has begun to integrate student-driven inquiry experiences into all of the biology laboratory courses in response to the growing national call for student-centered instruction. Given the current biology requirement for graduation, this approach leads to

nearly every undergraduate (both students majoring in a science discipline and students major in non-science disciplines) having at least one inquiry experience before they graduate. Given the number of laboratory sections offered, the use of teaching assistants in these courses is imperative for their implementation and success. Each academic year, about ~100 teaching assistants (TAs) are employed to teach in 115 lab sections. The TAs for our introductory biology courses have quite a bit of autonomy in determining how they run their classrooms and are the primary points of contact for 16-24 students per laboratory section. The TAs prepare for each session, run all laboratory activities, grade assignments, help develop lesson plans, and also implement multi-week, open-ended inquiry labs.

In the process of designing inquiry lab exercises as described above, we realized that in order to obtain the high level of student success that we expect to achieve from these experiences, we must better support the TAs in charge of facilitating these lab experiences. Therefore, we designed the *Building Excellence through Scientific Teaching* (BEST) program as a workshop series to support the implementation of Scientific Teaching (as defined by Handelsman et al., 2004) by TAs. After completing this program, each TA will be well equipped to lead their students through a scientific-inquiry experience and also develop as a scientist and science educator.

Description of Focal Course

The first offering of BEST centered on TAs for one—of several—non-majors introductory biology course. This course, "The Evolution and Biology of Sex," is a theme-based course that approaches the study of biology from the lens of the evolution of sexual reproduction and includes discussion of reproductive biology, sexual orientation, operational sex ratios, sexual selection, and mating systems. The "Sex Class," as it is called, has the dubious distinction of consistently enrolling the most science-phobic students at the institution. However, presumably due to the appeal of the content, it is the most popular of the non-majors offerings, filling to capacity before any other course. Students in the Sex Class tend not to have much interest in science in general, and rarely have extensive experience with advanced science courses in high school, for example, or science-focused extracurricular activities.

Sex Class TAs lead between two and four, 24-person lab sections, in a curriculum that includes single-week (or two-hour) inquiry labs (e.g. testing hypotheses about condoms, human sperm competition, and human population growth), a few "cookbook-style" labs, and one multi-week inquiry lab.

Description of Enrolled Teaching Assistants

In the first iteration of the BEST program, 4 undergraduate TAs voluntarily enrolled in the program. All four participants had no prior teaching experience at the University, although one of the TAs had previous taught in a summer language program. Three participating TAs were female and one TA was male. One TA was an international student. To preserve the anonymity of the participants, gender neutral pronouns are used throughout the text and no pseudonyms are assigned, instead we present the quotes from TAs in an unidentifiable manner.

Given that participation in the BEST program was voluntary and additional to the standard weekly meetings that TAs attend to review laboratory logistics, safety instructions, and review the content to be presented in the laboratory the next week. In order to encourage participation and honor the time TAs were devoting to their work, it was decided by the design team that TAs should be compensated for their participation. We decided on a rate of $500 per semester of participation based on the expectation that TAs spend about 2-3 hours per week on BEST programing over the 12 weeks of programing.

Program Design Process

Identification of Problem of Practice

CBS relies heavily on undergraduate and graduate student teaching assistants (TAs) to facilitate the course-based research experiences. However, historically, our undergraduate TAs have not been trained in pedagogy. This has left many TAs struggling to effectively facilitate inquiry in the laboratory. For these teaching assistants to achieve the goals of these inquiry-based laboratory courses they must understand both (a) the philosophical underpinnings of Scientific Teaching (b) strategies for facilitating student learning in an inquiry-based laboratory.

Literature Review and Theoretical Grounding

In order to ground our design choices within the current state of knowledge, we conducted a literature search to identify the most important aspects of a successful professional development program. Given, the relative paucity of information on specific design structures important to TAs in biology, we expanded our literature search to include programming designed for graduate teaching assistants in various sciences and also K-12 teacher professional development programing. This literature led us to implementing 4 specific design features in the BEST programming: weekly professional development activities, mentorship and coaching from an experienced teacher, participation in an action research project, and periodic classroom observations. The theoretical grounding and literature support for each of these design structures chosen is summarized in **Table 1**.

The Building Excellence through Scientific Teaching Program

Guiding Principles

The overall guiding principles for BEST were largely modeled on content in the book *Scientific Teaching* (Handelsman, Miller, & Pfund, 2007), dividing the first semester programming into three equal parts to cover the basic tenets laid out in the textbook: active learning, assessment, and inclusive teaching. Within each of the topics, TAs were provided with reading materials to increase awareness and understanding of the specific topic, asked to do a reflective writing to connect the readings to their own practice in the classroom, and finally participated in an in-person group discussion facilitated by an experienced practitioner of scientific teaching to provide mentorship and strategies for implementing scientific teaching practices. The majority of the program was delivered to participants using the learning management system Moodle. The general schedule for the program is shown in **Table 2** and a more detailed description of the weekly activities follows.

