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Ecosystem responses to increased organic carbon concentration: comparing results based on long-term monitoring and whole-lake experimentation

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Abstract

Recent increases in terrestrial dissolved organic carbon (DOC) concentrations in northern inland waters have many ecological consequences. We examined available data on carbon cycles and food webs of 2 boreal headwater lakes in southern Finland. Basic limnology and catchment characteristics of a pristine lake, Valkea-Kotinen (VK), were monitored over the past 25 years while the lake has undergone browning and DOC increased from ~11 to 13 mg L⁻¹. Pronounced changes in the early 2000s represent a regime shift in DOC concentration

and color. Lake Alinen Mustajärvi (AM) was manipulated for 2 years by additions of labile DOC (cane sugar), raising the DOC concentration from ~10 to 12 mg L⁻¹, but not changing light conditions. The 2 different approaches both revealed increased concentrations and efflux of carbon dioxide (CO₂) from the lakes and thus net heterotrophy and changes in the pelagic community structure following an increase in DOC concentration. Long-term monitoring of VK revealed a decline in phytoplankton primary production (PP) along with browning, which was reflected in retarded growth of young (1–2-year-old) perch. In the experimentally manipulated lake (AM), PP was not affected, and the growth of young perch was more variable. The results suggested the importance of a pathway from labile DOC via benthic invertebrates to perch. Although provided with this extra resource, the food chain based on DOC proved inefficient. Long-term monitoring and whole-lake experimentation are complementary approaches for revealing how freshwater ecosystems respond to climate and/or atmospheric deposition-induced changes, such as browning.

KEYWORDS boreal lakes, browning, CO₂ flux, food webs, lake metabolism, terrestrial organic carbon

Introduction

During recent decades increased concentrations of terrestrial dissolved organic carbon (DOC) and associated water browning have been reported in many lakes in the Northern Hemisphere (e.g., Jennings et al. 2010, Couture et al. 2012). This phenomenon has been related to various processes, including recovery from earlier acidification, increasing precipitation and runoff, climate warming connected with increase in vegetation cover and prolonged frost-free period of soils, and land use changes (Vuorenmaa et al. 2006, Monteith et al. 2007, Lepistö et al. 2014, Finstad et al. 2016). In addition to colored terrestrial DOC, increased concentration of iron can also contribute to water browning (Krizberg and Ekström 2012, Weyhenmeyer et al. 2014). The future impact of browning on boreal lakes is difficult to predict because of several

potential impacts on lake properties and processes (Solomon et al. 2015, Creed et al. 2018).

First, the increased water color reduces light penetration affecting both light quantity and
55 quality, which in turn may reduce both pelagic and benthic primary production as well as the
balance between these processes (e.g., Karlsson et al. 2009). Second, terrestrial organic matter
entering lakes provides additional substrate for bacteria (Tranvik 1988, Tulongen et al. 1992),
increasing production of CO₂ and shifting lake metabolism toward net heterotrophy (e.g., del
Giorgio and Peters 1994, Hanson et al. 2003, Sobek et al. 2003, Ask et al. 2009). These
60 changes may also have implications for food web structure and energy transfer efficiency
(Jansson et al. 2007, Carpenter et al. 2015). Third, terrestrial organic matter loading will also
bring nutrients such as phosphorus (P) and nitrogen (N), potentially available for primary
producers (Jones 1992, Seekell et al. 2015). Disentangling the consequences of these parallel
potential effects of terrestrial DOC increase has been extremely challenging and will require
65 different but complementary approaches.

Three basic approaches have been used to investigate the effects of lake browning and
DOC increase. Spatial surveys comparing the properties of lakes that currently exhibit
different concentrations of DOC assume that future temporal change will reflect present
spatial variation, but the assumptions underpinning this “space-for-time” approach are likely
70 at best weak and at worst invalid (e.g. Clark et al. 2010, Solomon et al. 2015, Kelly et al.
2016). Monitoring the properties of particular lakes over a long time span can reveal the
impact of browning on lake properties, but the generality of the findings will be uncertain.
Moreover, neither of these approaches can properly identify the mechanisms underpinning the
patterns revealed, a process that will require experimentation. However, experiments using
75 mesocosms to investigate the impact of DOC on lake properties (e.g., Arvola et al. 1996,
Faithfull et al. 2012, Jones and Lennon 2015) suffer from lack of realism or relevance because
mesocosms only represent a part (usually the open water pelagic) of an entire lake ecosystem,
are hydrologically isolated from the critical terrestrial loadings from the catchment, and only

yield meaningful results for short (days to few weeks) time periods. Hence, experimental
80 investigation of the effects of DOC increase are needed at the whole-lake scale, which is
logistically challenging and has only rarely been attempted (e.g., Blomquist et al. 2001, Peura
et al. 2014, Kelly et al. 2016).

