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. Each quartz tube was filled to a total volume of 30 mL. The quartz tubes had an

221 average transmittance of 96% of solar UV-A and 87% of solar UV-B, which allowed for an
222 accurate representation of *in-situ* solar radiation levels (SFig. 1, Morris and Hargreaves, 1997).
223 However, the quartz tubes were not gas tight, so samples for dissolved inorganic carbon (DIC)
224 and dissolved oxygen (DO) analysis were deployed in gas tight borosilicate exetainer vials
225 (138W; Labco, Ceredigion, UK). The borosilicate vials had a volume of 12 mL but were filled to
226 10 mL (i.e. 2 mL of headspace) due to safety concerns with mercury chloride (i.e. corrosive and
227 acute toxicity). A clean 10 mL pipette was used to carefully transfer water into the borosilicate
228 vials. Borosilicate glass has a sharp cut-off at 320 nm and transmits <5% UV-B, but it transmits
229 an average of 63% of UV-A radiation and 90% of PAR (SFig. 1, Reche et al., 1999). The field
230 station at Lacawac is a mixed use facility that is open to the public and supports researchers from
231 a variety of disciplines.
232 Water samples for all of the treatments were initially filtered through pre-combusted 0.7
233 µm Whatman GF/F filters one day prior to the start of each monthly experiment. For the
234 photodegradation and control treatments detailed below, samples for DO and DIC analysis were
235 treated with 0.35 mL of 1% mercury chloride ($HgCl_2$) to kill the microbial community. $HgCl_2$
236 was added with a pipette. All prep work for samples occurred in the laboratory. Samples for
237 DOC concentration and CDOM analysis ($SUVA_{320}$ and S_r) for the same treatments were sterile
238 filtered with a 0.2 µm membrane filter (Sterivex MilliporeSigma, Burlington, MA USA) pre-
239 rinsed with 100 mL of DI water and 50 mL of sample water instead of using $HgCl_2$ because
240 adding $HgCl_2$ altered the optical scans. Absorbance scans conducted prior to this experiment
241 using water from Lacawac and Annie showed increased absorbance in samples spiked with 1%
242 $HgCl_2$ (compared to non-spiked samples). There was a slight increase in absorbance from 800-
243 350nm and then a notable increase in absorbance from 350-200 nm. 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; 30 mL total volume). 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 DOC, while $SUVA_{320}$ can be used as a proxy for DOC color and aromatic carbon content (Helms et al., 2008, Williamson et al., 2014).

Due to differences between the borosilicate vials and quartz tubes, the DIC and DO samples were spectrally corrected for the amount of light they received (SI, SFig. 1). Total cumulative energy exposure over the monthly incubations was calculated from a BSI Model GUV-521 (Biospherical Instruments, San Diego, CA) radiometer with cosine irradiance sensors that have a nominal bandwidth of 8 nm for 305 nm, 320 nm, 340 nm, 380 nm, and 400-700 nm (PAR). Daily irradiance for UV-B, UV-A, and PAR were calculated using 15-minute averages of 1-second readings from a GUV radiometer located near Lake Lacawac over the 7-day experiments. The area under the curve was calculated by multiplying the measurement frequency (900 sec) by the average of two adjacent time step readings. These values were then summed over the exposure period to calculate the total cumulative energy exposure for each sample. Readings from a profiling BIC sensor (Biospherical Instruments, San Diego, CA) were then used to calculate the percent of the deck cell at the surface rack incubation depth (0.02 m) in Lake Lacawac.

1.3 *Explanation of Calculations and Statistical Analysis*

To determine the fate of terrestrial DOC in the four lakes, we used the measured changes (i.e. final – control) in DOC and DIC concentrations to identify four pools of DOC: photomineralized, partially photodegraded, biodegraded, and unprocessed. The amount of carbon photomineralized (converted to CO_2) was calculated as the concentration of DIC produced by sunlight (i.e. carbon that was completely oxidized by sunlight). The amount of carbon partially 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 photodegradation only treatments, exposure to sunlight resulted in significant production of DIC and increases in S_r, as well as significant decreases in DO, DOC, and $SUVA_{320}$ relative to the biodegradation treatments. The only significant effect of biodegradation on terrestrial DOC was a reduction in DO concentrations compared to the dark control (Fig. 1c). In all other cases, the biodegradation treatments were not significantly different than the control, and the average percent change was close to 0.

