Title: The relative importance of photodegradation and biodegradation of terrestrially derived dissolved organic carbon across four lakes of differing trophic status

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Abstract

Outgassing of carbon dioxide (CO_2) from freshwater ecosystems comprises 12-25% of the total carbon flux from soils and bedrock. This CO_2 is largely derived from both biodegradation and photodegradation of terrestrial dissolved organic carbon (DOC) entering lakes from wetlands and soils in the watersheds of lakes. In spite of the significance of these two processes in regulating rates of CO_2 outgassing, their relative importance remains poorly understood in lake ecosystems. In this study, we used groundwater from the watersheds of one subtropical and three temperate lakes of differing trophic status to simulate the effects of increases in terrestrial DOC from storm events. We assessed the relative importance of biodegradation and photodegradation in oxidizing DOC to CO_2. We measured changes in DOC concentration, colored dissolved organic carbon ($SUVA_{320}$ and S_r), dissolved oxygen, and dissolved inorganic carbon (DIC) in short-term experiments from May-August, 2016. In all lakes, photodegradation led to larger changes in DOC and DIC concentrations and optical characteristics than biodegradation. A descriptive discriminant analysis showed that in brown-water lakes, photodegradation led to the largest declines in DOC concentration. In these brown-water systems, ~30% of the DOC was processed by sunlight and a minimum of 1% was photo mineralized. In addition to documenting the importance of photodegradation in lakes, these results also highlight how lakes in the future may respond to changes in DOC inputs.

Introduction

Lakes are closely linked to their surrounding terrestrial ecosystems. As the lowest point in the landscape, they receive a significant influx of terrestrially-derived dissolved organic carbon (DOC) and nutrients (Williamson et al., 2009; Wilkinson et al., 2013). Climate and land use changes are altering the link between lakes and their surrounding landscapes by strengthening the flow of material during extreme rain events and large wildfires, or weakening it during extended periods of drought (Strock et al., 2016; Williamson et al., 2016). Long-term changes in DOC concentrations are variable and appear to be regionally controlled. In northeastern North American and western European lakes, there has been as much as a doubling of DOC concentrations due to recovery from anthropogenic acidification and climate change (Monteith et al., 2007; Williamson et al., 2015; de Wit et al., 2016). However, DOC concentrations in Greenland lakes (Saros et al., 2015) and the Mississippi River (Duan et al., 2017) have been decreasing. A long-term study of the Florida Everglades showed that some study sites were decreasing in DOC concentration, but the majority of sites were not changing (Julian et al., 2017). As DOC inputs into aquatic ecosystems have increased, stabilized, or decreased, long-term studies have focused on understanding the mechanisms behind the change, but less research has addressed the fate of DOC once it enters a lake.

By attenuating light in the water column and also providing a source of energy, DOC serves an important role in lakes by regulating the balance between photosynthesis and respiration (Williamson et al., 1999), and thus the flux of CO_2 to the atmosphere (Cole et al., 1994). Previous studies indicated that most lakes are net heterotrophic, where the breakdown of organic carbon exceeds production (Kling et al., 1991; Cole et al., 1994). Estimates suggest that lakes respire about half of the annual 2 gigaton flux of carbon to the oceans each year as CO_2

(Cole et al., 1994; Tranvik et al., 2009; Tranvik, 2014). The traditional paradigm has been that the dominant mechanism causing the release of excess CO_2 from lakes is bacterial respiration of DOC (biodegradation), with photomineralization (conversion of DOC to CO_2) accounting for only 10% of bacterial rates (Granéli et al., 1996; del Giorgio et al., 1997; Jonsson et al., 2001). However, research on over 200 Arctic lakes, rivers, and streams revealed that sunlight dominated the processing of DOC, and photomineralization rates were on average 5x greater than dark bacterial respiration rates (Cory et al., 2014). In addition, the source of inland water CO_2 remains uncertain, due in large part to a lack of measurements (Raymond et al., 2013; Lapierre et al., 2013; Weyhenmeyer et al., 2015) and predicting DOC reactivity has been challenging (Evans et al., 2017). Quantifying the dominant degradation pathways for terrestrial DOC from a range of lakes will improve estimates of carbon fluxes, particularly mineralization rates that currently have a high degree of uncertainty (Hanson et al., 2014).

Many past studies have focused on testing the effects of photodegradation and biodegradation on DOC quantity individually, but they have not simultaneously evaluated how these two processes alter the colored dissolved organic carbon (CDOM) (Granéli et al., 1996; Koehler et al., 2014; Vachon et al., 2016a). CDOM is the fraction of dissolved organic matter that is capable of absorbing light. The effects of sunlight on DOC are not isolated to only increasing mineralization rates. Photodegradation can also decrease the color and molecular weight of DOC, which can increase light availability and the subsequent bacterial respiration of DOC (Bertilsson and Tranvik, 2000; Amado et al., 2003; Chen and Jaffé, 2016). Cory et al. (2014) found the dominant degradation process for Arctic lakes to be partial photodegradation, suggesting that in lakes, sunlight-driven changes in CDOM without undergoing complete mineralization may dominate DOC processing.

Since light attenuation varies so strongly among lakes of differing trophic status, testing the relative importance of DOC processing via photodegradation or biodegradation with mechanistic experiments is needed. Previous research on DOC degradation has primarily occurred in high DOC lakes, but in clear-water lakes, 1% of surface UV-A and photosynthetically active radiation (PAR), which are the primary wavelengths active in photodegradation (Osburn et al., 2001) can reach significant depths. In some oligotrophic lakes UV-A may reach up to 7 m for UV-A and 14 m for PAR. In some of the clearest lakes in the world, such as Lake Tahoe, PAR can reach depths > 45 m (Rose et al., 2009a; Rose et al., 2009b). Geographic location and time of year influence the amount of solar radiation lakes receive. In the subtropics, PAR and UV light have high intensity across the spectrum year-around, whereas in temperate regions those wavelengths are strongest during the summer months.

Watershed land use and lake trophic status have also been shown to influence DOC composition and reactivity (Lu et al., 2013; Hosen et al., 2014; Larson et al., 2014; Evans et al., 2017). DOC from forested systems was more reactive and had different CDOM properties when compared to disturbed environments (Lu et al., 2012; Williams et al., 2015; Evans et al., 2017). Studies examining how terrestrial DOC inputs are processed in lakes are needed, especially with the increasing frequency of extreme rain events (Rahmstorf and Coumou, 2011; Westra et al., 2014; Fischer and Knutti, 2015). Future climate change projections suggest that for northern ecosystems a 10% increase in precipitation could lead to a 30% increase in the mobilization of soil organic matter (de Wit et al., 2016). Extreme rain events deliver fresh DOC not exposed to prior sunlight into lakes, which can lead to significant reductions in light availability, as well as increases in thermal stability and lake heterotrophy (Jennings et al., 2012; Klug et al., 2012; de

Eyto et al., 2016; Zwart et al., 2016). As DOC concentrations change globally, understanding the processes that determine the fate of DOC will help predict the systems most likely to release more CO_2.

Here our aim was to 1) determine the relative importance of photodegradation and biodegradation for altering terrestrial DOC quantity and CDOM from lakes of varying trophic status, 2) quantify the percentage of the initial DOC pool that was photomineralized, partially-photodegraded, biodegraded or remained unprocessed, and 3) compare the effects of photodegradation on DOC quantity and CDOM across four lakes to understand differences in how terrestrial DOC from the watersheds of different lake types responds to photodegradation. Since lakes are closely linked to their surrounding landscape (i.e. soils and vegetation), we collected terrestrial DOC from the watershed of three temperate lakes and one subtropical lake, all varying in trophic status. This soil organic matter represents the current and future inputs of organic material. We studied changes in the concentration of DOC, dissolved inorganic carbon (DIC), and dissolved oxygen (DO) and measured changes in CDOM. We hypothesized that photodegradation would be more important than biodegradation in all lakes, but the strongest responses to sunlight would be observed in the brown-water lakes.

1. Methods

1.1 *Study Sites and Samplers*

Groundwater samples were collected from the watersheds immediately adjacent to four lakes used in this study (Table 1). All of the lakes are small, with a surface area ≤ 0.48 km^2 and a maximum depth ranging from 12.5 m in Lake Waynewood to 24 m in Lake Giles. The three temperate lakes (Giles – oligotrophic; Lacawac – brown-water; Waynewood – eutrophic) are in

close proximity, located on the Pocono Plateau in northeastern Pennsylvania. Lake Annie (brown-water) is a subtropical, sinkhole lake located on the Lake Wales Ridge in south-central Florida. These lakes were selected because of their variability in the dominant vegetation types in their watersheds that lead to differences in DOC concentration and quality (Table 1). Annie, Giles, and Lacawac are all seepage lakes within protected watersheds, and there have been no significant changes in land use or land cover over the past thirty years. The watersheds of Giles and Lacawac have > 90% cover of mixed and northern hardwood-conifer forests, with oak trees dominating the watershed at Giles, while hemlocks represent the highest proportion of Lacawac's watershed (Moeller et al., 1995). Annie is surrounded by well-drained sandy soils and the major vegetation types include a mixed-scrub community, pinelands, and oak forests (Gaiser, 2009). Both Annie and Lacawac are brown-water lakes with moderate DOC concentrations and lower transparency (Table 1). A higher percentage of wetlands (7% for Annie and 25% for Lacawac) in their watersheds likely contribute to their darker color compared to the other lakes (Moeller et al., 1995; H. Swain *unpublished data)*. Waynewood is the most eutrophic lake and has the largest watershed with runoff from dairy farms upstream that feeds into the lake through an inlet stream. The forest surrounding Waynewood is similarly dominated by oak and hemlock trees, but there is overall less total forest cover in the watershed than Lacawac and Giles, and there are more homes adjacent to the lake (Moeller et al., 1995). Detailed information about lake residence time calculations and annual precipitation trends can be found for the Pocono lakes (Moeller et al, 1995) and Lake Annie (Swain, 1998; Sacks et al, 1998).

Table 1. Summary characteristics of the four study lakes in May-August 2013–2016 (mean ± SD). Abbreviations: Chl-*a* (chlorophyll-a), DOC (dissolved organic carbon), GW DOC (initial groundwater DOC), PAR (photosynthetically active radiation, 400-700 nm), UV-A (ultraviolet A radiation, 380 nm), UV-B (ultraviolet B radiation, 320 nm), RT (residence time).