Here we used data available from 2 small boreal headwater lakes in southern Finland
to compare how an increase in DOC concentration affected their carbon cycles and food web
85 structures. The data reflect 2 different approaches: long-term monitoring and whole-lake
manipulation experiments. Since 1990, Lake Valkea-Kotinen has been monitored for several
limnological variables, primary production (PP), and the food web up to fish. During this
period, the DOC (measured as C) concentration of the lake increased from ~11 to 13 mg L⁻¹
and water color values increased from ~100–150 to 150–200 mg Pt L⁻¹, whereas the mean
90 daily PP (measured as C) in the photic layer (0–1 m) declined from ~130 to 70 mg m⁻³ d⁻¹
(Arvola et al. 2014). Lake Alinen Mustajärvi was experimentally manipulated by increasing
the epilimnetic DOC concentration from ~10 to ~12 mg L⁻¹ for 2 ice-free periods by adding
cane sugar to the lake. The sugar addition was intended to increase the availability of labile
bacterial substrate without affecting either light penetration or nutrient concentrations, and
95 hence to isolate and study one of the parallel potential effects of lake browning. In addition,
the fate of the added DOC could be traced in organic and inorganic carbon fractions and
onward through the pelagic and benthic food webs up to fish (Peura et al. 2014, Jones et al.
2018) based on the $\delta^{13}\text{C}$ differences between organic carbon from a C4 plant (cane sugar) and
local terrestrial organic carbon from C3 plant origin. Here we (1) evaluate whether the
100 experimental whole-lake manipulation results can help us better understand the long-term
ecosystem changes in response to changes in color and DOC concentrations and (2) discuss
the relative merits of different but complementary approaches in the study of lake carbon
cycles and food webs.

105 **Methods**

Lake Valkea-Kotinen (VK) in southern Finland (61°15'N; 25°04'E) is a small boreal headwater lake located on a pristine old-forest nature conservation area (Ruoho-Airola et al. 2014). The lake and its catchment are part of the Finnish Long-Term Socio-Ecological Research network (FinLTSER) and have been continuously monitored for over 25 years (Rask et al. 2014). This lake has an area of 0.04 km², a catchment area of ~0.3 km², a maximum depth of 6.5 m, and an estimated volume of 103 × 10³ m³. The whole-lake manipulation was conducted in Alinen-Mustajärvi (AM), another small boreal headwater lake just 4 km from VK (61°12'N; 25°06'E). During the study years no forestry operations were carried out in the catchment area. The lake has an area of 0.007 km², a catchment area of ~0.04 km², a maximum depth of 6.5 m, and an estimated volume of 31 × 10³ m³. Both lakes are typically ice covered from around late November to late April and during summer develop a steep and stable stratification with an epilimnion depth of ~2 m and an anoxic hypolimnion. Littoral macrophyte vegetation is highly restricted in both lakes because light penetration into these humic lakes is poor; however, floating littoral vegetation mats with associated periphyton can be highly productive in such lakes (Vesterinen et al. 2016) and can harbor abundant macroinvertebrates. Both lakes contain reproducing populations of European perch (*Perca fluviatilis*) and Northern pike (*Esox lucius*). Further information about the characteristics of the 2 lakes can be found elsewhere (e.g., Peltomaa 2013, Arvola et al. 2014, Peura et al. 2014, Rask et al. 2014).

125 In AM, water chemistry and carbon cycle variables were measured during one open-water period (2007) before cane sugar was added. During the open-water periods of 2008 and 2009, 66 kg of cane sugar was added monthly to the epilimnion (May–Oct), equivalent to a concentration of 2 mg L⁻¹ of DOC, or a mean daily loading of 0.07 mg L⁻¹ to the epilimnion (22 g m⁻² yr⁻¹). The carbon addition was made assuming the proportion of labile DOC in the lake to be ~15% of the total DOC (e.g., Tranvik 1998) and represents an increase in labile

DOC to the level expected for the fourth quartile of boreal lakes according to the Nordic Lake Survey (Henriksen et al. 1998). Prior to the addition to the lake, the cane sugar was dissolved in lake water, and the sugar solution was then distributed evenly across the lake surface from a row boat.

