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Absence of a priming effect on dissolved organic carbon degradation in lake water

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Abstract

The idea that small amounts of labile organic carbon might trigger the degradation of previously unreactive organic matter has attracted increasing scientific interest across multiple disciplines. Although this phenomenon, referred to as priming, has been widely reported in soils, evidence in freshwater systems is scarce and inconclusive. Here, we use a multifactorial microcosm experiment to test the conditions under which priming may be observed in freshwater ecosystems. We assessed the effect of pulse additions of three labile carbon sources (acetate, glucose, and cellobiose) on dissolved organic carbon (DOC) consumption using water from lakes with different trophic states (eutrophic to oligotrophic and clear to brownwater lakes). We further analyzed the effect of nutrient availability and the role of attachment of cells to surfaces. Despite the range of conditions tested, we found no clear evidence of a priming effect on DOC degradation, indicating that priming in freshwater systems may be of limited importance.

A substantial amount of organic carbon (OC) in inland waters is either buried or passively transported towards the sea, but a considerable fraction is lost to the atmosphere by mineralization within the freshwater conduit (Cole et al. 2007; Tranvik et al. 2009). An important constraint on mineralization is the ability of microorganisms to degrade the complex and diverse pool of organic matter from dissolved and particulate fractions in aquatic environments (Hedges 2002; Amon and Benner 1996). Despite extensive research on the degradability of aquatic organic matter, the factors that determine degradability remain unclear (del Giorgio and Davis 2003; Guillemette and del Giorgio 2011), and interactive effects, i.e., the interplay between several factors, are difficult to resolve. Among these possible interactive effects is priming, a mechanism hypothesized to stimulate the mineralization of less available organic matter.

The priming effect refers to the observation that changes in OC inputs may modify organic matter decomposition rates (Blagodatsky et al. 2010; Kuzyakov 2010). The considered inputs are generally labile carbon sources that trigger the degradation of previously unreactive organic matter (Kuzyakov 2010). Priming is considered positive if organic matter decomposition increases and negative if net organic matter decomposition decreases (Blagodatskaya and Kuzyakov 2008). Initially described for soils (Löhnis 1926) and later suggested for aquatic environments (de Haan 1977), priming has recently attracted renewed interest (Guenet et al. 2010; Bianchi 2011). Although it has been intensively studied and is currently a broadly accepted process in soils (Fontaine et al. 2007; Blagodatskaya and Kuzyakov 2008; Schmidt et al. 2011), there is little experimental evidence in the literature to support or refute priming occurrence in freshwater ecosystems. The studies that report significant priming in freshwater ecosystems exclusively use biofilm assemblages (Danger et al. 2013; Kuehn et al. 2014) and even in these assemblages, absence of priming has been recently described (Bengtsson et al. 2014).

As priming has never been reported under sterile conditions (Kuzyakov 2010), the main mechanisms involved are thought to be microbially mediated (Blagodatskaya and Kuzyakov 2008; Bianchi 2011). Soil scientists have distinguished between real priming, describing the enhanced turnover of organic matter, and apparent priming, reflecting higher microbial biomass turnover but no effects on organic matter decomposition (Kuzyakov 2010). Both real and apparent priming are likely to occur in natural systems. Microbes may use labile carbon for population sustenance and invest energy derived from labile carbon inputs to synthesize

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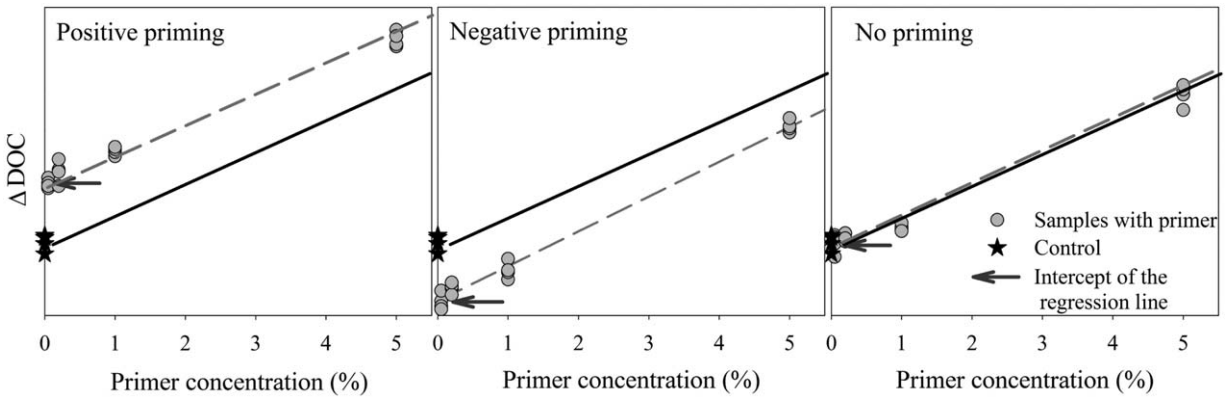

Fig. 1. Conceptual approach, $\Delta DOC = DOC_{initial} - DOC_{final}$. Under a positive priming scenario, the intercept of the regression line of the samples with primer (arrow) is higher than the mean value of the control samples. In the negative priming case the intercept of the regression line is lower than the mean value of the control samples. When no priming is detected no differences are found between the intercept and the control samples. The continuous black line represents the DOC consumption of the controls ($\Delta DOC_{Control}$) plus the amount of primer added at each concentration.