Lake	Lat. (°)	Long. (°)	Lake area (km^2)	Max. depth (m)	Chl-*a* ($\mu g\ L^{-1}$) ± (SD)	Lake DOC ($mg\ L^{-1}$) ± (SD)	GW DOC ($mg\ L^{-1}$) ± (SD)	pH ± (SD)	1% UV-B depth (m) ± (SD)	1% UV-A depth (m) ± (SD)	1% PAR depth (m) ± (SD)	RT (yr)
Lacawac	41° 22′ N	75° 17′ W	0.21	13	1.9 (1.4)	5.2 (0.8)	59.4 (6.1)	6.6[+]	0.4 (0.1)	0.9 (0.2)	5.7 (0.6)	3.3
Annie	27° 12′ N	81° 20′ W	0.36	20.7	4.0 (1.5)	9.4 (2.5)	20.7 (0.5)	5.5 (0.3)	0.5*	1.3*	4.5 (1.6)	2
Wayne-wood	41° 23′ N	75° 21′ W	0.28	12.5	5.3 (3.7)	6.4 (1.0)	7.6 (0.3)	7.5[+]	0.3 (0.1)	0.7 (0.2)	4.3 (0.9)	0.42
Giles	41° 22′ N	75° 5′ W	0.48	24	1.1 (0.7)	2.3 (0.3)	6.0 (0.6)	6.2[+] (0.3)	2.0 (0.5)	4.7 (1.2)	14.4 (2.1)	5.6

[*]Indicates estimates from a single profile in March 2012. [+]pH data in Lacawac and Waynewood are from 2015 only and from 2015-2016 in Giles.

Samplers were used to collect groundwater as a proxy for terrestrial DOC runoff entering the lakes. Storm events have been shown to mobilize DOC from shallow groundwater pools into aquatic ecosystems (Boyer et al, 1997). The samplers were installed in close proximity to the Pocono lakes near small inlet streams in sandy or bog areas on 6 July 2015 (~1 year prior to experiments). The groundwater sampler consisted of 1m sections of 7.6cm diameter PVC pipe installed to a depth of 60-81cm below ground. 0.5cm holes were drilled in the sides with a fine mesh covering the holes to let shallow groundwater in but exclude large particulates. At Lake Annie, a groundwater sampler was installed on 17 March 2016 on the south side of the lake near a small, intermittent inlet stream. The groundwater sampler near Lake Annie was a 3m section of PVC pipe installed slightly deeper to 2m below ground to allow continuous access to groundwater during the dry season.

On 7 May 2016, 10 L of water was collected using a peristaltic pump from the groundwater samplers at all of the Pocono lakes in acid-washed 18 L bottles. Groundwater samples from Annie were collected from the sampler monthly (25 April, 31 May, 27 June, and 1 Aug 2016) prior to starting the experiments and shipped overnight on ice to Pennsylvania. All groundwater samples were kept cold (4 °C) and dark until filtered to avoid sunlight exposure prior to the start of the experiments. Samples for the May experiments were filtered on May 8, 2016 through pre-combusted (450 ºC) 0.7 µm Whatman GF/F filters. The remaining 8 L of groundwater for the June, July, and August experiments for each Pocono lake were filtered in a similar manner over the next 14 days. Samples were kept cold and dark until the experiments started. Samples for June, July, and August were re-filtered with a pre-combusted 0.7 µm Whatman GF/F filter prior to the start of those experiments. The initial DOC concentration of the groundwater for each lake varied at the start of each experiment, but it was always higher than the in-lake DOC concentration (Table 1).

1.2 *Sampling Design and Variables Analyzed*

To determine the relative importance of photodegradation and biodegradation for processing DOC, we designed three treatments in a manner similar to Cory et al., (2014): 1) photodegradation only, 2) biodegradation only, and 3) control. From each treatment, five different variables were measured including DOC concentration, DIC concentration, DO concentration, $SUVA_{320}$, and S_r. The different variables measured in each treatment required the use of different containers for the sample water. Samples for DOC analysis (concentration and CDOM) were deployed in acid-washed, muffled 35 mL quartz tubes sealed with silicone stoppers. The quartz tubes had an average transmittance of 96% of solar UV-A and 87% of solar

UV-B, which allowed for an accurate representation of *in-situ* solar radiation levels (SFig. 1, Morris and Hargreaves, 1997). However, the quartz tubes were not gas tight, so samples for dissolved inorganic carbon (DIC) and dissolved oxygen (DO) analysis were deployed in gas tight borosilicate exetainer vials (138W; Labco, Ceredigion, UK). The borosilicate vials had a volume of 12 mL but were filled to 10 mL (i.e. 2 mL of headspace) due to safety concerns with mercury chloride (see below). A clean 10 mL pipette was used to carefully transfer water into the borosilicate vials. Borosilicate glass has a sharp cut-off at 320 nm and transmits <5% UV-B, but it transmits an average of 63% of UV-A radiation and 90% of PAR (SFig. 1, Reche et al., 1999).

Water samples for all of the treatments were initially filtered through pre-combusted 0.7 µm Whatman GF/F filters one day prior to the start of each monthly experiment. For the photodegradation and control treatments detailed below, samples for DO and DIC analysis were treated with 0.35 mL of 1% mercury chloride ($HgCl_2$) to kill the microbial community. Samples for DOC concentration and CDOM analysis ($SUVA_{320}$ and S_r) for the same treatments were sterile filtered with a 0.2 µm membrane filter (Sterivex MilliporeSigma, Burlington, MA USA) pre-rinsed with 100 mL of DI water and 50 mL of sample water instead of using $HgCl_2$ because adding $HgCl_2$ altered the optical scans. Sterile filtering has previously been shown to remove the majority of microbes present, and water samples remained sterile for one week following this procedure (Moran et al., 2000; Fasching and Battin, 2011). For the biodegradation treatment, water samples were inoculated with 100 µL of unfiltered groundwater that was collected 1 day prior to the start of each monthly experiment. By adding a fresh inoculum of groundwater each month, we aimed to re-stimulate the microbial community and assess the short-term response of biodegradation. In the biodegradation treatments, we did not correct for differences in vial size (i.e. 100 µL was added to both the 12 mL vials and the 35 mL tubes). Treatments were deployed

in triplicate for each lake (i.e. 3 DOC quartz tubes, 3 DO borosilicate vials, and 3 DIC borosilicate vials for each treatment). Here, we included a summary of the three experimental treatments that were designed as follows:

a) *Photodegradation Only*: Water for DOC concentration and CDOM analysis ($SUVA_{320}$ and S_r) was sterile filtered and stored in quartz tubes (n = 3 replicates). Water for DIC and DO analysis was treated with 1% $HgCl_2$ and stored in borosilicate vials (n = 6 replicates; 3 replicates for DIC and 3 replicates for DO analysis).

b) *Biodegradation Only*: Water for all analyses was inoculated with 100 µL of unfiltered groundwater. Water samples for DOC concentration and CDOM analysis were stored in quartz tubes (n = 3 replicates). Water samples for DIC and DO analysis were stored in borosilicate vials (n = 6 replicates; 3 replicates for DIC and 3 replicates for DO analysis). Both the quartz tubes and borosilicate vials were wrapped with multiple layers of aluminum foil to eliminate light exposure.

c) *Control*: Water for DOC concentration and CDOM analysis was sterile filtered and stored in quartz tubes (n = 3 replicates). Water for DIC and DO analysis was treated with 1% $HgCl_2$ and stored in borosilicate vials (n = 6 replicates; 3 replicates for DIC and 3 replicates for DO analysis). All samples were wrapped in aluminum foil (dark).

The experimental treatments for each lake were deployed for seven days at the surface of Lake Lacawac in May, June, July, and August of 2016 (for exact sampling dates see SI, Table 1). Mean surface lake temperature for each experiment are reported in SI Table 1. Samples were kept at the lake surface using floating racks, and samples from each lake were randomly distributed across the racks. The deployment design ensured that samples stayed at the surface

and dipped no deeper than 2 cm in the water column. After the one-week exposure, racks were collected from the surface of Lake Lacawac and samples were immediately transferred into coolers and returned to the lab. We assessed the response of terrestrially derived DOC to photodegradation and biodegradation by measuring changes in the concentrations of DOC, DIC, and DO, and the absorbance properties ($SUVA_{320}$ and S_r) of the CDOM. All samples were analyzed within 72 hours of collection.

Dissolved organic carbon concentrations and standards were analyzed using a Shimadzu TOC-V_{CPH} Total Organic Analyzer with an ASI-V auto sampler. External acidification was used for each sample and triplicate measurements were performed following the methods of Sharp (1993). Diluted 50 ppm DOC standards (Aqua Solutions) were used to calibrate the TOC Analyzer and standards were regularly analyzed with the samples. Dissolved inorganic carbon concentrations (as CO_2) were measured with a Shimadzu GC-8A Gas Chromatograph using helium as the carrier gas. Samples were acidified using 0.1 N H_2SO_4 and then stripped with nitrogen gas prior to injection. Dissolved oxygen was measured using a modified Winkler titration (Parson et al., 1984). Samples for gas measurements (DO and DIC) were kept in a 21°C water bath for 30 minutes prior to analysis. These samples were well mixed just prior to analysis. The absorbance properties of CDOM were analyzed using a Shimadzu UV 1800 scanning spectrophotometer at 25°C. Raw absorbance scans were generated from 800 to 200 nm using a 1 cm cuvette and were blank corrected with ultra-pure DI water. From the absorbance scans, the spectral slope ratio (S_r; 275-295 : 350-400 nm) was calculated following Helms et al. (2008). The DOC specific ultraviolet absorbance at 320 nm ($SUVA_{320}$) was calculated following methods in Williamson et al., (2014). S_r can be used as a proxy for the molecular weight of the

290 DOC, while $SUVA_{320}$ can be used as a proxy for DOC color and aromatic carbon content (Helms
291 et al., 2008, Williamson et al., 2014).
292 Due to differences between the borosilicate vials and quartz tubes, the DIC and DO
293 samples were spectrally corrected for the amount of light they received (SI, SFig. 1). Total
294 cumulative energy exposure over the monthly incubations was calculated from a BSI Model
295 GUV-521 (Biospherical Instruments, San Diego, CA) radiometer with cosine irradiance sensors
296 that have a nominal bandwidth of 8 nm for 305 nm, 320 nm, 340 nm, 380 nm, and 400-700 nm
297 (PAR). Daily irradiance for UV-B, UV-A, and PAR were calculated using 15-minute averages of
298 1-second readings from a GUV radiometer located near Lake Lacawac over the 7-day
299 experiments. The area under the curve was calculated by multiplying the measurement frequency
300 (900 sec) by the average of two adjacent time step readings. These values were then summed
301 over the exposure period to calculate the total cumulative energy exposure for each sample.
302 Readings from a profiling BIC sensor (Biospherical Instruments, San Diego, CA) were then used
303 to calculate the percent of the deck cell at the surface rack incubation depth (0.02 m) in Lake
304 Lacawac.
305
306 **1.3** ***Explanation of Calculations and Statistical Analysis***
307 To determine the fate of terrestrial DOC in the four lakes, we used the measured changes
308 (i.e. final – control) in DOC and DIC concentrations to identify four pools of DOC:
309 photomineralized, partially photodegraded, biodegraded, and unprocessed. The amount of carbon
310 photomineralized (converted to CO_2) was calculated as the concentration of DIC produced by
311 sunlight (i.e. carbon that was completely oxidized by sunlight). The amount of carbon partially
312 photodegraded represents the remainder of the carbon pool that was processed by sunlight (but

not completely oxidized to CO_2) and was calculated as the total DOC processed by sunlight minus the amount photomineralized (Eq 1).