The terrestrial DOC from the brown-water lakes (Lacawac and Annie) typically followed similar patterns to each other, while the terrestrial DOC from the oligotrophic and eutrophic lakes (Giles and Waynewood) responded more similarly to each other. In the brown-water lakes, we observed a stronger response in DOC quantity (i.e. DOC, DIC, and DO), while the changes in DOC quantity were much more muted in the oligotrophic and eutrophic lakes. The responses of S_r changes in each lake due to sunlight did not differ significantly. All four lakes showed a strong response to changes in terrestrial CDOM (i.e. $SUVA_{320}$ and S_r).

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: $F_{1,3} = 3.1$, $p = 0.04$).

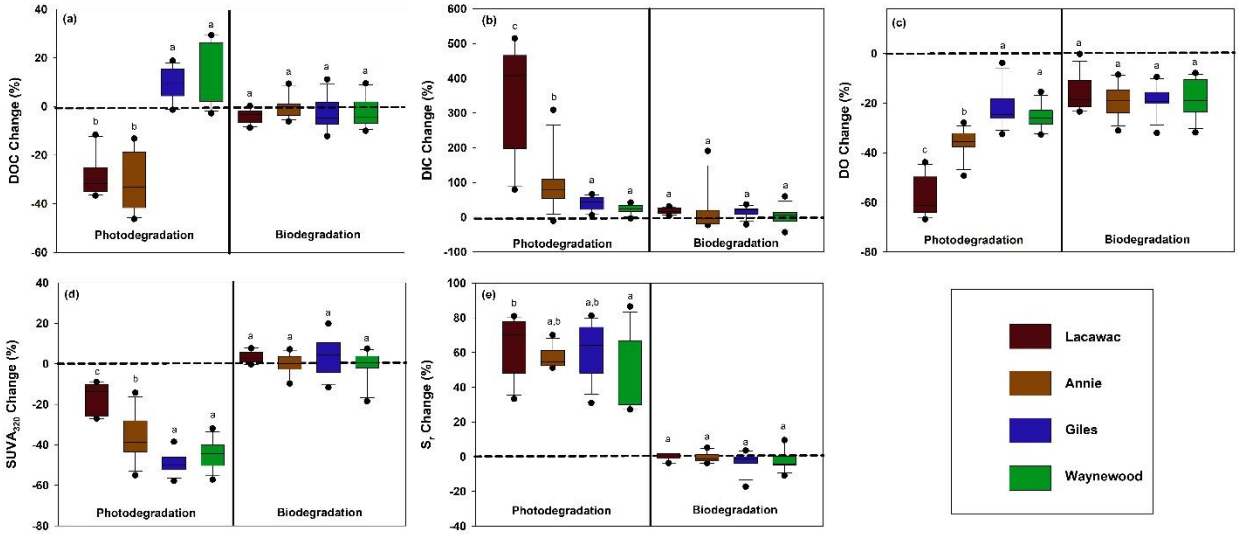