Weeks 1-4: Active Learning

Week one: Introduction to Active Learning. The first topic covered in BEST was active learning. TAs were first asked to introduce themselves using an online video posting service called Flipgrid prior to the end of the first week of the semester. During their

introduction, TAs were asked to describe their motivation for teaching as well as any previous teaching experience they had in other settings. Student were also assigned two readings to complete during the first week: *Scientific Teaching* by Handelsman et al (2004) and *Active learning increases student performance in science, engineering, and mathematics* by Freeman et al (2014). TAs were required to submit a 1-page reflective writing using the two readings and their experience in the first week of teaching as guidance in answer the following questions: 1) When did you feel that students were engaged in active learning? 2) When did you feel that students were not engaged in active learning? 3) Are there certain lab periods that were more active than others? 4) Did students behave differently during different lab activities? 5) What problems or struggles did you encounter when trying to facilitate active learning activities?

Week Two: Envisioning Growth. In week two, TAs were asked to watch the TED talk by Carol Dweck *The power of believing that you can improve* and submit three questions about teaching that they had been thinking about to an online forum to help prepare the agenda for the future in person discussion. These questions could be anything related to their teaching experience (things they noticed in their classroom, the prior week's readings, general questions about teaching, etc.). TAs were also provided materials specifically outlining how to implement a jigsaw activity (Smith, 1996) and think-pair-share (https://www.schreyerinstitute.psu.edu/pdf/alex/thinkpairshare.pdf) to help link the conceptual topics from week 1 into concrete teaching strategies.

Week Three: Mentoring Meeting. In the third week, TAs had their first of three mentorship meetings. TAs met with their teaching mentor (an experienced practitioner of ST) to discuss active learning. Discussion topics focused on the application of active learning strategies into the teaching laboratory. The mentorship meeting started by allowing TAs the opportunity to raise any questions or comments they had about their experiences in the teaching laboratories so far. After that initial question period, the teaching mentor facilitated a discussion using the TAs questions that were submitted in week 2. In the first interaction of BEST, TAs questions about active learning generally grouped into two categories: questions about implementing specific instructional strategies (i.e. a think pair share) or questions about student engagement and motivation as a barrier for active learning (through lack of participation). To address these concerns, TAs were asked to

share approaches they had used in the classroom to increase student participation and were allowed to discuss which strategies worked and which strategies did not work with their peers. The teaching mentor also provided feedback on the strategies that TAs were trying as well as other examples of techniques to implement to increased student participation. Next, the teaching mentor led a conversation on the literature readings and video from weeks 1 and 2. The following questions were used to guide the conversation: 1) Did you find the evidence provided in the readings persuasive? Why or why not? 2) How have your students responded to active learning so far? 3) How does active learning related to the video about growth mindset? The mentor meeting ended with the teaching mentor using instructional modeling to demonstrate how TAs could facilitate a think pair share in the teaching laboratory.

Week Four: POGIL as a Teaching Tool. The final week of the active learning sessions was used to provide materials responsive to the concerns and questions raised by the TAs in the first 3 weeks. In our case, TAs had many concerns about facilitating group work and assigning groups, so materials in week four focused on that area (but in future iterations this focus could change based on TAs need). TAs were introduced to the process oriented guided inquiry learning (POGIL; pogil.org) framework as a technique for facilitating group work within the teaching laboratories. TAs were asked to spend some time exploring the POGIL website and reviewing the resources that were available to them there. Specifically, they were asked to watch the "What is POGIL" and "What makes POGIL different" videos posted on the website. Afterwards, TAs were asked to submit a response to an online forum to the following prompt: "For this week's discussion points, please post 1 way you imagine you could use POGIL in your lab sections and 1 barrier you anticipate encountering."

Week 5-8: Assessment in the Classroom

Week 5 & 6: The Importance of Assessment. In week 5, participants were provided a reading by Black & Wiliam (1998) entitled "Inside the Black Box: Raising Standards Through Classroom Assessment" and were asked to reflect on opportunities they had in their own classrooms for assessment of their students. Additionally, TAs had a lab section video recorded for the first time in this week. Each TA was given a copy of their

own lab section and asked to watch it before the end of Week 6. Each TA was asked to evaluate their video using a classroom observation worksheet adapt from the University of Nebraska -Lincoln's resources for graduate teaching assistants (https://www.unl.edu/gradstudies/current/teaching/Classroom_Observation_Form.pdf). TAs were instructed to pay particularly close attention to the sections labeled "Presentation" and "Interactions" to assess their performance.

Week 7: Individual Mentor Meetings to Review Video Observations. After completing their own self-evaluation of their video recording, each TA met individually with a teaching mentor (author 1) to discuss their observations. Each meeting starting by having the TA explain how they had scored their own video section. TAs were asked to identify one area of strength based on their video analysis and also one area for future growth. After that, the teaching mentor discussed their own evaluation of the video recording with each TA. The teaching mentor provide examples from the video recording of both instances where TAs were actively facilitating inquiry and pointed out opportunities that the TA had missed or not fully utilized. The teaching mentor and the TAs then discussed specific strategies that could be implemented by the TA in order to improve their facilitation of student inquiry in the teaching lab.

Week 8: Whole Group Mentor Meeting on Assessment. The second whole group mentor meeting took place in the 8th week and primarily focused on discussing the paper that was read in week 5. The meeting started with an opportunity to bring up concerns or problems that had occurred in the teaching lab so far. The teaching mentor facilitated a conversation between the TAs as each of them brought up issues they had encountered. This initial period of the meetings proved to be very valuable in creating a culture of professional support among the TAs as they were able to discuss things happening in their lab sections with their peers. The teaching mentor primarily served as sounding board for ideas and help encourage other TAs to provide their own perspective on the topics brought to light. Occasionally the teaching mentor contributed some thoughts to the conversation, but mostly this time was used to strengthen the relationship between the TAs themselves.