extracellular enzymes to degrade organic matter. Although the mechanisms involved in priming are not well understood, at the ecosystem level they are likely driven by nutrient stoichiometry and energy constraints (Kuzyakov 2010). For example, nutrient limitation has been suggested to favor priming as dissolved organic matter (DOM) decomposition will be favored to obtain nutrients from complex DOM molecules (i.e., nutrient mining; Guenet et al. 2010). Regarding energy constraints, cometabolism strategies might facilitate the use of energy provided by labile DOM for the synthesis of enzymes hydrolyzing less available DOM (Blagodatskaya and Kuzyakov 2008). As priming has been demonstrated in soils and initial evidence in biofilms is appearing, the spatial organization of the microbial assemblages might play another important role for the occurrence of priming. Although attached cells and nearby clone mates profit from extracellular enzyme release (Drescher et al. 2014), planktonic cells might be less likely to invest energy from labile carbon degradation in extracellular enzyme production.

The objective of this work is to evaluate the conditions for priming occurrence in freshwater planktonic systems. To do so, we explored several conditions where priming may be observed, by performing a multifactorial microcosm experiment. We used water from three lakes and a concentrate of DOM from a humic river. These waters included contrasting nutrient and dissolved organic carbon (DOC) concentrations. We added three labile carbon sources, or potential "primers", along a concentration gradient, as it has been reported that priming is strongly dependent both on the concentration and composition of the primer used (Smith et al. 2007). We manipulated nutrient availability by N and P additions to obtain scenarios where either nutrient or carbon were limiting, as both scenarios (reduced stoichiometric constraints and conditions favoring a nutrient mining strategy) could facilitate bulk water DOM degradation. Finally, we tested the role of increased surface availability as we hypothesized that attached microbial cells may be more likely than free-floating ones to produce and utilize the products derived from extracellular enzymatic activity, increasing the probability of observing positive priming.

Methods

Conceptual approach

To test the likelihood of observing priming under a variety of scenarios, DOC consumption was measured in different waters amended with various concentrations of potential primers. Linear regressions of the consumed DOC vs. concentrations of primer were used as proposed in Levi-Minzi et al. (1990). The intercept of the regression line, may be used as an estimate of the DOC consumed in the absence of primer. We, thus, tested for priming by comparing the intercepts of linear regressions with the measured DOC consumption in control treatments which did not receive a labile carbon source (*see* Fig. 1). A significant difference between the intercept and DOC consumption in the control indicates either a positive or a negative priming effect (intercept higher or lower than the control DOC consumption, respectively). An underlying assumption of this approach is that the magnitude of priming is a linear response of the labile DOC addition. We further confirm the results obtained through this procedure by comparing the slopes of each primer and performing unilateral tests between DOC consumption in samples with primer and the corresponding controls (*see* section Statistical approach for further details).

Characteristics of the experimental waters

The experimental waters—lakes Ljustjärn, Svarttjärn, and Valloxen and a DOM extract (Table 1) were chosen to represent various trophic states and pools of DOM. The lakes sampled are located in central Sweden: Ljustjärn is a clear-water oligotrophic lake in a forested catchment, with low DOC, aromaticity, and color. Lake Svarttjärn is a polyhumic and mesotrophic lake, located also in a forested landscape. Svarttjärn is smaller than Ljustjärn and has high DOC,

Table 1. Location and characteristics of the studied freshwater systems: latitude, longitude, surface area, Chlorophyll *a* concentration, DOC concentration, total phosphorous (TP), specific absorbance at 254 nm (an indicator of aromaticity, Weishaar et al. 2003) and absorbance at 420 nm

Water source	Location	Area (km^2)	Chl *a** ($mg\ L^{-1}$)	DOC ($mg\ L^{-1}$)	TP ($\mu g\ L^{-1}$)*	TN ($mg\ L^{-1}$)‡	$SUVA_{254}$ ($L\ mg\ C^{-1}\ m^{-1}$)	A_{420} (m^{-1})
Lake Ljustjärn	59°55′N/15°26′E	0.12	0.8±0.5	4.18±1.1	11.01±1.9	0.22±0.02	1.56	0.38
River Örälven (DOM extract)	64°10′N/18°55′E	—	—	8.9†	<0.08	—	3.65	3.17
Lake Valloxen	59°44′N/17°49′E	2.9	16.4±21.8	16.31±1.5	46.83±12.9	0.93±0.10	2.55	2.05
Lake Svärttjärn	59°53′N/15°15′E	0.07	0.8±0.9	22.83±6.7	15.1±6.1	0.49±0.02	4.49	6.26

*Values are means ± standard error of reported data (Koehler et al. 2012; Gudasz et al. 2012).
†Initial value measured during the incubations.
‡Values from the Swedish Agricultural University database (SLU, http:// www.slu.se/vatten-miljo).