Most basic data used in this paper have been reported previously, and the methods used to collect the data have been described in detail elsewhere and will not be repeated here. For VK monitoring, see Vuorenmaa et al. (2014) for chemistry, Arvola et al. (2014) for plankton metabolism, Peltomaa et al. (2013) for phytoplankton, Lehtovaara et al. (2014) for zooplankton, and Rask et al. (2014) for fish. Respective methods used in AM studies are described by Peura et al. (2014) and Jones et al. (2018). Only the results of variables measured during the summer stratification period (Jun–Sep) in both lakes using the same methods and the same instruments at Lammi Biological Station were compared.

To detect possible abrupt changes in DOC concentration and/or water color during 1990–2009 in VK, a regime shift index (RSI) was calculated with SRDS Software (www.climatelogic.com) according to Rodionov (2004), based on monthly mean values (May–Sep) of the variables measured at 1 m depth. The SRDS algorithm is based on sequential Student *t*-tests for detecting time points of significant changes in the variable mean in a time series. For the RSI calculation we set the probability level at 0.05 and cut-off length as 50, corresponding to a 10-year period. A clear rise in DOC concentration occurred around 2000 and 2001 (Fig. 1), which was also detected by RSI. Thus, we compared VK data separately for the period of “lower-DOC” years 1990–2000 and of “higher-DOC” years 2001–2009 (later referred to as low-DOC_{period} and high-DOC_{period}, respectively). We also compare specifically data pooled for the 3 years with the lowest (1990, 1998, 2000; low-DOC _{yrs}) and the highest (2002, 2004, 2005; high-DOC _{yrs}) DOC concentration. Sampling for most variables took place weekly or at two-week intervals, and thus $n = 8–16$ per ice-free season (i.e., Jun–Sep). Because normal distribution and data independence could not be

ensured, a nonparametric Mann-Whitney U test (SigmaPlot 13 software) was applied to test for statistical differences in the measured variables within the lakes between low-DOC_{period} and high-DOC_{period} as well as between low-DOC_{yrs} and high-DOC_{yrs}. In AM, the pre-DOC addition data from June to September 2007 were compared with those of the DOC addition years (2008 and 2009) and tested with Mann-Whitney U test, similar to the approach with VK data. During 2007 and 2008, most samples in AM were taken twice a month but in 2009 only once a month. Thus, the results of both DOC addition years were pooled.

Although some differences in chemical properties (color, original DOC, total nitrogen [TN], total phosphorus [TP]) between VK and AM were noted (Tables 1, 2), we estimated possible changes caused by cane sugar DOC addition on epilimnetic metabolism and phytoplankton community composition in AM by using VK as a “reference lake” before (2007) and during the DOC addition years (2008–2009). Because the number of measurements per month varied (1–4) and the sampling did not always take place during the same day, monthly mean values of the variables were compared with a paired t -test (SigmaPlot 13). In a case of failed normal distribution (4 of 24), a nonparametric Wilcoxon Signed rank test was used. We compared basic results of water chemistry (samples from 1 m depth, except for dissolved inorganic carbon [DIC] from the 0–0.30 m layer), phytoplankton PP, (0–1 m layer), epilimnetic community respiration (CR, 0–1 m layer), and carbon dioxide (CO₂) efflux to the atmosphere collected during the summer stratification period (Jun–Sep). Phytoplankton biomass based on microscopical counts and crustacean zooplankton abundance in VK were from the epilimnion (0–1 m), but in AM these abundances were averaged for the epilimnion and metalimnion (0–3 m). Thus, for these variables only within-lake differences between low-DOC and high-DOC periods and years were tested. Non-metric multivariate scaling (NMS) was used to visualize differences in monthly phytoplankton community composition between different years (2007–2009) in VK and AM. The variables were plotted in the NMS figure as vectors. Differences in within-year variation of phytoplankton community composition were

tested with the homogeneity of multivariate dispersion (PERMDISP)-routine in PERIMER 6 and PERMANOVA+ add-on (Anderson 2006).