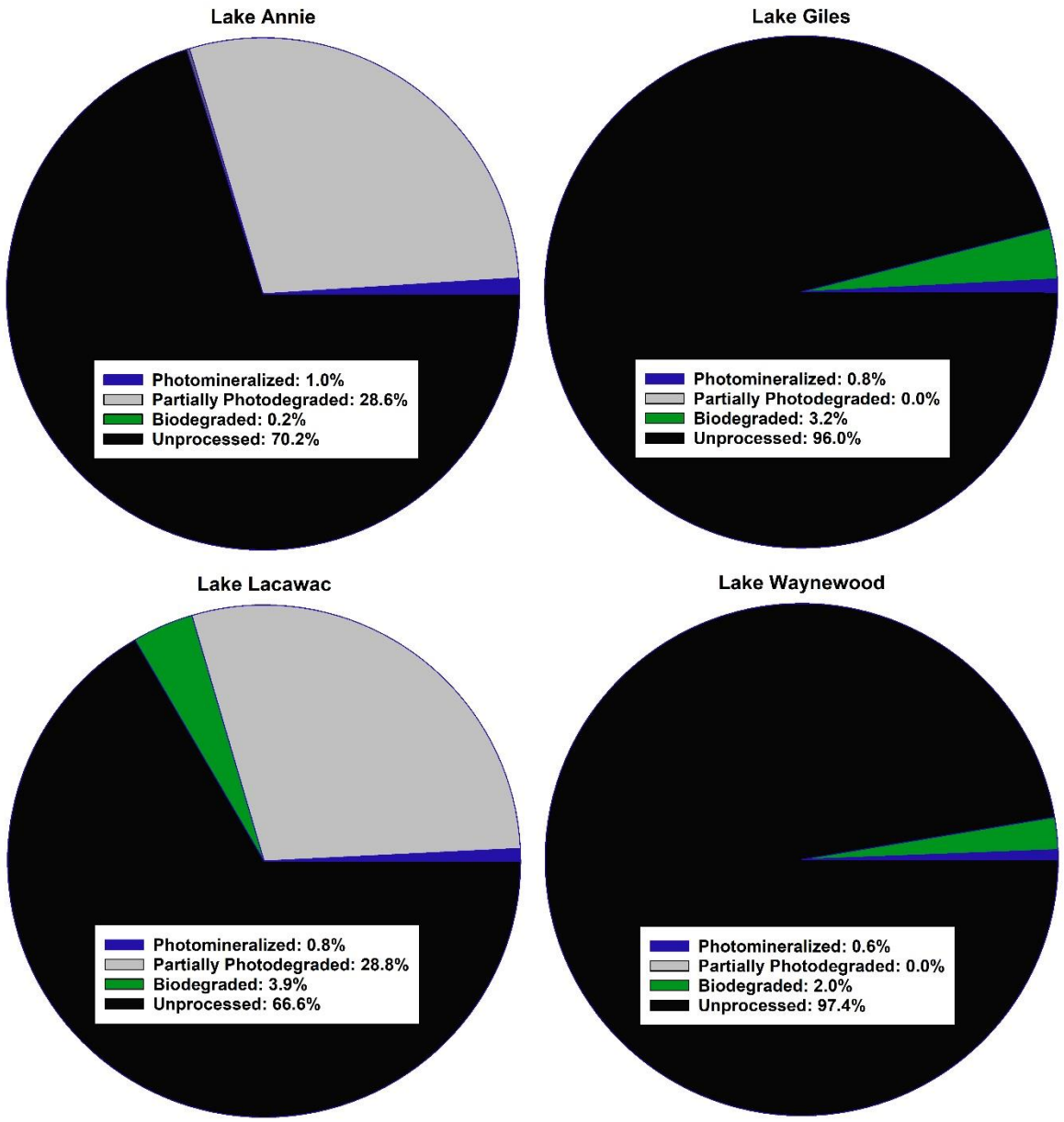


Figure 1. The monthly average percent change from the dark and killed control treatments (dashed line) in each lake for photodegradation (left) and biodegradation (right) for (a) DOC, (b) DIC, (c) DO, (d) $SUVA_{320}$, and (e) S_r. Statistical differences ($p < 0.05$) between lakes are indicated by different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

437 lakes (ranging from 0.2–4%). The fraction of the unprocessed carbon pool ranged from a low of
438 66% for Lacawac to a high of 97% for Waynewood. An average of 2.6 to 33% of the carbon
439 pool was processed in one week. The photomineralization data represents a minima value for
440 each lake due to some of the DIC partitioning into the headspace of each vial.