Equation 1. Partially Photodegraded = [Total Photodegraded – Photomineralized]

The amount of carbon biodegraded was calculated as the concentration of DOC lost in the biodegradation treatments. The unprocessed carbon was calculated as the fraction of the carbon pool that was not processed by either sunlight or microbes as shown in Eq. 2

Equation 2. Unprocessed = [Control DOC – Photomineralized – Partially Photodegraded – Biodegraded].

Each process was determined for each lake and each month. Here we report the average response across all four months for each DOC pool.

While we carried out monthly experiments (May-August), here we report the average response across the open-water season (i.e. all four months) to provide a more complete picture of DOC processing. The downside of this approach is that it potentially increases variation in variables associated with DOC processing, since such processing may vary across the season. However, there was not a strong seasonal response to photodegradation or biodegradation in all of our study variables (SI Fig. 3). Furthermore, the majority of the terrestrial DOC was collected on a single date and time (except for Lake Annie).

Final treatments were compared relative to the dark and killed (1% $HgCl_2$) control treatments, as those samples were deployed at the surface of the lake with the photodegradation and biodegradation treatments. We used a t-test to determine whether the photodegradation samples for all of the variables were significantly different from the biodegradation samples (n = 12 for each treatment) in each lake (Table 2). Photodegradation and biodegradation samples were analyzed separately using a one-way ANOVA to assess differences between lakes. A post-hoc

Tukey's multiple comparison test (Sigma Plot 14.0) was used to determine if there were significant differences in the response variables between the lakes to the photodegradation and biodegradation treatments (Fig 1). A descriptive discriminant analysis (DDA) was used to classify the four lakes based on changes in DOC, DIC, DO, $SUVA_{320}$, and S_r measurements due to photodegradation (Fig 3). Since these five measures are likely to be highly correlated with one another, DDA is a good choice since it considers these relationships simultaneously in the analysis (Sherry 2006). In this case, DDA, works by producing linear combinations of the five measured variables (DOC, DIC, DO, $SUVA_{320}$, and S_r). The first linear combination provides the best separation of the four lakes, followed by subsequent linear combinations for axes that are orthogonal (Sherry, 2006). Linear combinations are weighted more heavily by variables that are better able to discriminate between the lakes. In the figures and tables below, we report these data as either average measured changes (i.e. concentrations) or average percent changes and have indicated where appropriate. Data for this experiment were analyzed in either Sigma Plot 14.0 (Fig. 1, Table 2) or Systat version 10.2 (Fig. 4).

2. Results

Throughout the results and discussion, the use of the lake names is to present the data in a meaningful manner, but it is important to recognize that the actual water samples originated from groundwater samples adjacent to each lake.

2.1 *Photodegradation and biodegradation responses in each lake*

Photodegradation altered DOC quantity and CDOM significantly more than biodegradation for terrestrial DOC from the watersheds of all four lakes (Table 2, Fig. 1). For the

359 photodegradation only treatments, exposure to sunlight resulted in significant production of DIC
360 and increases in S_r, as well as significant decreases in DO, DOC, and $SUVA_{320}$ relative to the
361 biodegradation treatments. The only significant effect of biodegradation on terrestrial DOC was
362 a reduction in DO concentrations compared to the dark control (Fig. 1c). In all other cases, the
363 biodegradation treatments were not significantly different than the control, and the average
364 percent change was close to 0.
365 The terrestrial DOC from the brown-water lakes (Lacawac and Annie) typically followed
366 similar patterns to each other, while the terrestrial DOC from the oligotrophic and eutrophic
367 lakes (Giles and Waynewood) responded more similarly to each other. In the brown-water lakes,
368 we observed a stronger response in DOC quantity (i.e. DOC, DIC, and DO), while the changes in
369 DOC quantity were much more muted in the oligotrophic and eutrophic lakes. The responses of
370 S_r changes in each lake due to sunlight did not differ significantly. All four lakes showed a strong
371 response to changes in terrestrial CDOM (i.e. $SUVA_{320}$ and S_r).
372

Table 2. A summary of the mean (± SD) final concentration of DOC, DIC, DO, $SUVA_{320}$ and S_r in photodegradation (Photo), biodegradation (Bio), and control experimental treatments in groundwater samples from the watersheds of lakes Lacawac, Annie, Giles, and Waynewood. The mean (± SD) initial concentration for each variable is also depicted. The P/B column list the results of a t-test to determine whether photodegradation samples were significantly different from the biodegradation samples (n = 12 for each treatment for the four months). Bolded values indicate the Photo treatments that were statistically different from the Bio treatments ($p < 0.05$).

Analysis	Treatment	Lacawac (Mean ± SD)	P/B p-value	Annie (Mean ± SD)	P/B p-value	Giles (Mean ± SD)	P/B p-value	Waynewood (Mean ± SD)	P/B p-value
DOC (μmoles L^{-1})	Photo	3600 ± 330	**p < 0.001**	1270 ± 211	**p < 0.001**	692 ± 123	p = 0.08	883 ± 73.3	**p =0.002**
	Bio	4910 ± 674		1810 ± 45.7		608 ± 99.0		765 ± 93.8	
	Control	5110 ± 628		1820 ± 76.9		630 ± 102		783 ± 73.8	
DIC (μmoles L^{-1})	Photo	54 ± 8.2	**p < 0.001**	41.9 ± 11.4	**p < 0.001**	20.4 ± 1.9	**p = 0.02**	32.2 ± 7.3	**p = 0.04**
	Bio	16.1 ± 5.0		25.3 ± 7.2		17.7 ± 3.0		27.1 ± 8.0	
	Control	13.8 ± 4.6		30.4 ± 18.2		15.3 ± 2.1		27.8 ± 3.5	
DO (μmoles L^{-1})	Photo	278 ± 62.4	**p < 0.001**	419 ± 25.9	**p < 0.001**	536 ± 35.6	p = 0.16	522 ± 49.0	**p = 0.05**
	Bio	556 ± 46.4		533 ± 42.2		556 ± 34.3		577 ± 76.9	
	Control	660 ± 29.4		656 ± 32.1		688 ± 60.9		702 ± 57.3	
$SUVA_{320}$ (m^{-1} /mg L^{-1})	Photo	4.3 ± 0.4	**p < 0.001**	2.4 ± 0.4	**p < 0.001**	2.4 ± 0.2	**p < 0.001**	1.8 ± 0.2	**p < 0.001**
	Bio	5.3 ± 0.2		3.8 ± 0.1		4.8 ± 0.3		3.2 ± 0.2	
	Control	5.1 ± 0.2		3.8 ± 0.1		4.7 ± 0.2		3.2 ±0.1	
S_r	Photo	1.1 ± 0.0	**p < 0.001**	1.3 ± 0.1	**p < 0.001**	1.4 ± 0.1	**p < 0.001**	1.2 ± 0.1	**p < 0.001**
	Bio	0.7 ± 0.1		0.8 ± 0.0		0.9 ± 0.1		0.8 ± 0.1	
	Control	0.7 ± 0.1		0.8 ± 0.0		0.9 ± 0.1		0.9 ± 0.1	

Sunlight caused average (± SD) DOC losses relative to the control treatments of 30.5 ± 11.5% and 28.9 ± 8.3% in Lacawac and Annie, respectively (Fig. 1a). In Giles and Waynewood, we observed an average of 9.6 ± 6.5% and 13.4 ± 6.2% increase in DOC concentration, respectively following exposure to sunlight. When we compared lakes within each treatment, there were no significant differences in DOC concentration due to sunlight in Giles vs. Waynewood, whereas Annie and Lacawac were significantly different from the prior two lakes and from each other (ANOVA: $F_{1,3} = 70.9$, $p < 0.001$).

Decreases in DOC concentration due to photodegradation could lead to mineralization (i.e. DIC production; Fig. 1b) and therefore oxidation (i.e. DO consumption; Fig. 1c). We

observed the production of DIC due to sunlight in all of our lakes (Fig. 1b). In Lacawac and Annie, the average (± SD) percent increases in DIC relative to the control treatments were 350 ± 160% and 96.0 ± 79.0%, respectively. The average percent increases relative to controls in Giles and Waynewood were 40.7 ± 19.4% and 23.2 ± 12.7% respectively. The DIC percent change was similar between Giles and Waynewood, and both were statistically different from Annie and Lacawac. The percent DIC change in Lacawac was significantly higher than Annie (ANOVA: $F_{1,3} = 36.4$, $p < 0.001$).

In all lakes, both photodegradation and biodegradation led to decreases in DO concentrations (Fig. 1c). Average DO losses due to biodegradation for all four lakes ranged from 15 to 18%. DO losses due to photodegradation were more variable. The average DO loss from sunlight in Lacawac and Annie was 58.2 ± 7.8% and 35.9 ± 5.4%, respectively. In Giles and Waynewood, we observed average DO losses of 21.6 ± 7.9% and 25.6 ± 4.7% respectively. While the largest losses of DO due to sunlight were observed in Annie and Lacawac, there was no significant difference between Annie and Waynewood. Giles and Lacawac were significantly different from the other two lakes and from each other (ANOVA: $F_{1,3} = 73.9$, $p < 0.001$).