CO₂ efflux from each lake was estimated with the boundary layer (BL) method (Cole and Caraco 1998, details in Kankaala et al. 2013) based on DIC concentration and pH measured from the 0–30 cm top layer of the lakes and wind speed measurements at VK (Huotari et al. 2011). Although shown to underestimate CO₂ fluxes in VK (Huotari et al. 2011, Gålfalk et al. 2013, Erkkilä et al. 2018), the BL method was used because high-frequency measurements of water temperature and meteorological drivers were lacking during the 1990s in VK as well as in AM studies and are required for more accurate model estimations. The BL method does not take into account possible changes in physical processes in the surface mixing layer related to DOC and color increase. Especially in the gas exchange of small lakes, the relative importance of convection may exceed that by wind mixing during summer stratification (Read et al. 2012). Although the bulk of the CO₂ effluxes from these small headwater lakes usually occurs during the autumn turnover period (Huotari et al. 2011), the data for October and November during the whole study period (1990–2009) were too sparse for reliable estimates for the whole open-water period. However, the results should provide a reliable indication of the potential direction of changes related to DOC increase.

Perch growth was back-calculated from opercular bones in both lakes, and the results provided here are for 3 years (2005–2007) prior to DOC addition to AM, during the DOC addition years (2008–2009), and for years 2010 and 2011 after the manipulation (for detailed methods see Rask et al. 2014). Between-lake differences in the growth of young (first- and second-year) perch were tested with a Mann-Whitney *U* test, as well as for those in AM before (2007) and during (2008 and 2009) the sugar manipulation.

Results and discussion

Long-term and short-term changes in VK

The long-term increase in DOC concentration and water color (Fig. 1) in VK from the 1990s to the next decade (from ~11 to 13 mg L⁻¹ and from 140 to 160 mg Pt L⁻¹, respectively) was strongly related to recovery from acidification of the lake and its catchment, indicated by a significant increase in alkalinity (Vuorenmaa et al. 2014, Lehtovaara et al. 2014; Table 1). Water pH varied more than alkalinity and showed only a minor increase, presumably because of the impact of organic acids transported from the catchment by inflow (Arvola et al. 2010). The RSI for DOC indicates that a clear sudden increase took place around 2000–2001 (Fig. 1) but not simultaneously with the increase in color values (most pronounced shift in 2004). In this long-term dataset, color explained only 56% of the DOC variation by a linear regression equation: $\text{DOC (mg L}^{-1}\text{)} = 0.04 \times \text{color (mg Pt L}^{-1}\text{)} - 6.05$ ($R^2 = 0.56$, $n = 100$). Thus, as shown in many previous studies, browning is also influenced by other factors such as iron leaching from soils and its complexation with DOC (Krizberg and Ekström 2012, Weyhenmeyer et al. 2014) as well as differences in molecular structure and the proportion of colored substances in DOC, shown by spectral absorbance characteristics (e.g., Köhler et al. 2013, Adams et al. 2018).

In a 3-year study (2007–2009) at the riparian zone and in the lake, Rasilo et al. (2015) observed that DOC concentration in VK was positively correlated with catchment runoff but negatively with weekly rainfall, air, soil, and lake-surface water temperatures. During the whole study period (1990–2009) the annual variation in DOC concentration in VK was not clearly related to the annual variation in mean precipitation and air temperature (Supplemental Fig. S1). For example, of the 3 years with the highest annual precipitation (1998, 2004, and 2008), only 2004 was one of the high-DOC_{yr}s in our dataset. In whole ecosystem-scale carbon flux measurements during 15 years in 2 small boreal forest catchments, Pumpanen et al. (2014) showed that precipitation largely determined DOC fluxes, but the forest (~50 year old Scots pine [*Pinus sylvestris*] stand) net ecosystem exchange and litter production during the

previous year also had a small but significant effect on DOC runoff. Thus, presumably time lags in DOC leaching from the soil and reaching the lake also affected the DOC concentration variation in the headwater lake VK.