Week 9-12: Inclusive Teaching Practice

Week 9: Introduction to Inclusive Teaching Practice. To start the unit on inclusive teaching, TAs were directed to review the Yale Center for Teaching and Learning's webpage on Diversity and Inclusion (http://ctl.yale.edu/FacultyResources/Diversity-Inclusion). Specifically, TAs were tasked with reviewing the materials under the "Inclusive Teaching Strategies" section header. This website provides a number of very useful strategies that TAs could implement in own classroom, including soliciting student feedback on classroom climate and cultivating a feeling of inclusion within the teaching lab.

Week 10 and 11: Reflecting on Creating an Inclusive Classroom. Following their review of the diversity and inclusion website, TAs were given a handout from the University of Michigan on creating inclusive classrooms (http://www.crlt.umich.edu). This document helps TAs identify typical problematic assumptions in STEM classrooms and provides practical ways to address these through teaching. TAs were asked to respond to this material in a 1 page reflective writing assignment addressing the following four questions: 1) Why do some types of students seem to participate more frequently and learn more easily in my course or field? 2) How might my cultural assumptions influence my interactions with students? 3) How might the identities, ideologies, and backgrounds of students influence their level of engagement in my classroom? 3)How can I change my course (activities, assessments, etc.) to encourage full participation and provide accessibility to all types of students?

Week 12: Whole Group Mentor Meeting on Inclusive Teaching. The final whole group meeting for the first semester occurred during the 12th week of the program. As with previous in person meetings, TAs were first given the opportunity to raise potential concerns or issues they were dealing with in the teaching lab. They leveraged the experiences of their peers to normalize and address these situations as needed. After this preliminary activity, the teaching mentor facilitated a conversation about the value of inclusive teaching, drawing heavily on the materials presented to the TAs in the previous weeks. TAs were asked to reflect on their experience from the semester and comment things they had done to promote an inclusive environment in their lab as well as comment on opportunities they may have missed. This reflective discussion allowed the TAs to acknowledge the complexity of creating inclusive spaces and talk with their peers about

strategies that had been useful for them. TAs commented during the discussion that inclusive teaching was the aspect of programming that they felt least confident in and were least knowledgeable about.

Assessment of Pilot Program

Several artifacts were collected during BEST to assess TAs' learning around program goals and inform design decisions. These include: written reflections, transcripts from whole group mentor meetings, and video recordings (recorded with consent of the participating TAs) of three laboratory sections. A summary of the learning artifacts collected for each program goal is provided in **Table 3**.

Written Reflections

TAs submitted three written reflections during BEST, one reflection for each of the core topics. The reflections were valuable opportunities for the TAs to surface their attitudes and understandings of each core topic. The writings also provide the design team useful metrics for assessing how TAs were thinking about these topics and approaching the challenges of implementing them into their classrooms, as well as guiding the agenda for upcoming whole-group mentor meetings. For example, in the first reflective writing about facilitating active learning, TAs voiced having struggled with facilitating a think-pair-share in their class. In response to this, the teaching mentor specifically included an instruction modeling exercise for facilitating think-pair-shares into the first whole-group mentor meeting. TAs also pointed out instances that they had seen active learning work well in their lab sections, such as "When we went into our lab activity as well, I noticed that they retained information better if they were able to practice using it themselves, rather than listening in theory. They asked me more intuitive and in-depth questions, rather than basic." This allowed the teaching mentor to also highlight these success for the other TAs during the whole group mentor meeting.

Despite an appreciation for the value of active learning, the TAs also reported challenges with facilitating active learning in their lab sections. As an example, one TA mentioned having trouble getting students to participate: "When trying to facilitate active learning methods, I often encountered lack of participation or the idea that they were all safe due to the size of the lab. This often caused me to 'wait it out' when asking them a

question." This TA was trying to involve the students in more active discussion to enhance their learning, but successful facilitation was limited by a lack of experience and familiarity with strategies for overcoming such problems. By surfacing this struggle in the written reflection, the TA provided vital information to their teaching mentor for identifying the most useful resources to provide in order to support the TA. In this case, the teaching mentor used the reflection to start a brainstorming session with TAs during the whole group mentor meeting about how to encourage participation from more students.

TA Lab section video recordings

TAs had one lab section recorded three times throughout the 12-week programming. TAs agreed to have a SWIVL device placed in their classroom to facilitate the recordings. The SWIVL devices were chosen by the design team for video recording because they provided high quality audio and video that tracked the movements of the TAs within the lab. They also required less human effort to collect compared to a traditional video camera, because the SWIVL robot rotated the recording device to follow the TA, eliminating the need for an additional person to disturb the class by following the TA around with a video recorder.

After each of the record lab sections, the videos where shared about with the TAs (each TA only received their own video) for them to review and reflect on their teaching. Because the expectation was set that TAs would only spend an additional 2-3 hours a week on BEST programing, TAs were only required to meet with their teaching mentor once during the 12-weeks to have a specific review session about on of their teaching videos. These individual meetings with the teaching mentor were excellent for addressing the specific concerns that each TA had raised through previous assignments and provide feedback in response to very specific teaching strategies observed on the video recordings.

Whole Group Mentor Meeting

Whole group mentor meetings were used as summative sessions for each of the three core topics. The agenda for the whole group mentor meeting was set by the teaching mentor based on the issues that had surfaced during the reflective writing for the topic

and the TAs interactions. In this manner, the whole group mentor meetings provided highly flexible capstone experience for each topic.