Table 2. Summary of the treatments

Variable	Treatments
Water (Bulk DOM source)	Lake Ljustjärn, Lake Svarttjärn, Lake Valloxen, DOM extract
Primer added*	Acetate (Ace), glucose (Glu), cellobiose (Cel), without primer (control)
Primer concentration†	0.05%, 0.2%, 1%, 5%
Nutrients (N and P addition)‡	Without (−NP), with (+NP)
Glass beads (Surface availability)‡	Without (−BEADS), with (+BEADS)

*Four replicates were set for samples with primer; five replicates for control samples.
†Added at relative carbon concentrations of the bulk DOC concentration in the studied waters.
‡Treatments applied only in the lakes Ljustjärn and Svarttjärn.

aromaticity, and color. Valloxen, located in an agricultural catchment, is a eutrophic lake with intermediate DOC concentrations and likely to be of higher lability. All lakes were sampled in February 2012 at one meter depth. The DOM extract was prepared from a sample taken from the river Örälven. DOM from the river was concentrated using reverse osmosis, and aged for 12 yrs in darkness at 4°C, to ensure a very recalcitrant DOC source. The concentrate was filtered through a 0.2 μm filter (Supor, Pall, Lund, Sweden) and diluted in artificial lake water prepared according to Lehman (1980) to reach a final concentration of 10 mg C L^{-1}.

Experimental design

We performed a factorial experiment with the four experimental waters, with primer as a factor (three labile carbon sources: acetate [Ace], glucose [Glu], and cellobiose [Cel]) and the primer concentration as a concomitant variable (each primer was added at four concentrations: 0.05%, 0.2%, 1%, and 5% of the bulk DOC concentration in the studied waters). Each treatment was replicated four times and had five control replicates without primer. In the case of Ljustjärn and Svarttjärn, two additional factors were added: nutrients, as inorganic nitrogen and phosphorus (two levels: ± NP) and surface availability, provided by open-pore glass beads (two levels: ± BEADS). The experiments were conducted in 40 mL microcosms, totaling 1060 experimental units. The microcosms were incubated in the dark at 15°C and submersed in deionized water for five weeks, a period chosen following previous studies in DOM biodegradability (Amon and Benner 1996; Guillemette and del Giorgio 2011). A summary of the treatments and abbreviations used to designate them can be found in Table 2.

Experimental setup and measurements

Water was stored in the dark at 4°C until filtering through 0.7 μm precombusted GF/F filters and prerinsed 0.2 μm membrane filters (Supor, Pall). Treatments were prepared as a batch of filtered water and sequentially amended with nutrients and primer according to the treatments. Thereafter, an inoculum prepared as unfiltered lake water was added in a 1 : 10 proportion. A mixed inoculum from the three unfiltered lake waters was prepared for the DOM extract. In treatments with nutrients (+NP), nitrogen and phosphorus were added as KNO_3 and Na_2HPO_4 to final C : N : P ratios of 45 : 7.4 : 1 to ensure conditions where C is the limiting factor. In treatments with increased surface availability (+BEADS), surface area was increased 20 times by adding two milliliter of open-pore glass beads with a large surface area (surface : volume ratio of 90,000, Siran TM Carriers, Jaeger Biotech Engineering).

After adding the inoculum, the water was distributed into acid-washed, precombusted (450°C for four hours) 40 mL glass vials, which were sealed headspace free with Teflon coated septa. Oxygen was measured with a Microx system (PreSens) to ensure oxic conditions along the experiment (values were never lower than 6.9 mg L^{-1}). To avoid gas exchange and contamination, the initial and final measurements correspond to two different vials prepared simultaneously from the same batch. One was sampled at the start

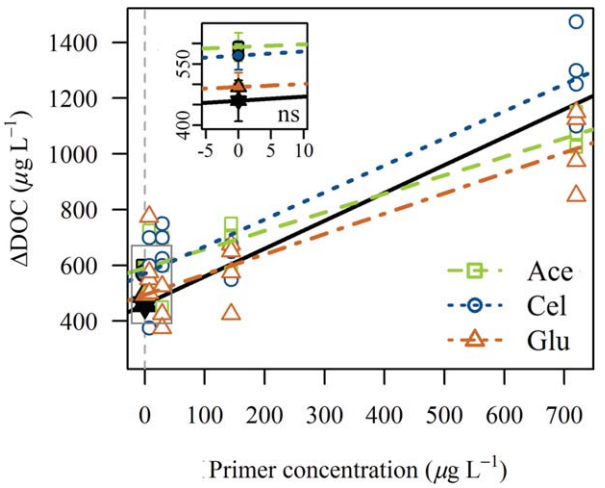

Fig. 2. DOC consumed during the incubation period as a function of the concentration of primer added for the eutrophic lake Valloxen. The inset shows in detail the values of the intercept and the control with the corresponding standard error. ns indicates no significant differences between the mean value of the control and the intercepts of the regression line. The legend indicates the three primers used: Acetate (Ace), Cellobiose (Cel), and Glucose (Glu). The continuous black line represents the values of the DOC consumed by the control ($\Delta DOC_{Control}$) plus the amount of primer added.

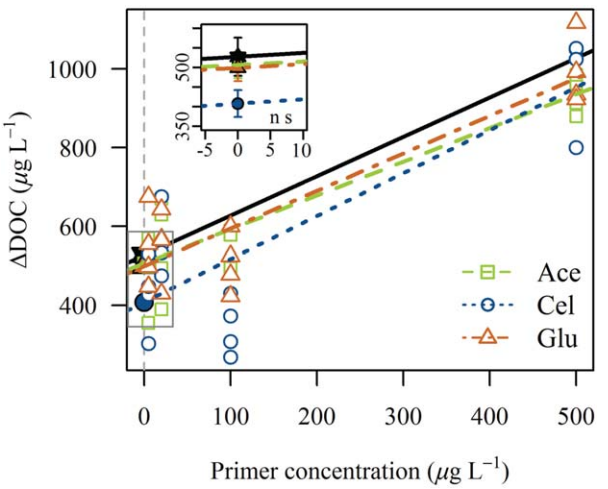

Fig. 3. DOC consumed during the incubation period as a function of the concentration of primer added for the DOM extract. Symbols and codes as in Fig. 2.

and the other at the end of the incubations. We measured initial and final DOC concentrations and evaluated DOC consumption (ΔDOC) as the difference. Concentrations of DOC were measured using a Sievers 900 Total Organic Carbon Analyzer (General Electric Analytical Instruments), which determines Total Organic Carbon in a range from 0.3 μg L^{-1} to 50 mg L^{-1} ppm with a precision of <1% relative standard deviation and an accuracy of ± 2% or ± 0.5 μg L^{-1}.