The concentrations of DIC in the lake surface layer and CO₂ efflux to atmosphere as well as CR in the epilimnion were significantly higher (Mann-Whitney *U* test, $p = 0.021$, 0.029, 0.024, respectively; Table 1, Supplemental Table S1) and oxygen concentration lower ($p < 0.001$) during high-DOC_{period} than values during low-DOC_{period}. However, when only the measurements from low-DOC_{yrs} (1990, 1998, 2000) and high-DOC_{yrs} (2002, 2004, 2005) were compared, no significant differences were detected except for oxygen concentration ($p < 0.001$; Supplemental Table S1), suggesting that interannual variation in CO₂ efflux may not be directly coupled with DOC and CR. The bulk of annual CO₂ release takes place during erosion of stratification and water column mixing in late summer and autumn (Huotari et al. 2011), which were not fully covered in our dataset of samples every two weeks from June to September. Moreover, CO₂ originating from catchment and riparian soil respiration also contributes to CO₂ concentrations and fluxes from headwater streams and lakes (Rasilo et al. 2012, Dinsmore et al. 2013), uncoupled from DOC decomposition in the lake itself.

No significant changes in TN or TP concentration took place during the study period in VK (Mann-Whitney *U* test, $p > 0.05$; Table 1), although the concentration of dissolved inorganic nitrogen (DIN; i.e., NO₃-N, NO₂-N, and NH₄-N) slightly increased (Vuorenmaa et al. 2014). DIN increase was presumably more related to in-lake processes such as changes in phytoplankton nutrient uptake in the light-limited environment and to hydrology (Vuorenmaa et al. 2014, Arvola et al. 2014) than to processes taking place in the catchment; N deposition declined (Ruoho-Airola et al. 2014) and net retention of inorganic N in the catchment was high (Forsius et al. 2005). However, the TN concentrations were significantly higher during high-DOC_{yrs} than N concentrations during low-DOC_{yrs} ($p < 0.001$; Supplemental Table S1).

Thus, although not clearly seen in VK long-term data, TN input seemed to be connected with

DOC during high-DOC_{yrs}. In VK, the relationship between N and DOC as well as between lake DIN and atmospheric N deposition differed from those observed on broad regional and/or time-scale surveys in the same climatic and vegetation zone. In data collected from unmanaged boreal forest catchments from southern to northern Finland ($n = 21$) during several years, annual median and/or mean export estimates for total organic carbon and N were strongly correlated, and TN consisted of, on average, 85% of organic N (Kortelainen et al. 2006). However, in the long-term parallel measurements in VK, DOC and organic N concentrations were not correlated (Vuorenmaa et al. 2014). In a large dataset, covering 78 headwater streams and 95 nutrient-poor lakes in Sweden sampled during 1998–2013, average epilimnetic (Jul–Sep) DIN concentrations decreased, and DIN:TP declined in a strong correlation with diminishing atmospheric N deposition (Isles et al. 2018). This phenomenon was not seen in VK during the study period 1990–2009, but no obvious explanation exists. Presumably, several counteracting, nonsynchronous, and catchment-specific factors such as variable organic matter loading, decreased N deposition, and changes in-lake processes influenced N processing in the old-forest catchment and the lake.

Along with browning and DOC increase, a long-term decrease in PP was evident in VK (Arvola et al. 2014, Peltomaa 2013), as also seen in the data grouped for low-DOC and high-DOC periods and years (Table 1, Supplemental Table S1). Seekell et al. (2015) detected nonlinearity in the response of PP to allochthonous DOC in small arctic and boreal lakes. Below a DOC concentration of $\sim 5 \text{ mg L}^{-1}$, primary producers benefit from additional N and P transported with allochthonous organic matter, but at higher DOC concentrations the effect is negative. In VK the DOC concentration was already $> 8 \text{ mg L}^{-1}$ at the beginning of the monitoring period, thus supporting the conclusion drawn by Seekell et al. (2015). However, the long-term change in VK was not clearly detected in short-term measurements. In weekly measurements during 3 years (2002–2004), Peltomaa and Ojala (2016) observed no clear immediate response in pelagic metabolism (PP, CR, and ^{14}C -leucine uptake) after sudden rain

events. After a long rainy period with some extreme rain peaks (20–50 mm d⁻¹) in 2004, water color in the epilimnion increased markedly, but no clear immediate increase was observed in DOC concentration. The photic layer of the lake became shallower, which caused a decrease in PP at 1 m depth but did not affect PP in the uppermost 0.5 m layer where most PP occurred.