441

442 **Figure 2.** A summary of the average fate of carbon in the groundwater samples from our study
443 lakes (see methods section for explanation of calculations). All terms were converted to a carbon
444 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

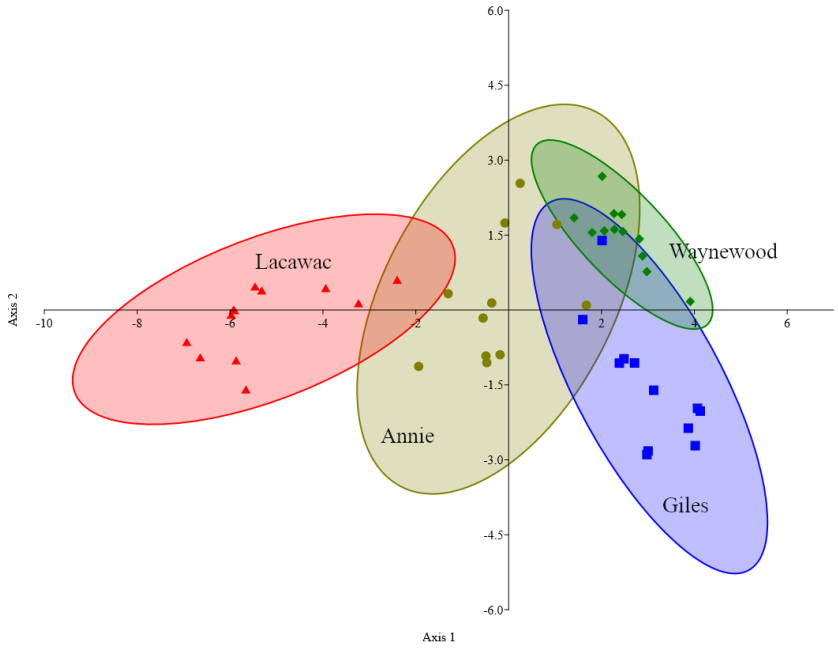


Figure 3. Canonical plot scores and 95% confidence ellipses from descriptive discriminant analysis of the measured changes (i.e. treatment minus control) in the five variables (DOC, DIC, DO, $SUVA_{320}$, and S_r) and four lakes: Annie (olive circles), Giles (blue squares), Lacawac (red triangles), and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

waters responsible for degrading terrestrial DOC from the watershed of all four lakes. Biodegradation played a minimal role in changing the DOC quantity and CDOM. We observed decreases in DOC, DO, and $SUVA_{320}$ due to sunlight and saw increases in DIC and S_r. The loss of DOC, as well as a shift to more photobleached, and lower molecular weight organic material is consistent with prior studies on these lakes that evaluated just the effects of sunlight (Morris and Hargreaves, 1997). Exceptions to DOC loss due to photodegradation occurred in Giles and Waynewood. In these lakes, we observed an increase in average DOC concentrations. In Giles, there was significant production of DOC in June and July. In Waynewood, significant production occurred in May and July. We speculate that this production may be due to the lysing of any microbes remaining in solution. Increases may also be attributed to interactions with iron. We have no measurable evidence, but a number of samples from Giles and Waynewood contained a red precipitate at the conclusion of the one-week experiments. Iron-bound DOC could have been released back into the water. Subsequent photodegradation experiments using water from Giles and Waynewood have also indicated DOC production (Dempsey, unpublished).

Dissolved oxygen was the lone variable where biodegradation led to decreases relative to the controls, but the differences between lakes were not significant. We attributed the changes in DO to the "sloppy feeding" of bacteria, where they produce DOC through exudates and then assimilate it (Evans et al., 2017). The above results are similar to observations in Arctic and tropical waters in that photodegradation was more important than biodegradation on short time scales (Cory et al., 2014; Chen and Jaffé, 2014; Amado et al., 2003). Interestingly, we found that terrestrial DOC from the watersheds of lakes of different trophic status was processed differently, resulting in DIC production and DOC degradation for the brown-water lakes (Lacawac and Annie), but greater changes in $SUVA_{320}$ for the oligotrophic and eutrophic lakes

(Giles and Waynewood). This highlights the need to account for lake trophic status in predicting DOC processing and CO_2 emissions from lakes.