Statistical approach

To test differences between the intercept of each treatment with primer and DOC consumption in the controls ($\Delta DOC_{Control}$), we used analysis of covariance to analyze DOC consumption (y) with the primer concentration as a numeric variable (x) and the primer used as a discrete factor (i). The following models were fitted:

H_0: $y_{i,j} = \mu + \beta_i x_{i,j} + \varepsilon_{i,j}$ if i = Ace, Cel, Glu and $y_{i,j} = \mu + \varepsilon_{i,j}$ if i = Control

H_1: $y_{i,j} = \alpha_i + \beta_i x_{i,j} + \varepsilon_{i,j}$ if i = Ace, Cel, Glu and $y_{i,j} = \alpha_i + \varepsilon_{i,j}$ if i = Control

where α_i are the intercepts of regression lines for the alternative hypothesis, β_i the slopes of the regressions, μ the common intercept of the regressions under H_0, and j the replicates of each treatment. The null hypothesis was accepted if no significant differences between the intercepts of the three primers and the control were found ($H_0: \alpha_{Ace} = \alpha_{Cel} = \alpha_{Glu} = \mu$), which we interpret as the absence of priming. Each of the blocks of design was analyzed independently. The differences between the slopes of the regression lines (β) were also tested using a similar approach, to evaluate changes in the DOC consumption pattern as a function of the primer added. We also tested nonlinear models, however, none exhibited a better fit as compared to the linear model. Moreover, to study the influence of high primer concentrations on the position of the intercept (leverage), we inspected plots of leverage against standardized residuals and Cook's distance. None of the experimental units had high leverage and large standardized residuals in the regression model.

Finally, the difference between DOC consumed in each treatment (ΔDOC_i) was compared to the DOC consumed in the controls plus the amount of primer added (i.e., we tested if ΔDOC_i was higher or lower than $\Delta DOC_{Control} + DOC_{primer}$) using Student's *t* comparisons corrected for multiple comparisons using the Benjamini–Hochberg false discovery rate correction (Benjamini and Hochberg 1995). To visually identify this difference, a regression line was added in each graph representing the $\Delta DOC_{Control}$ plus the amount of primer added at each concentration (the black line in Fig. 1). The assumptions for general linear models, such as normality, homoscedasticity and leverage were checked by inspection of diagnostic plots and applying Shapiro–Wilks and Levene's tests. All analyses were run using R version 2.15.0 (R Development Core team 2012).

Results

DOC consumption in the controls

The mean amount of DOC degraded during the five-week incubation without primer addition (i.e., in the controls) and without nutrients or glass beads in Lake Valloxen was 460 ± 120 μg L^{-1} C, corresponding to 3.14% of the initial