Plankton community

Total phytoplankton biomass, based on microscopical counts, was significantly lower in VK during the high-DOC years and periods (Mann-Whitney *U* test, $p = 0.002$, $p < 0.001$, respectively; Table 1, Supplemental Table S1), although the differences based on chlorophyll *a* were significant only when low-DOC_{yr} and high-DOC_{yr} were compared ($p = 0.013$; Supplemental Table S1). Changes in phytoplankton community composition also took place: the biomass of chlorophytes declined whereas that of cryptophytes and chrysophytes slightly increased during high-DOC_{period}. Many taxa among cryptophytes and chrysophytes are potentially mixotrophic (e.g., Jones 2000), and thus presumably benefitted from high-DOC conditions and associated bacteria. Flagellate taxa, capable of diurnal vertical migration for nutrient uptake from the dark nutrient-rich hypolimnion and photosynthesis in the euphotic zone, are typical in humic lakes (Salonen et al. 1984, Lepistö and Rosenström 1998). Drakare et al. (2002) observed that the proportion of autotrophic and mixotrophic flagellates in phytoplankton increased along a DOC gradient from 3 to 31 mg L⁻¹ in small lakes in northern Sweden.

In VK, the raphidophyte *Gonyostomum semen* formed ~40% of the total phytoplankton biomass while its annual and seasonal contribution was highly variable. For 1990–2003, Peltomaa et al. (2013) detected a slight decline of *G. semen* biomass, along with TP concentration, but in the data for the whole study period (1990–2009) no clear trend was seen. Annual median biomass variation of this taxon was not related to DOC concentration.

G. semen is typically abundant in humic lakes (Cronberg et al. 1988, Angeler et al. 2012). In monitoring data collected from the epilimnion of 95 Swedish lakes in August over 5 years, blooms were typical in acidic brown-water lakes with TP concentration $>15 \mu\text{g L}^{-1}$ (Lebret et al. 2018). However, the blooms were more associated with high iron concentration ($\sim 200 \mu\text{g L}^{-1}$) than with total organic carbon contributing to lake browning (Lebret et al. 2018). In VK, pH and TP (Table 1) as well as epilimnetic iron concentrations (average [SD] = 225 [43] $\mu\text{g L}^{-1}$, measured at 1 m depth in Jun–Aug 1994–2005 and 2009; Finnish Environment Institute, open data, <https://www.syke.fi/avointieto>) were similar to those in Swedish lakes with *G. semen* blooms. However, this vertically migrating species also efficiently utilizes N and P resources from the anoxic hypolimnion in VK (Salonen and Rosenberg 2000), where the concentration of iron is also higher (677 [SD 128] $\mu\text{g L}^{-1}$ at 5–6 m depth, the same period and data source noted earlier) than in the epilimnion.

During the study period 1990–2009, changes in crustacean zooplankton species dominance were recorded both in cladocerans and copepods. *Ceriodaphnia quadrangula* was the dominant cladoceran in most years in the 1990s but was gradually replaced by *Bosmina longirostris* during the years of regime shift (2002–2004) for DOC and water color. Among copepods, *Mesocyclops leuckarti* was most abundant in the early 1990s but subsequently *Thermocyclops oithonoides* dominated (Lehtovaara et al. 2014). The changes in zooplankton community were associated with increasing carbon load and recovery from acidification, but changes in the microbial community and phytoplankton, including the decrease in primary production (Arvola et al. 2014), may also have had an influence. Within the food web, bottom-up regulation of zooplankton seemed to exceed the importance of top-down control (Lehtovaara et al. 2014). However, no significant differences in the total abundance of cladocerans and copepods were noted between high-DOC and low-DOC periods and/or years ($p > 0.05$; Table 1, Supplemental Table 1). This finding differs from the results obtained for 2 small lakes in northeastern Pennsylvania, USA, undergoing recovery from acidification and

browning during a 27-year monitoring period. In these lakes, the abundance of *Daphnia* and
calanoids decreased, whereas the abundance of cyclopoids increased or was unchanged
(Williamson et al. 2015). The DOC concentration in these lakes was originally much lower
than in VK and increased $\sim 1 \text{ mg L}^{-1}$ (from ~ 1 to 2 mg L^{-1} and from 5 to 6 mg L^{-1} ,
respectively) during the monitoring period.

Fish

In VK, long-term changes took place in the population dynamics of perch, evidenced by the
occurrence of strong year-classes every 4 years in the 1990s levelling off in the 2000s (Rask
et al. 2014; Table 1). Around 2000–2005 (i.e., the years of the regime shift discussed earlier),
a significant decrease was also recorded in perch growth in the first 2 years of life. This
decrease was related to the increase in water color and subsequent decrease of the general
productivity of the lake, decrease in volume of oxygenated habitat, and deterioration of light
conditions for perch, which is a visual feeder (Rask et al. 2014). The slight increase in the
growth of older and larger perch in the same years suggested their lower dependence on the
pelagic food web and feeding on macroinvertebrates (Rask et al. 2014).