Overall, TAs found the whole group mentor meetings to be the most helpful type of experience they had. These sessions provided TAs an opportunity to not only interact with Author 1 and get feedback on specific types of experiences they had in the lab, but these sessions also provided a structured time for TAs to meet with their peers. Having this time for TAs to discuss their laboratory sections and reflect on how they approached their teaching with their peers promoted a very positive culture of professional support.

The beginning of each session was reserved for TAs to drive the conversation and granting them this time together allowed the teaching mentor to structure the rest of the discussion time to directly meet the needs of the TAs as identified by them. All of the TAs expressed an interest in increasing the number of in person meetings, but the logistics of scheduling these meetings were challenging. Aligning TA schedules with the teaching mentors was not always possible. Some of these logistical problems could be overcome if TAs were required to hold a specific period of time during the week for mentor meetings (i.e. using a course-like structure where a condition of participation is availability for the meeting time), but in our initial population this approach would have excluded too many potential participants.

TA Confidence

TAs also completed a survey prior to starting their professional develop and again at the end of their experience. The survey measured TA's confidence in their own abilities to perform several science process skills (understand literature, analyze data, pose questions, develop hypotheses, design experiments, make predictions, collect data, use statistics, draw conclusions, explain things orally, and explain things orally and in writing) as well as their confidence in their abilities to facilitate learning such that their students could perform the same tasks. Generally, TAs were more confident in their own abilities to do a task then to facilitate their students learning (Table 4). This "confidence gap" tended to persist over the course of the first semester of teaching as 3 out of 4 TAs still showed an overall confidence differential (Table 5). In one case, the TAs confidence to facilitate student learning relative to their own skills dramatically decreased (see Ta-

bles 4 and 5; TA 2) most likely because their initial confidence in their facilitation skills was overestimated and a semester in the classroom brought on this realization. The fact that TAs confidence gap was persistent after a semester of teaching and targeted professional development suggest that TAs require more time to develop confidence in their own facilitation skills. Therefore, for Universities to get the most value from their TAs, they will need to invest in multi-semester teaching opportunities that allow TAs to ad equating develop their skills and confidence in facilitating student inquiry.

Positive Program Outcomes

Overall, participating TAs found the extra professional development to be a positive experience. All four TAs reported the additional training as highly valuable for the professional and career development and that they had learned many transferable skills that would benefit outside of the teaching laboratory. TAs benefited from an enhanced sense of community with their colleagues, which led to greater feelings of professional support. Having regular interactions with a teaching mentor encouraged TAs to be more thoughtful and reflective about their practice. Specifically, the pilot programming of BEST helped TAs gain confidence in their teaching practice and allowed them to identify specific areas for growth.

TAs gained confidence in teaching and acquired new skills for facilitating student inquiry

Over the course of their first semester of teaching, TAs developed a greater appreciation for the importance of implementing evidence based teaching practices. When discussing strategies for active learning in the first mentor meeting, one TA mentioned “Well usually I try to like incorporate like think-pair-share in my lectures and ask them questions along the way. Um. I usually like go through lecture slides before class to like learn about any questions that I could ask them.” This TA was trying to incorporate active learning into their class, even at a very early stage (only 3 weeks of teaching experience at this point), although they admitted that “I think that like a big problem is that once again people are afraid to be wrong in front of their peers” when describing that the think-pair-share was not always successful at engaging students. In this way, TAs were communicating a desire to improve their teaching, but also a need for

continued support and training on specific strategies to improve their implementation of new teaching methods. By the end of the semester, TAs began to see themselves more as facilitators of student learning, rather than distributors of content to students. This shift was evident in the TAs final reflective writing, as one TA put it "Usually, students are more likely to volunteer ideas when they are in a small group. *It will be the instructor's role to facilitate that discussion* and choose suitable topics for students to discuss (emphasis added)."

Reviewing Videos with TAs identified specific areas for improvement

During their second whole group mentor meeting, TAs commented on reviewing their video recorded lab sections as one of the most valuable experiences in training. When asked about the most valuable experience, one TA replied with "I liked the video you had us watch (referring to their video recorded lab section)" and the remaining three TAs all agreed with this. Another TA interjected that it was not only watching the videos of their own teaching, but also the opportunity to specifically meet with the teaching mentor to receive feedback by commenting "just the video, it wasn't that helpful...but (the teaching mentor) really helped us see where we could improve."

During the week 7 video analysis sessions, TAs were able to observe their strengths and weakness in regards to facilitating inquiry within the labs. All four TAs demonstrated the ability to use open-ended questions to engage their students during the lab and presented the course material at an appropriate level for their students. Three of the Four TAs actively encouraged collaborative learning during their observed section and two TAs effectively differentiated their instructional methods to explain complex material to different groups of students.

Video review sessions also revealed some clear challenges for participating TAs. All of the TAs struggled with allowing for appropriate wait time for students to responded to open-ended questions that were posed. Additionally, three of the four TAs struggled with implementing formative assessment strategies such as checking for student's understanding using probing questions. Instead, these TAs often offered a vague confirmation of understanding like "Do you have an questions?" or "Everything going okay?" During the individual video review meetings, the teaching mentor was able

to point to these situations for TAs to improve their use of probing questions and other strategies for more accurately gauging student understanding.