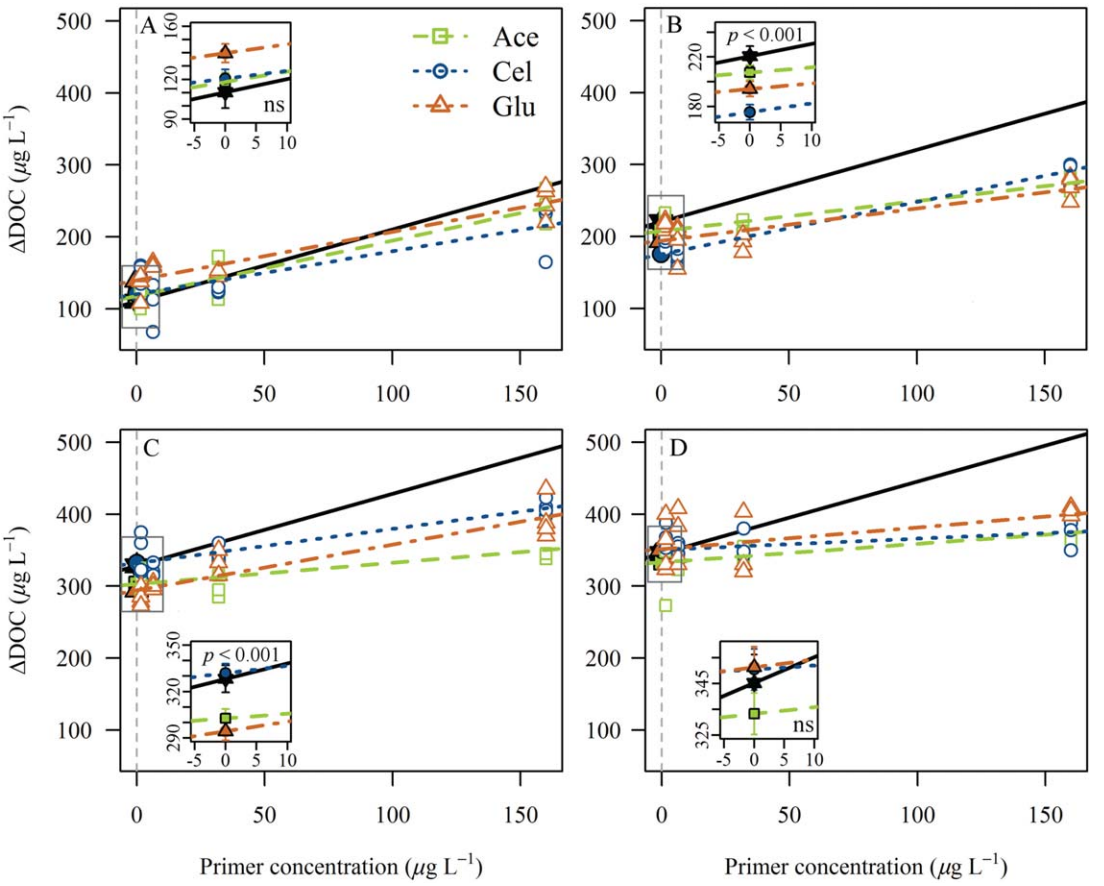

Fig. 4. DOC consumed during the incubation period as a function of the concentration of primer added for the oligotrophic lake Ljustjärn. Treatments (a) without nutrients or glass beads $_{-NP\ -BEADS}$, (b) with nutrients and without glass beads, $_{+NP\ -BEADS}$, (c) with glass beads without nutrients, $_{-NP\ +BEADS}$, and (d) with nutrients and glass beads, $_{+NP+BEADS}$ (d) are shown. Symbols and codes as in Fig. 2.

DOC (Fig. 2). For the DOM extract, mean DOC consumption was 527 ± 58 μg L^{-1} C, or 6.57% of the initial DOC (Fig. 3). In Ljustjärn and Svarttjärn, 110 ± 34 μg L^{-1}C and 608 ± 140 μg L^{-1} C was degraded, corresponding to 3.36% and 4.37% of the initial DOC, respectively. DOC consumption increased in the control treatments with nutrients and increased surface availability in Ljustjärn (Fig. 4) and in the control treatment with increased surface availability in Svarttjärn ($_{-NP\ +BEADS}$; Fig. 5c). However, nutrients did not enhance DOC consumption in Svarttjärn ($_{+NP\ -BEADS}$ and $_{+NP\ +BEADS}$; Fig 5b,d).

Effects on DOC degradation

Primers

The effect of the different primers on bulk DOC consumption varied between the four lakes. For Valloxen, significant differences were found between the slopes of the regression lines for the different primers (Fig. 2; $F_{2,49} = 5.78$, $p = 0.0056$), with cellobiose showing the steepest slope. In this lake, even if DOC consumption with primer was generally higher than the control consumption plus the amount of primer added ($\Delta DOC_{Cellobiose} > \Delta DOC_0 + DOC_{primer}$), this difference was not significant. For the DOM extract, no significant differences were found among the slopes of the regression lines of acetate, cellobiose, and glucose ($F_{2,49} = 0.18$, $p > 0.1$; Fig. 3). Similar results were found for Ljustjärn $_{-NP\ -BEADS}$, with no differences in the regression lines of the three primers ($F_{2,48} = 2.38$, $p > 0.1$; Fig. 4a). The slopes of the regression lines in Svarttjärn $_{-NP\ -BEADS}$ were significantly different ($F_{2,30} = 5.06$, $p = 0.013$), cellobiose had the highest DOC consumption and the steepest regression slope (Fig. 5a). However, although DOC consumption in the cellobiose treatment was generally higher than the control consumption plus the amount of primer added ($\Delta DOC_{Cellobiose} > \Delta DOC_{Control} + DOC_{primer}$), this difference was not significant at any cellobiose concentration ($p > 0.05$).

Nutrients

Nutrients were added to samples from Ljustjärn and Svarttjärn, resulting in different effects. For Ljustjärn, DOC consumption in the controls increased with nutrients (treatment$_{+NP\ -BEADS}$; $F_{3,15} = 69$, $p < 0.001$; Fig. 4b compared to Fig. 4a). However, DOC consumption in the samples with