Short-term effects in AM in comparison to VK

Water chemistry and carbon flux

During the AM study (2007–2009), the reference lake VK was already in the “high-DOC”
phase. Rasilo et al. (2015) estimated that during these years the annual load of terrestrial DOC
to VK varied between 1500 and 4100 kg, which means $\sim 37\text{--}100 \text{ g m}^{-2} \text{ yr}^{-1}$ per lake surface
area. Assuming that terrestrial DOC input to AM was of the same order of magnitude as that
to VK, the cane sugar addition in 2008 and 2009 was $\sim 18\text{--}37\%$ of the annual DOC input to
the lake. However, when the labile low-molecular weight fraction is generally $<20\%$
terrestrial DOC (Münster 1993, Jonsson et al. 2007, Hulatt et al. 2014), the cane sugar

addition most likely exceeded the input of labile DOC transported from the surrounding catchment; the DOC concentration increased by 2 mg L^{-1} , thus reaching the level prevailing in VK during those years (Table 2).

Because monthly mean temperatures, measured at 1 m depth in both lakes, did not differ significantly (paired t -test, $p > 0.05$; Table 2), we assumed that seasonal weather-induced variations in both lakes were similar, and thus the paired mean monthly data could be used for the comparisons. During the pre-DOC addition year 2007, CO_2 efflux, PP, CR, and water pH did not differ significantly ($p > 0.05$) between the lakes, even though DOC, TP, TN and chlorophyll a concentrations as well as color values were significantly higher in VK, (all $p < 0.05$; Table 2). Cane sugar caused no change in water color, as expected, and no marked changes in PP took place during the study years (Peura et al. 2014; Table 2, Supplemental Table S2), as expected.

In AM, significantly greater surface DIC concentrations and CO_2 effluxes were measured during the DOC addition years (paired t -test, $p < 0.001$ for both), suggesting immediate increase in DOC decomposition. However, this increase was not clearly seen in the measurements of epilimnetic CR, bacterial production, or oxygen (O_2) concentration (Supplemental Table S2; Peura et al. 2014). The measurements were taken 2 weeks after DOC amendments, and presumably some microbial activity peaks were missed. However, a slight but significant decrease in pH and alkalinity in AM during the DOC addition years was presumably related to increased concentration of CO_2 (Mann-Whitney U test, $p < 0.001$, 0.017; Supplemental Table S2, Table 2), thus in the opposite direction from measurements taken in VK.

Plankton community

Phytoplankton community composition in VK and AM clearly differed. In June–September 2007–2009, 58–81% of the biomass in VK consisted of the raphidophyte *G. semen* (on

average 67%), and in only one case was the proportion of chrysophytes higher (69%) than that of *G. semen*. Cryptophytes, dinophytes, chrysophytes, diatomophytes, and chlorophytes averaged 4%, 9%, 15%, 2%, and 1%, respectively, of the biomass. In AM, before cane sugar addition in 2007, chrysophytes and chlorophytes averaged 60% and 39%, respectively, of the phytoplankton biomass. The proportion of cryptophytes, dinophytes, and *G. semen* was then ~1–2% of the biomass. During the DOC addition years the total phytoplankton biomass was at the same level as in 2007 (Supplemental Table S2). However, changes in the community composition occurred; the proportion of chrysophytes decreased and that of *G. semen* increased. In the latter half of the second DOC manipulation season (Aug–Sep 2009), 37–75% of the phytoplankton biomass consisted of this taxon. Thus, although no water browning took place, *G. semen* was favored by labile DOC increase and presumably also by the lower pH (see Lebre et al. 2018 and earlier discussion). The biomass of cryptophytes was slightly but significantly higher during the DOC addition years than that in 2007 (Mann-Whitney *U* test, $p = 0.028$; Supplemental Table S2). Also, the proportion of heterotrophic taxa (e.g., *Bicosoeca* spp., *Petalomonas* sp., *Katablepharis ovalis*) increased from ~1% in 2007 to 4% during the DOC addition years. PERMDISP analysis indicated that the seasonal variation in phytoplankton community composition increased in AM during the DOC addition years ($F_{2,9} = 9.069$, $p = 0.02$; Fig. 2) while the variation in VK did not change ($F_{2,9} = 2.981$, $p = 0.299$). Hence, the phytoplankton community seemingly became more stochastic in AM following the DOC additions.