Areas for Program Growth

The initial iteration of the BEST program identified a number of areas for further growth. For new TAs support was most needed on logistics and classroom management at the beginning of their first teaching semester. Our first iteration underestimated this need and did not provide any of this type of support for new TAs, instead we started immediately by focusing on the philosophical underpinnings of scientific teaching and how to implement evidence based teaching strategies in the teaching labs. During one of the mentor meetings later in the first semester, TAs expressed that they felt unprepared for the material early in the semester and would have preferred to have done those readings later when they had more experience to reflect on and were better able to connect with the material. In this manner, the emphasis on scientific teaching principles early on caused a disconnect between content and context because the assigned readings did not related to TA experiences (or preempted experiences).

We also identified a strong preference by TAs to participating in professional development in person rather than through online activities. For example, TAs found the in person discussions with their teaching mentor much more helpful than reflective writings. One TA commented "When you give us a reading and have us submit a reflection, I think I forget it right after submitting." The TAs all expressed a strong desire to meet more often with their teaching mentor than the once a month that we offered in our first implementation of BEST. This has important design and logistical implications as scheduling in person meetings with TAs can be challenging if the schedule for those meetings is not set early on. However, if the logistical barriers can be overcome, our experience suggests TAs benefit more from in person meetings.

Conclusions and next steps

Scaffolding material to meet TA needs

Perhaps the most important lesson learned from the initial implementation of the BEST program was that TA support needs are dynamic over time and professional development opportunities must be closely aligned to fill those needs. Our group of new

TAs started out primarily needing support in the logistical aspects of running a lab and performing their day to day duties (such as taking attendance and grading in the course management software program). After 3-5 weeks, TAs became more comfortable in the teaching labs and were more confident in testing new teaching strategies. At this point, they were more engaged with materials examining the theory behind Scientific Teaching strategies. Future training programs should focus primarily on logistical support and building TA confidence in their classroom early on in the first semester of teaching. After TAs have gained confidence and feel that they have their classrooms under control, then they should begin receiving coaching and training on the fundamentals of scientific teaching.

Opportunities for peer observations to learn from colleagues

TAs all had a positive experience reviewing their own video recorded lab sections to do self-evaluations of their teachings, but all of the TAs felt it would have been helpful if they had also been given the opportunity to observe and learn from their colleagues. By having the video observations occur in the middle of the first semester, TAs had already built a strong sense of supportive community with their fellows TAs. This community building would be pivotal for being able to facilitate a constructive peer observation system. Incorporating this opportunity for peer feedback could also have an additional positive impact on the feelings of support from colleagues. This type of opportunity could be facilitated in a group setting at first, with the teaching mentor leading a group observation session on videos from each TAs lab section. That would help promote a positive and constructive atmosphere. Once this has occurred, it would then be possible to set up live observations for TAs that wanted to continue learning form their peers.

Universities need a shift in culture around TAs training and expectations

Working with departments and or colleges to shift the culture around the value of teaching experience is paramount for the success of any of the proposed training experiences. In order for TAs to invest time in improving their teaching practice, they need to feel like teaching is a skill that is valued at their institution. Shifting this culture can be particularly tricky at large research universities, where many programs have been designed to get students involved in research experiences. Institutional investment into

undergraduate research is an important endeavor, but it can lead to the devaluing of undergraduate teaching opportunities as important professional development. By shifting the cultural around teaching towards a more professional and supportive community, where TAs are expected to work hard to improve their teaching but also given the support system they need to meet these expectations, TAs will gain a lot more from their experience. Additionally, the students in the classrooms will also greatly benefit from the improved teaching of their TAs. In this manner, creating a culture focused on providing excellent teaching and learning opportunities will be imperative for helping colleges and universities fulfill their primary missions of educating their students.

Table 3-1: Theoretical grounding table for the BEST program.

Each design structure was chosen to specifically achieve a desired program goal based upon our review of the literature.

BEST Program Goal	Design Structure	Theoretical Grounding
Skills for Facilitating inquiry Increased confidence in teaching skills	Weekly professional development programming focusing on Scientific Teaching	1. National call for increased engagement in Science, Technology, Engineering, and Mathematics (Olsen & Riordan, 2002) 2. Inquiry drives conceptual understanding for students (Minner, Levy, & Century, 2010) 3. PD is more effective when sustained over time (Garet et al., 2001) 4. Effective PD is imperative for promoting inquiry-based teaching approaches (Pozuelos, Travé González & Cañal de León, 2001)
Incorporation of summative and formative assessments	Coaching and mentorship from experienced facilitators of scientific teaching throughout the 1st and 2nd teaching semester	1. Formative assessment promotes deeper learning by students (Higgins, Hartley, & Skelton, 2002). 2. Effective relationships with a teaching mentor promote evidence-based teaching practices (Bradbury, 2010) 3. Metacognitive-guided inquiry can enhance gains in inquiry skills (Brewer & Smith, 2011)
Increased confidence in teaching skills	Action Research Project	1. National call for Student-Centered learning (Brewer & Smith, 2011) 2. National call for increased engagement in Science, Technology, Engineering, and Mathematics (Olsen & Riordan, 2002)
Active Facilitation of Inquiry in the laboratory Demonstration of Inclusive Teaching	Classroom Observations	1. Classroom practices are major contributors to student learning (Smith, Jones, Gilbert, & Wieman, 2013) 2. National call for increased engagement in Science, Technology, Engineering, and Mathematics (Olsen & Riordan, 2002)

Table 3-2: Schedule of activities for the BEST program.