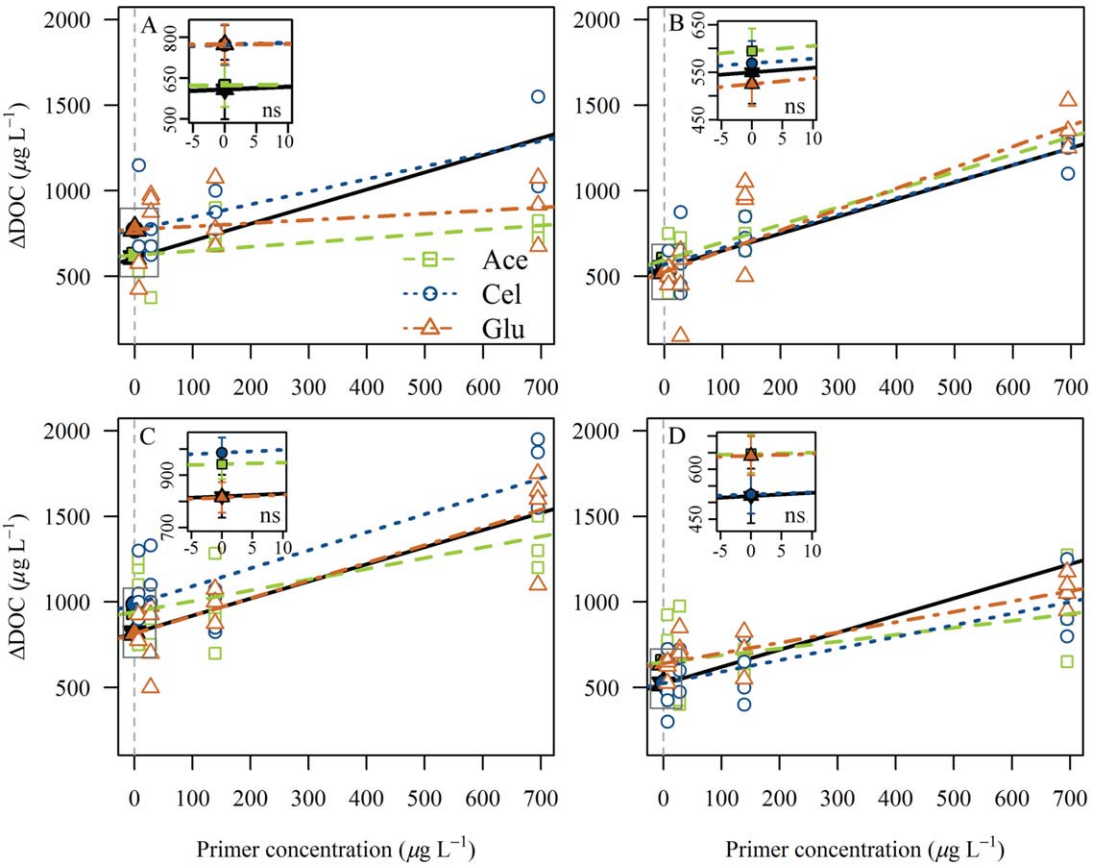

Fig. 5. DOC consumed during the incubation period as a function of the concentration of primer added for the polyhumic mesotrophic lake Svarttjärn. Treatments (a) without nutrients or glass beads $_{-NP\ -BEADS}$, (b) with nutrients and without glass beads, $_{+NP\ -BEADS}$, (c) without nutrients with glass beads $_{-NP\ +BEADS}$, and (d) with nutrients and glass beads $_{+NP\ +BEADS}$ are shown. Symbols and codes as in Fig. 2.

added primer was lower than the consumption of the controls plus the amount of primer added ($\Delta DOC_i < \Delta DOC_{Control} + DOC_{primer}$) and no differences among the regression lines of the three primers were found ($F_{2,48} = 1.01$, $p > 0.05$). In Svarttjärn, nutrients had no effect on DOC consumption in the controls or the samples with primer ($F_{3,186} = 18.55$, $p > 0.1$; Fig. 5b compared to 5a). No differences in the regression lines of the three primers were found ($F_{2,47} = 0.74$, $p > 0.1$).

Surface availability

In both Ljustjärn and Svarttjärn, adding glass beads (treatment $_{-NP\ +BEADS}$) increased DOC consumption by 30–200% over treatments without beads ($_{-NP\ -BEADS}$ and $_{+NP\ -BEADS}$; Figs. 4c, 5c compared to Figs. 4a,b, 5a,b; $F_{3,206} = 293$, $p < 0.001$ and $F_{3,186} = 18.55$, $p < 0.001$). Differences between the slopes of the regression lines of the primers were found for Ljustjärn $_{-NP\ +BEADS}$ ($F_{2,49} = 8.05$, $p < 0.01$). Samples with glucose had the highest slope, but samples in the cellobiose treatment exhibited the highest DOC consumption (Fig. 4c). However, the DOC consumption was lower in treatments with primer than in the controls plus the amount of primer added ($\Delta DOC_i < \Delta DOC_{Control} + DOC_{primer}$; $p > 0.05$). In Svarttjärn$_{-NP\ +BEADS}$ (Fig. 5c), the regression lines of the three primers were significantly different ($F_{2,47} = 3.58$, $p = 0.035$). DOC consumption in the cellobiose treatment was higher than the amount consumed by the control plus the amount of primer added ($\Delta DOC_i > \Delta DOC_{Control} + DOC_{primer}$) but this difference was not significant ($p > 0.05$).

Nutrient and surface availability interactions

The treatment with both nutrients and glass beads had different effects in Ljustjärn and Svarttjärn waters (Figs. 4d, 5d). DOC consumption was greater in the control samples of Ljustjärn $_{+NP\ +BEADS}$ than in the treatments without beads ($_{-NP\ -BEADS}$; $_{+NP\ -BEADS}$; Fig. 4d compared to Fig. 4a; $F_{3,15} = 69$, $p < 0.001$). However, samples with primer had lower DOC consumption compared to the controls plus the amount of primer added ($\Delta DOC_i < \Delta DOC_{Control} + DOC_{primer}$). No significant differences in the slopes of the three primers were found ($F_{2,49} = 1.79$, $p > 0.05$). In Svarttjärn$_{+NP\ +BEADS}$, DOC consumption decreased compared to the other treatments ($_{-NP\ -BEADS}$; $_{+NP\ -BEADS}$ and $_{-NP\ +BEADS}$) both in the controls and in the samples with primer (Fig. 5d compared

to Fig. 5a,b,c). No differences in the regression lines of the primers were found ($F_{2,48} = 0.58$, $p > 0.05$).