Changes in CDOM due to biodegradation were minimal in all of the lakes (Fig. 1d & 1e). In contrast, photodegradation caused significant changes in all of the lakes, but the magnitude of the change varied by lake. $SUVA_{320}$ decreased in all lakes due to sunlight, but the largest changes were observed in the oligotrophic and eutrophic lakes (Fig. 1d). Average $SUVA_{320}$ values decreased between 16.8% in Lacawac and 48.9% in Giles. The response in Annie and Waynewood were similar, whereas Lacawac and Giles were significantly different from the prior two lakes and each other (ANOVA: $F_{1,3} = 39.7$, $p < 0.001$). In all lakes, S_r increased due to sunlight (Fig. 1e). Average percent increases for the lakes ranged from 46.4% in Waynewood to

65.1% in Lacawac. For S_r, the response between Lacawac and Waynewood were significantly different, but those lakes were no different compared to the remaining lakes (ANOVA: $F_{1,3} = 3.1$, $p = 0.04$).

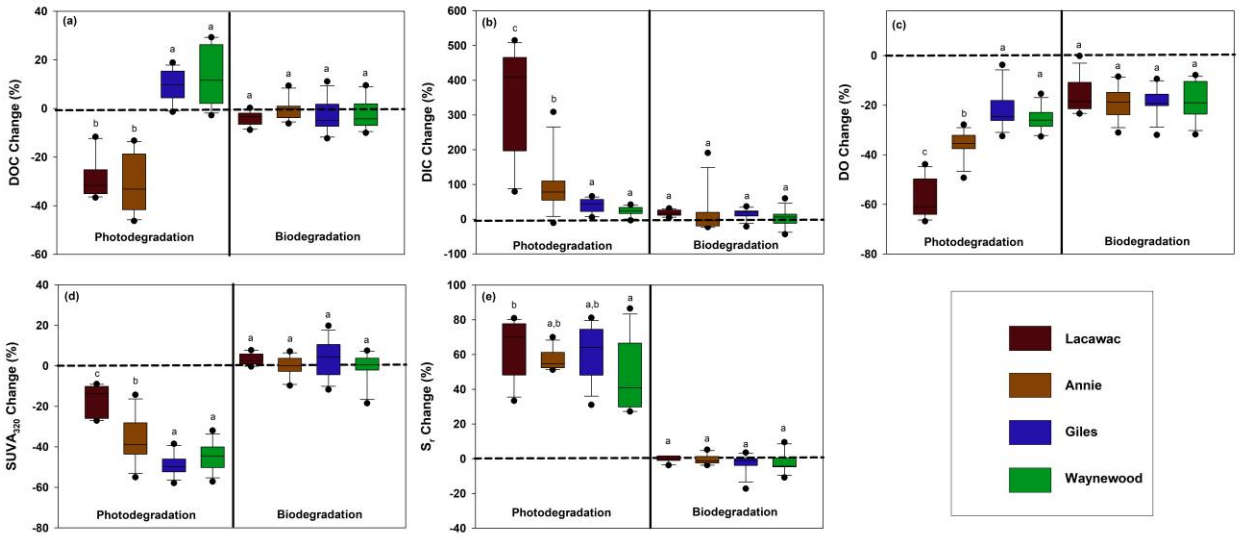

Figure 1. The monthly average percent change from the dark and killed control treatments (dashed line) in each lake for photodegradation (left) and biodegradation (right) for (a) DOC, (b) DIC, (c) DO, (d) $SUVA_{320}$, and (e) S_r. Statistical differences ($p < 0.05$) between lakes are indicated by different letters above each boxplot. For each boxplot n =12 replicates.

2.2 *Fate of DOC*

Of the four pools of carbon we identified in the groundwater samples entering our study lakes, we found the average amount of carbon processed by sunlight ranged from 0.6% to ~30% (Fig. 2). Carbon in Giles and Waynewood (< 1%) showed little response to sunlight, whereas the response in Annie and Lacawac (~30%) was much higher over the 7-day experiments. The dominant pathway through which sunlight interacted with DOC was through partial photodegradation in these latter two lakes. About 1% of the carbon pool was photomineralized in the brown water lakes. The amount of carbon processed via biodegradation was minimal in all

lakes (ranging from 0.2–4%). The fraction of the unprocessed carbon pool ranged from a low of 66% for Lacawac to a high of 97% for Waynewood. An average of 2.6 to 33% of the carbon pool was processed in one week. The photomineralization data represents a minima value for each lake due to some of the DIC partitioning into the headspace of each vial.

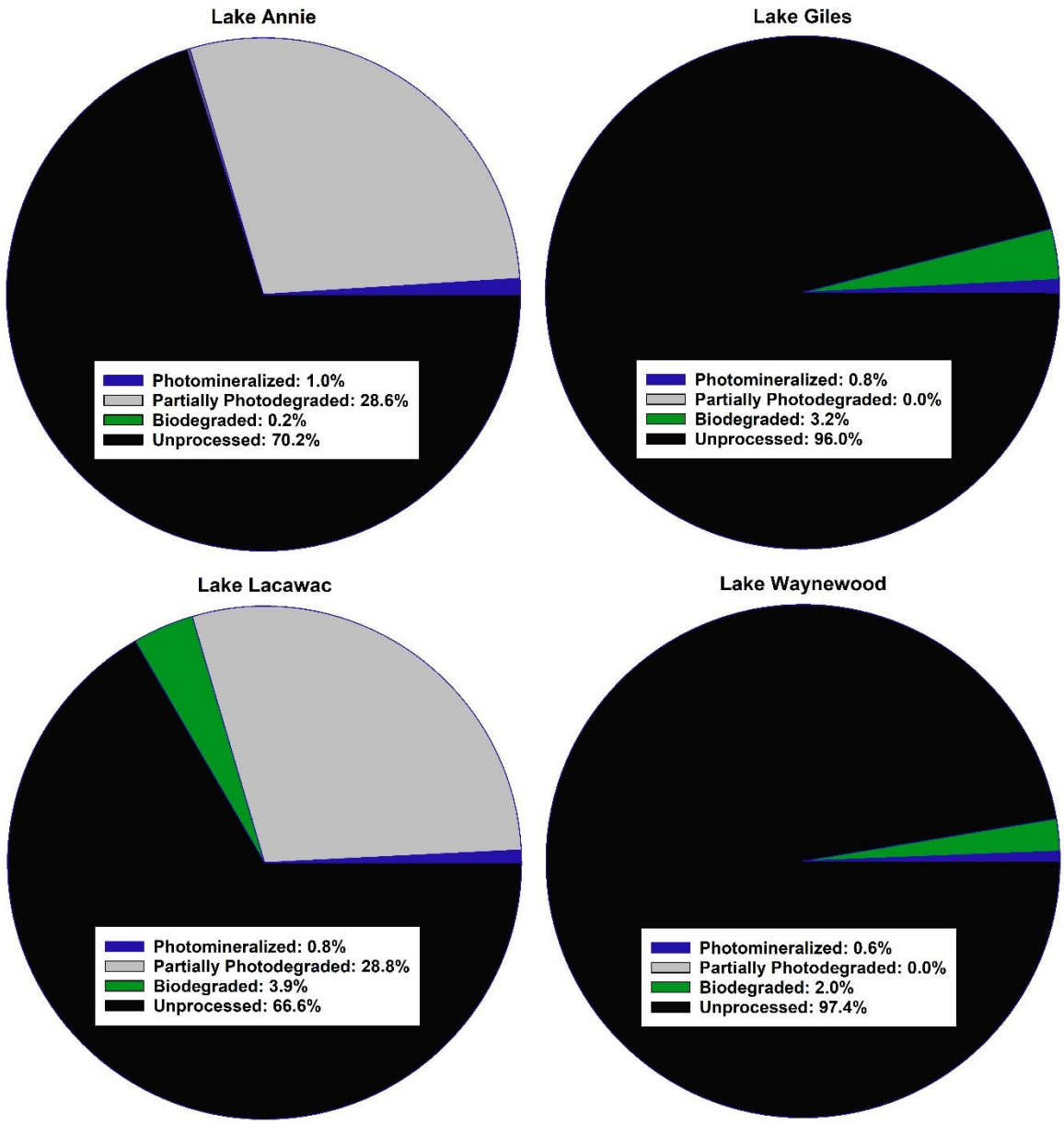

Figure 2. A summary of the average fate of carbon in the groundwater samples from our study lakes (see methods section for explanation of calculations). All terms were converted to a carbon basis. Photomineralized describes the amount of carbon completely mineralized to CO_2 by

sunlight. Partially photodegraded describes the amount of carbon processed by sunlight minus the amount photomineralized. Biodegraded describes the amount of carbon lost through biodegradation. Unprocessed carbon describes the remaining carbon that was not processed by photodegradation or biodegradation.

2.3 *DOC response by lake trophic status*

For the descriptive discriminant analysis (DDA) to classify the lakes, we found that the five metrics were strongly correlated with one another (Table 3). In general, the changes in DOC, DIC, and DO were more strongly correlated with one another than with $SUVA_{320}$ and S_r and vice versa (Table 3). We will refer to the changes in DOC, DIC, and DO as "DOC quantity" and the changes in $SUVA_{320}$ and S_r as "CDOM" for brevity.

Table 3. Pearson correlations between the measured changes in the five metrics: DOC, DIC, DO, $SUVA_{320}$, and S_r.

	DOC	DIC	DO	$SUVA_{320}$
DIC	-0.934			
DO	0.869	-0.837		
$SUVA_{320}$	-0.705	-0.671	-0.666	
S_r	-0.027	0.021	0.163	-0.319

DDA produced three functions (axes) with canonical correlations of 0.961, 0.753, and 0.181 (Fig. 3). Collectively, the entire model was significant (Wilks' $\lambda = 0.032$; $F_{15, 108} = 17.79$; $p < 0.001$). Effect size was calculated following Sherry and Henson (2010) as 1 – Wilks' λ, and therefore the overall model explains 96.8% of the variation among lakes. Functions 1 through 3 and 2 through 3 were significant ($p < 0.001$ for both). Function 3 was not significant ($p = 0.710$) and therefore is not discussed further. Functions 1 through 3 collectively explain 92.4% of the shared variance while functions 2 through 3 collectively explain 56.7% of the shared variance.

Function 1 represents a new variate that is a linear combination of the changes in the five variables that best discriminates the lakes from one another. This new variate is composed

mainly of DOC, with a function coefficient of 0.465 and a structure coefficient of 0.821 (Table 4). Of note are also DIC, DO, and $SUVA_{320}$ that had smaller function coefficients (< 0.45), but had large structure coefficients (> 0.45). This result suggests that Function 1 is mainly related to DOC quantity. Function 2, also a new variate that is a linear combination of the five measured changes, is composed mainly of $SUVA_{320}$ (function coefficient = 0.985 and structure coefficient = 0.719; Table 4). Function 2 is orthogonal to Function 1 and together they discriminate the four lakes (Fig. 3).