The semester was broken into three large chunks, each focusing on a different aspect of Scientific Teaching. Within each topic, participants were asked to complete a number of readings and assignments. Each topic was capped off with an in person group meeting with the teaching mentor to debrief on the material and transition to the next topic.

Week	Scientific Teaching Dimension	Subtopic	Readings and Assignment(s) Due
1	**Active Learning**	Introduction to Active Learning	**Readings** 1. *Scientific Teaching* (Handelsman et al., 2004) 2. *Active learning increases student performance in science, engineering, and mathematics* (Freeman et al., 2014) **Assignments:** 1-page reflective writing
2		Envisioning Growth	**Readings** 1. *The power of believing that you can improve* (Carol Dweck Ted Talk) 2. *Cooperative Learning: Making "Groupwork" Work* (Smith 1996) 3. Think-Pair-Share handout **Assignments:** Submit 3 questions about teaching
3		Active Learning Mentor Meeting	TAs meet with Author 1 in person for discussion and feedback on facilitating active learning
4		*Process Oriented Guided Inquiry Learning* (POGIL) as a Teaching Tool	**Readings:** *Implementing POGIL* **Assignments:** Online forum submission on using POGIL

Week	Scientific Teaching Dimension	Subtopic	Readings and Assignment(s) Due
5	**Assessment**	The Importance of Assessment	**Readings**: *Inside the Black Box: Raising the Standards Through Classroom Assessment* (Black and Wiliam 1998) **Assignments:** 1. Written reflection on assessment opportunities in their classroom 2. Review of laboratory recording
6 & 7		Individual Mentor Meeting	Each TA met individually with Author 1 to review the video recording of their lab section form the previous week
8		Assessment Group Mentor Meeting	TAs meet as a group with Author 1 for discussion and feedback on using assessment in the classroom and to reflect on the usefulness of self-assessment in their teaching
9	**Inclusive Teaching**	Introduction to Inclusive Teaching	**Readings** 1. Yale Center for Teaching and Learning Diversity and Inclusion Website 2. *Inclusive Teaching Strategies* from same website
10 &11		Creating an Inclusive Classroom	**Readings:** Creating Inclusive Classrooms handout from University of Michigan **Assignments:** 1-page reflective writing
12		Inclusive Teaching Group Mentor Meeting	TAs meet as a group with Author 1 to discuss strategies for creating inclusive classrooms. TAs are asked to reflect on missed opportunities for inclusion and ways to improve in future semesters.

Table 3-3. List of learning artifacts collected to address each of the BEST program goal

BEST Program Goal	Artifact Collected
Skills for Facilitating inquiry	Reflective Writings, TA lab section video
Increased confidence in teaching skills	Reflective writings, whole group meeting transcripts
Incorporation of summative and formative assessments	TA lab section video, whole group meeting transcripts
Active Facilitation of Inquiry in the laboratory	TA lab section video, reflective writings
Demonstration of Inclusive Teaching	TA lab section video, reflective writings

Table 3-4. Confidence differential for TAs prior to their first semester of teaching.

TAs each took a survey before their first semester of teaching and rated their confidence in doing a variety of science tasks as well as their confidence in facilitating the same tasks with their students (on a 5 point Likert scale). Confidence differential was calculated for each TA by subtracting a TAs confidence in facilitating a task from their confidence in during a task themselves. In this manner, positive values mean that TAs were more confident in their own abilities to do a task, whereas negative values mean that a TA is more confident in facilitating the task with their students. Total differential was calculated by summing the differential for each question.

Question	TA 1	TA 2	TA 3	TA 4
Understand Literature	**0**	**0**	**1**	**0**
Analyze Data	**1**	**1**	**1**	**-1**
Pose Questions	**1**	**0**	**1**	**0**
Develop Hypothesis	**1**	**0**	**1**	**1**
Design Experiments	**1**	**0**	**1**	**0**
Make Predictions	**1**	**0**	**1**	**0**
Collect Data	**1**	**1**	**1**	**0**
Use Statistics	**0**	**1**	**1**	**0**
Draw Conclusions	**1**	**0**	**1**	**-1**
Explain Orally	**0**	**-1**	**1**	**0**
Explain Orally and Written	**1**	**0**	**1**	**0**
Total Confidence Differential	**8**	**2**	**11**	**-1**

Table 3-5. Confidence differential for TAs after their first semester of teaching.

TAs repeated the confidence survey after their first semester of teaching and rated their confidence in doing a variety of science tasks as well as their confidence in facilitating the same tasks with their students (on a 5 point Likert scale). Confidence differential was calculated for each TA by subtracting a TAs confidence in facilitating a task from their confidence in during a task themselves. In this manner, positive values mean that TAs were more confident in their own abilities to do a task, whereas negative values mean that a TA is more confident in facilitating the task with their students. Total differential was calculated by summing the differential for each question.