Priming effect

No significant differences were detected between DOC consumption in controls and the intercept of the regression in 8 of the 10 blocks of design, i.e., we accepted the null hypothesis of equal values between the intercept for the treatments with primer and the controls. The two blocks showing significant differences were Ljustjärn $_{+NP-BEADS}$ and Ljustjärn $_{-NP\ +BEADS}$ for all three primers used (*see* Table 2 for nomenclature). However, in both cases the values of the intercepts were lower than the controls, indicating lower DOC consumption in the samples amended with primer than in the controls (Fig. 4b,c). Thus, the labile C addition had a significant negative effect on DOC consumption both in Ljustjärn $_{+NP-BEADS}$ and in Ljustjärn $_{-NP\ +BEADS}$ treatments.

Discussion

The aim of this study was to test the likelihood of observing priming in a range of freshwater ecosystems and the results presented in this study suggest that priming is unlikely to have a significant effect on bulk DOC degradation in these systems. Only 2 out of 10 cases (Ljustjärn $_{+NP\ -BEADS}$ and Ljustjärn $_{-NP\ +BEADS}$) showed a significant difference in DOC consumption between the controls and the samples with primer and in both the derived DOC consumption was lower than the actual control DOC consumption, implying that priming was weak and negative. However, no consistent sign for a positive priming effect was found using three different primers, lake waters of different trophic state, under carbon or nutrient limitation or if the bacterial communities were attached to a surface.

If significant priming would have occurred, it should affect the carbon budget of the samples, as the increase in DOC decomposition should be higher than the input of labile carbon (Guenet et al. 2010). The basal consumption (% of initial DOC consumed) was similar in the four water types including the DOM extract, and was within the range expected for similar incubation times and conditions for lake waters (e.g., Guillemette and del Giorgio 2011). DOC consumption increased with labile carbon amendment in all cases, but the additional consumption could in all cases be attributed to degradation of the added primer. To evaluate the occurrence of priming, it is necessary to assess the effect of the primer addition on the carbon budget. Some soil studies using labeled substrates to detect priming have found increased consumption of the soil OC after the labile C addition (identified as positive priming) but did not report if this increased consumption is higher than the labile C input rate (i.e., if ΔDOC_i was higher or smaller than $\Delta DOC_{Control} + DOC_{primer}$; Kuzyakov 2010).

Negative or nonsignificant priming is generally attributed to a preferential use of the added primer instead of the bulk DOC, resulting in a decreased overall consumption of OC (Guenet et al. 2010). This is likely the case in Ljustjärn $_{+NP\ -BEADS}$ and $_{-NP\ +BEADS}$, where we found a significant negative priming effect. Indeed, the proposed mechanisms underlying priming involve different fates of the amended labile carbon (Blagodatskaya and Kuzyakov 2008; Guenet et al. 2010; Bianchi 2011). First, the labile carbon could be used for population maintenance (respiration) or growth. In this case, priming will not occur, as no labile carbon will be available for enzymatic anabolism (Guenet et al. 2010). Second, if the catabolism of the labile carbon provides energy to produce additional extracellular enzymes to further degrade bulk DOM, enhanced DOC degradation on addition of labile carbon might be observed and identified as priming (Kuzyakov 2010). Finally, the hydrolysis products of such extracellular enzymatic reactions might stimulate auxiliary populations to produce new sets of enzymes and this cometabolism pathway might also lead to positive priming (Guenet et al. 2010). In any case, complex communities such as those present in natural waters likely use the aforementioned strategies simultaneously, with DOC consumption being the net result of a variety of metabolic pathways. We did not find any evidence that these strategies result in enhanced DOC consumption.

Effect of the source of labile C

Different microbial populations might thrive and different enzymatic activities may be expressed depending on the labile C source (Blagodatskaya and Kuzyakov 2008). Initially, we hypothesized that simple substrates such as acetate or glucose, commonly used as labile C sources in priming experiments in soils (Fontaine et al. 2007; Kuzyakov 2010), would be easily catabolized, supplying accessible energy. Cellobiose, a disaccharide, requires cellulase activity to be hydrolyzed, and thus, might induce cometabolic reactions. Furthermore, the catabolism of cellobiose might be more energetically efficient than the utilization of simple oxidized substrates such as acetate (del Giorgio and Cole 1998). Accordingly, we found differences in DOC consumption in samples with cellobiose in different experimental waters and treatments, but these differences were not systematic and priming was not detected. Farjalla et al. (2009) and Guenet et al. (2012) propose using labile substrates of higher complexity, such as arginine or macrophyte leachates, which might be more likely to induce priming, as they promote the growth of a wide variety of microbial functional groups (Farjalla et al. 2009; Guenet et al. 2012). However, they also provide a matrix of nutrients that confound the identification of the mechanisms enhancing OC degradation and limit the experimental power to sort out the actual effect of primer addition. To avoid this, we used labile substrates that were exclusively C sources, treating nutrients as a separate factor affecting priming.

Effect of nutrients: Do limiting conditions promote priming?