Table 4. The solution for changes in measured independent variables that predict the dependent variable, lake. Structure coefficients (r_s) and communality coefficients greater than $|0.45|$ are in bold. Coeff = standardized canonical function coefficient; r_s = structure coefficient; r_s^2 = squared structure coefficient.

	Function 1			Function 2		
Variable	Coeff.	r_s	r_s^2(%)	Coeff.	r_s	r_s^2(%)
DOC	0.465	**0.821**	67.40	0.639	0.278	40.83
DIC	-0.337	**-0.703**	11.36	-0.059	-0.216	0.35
DO	0.440	**0.679**	19.36	-0.124	0.009	1.54
$SUVA_{320}$	-0.139	**-0.473**	1.93	0.985	**0.719**	97.02
S_r	0.244	0.068	5.95	-0.238	-0.434	5.66

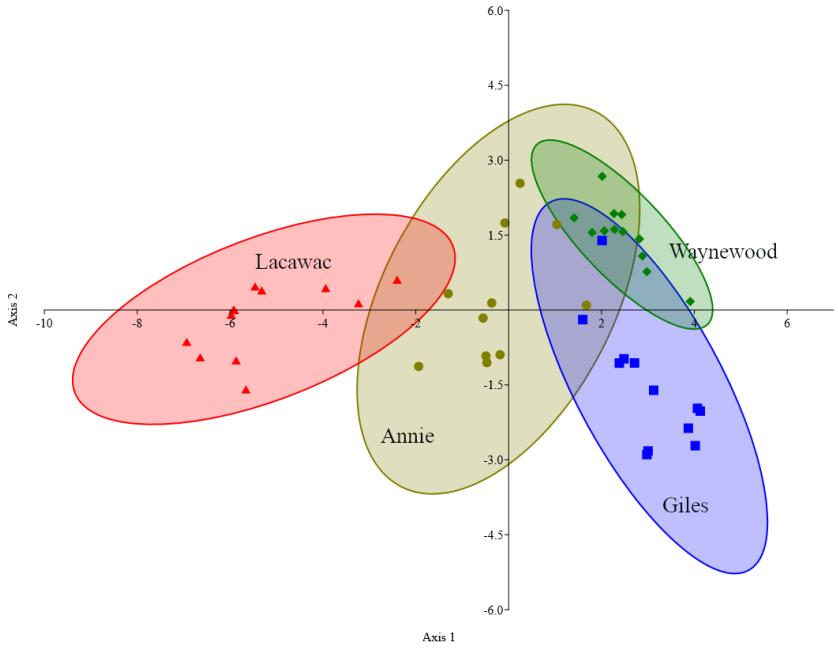

Figure 3. Canonical plot scores and 95% confidence ellipses from descriptive discriminant analysis of the measured changes (i.e. treatment minus control) in the five variables (DOC, DIC, DO, $SUVA_{320}$, and S_r) and four lakes: Annie (olive circles), Giles (blue squares), Lacawac (red triangles), and Waynewood (green diamonds). Only photodegradation samples were included in this analysis.

DDA correctly classified 89.4% of the samples to their collection site (Fig. 3). One sample from Annie was incorrectly assigned to Waynewood, two samples from Giles were incorrectly assigned to Waynewood, and two samples from Lacawac were incorrectly assigned to Annie. All of the Waynewood samples were correctly classified.

3. Discussion

3.1 *Comparing the relative importance of photodegradation and biodegradation*

Despite a large number of studies examining the effects of either photodegradation or biodegradation on DOC processing, very few have conducted simultaneous *in-situ* experiments of the relative importance of both processes for transforming DOC from the watersheds of a range of different lakes. Our results indicate that sunlight was the primary process in the surface

494 waters responsible for degrading terrestrial DOC from the watershed of all four lakes.
495 Biodegradation played a minimal role in changing the DOC quantity and CDOM. We observed
496 decreases in DOC, DO, and $SUVA_{320}$ due to sunlight and saw increases in DIC and S_r. The loss
497 of DOC, as well as a shift to more photobleached, and lower molecular weight organic material
498 is consistent with prior studies on these lakes that evaluated just the effects of sunlight (Morris
499 and Hargreaves, 1997). Exceptions to DOC loss due to photodegradation occurred in Giles and
500 Waynewood. In these lakes, we observed an increase in average DOC concentrations. In Giles,
501 there was significant production of DOC in June and July. In Waynewood, significant production
502 occurred in May and July. We speculate that this production may be due to the lysing of any
503 microbes remaining in solution. Increases may also be attributed to interactions with iron. We
504 have no measurable evidence, but a number of samples from Giles and Waynewood contained a
505 red precipitate at the conclusion of the one-week experiments. Iron-bound DOC could have been
506 released back into the water. Subsequent photodegradation experiments using water from Giles
507 and Waynewood have also indicated DOC production (Dempsey, unpublished).
508 Dissolved oxygen was the lone variable where biodegradation led to decreases relative to
509 the controls, but the differences between lakes were not significant. We attributed the changes in
510 DO to the "sloppy feeding" of bacteria, where they produce DOC through exudates and then
511 assimilate it (Evans et al., 2017). The above results are similar to observations in Arctic and
512 tropical waters in that photodegradation was more important than biodegradation on short time
513 scales (Cory et al., 2014; Chen and Jaffé, 2014; Amado et al., 2003). Interestingly, we found that
514 terrestrial DOC from the watersheds of lakes of different trophic status was processed
515 differently, resulting in DIC production and DOC degradation for the brown-water lakes
516 (Lacawac and Annie), but greater changes in $SUVA_{320}$ for the oligotrophic and eutrophic lakes

517 (Giles and Waynewood). This highlights the need to account for lake trophic status in predicting
518 DOC processing and CO_2 emissions from lakes.
519
520 **3.2 *Dominant degradation process***
521 Based on our study design we were able to identify four pools of carbon:
522 photomineralized, partially photodegraded, biodegraded, and unprocessed. The dominant
523 degradation pathway across all lakes was partial photodegradation (i.e. loss of DOC, but no
524 mineralization), although the size of each carbon pool varied by lake. In the brown-water lakes,
525 ~28% of the total carbon pool was partially photodegraded and ~1% was photomineralized. In
526 the oligotrophic and eutrophic lakes ~0.7% of the carbon was photomineralized and none of the
527 carbon was partially photodegraded. The values reported here for photomineralization are
528 underestimates. Actual values are likely to be higher since we did not account for DIC that
529 partitioned into the headspace of the exetainer vials. If we assume a 1:1 (O_2: CO_2) respiration
530 quotient (RQ) (Cory et al, 2014) and use our DO data in the Fig 2 calculations,
531 photomineralization in Annie and Lacawac could be as high as 13 and 7.5% of the carbon pool
532 respectively. Use of the oxygen data is less than ideal since several authors have reported RQ
533 values different than 1:1 (Allesson et al, 2016 and Xie et al, 2004).
534 Observations in Toolik Lake showed 70% of the total carbon pool being processed by
535 sunlight during the open water period (~3 months) (Cory et al., 2014). Other estimates have
536 found that photomineralization of DOC accounts for only 8-14% of total water column CO_2
537 production (Granéli et al., 1996; Jonsson et al., 2001; Koehler et al., 2014; Vachon et al., 2016b).
538 We observed ~30% of the carbon pool being processed by sunlight within one week in our lakes
539 and this was restricted to the brown-water lakes. Similar to Toolik Lake, the dominant

degradation process was partial photodegradation. Partial photodegradation can alter CDOM and stimulate subsequent bacterial respiration. Degradation of CDOM can have important effects for downstream ecosystems if it can be further processed and released as CO_2 or instead is buried or exported downstream (Weyhenmeyer et al., 2012; Catalan et al., 2016; Chen and Jaffe, 2014; Biddanda and Cotner, 2003). It is thus important to include all sunlight-driven degradation processes to fully account for its relative importance.

Differences between the responses observed in the Arctic and our temperate/subtropical lakes are most likely explained by the initial concentration and quality of terrestrially derived DOC and time. In the Arctic, glacial meltwater can be highly photolabile and dominated by seasonal inputs of DOC from shallow or deep soils (Cory et al., 2014; Spencer et al., 2014; and Kaiser et al., 2017). In temperate regions, DOC tends to contain more humic and fulvic acids derived from soils, which may be less photolabile than Arctic DOC. Additionally, we did not integrate our results over the entire water column because the samples were analyzed on the surface of a single lake. Over the entire water column, photodegradation could have processed additional carbon. In clear-water lakes, DOC may be photodegraded down to the 1% UV-A attenuation depth (Osburn et al., 2001), which ranged from 0.7-4.7 m in our study lakes (Table 1).