3.2 *Dominant degradation process*

Based on our study design we were able to identify four pools of carbon: photomineralized, partially photodegraded, biodegraded, and unprocessed. The dominant degradation pathway across all lakes was partial photodegradation (i.e. loss of DOC, but no mineralization), although the size of each carbon pool varied by lake. In the brown-water lakes, ~28% of the total carbon pool was partially photodegraded and ~1% was photomineralized. In the oligotrophic and eutrophic lakes ~0.7% of the carbon was photomineralized and none of the carbon was partially photodegraded. The values reported here for photomineralization are underestimates. Actual values are likely to be higher since we did not account for DIC that partitioned into the headspace of the exetainer vials. If we assume a 1:1 (O_2: CO_2) respiration quotient (RQ) (Cory et al, 2014) and use our DO data in the Fig 2 calculations, photomineralization in Annie and Lacawac could be as high as 13 and 7.5% of the carbon pool respectively. Use of the oxygen data is less than ideal since several authors have reported RQ values different than 1:1 (Allesson et al, 2016 and Xie et al, 2004).

Observations in Toolik Lake showed 70% of the total carbon pool being processed by sunlight during the open water period (~3 months) (Cory et al., 2014). Other estimates have found that photomineralization of DOC accounts for only 8-14% of total water column CO_2 production (Granéli et al., 1996; Jonsson et al., 2001; Koehler et al., 2014; Vachon et al., 2016b). We observed ~30% of the carbon pool being processed by sunlight within one week in our lakes and this was restricted to the brown-water lakes. Similar to Toolik Lake, the dominant

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

552 Differences between the responses observed in the Arctic and our temperate/subtropical
553 lakes are most likely explained by the initial concentration and quality of terrestrially derived
554 DOC and time. In the Arctic, glacial meltwater can be highly photolabile and dominated by
555 seasonal inputs of DOC from shallow or deep soils (Cory et al., 2014; Spencer et al., 2014; and
556 Kaiser et al., 2017). In temperate regions, DOC tends to contain more humic and fulvic acids
557 derived from soils, which may be less photolabile than Arctic DOC. Additionally, we did not
558 integrate our results over the entire water column because the samples were analyzed on the
559 surface of a single lake. Over the entire water column, photodegradation could have processed
560 additional carbon. In clear-water lakes, DOC may be photodegraded down to the 1% UV-A
561 attenuation depth (Osburn et al., 2001), which ranged from 0.7-4.7 m in our study lakes (Table
562 1).

563

564 **3.3** ***Response of lakes to photodegradation***

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

DOC sources to photodegradation. Interestingly, we found DOC from the watersheds of oligotrophic and eutrophic lakes showed stronger changes in CDOM, compared to DOC from the watersheds of the brown-water lakes that showed significantly larger changes in DOC quantity. This difference may be due to the more allochthonous nature of the brown-water DOC, which is highly photolabile, resulting in greater changes in DOC quantity due to its ability to absorb UV radiation (Bertilsson and Tranvik, 2000). The less allochthonous and more microbially derived DOC from the watersheds of the eutrophic and oligotrophic lakes may be less photolabile with fewer UV-absorbing chromophores. Results of the DDA may be helpful in predicting changes in other lakes based on their trophic status. $SUVA_{320}$ is the variable most likely to change due to photodegradation in eutrophic and oligotrophic lakes. In contrast, DOC concentration is the variable most likely to change in brown-water lakes due to photodegradation. Both results (DOC and $SUVA_{320}$) highlight how lakes of varying trophic status respond to photodegradation. These results can be used to predict how lakes not included in this study will respond to increased DOC concentrations (i.e. browning).