Question	TA 1	TA 2	TA 3	TA 4
Understand Literature	**1**	**0**	**1**	**0**
Analyze Data	**1**	**1**	**1**	**0**
Pose Questions	**0**	**1**	**1**	**0**
Develop Hypothesis	**0**	**1**	**0**	**0**
Design Experiments	**1**	**1**	**1**	**-1**
Make Predictions	**1**	**1**	**1**	**0**
Collect Data	**0**	**1**	**0**	**-1**
Use Statistics	**0**	**0**	**2**	**0**
Draw Conclusions	**0**	**1**	**1**	**0**
Explain Orally	**0**	**0**	**0**	**1**
Explain Orally and Written	**0**	**1**	**0**	**1**
Total Confidence Differential	**4**	**8**	**8**	**0**

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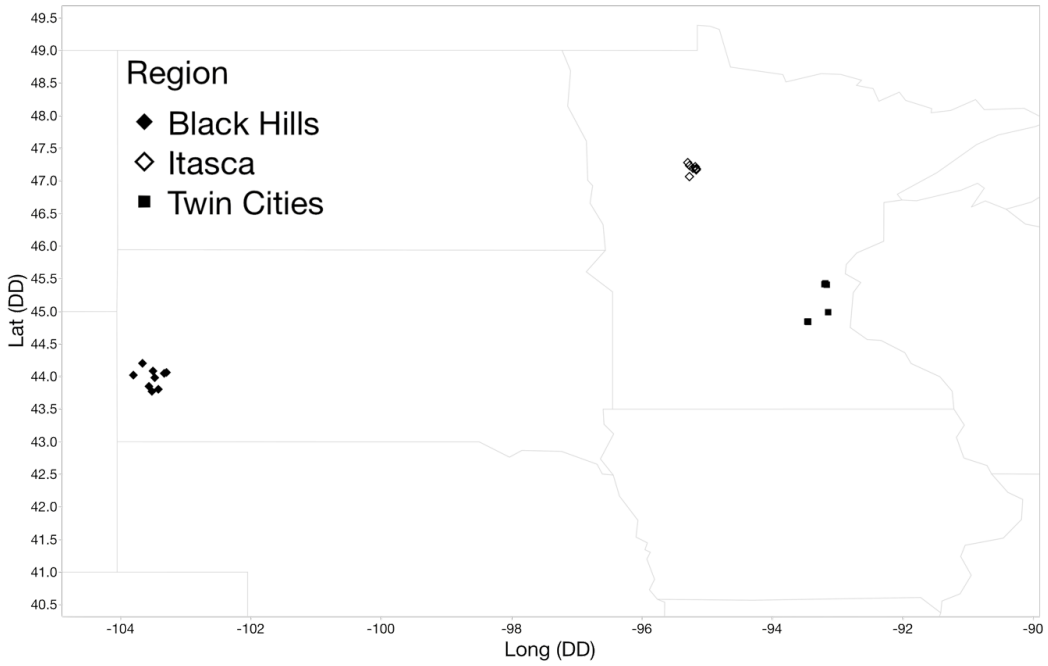
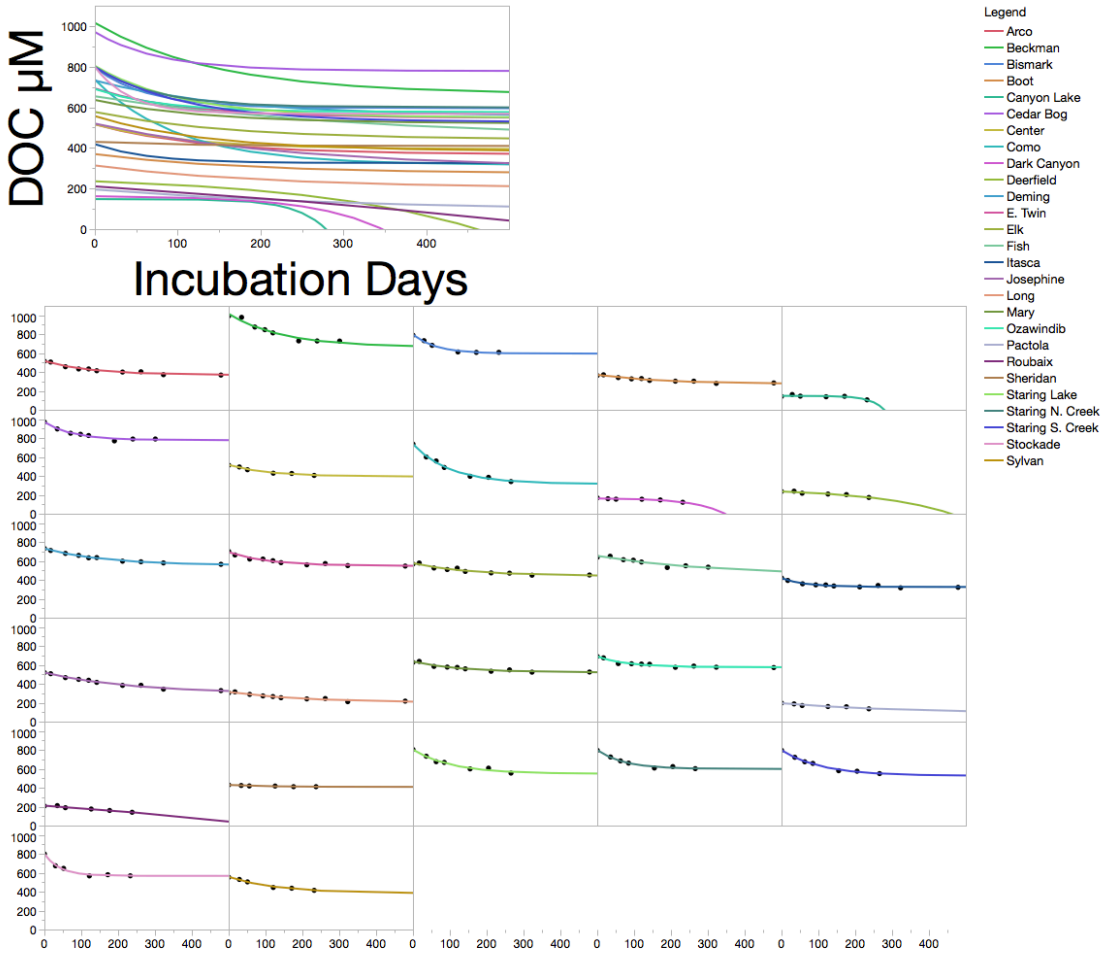
Appendix A – Chapter 1 Supplementary Material