3.3 *Response of lakes to photodegradation*

With an increase in extreme precipitation events, terrestrial DOC inputs are likely to increase in many aquatic ecosystems (Rahmstorf and Coumou, 2011; Westra et al., 2014). By using groundwater as a proxy of terrestrial inputs from the watersheds of different types of lakes, we simulated the effects of storm events and compared the sensitivity of different terrestrial

563 DOC sources to photodegradation. Interestingly, we found DOC from the watersheds of
564 oligotrophic and eutrophic lakes showed stronger changes in CDOM, compared to DOC from the
565 watersheds of the brown-water lakes that showed significantly larger changes in DOC quantity.
566 This difference may be due to the more allochthonous nature of the brown-water DOC, which is
567 highly photolabile, resulting in greater changes in DOC quantity due to its ability to absorb UV
568 radiation (Bertilsson and Tranvik, 2000). The less allochthonous and more microbially derived
569 DOC from the watersheds of the eutrophic and oligotrophic lakes may be less photolabile with
570 fewer UV-absorbing chromophores. Results of the DDA may be helpful in predicting changes in
571 other lakes based on their trophic status. $SUVA_{320}$ is the variable most likely to change due to
572 photodegradation in eutrophic and oligotrophic lakes. In contrast, DOC concentration is the
573 variable most likely to change in brown-water lakes due to photodegradation. Both results (DOC
574 and $SUVA_{320}$) highlight how lakes of varying trophic status respond to photodegradation. These
575 results can be used to predict how lakes not included in this study will respond to increased DOC
576 concentrations (i.e. browning).
577 Across our study lakes, changes in DIC production scaled linearly with initial
578 groundwater DOC concentration. Lacawac had the highest initial DOC concentration (59.4 ± 6.1
579 mg L^{-1}) and the highest average DIC production, while Giles had the lowest initial DOC
580 concentration (6.0 ± 0.6 mg L^{-1}) and the lowest average DIC production. This suggests that the
581 initial DOC concentration plays a critical role in determining the fate of DOC (Leech et al.,
582 2014; Lapierre et al., 2013). Lake temperature can also influence photodegradation. In this
583 study, average lake temperature increased from May through July (SI Table 1). Porcal et al.
584 (2015) showed that the largest loss of DOC occurred in warmer (i.e. 25 ºC) waters due to
585 photodegradation. Additionally, DIC production was higher in those waters compared to colder

586 water (9 ºC) (Porcal et al., 2015) Recent research has also reported that residence time controls
587 organic carbon decomposition across a wide range of freshwater ecosystems (Catalan et al.,
588 2016, Evans et al., 2017). However, extreme precipitation events may shorten the residence time
589 of lakes, effectively flushing out fresh DOC and preventing significant in-lake degradation from
590 occurring (de Wit et al., 2018). For the terrestrial DOC from the oligotrophic and eutrophic
591 lakes, a significant fraction was not degraded, which may mean that terrestrial inputs from these
592 watersheds undergoes less immediate in-lake processing and instead is exported downstream.
593 Our results indicate that differences in the fate and processing of DOC from the watersheds of a
594 range of lake types have important implications for determining which lakes may release more
595 CO_2 versus export DOC downstream (Weyhenmeyer et al., 2012; Zwart et al., 2015;
596 Weyhenmeyer and Conley, 2017).

597 Even though we observed similar responses to photodegradation in the brown-water lakes
598 (Fig. 1), the magnitude of the response varied and may have been related to the initial DOC
599 concentration. Initial concentrations (mg L^{-1}) of terrestrial DOC from Lacawac (59.4 ± 6.1) were
600 almost 3x higher than Annie (20.7 ± 0.5). Average DOC losses for both lakes due to
601 photodegradation were ~30%. The main difference between Lacawac and Annie was the DIC
602 percent change due to photodegradation (Fig. 1b). Average percent increases in DIC for Lacawac
603 were close to 400%, whereas in Annie it was ~85%. Despite the fact that both Annie and
604 Lacawac are brown-water lakes, their different DIC production rates indicate that certain types of
605 terrestrial DOC may be more photolabile than others and capable of outgassing large amounts of
606 CO_2. The DDA analysis did also pick out the separation between Lacawac and Annie primarily
607 on axis 1 (DOC). The responses in Annie shared similarities with the other 3 lakes while
608 Lacawac only overlapped with Annie. When put in the context of the entire DOC pool for each

609 lake, photomineralization accounted for 1% of the carbon loss. We anticipated that terrestrial
610 DOC from subtropical lakes would undergo additional microbial processing due to the higher
611 temperatures year-round. In a comparison between boreal Swedish and tropical Brazilian lakes,
612 Graneli et al., (1998) also found strong similarities in changes of DOC concentrations and DIC
613 production between lakes from the different latitudes. A weak significant correlation between
614 DOC concentration and DIC production has also been observed in Amazon clear water systems
615 (Amado et al., 2003)
616
617 **Conclusions**
618 Here we showed that photodegradation can be more important than biodegradation in
619 processing watershed inputs of terrestrial DOC on short time scales in the surface waters of a
620 lake. The responses that we observed varied with lake trophic status. Quantitative changes in
621 DOC, DIC, and DO were strongest in the terrestrial DOC from the watersheds of the brown-
622 water lakes, whereas the largest changes in $SUVA_{320}$ were observed in the terrestrial DOC from
623 the watersheds of the eutrophic and oligotrophic lakes. Consistent with prior studies, we found
624 that sunlight can impact not only changes in the concentration, but also CDOM characteristics.
625 We observed a range of 2.6 to 33% of the carbon pool processed in one week. As DOC
626 concentrations increase in some aquatic ecosystems, the potential for increased CO_2 outgassing
627 due to photo-mineralization also increases. On short time scales, sunlight had important impacts
628 on our study lakes. Future studies should focus on additional lakes, longer timescales, and
629 integrating DIC production throughout the water column.
630 Over the next century, DOC concentrations in northern boreal lakes are projected to
631 increase by 65% (Larsen et al., 2011). Thus, understanding the fate of terrestrial sourced organic

material will be essential for predicting the ecological consequences for lakes and downstream ecosystems (Solomon et al., 2015; Williamson et al., 2015; Finstad et al., 2016). Improving estimates of organic carbon processing in lakes will be an important component of creating more complete carbon budgets (Hanson et al., 2004; 2014) and global estimates of CO_2 emissions can be more accurately scaled to reflect the ability of lakes to act as CO_2 sinks or sources as browning continues (Lapierre et al., 2013, Evans et al., 2017).

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Data Availability:

Data and metadata will be made available in the Environmental Data Initiative repository. Data archiving will be led by C. Dempsey and J. Brentrup.

Author Contribution Statement

CMD, JAB, and CEW designed the study with help from LBK, EEG, and HMS. CMD, JAB, SM, and HMS collected the water samples and ran the experiments. DPM provided the analytical equipment for measuring DIC and DOC. CMD and JAB analyzed the data, and CMD and MTG conducted the statistical and DDA analyses. CMD and JAB wrote the manuscript with contributions from all of the authors.

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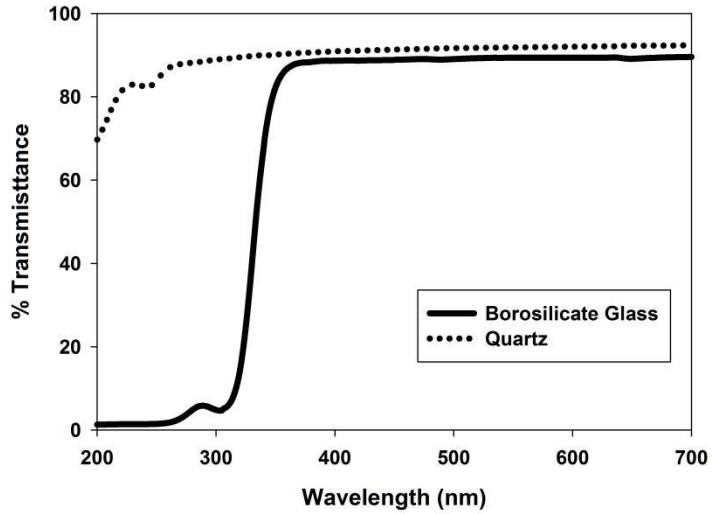
Supplemental Information

Table 1. Timeframe for when samples were deployed on the surface of Lake Lacawac, the total amount of light received by samples (280–700 nm), and the average surface (0.1 m) temperature of Lake Lacawac during each 7 day experiment

Month	Dates	Total Light (J km^{-2} nm^{-1})	Mean Surface Temp (° C) ± SD
May	May 9-16	299.9	15.1 ± **1.5**
June	June 7-13	365.4	20.1 ± **1.4**
July	July 7-13	320.9	25.4 ± **0.8**
August	August 8-14	365.4	26.2 ± **1.1**

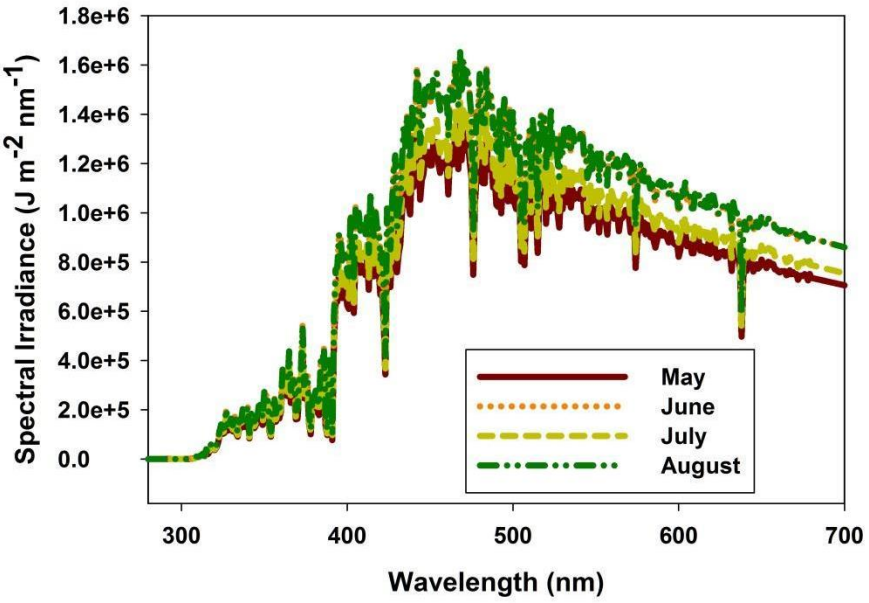


SFigure 1. Percent transmittance of the quartz and borosilicate glass that was used for the experiments.