Nutrient availability can constrain microbial carbon and energy sequestration strategies. Nutrient limitation, as in

oligotrophic systems like Ljustjärn, might stimulate the production of extracellular enzymes to obtain nutrients from DOM, with enhanced degradation of organic matter as a side effect (i.e., nutrient mining, as proposed by Guenet et al. 2010). Conversely, increasing nutrient levels to C-limiting conditions is also expected to enhance DOC degradation (Vrede et al. 2002). Even so, enhanced DOC consumption was not observed in the oligotrophic lake without nutrient addition (i.e., with nutrient limitation, Ljustjärn $_{-\mathrm{NP}\ -\mathrm{BEADS}}$) or in the C-limited treatment Svarttjärn $_{+\mathrm{NP}\ -\mathrm{BEADS}}$. Finally, although nutrients enhanced DOC consumption in the controls for Ljustjärn $_{+\mathrm{NP}\ -\mathrm{BEADS}}$, samples with primer had lower DOC consumption than the controls (Fig. 4b). Thus, similar to the findings of Carlson et al. (2002), nutrients increased the availability of the bulk DOM, but there was no evidence of priming effect.

Effect of glass beads: Does cell attachment promote priming?

Investing energy derived from labile C mineralization into extracellular enzyme production is not an adaptive strategy for free-floating cells, as they are unlikely to directly benefit from the release of extracellular enzymes (Beier and Bertilsson 2011). In the case of attached cells, members of the same population are found in close spatial proximity, increasing the likelihood of benefitting from released extracellular enzymes (i.e., kin selection; Drescher et al. 2014). These conditions might be facilitated in hotspots such as lake snow, vegetation debris or the sediment surface (Kuehn et al. 2014), all potential settings of freshwater systems where priming could be relevant (Guenet et al. 2010). We found increased DOC consumption in treatments with glass beads without nutrients ($_{-\mathrm{NP}\ +\mathrm{BEADS}}$) both in the oligotrophic Ljustjärn and the mesotrophic Svarttjärn lakes. However, despite higher DOC degradation in the controls with glass beads, priming was not detected (Figs. 4c, 5c).

Unconvincing evidence of priming in aquatic systems

Priming is assumed to occur under bioenergetic or stoichiometric limiting conditions, which constrain DOC consumption (Kuzyakov 2010). However, we did not find enhanced DOC degradation due to labile C amendment in lakes spanning a gradient of trophic states (i.e., testing a range of energetic and stoichiometric conditions). Despite the considerable effort to assess the likelihood of priming in freshwater pelagic ecosystems ($n = 1060$), our microcosm assays are limited in their temporal and spatial resolution. Both temporal and spatial turnover of microbial assemblages might foster priming, and long-term experiments, open for dispersal, might be able to address how changes in microbial community structure and biomass allocation interact with the priming phenomenon.

The negative results presented here corroborate findings by Koehler et al. (2012) that glucose additions at different concentrations caused no priming in incubations of DOM from two lakes. They further agree with the findings of Bengtsson et al. (2014) who did not detect priming in hyporheic biofilms. These findings differ from other biofilm studies (Danger et al. 2013; Franke et al. 2013; Kuehn et al. 2014) or from soil studies where priming is currently accepted as a significant pathway in carbon cycling (Blagodatskaya and Kuzyakov 2008; Schmidt et al. 2011). Ecological differences in the processing of labile and recalcitrant carbon sources between soils, biofilms, and planktonic systems may explain this discrepancy. In freshwater planktonic ecosystems, priming has been suggested to emerge on pulsed availability of labile carbon (Bianchi 2011) or as a consequence of spatial proximity between auto- and heterotrophic microbial populations (Guenet et al. 2010). However, in these systems, free-living bacterial cells that encounter a labile carbon pulse might allocate the energy directly into growth and biomass instead of investing it in the production of the extracellular enzymes required to increase the exploitation of recalcitrant carbon. In a structurally stable environment such as soil, the availability of organic matter is highly constrained (Jobbagy and Jackson 2000) and labile carbon pulses (e.g., leaf litter) might occur intermittently. In such an environment, priming might be a successful ecological strategy allowing heterotrophic microbial populations to endure periods of labile carbon shortage.

Finally, in biofilms, auto- and heterotrophic microbes are embedded in an extrapolymeric matrix that might provide both labile carbon from autotrophic production and recalcitrant carbon from the environment at a constant rate (Besemer et al. 2012). Increases in the mineralization rates in biofilms have been identified as priming and attributed to a stimulation of the heterotrophic community through algal exudates, although the quantitative relevance of those potential priming effects is not clear (Danger et al. 2013; Kuehn et al. 2014). Using heterotrophic biofilms, comparable to those in the glass-beads treatment of our study, Franke et al. (2013) concluded that priming might be a rare phenomenon of minor importance for carbon cycling in boreal systems. Additionally, in the first study in biofilms specifically designed to detect priming, Bengtsson et al. (2014) were unable to find evidence of priming. All together, scattered evidence of priming effect in biofilms has been documented (Danger et al. 2013; Franke et al. 2013).

The long absence of studies testing priming in aquatic environments compared to the early documentation of this phenomenon in soils (Löhnis 1926) suggests there may be a bias against the publication of negative results, a bias that is a topic of general concern in science (Gupta and Stopfer 2011; Schooler 2011). Our study was designed to address the likelihood of priming in freshwater environments under manifold ecological scenarios and we report the absence of a significant influence of priming on OC degradation: there is no such thing as a free lunch. The persistence of organic matter in aquatic systems is controlled by the composition of substrates and microbial communities, and a multitude of biotic and

abiotic conditions and their interactions. To achieve an integrated view of carbon processing, it is important to identify which of the various hypothesized factors and interactions are relevant. Here, we propose that priming, which is receiving increasing attention among investigators of aquatic carbon biogeochemistry (Guenet et al. 2010; Bianchi 2011; Danger et al. 2013) might be of limited importance in these systems.

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