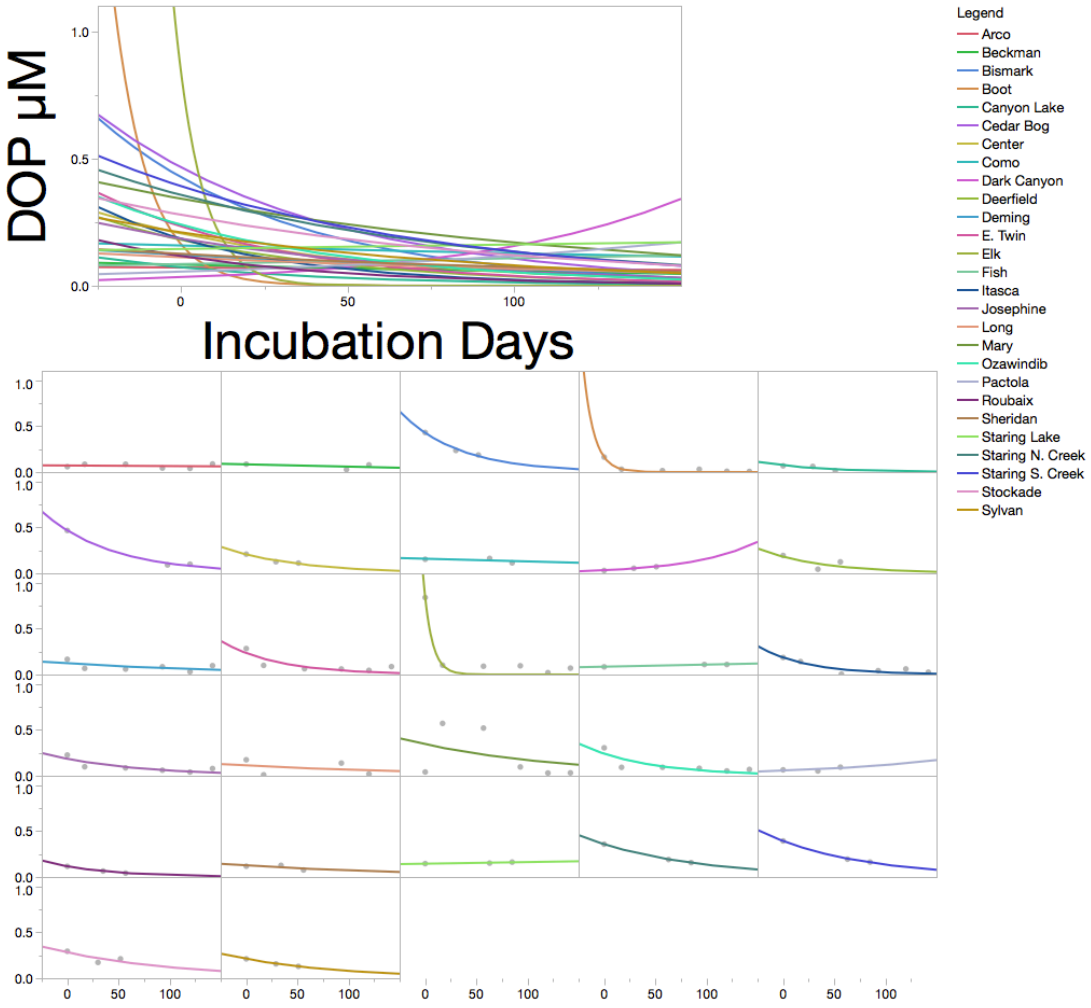
Supplementary Figure 1: Map showing the relative location of each of the study sites



Model Comparison

Model	AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Square
Exponential 3P	1788.8045	1		1958.5252	16077.058	122.72564	11.07816	0.9980936
Exponential 2P	2030.7257	2.934e-53		2175.8507	88652.153	561.08958	23.687329	0.9894877

Supplementary Figure 2: Long term incubation data showing the DOC degradation over time for each of the 27 systems studied. Degradation rates were calculated using an 3 parameter exponential fit model. Model comparisons between a 2 parameter and 3 parameter model is also shown.



Model Comparison

Model	AICc	AICc Weight	.2 .4 .6 .8	BIC	SSE	MSE	RMSE	R-Square
Exponential 2P	-80.54247	1		-48.74475	0.3825398	0.0069553	0.0833983	0.7731538
Exponential 3P	335.71441	4.083e-91		32.866472	0.2530235	0.0090366	0.0950608	0.849957

Supplementary Figure 3: Long term incubation data showing the DOP degradation over time for each of the 27 systems studied. Degradation rates were calculated using an 2 parameter exponential fit model. Model comparisons between a 2 parameter and 3 parameter model is also shown.