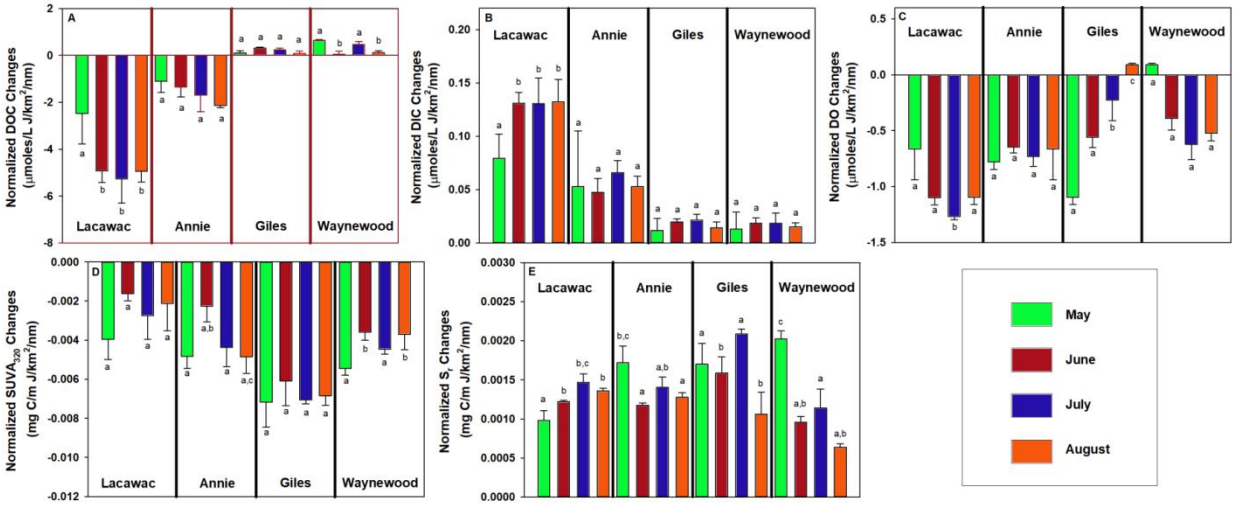
Supplemental Methods

The photodegradation treatments described above (DOC, DIC, DO, S_r, and $SUVA_{320}$) were normalized to the total amount of light received by the samples for each month. This allowed us to determine the impact of seasonality. To calculate the total amount of light (J m^{-2} nm^{-1}), a modeled solar spectrum (280-700nm) was created. The base spectrum was generated with the Quick TUV Calculator (version 5.2; http://cprm.acom.ucar.edu/Models/TUV/Interactive_TUV/) for June 21 through June 27, 2016 (Madronich 1993). The latitude and longitude of Lake Lacawac (Table 1) was provided and the ozone concentration from the Total Ozone Mapping

Spectrometer (TOMS; https://ozoneaq.gsfc.nasa.gov/tools/ozonemap/) for each day was entered. We then fit this modeled solar spectrum to our GUV data for each experimental timeframe using Solver in Microsoft Excel (version 2013). A best fit was determined by calculating the square of the difference between the measured GUV data and the values estimated by the model for the 305 and 340nm wavelengths. In the resulting modeled solar spectra (SI Fig. 2), the total amount of light (280-700nm) was summed for each month of the experiment and was used to standardize the concentration and optical data described above.



SFigure 2. Modeled solar spectra for each month plotted against wavelength (nm)



SFigure 3. Normalized photodegradation data (described in SI Fig 2) for each variable, lake, and month. Photodegradation samples were compared to controls (0 value on each panel). The panels are arranged as follows: A) DOC, B) DIC, C) DO, D) $SUVA_{320}$, and E) S_r. Statistical significance is indicated by the letter(s) above each bar. Months were compared using an ANOVA with a Tukey post-hoc test (CI = 95%). n = 3 for each bar.

Response to reviewer comments

The authors would like to thank the two anonymous reviewers for their thoughtful comments. We have incorporated many of the suggestions into the revised draft of the manuscript. Below we have provided specific comments in "red" too each line item.

Anonymous Referee #1

Received and published: 18 June 2020

The manuscript presents a very interesting study aimed at discerning processes within lakes that control the mineralization of DOC, whether biodegradation or photodegradation prevails. It's an important contribution to the field to quantify the possible load of CO2 emitted from lakes, in the current scenario of climate change, and studies in this respect are highly valuable. Moreover, studies addressing in-lake DOC processing as both biodegradation and photodegradation are scarce. Therefore, this manuscript is a timely contribution to this area of research. The manuscript is well written and despite being easy to follow it is not trivial, but instead of a high scientific soundness.

The aims are well described, the abstract is informative and well resumes the study.
I have some comments that are highlighted below, and I recommend to consider, in the discussion, mechanisms of primary production in lakes and DOC lability, as this may be related to the different biodegradation response. Overall I enjoyed reading this manuscript and I would like to see it published after some revision, as suggested by the following comments.

Introduction

84: I would suggest to add a few words clarifying mineralization, degradation, respiration
These were added into the introduction.

95: Just a guess but in the Arctic it might be that the low temperatures slow down bacterial activity and therefore, photomineralization prevails. I think it really depends on other bio-physical factors and external climate conditions, could you provide other examples?
There are so few studies that directly compare photo and biodegradation. The amount of time experiments are run appears to be important. Sunlight degrades material fairly quickly, whereas microbes need more time.

97: why does the source of inland water CO2 remain uncertain? What are the possible explanations besides bacterial activity and photomineralization?
Some of this is land use, but most of the uncertainty is due to the lack of measurements in different aquatic ecosystems.

104/105: I would talk a bit more on CDOM, giving some more information. As it can represent a great portion of DOC, I think it is a bit reductive to refer to it only as "absorbance characteristics".
We went back and forth on this in earlier drafts, but ended up using the term CDOM throughout the revised draft. We added some description into the manuscript.

107: do you mean the smaller MW, the less attenuation by DOC because of larger molecules? Or do you mean that light can make DOC otherwise unavailable to bacteria, available? Eg. Kieber et al., Nature 1989 (referred to marine systems, but could be probably applied to freshwater environments as well).
No. We are just trying to point out that sunlight effects DOC in other ways (i.e. not just mineralization)

111: in this study, does sunlight-driven degradation accelerate subsequent bacterial activity?
We unfortunately did not test that in this study.

115-119: this sentence is very long and hard to follow. Could you break it up into two sentences or rephrase it?
Yes.

125: when you say reactive, do you mean easily broken down/degraded/uptaken by bacteria?
Yes. More easily degraded or broken down. We could have phrased that to be clearer.

126: different optical properties how? Can you specify?
In the papers that were cited, the optical properties (i.e. $SUVA_{254}$, S_r, etc...) were different between forested and disturbed study sites.

Methods:

183: can you specify why groundwater can be a proxy for terrestrial DOC runoff? I have seen it afterwards in the discussion but I would just add a few words here as well.
Yes.

209: additionally to the 3 treatments, did you also check for combined photodegradation + biodegradation setting or would it have been too tricky to discern which process from which in that case? Maybe it could be some experiment to try in the future. What are possible limitations? You could add a few sentences in the discussion about that, if it's a feasible experiment.
We did not include it in this manuscript, but we did run a combined photodegradation and biodegradation treatment. The response in those treatments was similar to the photodegradation response, but greater. Those treatments did not fit well with the points we wanted to make here.

239: why did you inoculate the samples with GW only for the biodegradation experiment? Would it have been interesting to see the short-term response of photodegradation too, and check which one could be faster or prevailing? Just asking for curiosity.
For the biodegradation experiments, we wanted to provide a fresh source of bacteria. In hindsight, we should have used bacteria from the surface each lake, but we only had groundwater shipped from Lake Annie. For consistency, we opted to use groundwater for all biodegradation treatments.

We did not test the short-term response in this set of experiments, but we have run additional experiments since 2016 to test DOC photodegradation over 48 hours and over 90 days.

255: what was the temperature of the Lacawac Lake in the experimental months compared to each other lake, singularly? Was there a high difference?
The experiments were run only in Lacawac. Average lake temperature for each 7 day experiment has been added to SI Table 1.

277: I would explain better the meaning of Sr: how do you calculate it and that it increases with photodegradation, thus its increase is inversely related to MW. What about its relation to biodegradation instead?
The wavelengths used for calculated the ratio have been added. The full description is in the Helms 2008 paper.

299-308: it would be easier to see if presented in a suite of equations/formulas.
Good suggestion. These were added.

314: As "seasonal response" do you mean the monthly differences? June July and august seems more similar in DOC response compared to May in Lacawac lake. Could it be because of temperature? (Fig S3). It seems to me that brown water lakes with higher GW DOC background showed a more variable response. Were there any significant differences among different months?
Yes. Monthly differences is the same as seasonal response. It could be lake temperature, but it may have more to do with how long it took to filter the Lacawac groundwater. Annie water was shipped to us each month and there is not a strong seasonal response in that lake (SI Fig 3). For Lacawac, the groundwater was collected in early May, but we were unable to filter the remaining water until ~10 days later. There was a large amount of particulates in the samples which may have continued to degrade. It is not reported in the manuscript but for Lacawac the starting concentration of DOC in May, June, July, and August was ~48, ~63, ~63, and ~61 mg/L. While we did not assess the role of temperature, the starting concentration of DOC is important.
377 and Figure 1: it seems that biodegradation mostly affected DO concentration with respect to control with no evident differences between the lakes, while it didn't change much for DOC, DIC, SUVA and Sr. Why do you think the losses of DO due to photodegradation were higher with respect to biodegradation? Again, do you think temperature may have played a role? Together with light there must have been an increase in temperature in all samples exposed to light. This could not be much evident, probably, in the biodegradation samples (slighter differences among lakes). In general T increases bacterial activity and therefore DO loss. Is it possible that lakes with higher load of particulates may have experienced higher temperatures and therefore, more changes in the DOC and DO?
It is possible that temperature played a role in the seasonal differences. Since all experiments were conducted in Lake Lacawac, all treatments were at the same temperature each month. On the timescales in which we conducted the experiments, photodegradation acts on the DOC much more rapidly than biodegradation. Given longer time periods, bacteria likely become important in the degradation of the DOC. Porcal et al (2015) showed that temperature is important to

photodegradation. Higher temperatures caused larger losses of DOC compared to colder temperatures.

Discussion

487 and 493 (DOC and DO): It seems to me that where the DOC background is lower (eutrophic and oligotrophic) the bacterial response is higher. Could the higher DOC be the result of a bacterial turnover of carbon, producing different DOC over previous substrates? If looking at Sr, Gilles and Waynewood have the lowest values, possibly indicating a production of DOM of higher MW with respect from starting compounds. Maybe the differences seem minimal but if changing the scale in figure 1e for the biodegradation part, possibly differences are more evident. Maybe you could check with S (e.g. 275-295) or CDOM absorbance to further investigate into that process, whether it could actually be a bacterial contribution. I may think that in general, where the DOC background is lower, the partially photodegraded DOC is extremely easy to be processed and taken up by bacteria, therefore it's harder to detect it. In your samples you may not see this mechanism because samples exposed to photodegradation excluded bacterial activity by setup and viceversa, but if I understood the setup right, it may be that that the photodegraded fraction in the biodegradation experiments might have quickly been assimilated by bacteria already in situ (i.e. in the lake at samples collection), remaining with a fraction to be processed and a large unprocessed fraction. Thus, complex substrates need light as a catalyst for further bacterial assimilation. And time can also explain this process, the turnover rate for bacteria is longer than for light, depending on the complexity and reactivity of the starting material. Some more argumentation should be added in this respect (see comment below as well).

We kept the treatments separate in this manuscript and did not do a combined photo and biodegradation treatment. We also don't know if a combined treatment would kill or lyse bacteria due to UV exposure. Samples were kept at the surface of the lake for 7 days. It would be interesting to test this to determine whether bacteria can survive for 7 days. We do think that the length of time experiments are run is important.