Across our study lakes, changes in DIC production scaled linearly with initial groundwater DOC concentration. Lacawac had the highest initial DOC concentration (59.4 ± 6.1 mg L^{-1}) and the highest average DIC production, while Giles had the lowest initial DOC concentration (6.0 ± 0.6 mg L^{-1}) and the lowest average DIC production. This suggests that the initial DOC concentration plays a critical role in determining the fate of DOC (Leech et al., 2014; Lapierre et al., 2013). Lake temperature can also influence photodegradation. In this study, average lake temperature increased from May through July (SI Table 1). Porcal et al. (2015) showed that the largest loss of DOC occurred in warmer (i.e. 25 ºC) waters due to photodegradation. Additionally, DIC production was higher in those waters compared to colder

592 water (9 ºC) (Porcal et al., 2015) Recent research has also reported that residence time controls
593 organic carbon decomposition across a wide range of freshwater ecosystems (Catalan et al.,
594 2016, Evans et al., 2017). However, extreme precipitation events may shorten the residence time
595 of lakes, effectively flushing out fresh DOC and preventing significant in-lake degradation from
596 occurring (de Wit et al., 2018). For the terrestrial DOC from the oligotrophic and eutrophic
597 lakes, a significant fraction was not degraded, which may mean that terrestrial inputs from these
598 watersheds undergoes less immediate in-lake processing and instead is exported downstream.
599 Our results indicate that differences in the fate and processing of DOC from the watersheds of a
600 range of lake types have important implications for determining which lakes may release more
601 CO_2 versus export DOC downstream (Weyhenmeyer et al., 2012; Zwart et al., 2015;
602 Weyhenmeyer and Conley, 2017).
603 Even though we observed similar responses to photodegradation in the brown-water lakes
604 (Fig. 1), the magnitude of the response varied and may have been related to the initial DOC
605 concentration. Initial concentrations (mg L^{-1}) of terrestrial DOC from Lacawac (59.4 ± 6.1) were
606 almost 3x higher than Annie (20.7 ± 0.5). Average DOC losses for both lakes due to
607 photodegradation were ~30%. The main difference between Lacawac and Annie was the DIC
608 percent change due to photodegradation (Fig. 1b). Average percent increases in DIC for Lacawac
609 were close to 400%, whereas in Annie it was ~85%. Despite the fact that both Annie and
610 Lacawac are brown-water lakes, their different DIC production rates indicate that certain types of
611 terrestrial DOC may be more photolabile than others and capable of outgassing large amounts of
612 CO_2. The DDA analysis did also pick out the separation between Lacawac and Annie primarily
613 on axis 1 (DOC). The responses in Annie shared similarities with the other 3 lakes while
614 Lacawac only overlapped with Annie. When put in the context of the entire DOC pool for each

615 lake, photomineralization accounted for 1% of the carbon loss. We anticipated that terrestrial
616 DOC from subtropical lakes would undergo additional microbial processing due to the higher
617 temperatures year-round. In a comparison between boreal Swedish and tropical Brazilian lakes,
618 Graneli et al., (1998) also found strong similarities in changes of DOC concentrations and DIC
619 production between lakes from the different latitudes. A weak significant correlation between
620 DOC concentration and DIC production has also been observed in Amazon clear water systems
621 (Amado et al., 2003)
622
623 **Conclusions**
624 Here we showed that photodegradation can be more important than biodegradation in
625 processing watershed inputs of terrestrial DOC on short time scales in the surface waters of a
626 lake. The responses that we observed varied with lake trophic status. Quantitative changes in
627 DOC, DIC, and DO were strongest in the terrestrial DOC from the watersheds of the brown-
628 water lakes, whereas the largest changes in $SUVA_{320}$ were observed in the terrestrial DOC from
629 the watersheds of the eutrophic and oligotrophic lakes. Consistent with prior studies, we found
630 that sunlight can impact not only changes in the concentration, but also CDOM characteristics.
631 We observed a range of 2.6 to 33% of the carbon pool processed in one week. As DOC
632 concentrations increase in some aquatic ecosystems, the potential for increased CO_2 outgassing
633 due to photo-mineralization also increases. On short time scales, sunlight had important impacts
634 on our study lakes. Future studies should focus on additional lakes, longer timescales, and
635 integrating DIC production throughout the water column.
636 Over the next century, DOC concentrations in northern boreal lakes are projected to
637 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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