501-503: this is very interesting. Why do you think this mechanism occurs? How can it be related to primary production and the release of "fresh" and labile DOC that bacteria can assimilate more easily? I think some argumentation should be added here, and I believe it would be very useful to discuss about different carbon fractions and reactivities (labile, semi-labile, refractory)..

We think this occurs because the properties of the watershed control the lake. Lacawac and Annie have bog/wetland areas that contribute to their brown water description. There is likely a fraction of the DOC pool in each lake that is capable of being biodegraded, a fraction that is capable of being photodegraded, and a fraction that not able to be processed. Photodegradation and biodegradation are both important in aquatic ecosystems and the combination of both appears to more than each individually.

520: although for marine systems, I think here Kieber et al. 1989 could be added as a reference https://www.nature.com/articles/341637a0

Thank you.

547: it joins to my previous comment (501-503): “microbially derived” should be better explained as bacteria may channel a great portion of DOC produced by autotrophic organisms.

559: I suggest to better explain this concept: the more DOC the more DIC produced?
We restated this in the revised draft. In our data, the lakes with higher concentrations of initial DOC correspondingly saw the largest changes in DIC (i.e. Lacawac and Annie). The lakes with the lowest initial DOC concentration saw the least amount of DIC produced during photodegradation.

Reviewer 2 comments

Overview:
The manuscript presents data on the photo-oxidation of dissolved organic carbon (DOC) using groundwater inflow as the source waters to 4 freshwater lake ecosystems. While it is an interesting study, there are a number of issues with the experimental aspects of the work that need to be clarified at present as there is considerable variability in the DOC concentrations and the DIC yields are surprisingly low with an apparently large pool of missing carbon unaccounted for in the experimental analysis when viewed in terms of a C mass balance.
General Comments:

Carbon mass balance and potential loss of CO2 to headspace in exetainers:
The description of the DIC analysis raises a number of questions as to how well the measurements were made in this work. Measuring changes in DIC in the presence of large concentrations of DOC has always been a challenge (Granéli *et al.*, 1998; Granéli *et al.*, 1996) but there are methods available to do this reasonably well (Porcal *et al.*, 2015). However, in the present case the method description seems to indicate that for the DIC and DO measurements there was a headspace in the Exetainer Vials used. Having a headspace when measuring dissolved gases like O2 and CO2 is problematic as there will be a considerable amount of gas exchange to the headspace. This is likely the reason why the DIC yield from DOC photo-oxidation are so low in this work and inconsistent with previous studies. At the very least the description has to be improved in a revised manuscript so the analytical issues arising from this type of measurement can at least be understood in a reasonable framework.

Thank you for the suggestions. Our future work with DIC will be sure to acidify immediately and remove the headspace issue. We still think the DIC results are relevant as samples were mixed prior to analysis. It does seem likely that some of the CO_2 partitioned into the headspace. The results we present here represent minima for DIC production due to photodegradation.

In the manuscript there is no real attempt at an overall carbon balance as there were no measurements of POC taken. This is problematic from the point of view of the overall experimental design but it is made more complicated by a misreading of the Cory et al. (2014) work by which the authors have confused O2 and CO2 (see below for full details) stoichiometries and this unfortunately impacts the interpretation of the results considerably – at best it is a series of typos at worst a serious misunderstanding of the earlier work and of what this

paper itself was trying to achieve (i.e. why measure DIC if you can just use a relationship from elsewhere).
Correct. Our attempt was not develop an overall carbon balance. We did not measure POC. I did make a mistake in the calculations in regards to calculating the amount of carbon photomineralized. Thank you for finding my error.

Role of iron in the photochemical reduction of DOC:
Iron and pH have been identified previously as playing an important role in the photo-oxidation of DOC in freshwaters (Gu *et al.*, 2017; Molot *et al.*, 2005), it is a pity then that there are apparently no measurements of the iron content of these waters.
We did not collect iron data as a part of this experiment. Other researchers at the lake collected samples from the three Pocono lakes and from wetlands surrounding each lake in 2018. The concentrations they reported were low (unpublished data).

Specific comments:
Line 77: An additional reference of note on the Brownification of fresh waters is the recent review by Kritzberg *et al.* (2020).
Thank you.

Line 179: Where does the data for the residence time of the lakes come from? It would also be useful to include the estimates of sinks/sources and lake inventory that were used in estimating the residence time. In this context it would be useful to know what the catchment sizes were and the average rainfall to each lake. This would help the reader understand more the processes impacting DOC in the lakes.
The citations for the residence time have been added and details about the estimates can be found there. Precipitation data references have also been included.

Line 199: Please indicate if the GF/F filter was pre-combusted before use to remove any DOC on the filter itself.
All filters were pre-combusted at 450 ºC prior to filtering.

Line 218: Are these the usual Labco Exetainer vials? If so please provide the part number etc as these are commonly used for dissolved gas samples and so are well known to most researchers.
They are the Labco Exetainer 138W vials.

Line 218: So does this mean there was a 2 mL headspace in the Exetainers? This will impact the measurements of the DO and DIC considerably (Spötl, 2005; Waldron *et al.*, 2014), see also recommendations from the lab at UC Davis:
https://stableisotopefacility.ucdavis.edu/dictracegassamplepreparation.html
Yes. There was 2 mL of headspace. We aired on the side of safety with not overflowing the vials with mercury chloride. The samples water and headspace in each vial were mixed for 30 seconds. A syringe was used to carefully extract 5 mL of sample. Samples were acidified using 200 uL of 0.1N H2SO4. 5 mL of nitrogen gas was then added to the syringe and the syringe was then mixed prior to affixing to the GC.

Line 223: It would be useful to restate here that these are all groundwater samples and not water from the adjacent lake.
Agreed.

Line 233: This is 100 µL of groundwater to both the 35 mL Quartz tube and the 12 mL Exetainer? If this is the case how are the data then corrected for the differences in the additions between the DOC and DIC samples?
The data are not corrected for differences in the volumes.

Line 233: The bacterial community in the groundwater may be significantly different from that found in the lake, most notably in the presumably the abundance of photosynthetic organisms and the response to light. Could photoinhibition of bacterial activity also have been important here?
Yes. This is possible and can be added here.

Line 248: The samples wrapped in Al foil may have been exposed to greater temperatures during the course of the incubations due to solar heating. Some indication of the *in situ* temperatures and the solar irradiation received (e.g. the data from the radiometer) would be helpful then to gauge if this could have been an influence on the experiment.
We have *in situ* lake temperature for Lacawac, but we did not install temperature sensors inside of aluminum foil wrapped tubes. We also have radiometer data that is publically available.

Line 266: Please report the standards and/or Certified Reference Materials used for the DOC analysis.
We use a 50ppm TOC standard from Aqua Solutions and create dilutions to calibrate the TOC analyzer.

Line 268: Were the DIC samples acidified through the Exetainer septum to prevent gas exchange?
No. DIC samples were acidified in a syringe after extraction from the Exetainer (see above description)

Line 268: What was the volume of sulfuric acid added to each vial?
After removal from the exetainer, 200 µL of sulfuric acid was added to the syringe (see above description).

Line 271: What does well mixed mean in this case? That the headspace in the exetainers was shaken with the water layer. Where the samples acidified prior to this mixing? – see the comment also above regarding the acidification steps.
Yes. The headspace was mixed with the water layer prior to being acidified.

Line 298: Unfortunately this statement is incorrect as the original citation (Cory *et al.*, 2014) assumes a 0.5 mol O2 consumed to 1 mol DOC oxidized for partial photooxidation, not CO2 as stated in the present manuscript. The Cory et al. (2014) value is also not valid as other work has shown this value can vary depending on the river water itself (Xie *et al.*, 2004).
We fixed this issue in the revised manuscript.

Line 299: What does [DIC*2] signify here? Are you suggesting that half the photo-oxidized carbon turns into some other form of carbon? As the previous sentence in the manuscript linking CO2 production from DOC was erroneous, this sentence is also incorrect. It begs the question as to how the C balance is achieved if only 50% of the DOC photo-oxidized forms CO2 what happens to the other 50% of the C as normally CO production is only a small pathway.
The calculations have been corrected. A revised Figure 2 has been added to the manuscript.

Line 301: What is this pool of carbon then, if it is converted from DOC but it is not DIC it has to then be POC by default, unless the authors are arguing for a 3rd form of dissolved carbon? See the work Porcal *et al.* (2015) for more details on the carbon balance in these types of experiments.
We did not plan on putting together a carbon budget. The intent of Figure 2 was to show what happens to the DOC in each lake.

Line 366: Table 1 - I found some of the statistical relationships to be not credible here; given the data provided so it would be extremely useful to include more details on how the statistics were generated here and for the values to be rechecked. For example when the P/B column indicates a $p < 0.001$ values for DIC results but the data clearly overlap at the 1 or 2σ level then something is not right with regard to the p value reported: 49.1 ± 11.4 compared to 25.3 ± 7.2 and 20.4 ± 1.9 compared to 17.7 ± 3.0
There is some variability in the data since we opted to combine all months together. We re-ran the stats analysis and discovered some minor errors. Those have been corrected in the revised manuscript. The errors do not change the interpretation of the data.

Line 366: Table 1: The DIC yields from photo-oxidation are very low, for example only at Lacawac there is approximately a 30 µmol L-1 increase in DIC for a 1500 µmol L-1 decrease in DOC so either there is very large POC production or there is something very wrong with the DIC or DOC values. Given the issues noted above for the DIC measurements it is most likely those values that are questionable.
Agreed. The headspace is likely the issue.

Line 490: The red precipitate is likely iron oxide but was this found in the controls as well?
No. The red precipitate only occurs in the samples exposed to sunlight.

Line 491: If there is a precipitate then the iron is not being released 'back into the water', it is now precipitating out into the solid phase as the complexing agents responsible for solubilizing it have been destroyed.
The sentence will be re-worded to be more clear. We were suggesting a potential reason as to why DOC concentrations increased in Giles and Waynewood. The intent was to refer to the DOC being released back into the water as the iron precipitated out.

Line 495: Sloppy feeding is a term normally applied to zooplankton grazing on bacteria and not bacteria themselves. Why mention this in the context of O2? As producing DOC does nothing to the O2 content necessarily – the O2 is used up in respiration.

This sentence can be reworded to be clearer. We were proposing respiration as mechanism for the loss of DO in the biodegradation experiments.

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