Zooplankton community composition also differed between VK and AM. In VK copepods were more abundant than cladocerans (Table 2). In AM the crustacean zooplankton community was dominated by the cladocerans *Holopedium gibberum*, *C. quadrangula*, and *Daphnia* sp. and by small cyclopoids. Enriched stable carbon isotope values ($\delta^{13}\text{C}$) of particulate organic matter and zooplankton clearly showed transfer of cane-sugar DOC to zooplankton during the DOC addition years (Peura et al. 2014), although no significant

differences were observed in the abundance of cladocerans and copepods during the study years (Mann-Whiney U test, $p > 0.05$; Supplemental Table S2). This result differs from that obtained by Kelly et al. (2016) in a DOC manipulation experiment of an oligotrophic temperate lake in which the catchment-origin DOC concentration in the treatment basin increased from 8 to 11 mg L⁻¹, whereas in the reference basin DOC concentration decreased from ~8 to 6 mg L⁻¹. The authors concluded that zooplankton in the treatment basin benefitted from improved resource quality because of an increase in PP associated with transported P (see also Seekell et al. 2015 for the positive impact on PP, but Williamson et al. 2015 for negative long-term changes in zooplankton in the lakes with DOC concentration <6 mg L⁻¹). In our study lakes, DOC concentration was originally higher (~10–11 mg L⁻¹), and the long-term effect on PP was negative in VK and the short-term effect insignificant in both lakes, resulting in no clear effect on crustacean zooplankton density.

Fish

During the DOC addition experiment in AM (2007–2009), the density and biomass of the perch population in both lakes (AM and VK) were similar (600–1500 and 1000–1700 ind. ha⁻¹, and 14–28 and 22–27 kg ha⁻¹, respectively).

The first year median growth of perch was higher in AM than in VK during 2005–2010 ($U = 36$, $p = 0.004$). In VK both the first- and second-year growth of perch showed less variation than in AM. The second-year growth of perch was similar in both lakes during 2005–2007, but during the years of DOC manipulation (2008 and 2009) the growth was significantly faster in AM (Mann-Whitney $U = 408$ and 352 , $p = 0.04$ and < 0.001 , respectively; Fig. 3). When only the “reference year” 2007 and DOC “manipulation years” 2008 and 2009 were considered, the first-year growth of perch in AM was significantly higher in the reference year (Mann-Whitney $U = 561$, $p = 0.005$) but the second-year growth during the manipulation years was greater (Mann-Whitney $U = 721$, $p < 0.001$). Enriched stable

carbon isotope values ($\delta^{13}\text{C}$) revealed the importance of the route of benthic invertebrates to fish, leading to a cane sugar carbon proportion of 18.7% of perch carbon biomass at the end of the sugar addition (Jones et al. 2018). The extra cane sugar DOC input seemed to temporarily stimulate more the growth of benthivorous second-year perch than that of primarily planktivorous young-of-the-year perch. The diet of perch was not examined in this study, but the increasing proportion of benthic food during the early years of perch has been recorded in other studies and lakes in the study area (Rask and Arvola 1985, Estlander et al. 2010). The slight increase in the growth of older and larger perch in VK (Rask et al. 2014) suggests that increasing DOC may positively affect benthivorous fish also in natural conditions.

Terrestrial DOC vs. cane sugar DOC and long-term monitoring vs. short-term manipulation

– What can be learned?

The extensive dataset collected during long-term monitoring shows clear ecosystem-scale changes and a regime shift due to DOC increase and browning in VK; poorer light penetration into the water column was followed by changes in community structure, a decrease in PP and in the growth of young perch, and an increase in CO_2 efflux to the atmosphere. In the short-term (2 years) manipulation with cane sugar in AM, the pathway of DOC up to fish could be traced by $\delta^{13}\text{C}$, and an increase in CO_2 efflux could also be observed. However, detecting significant changes in a strict statistical sense was limited by marked seasonal and interannual variation in the measured variables and rather low frequency of measurements. For example, epilimnetic bacterial cell number and production as well as CR were not clearly related to DOC addition to AM (Peura et al. 2014). However, in short (2–3 weeks) replicated mesocosm experiments, a significant increase in these variables indicating heterotrophy was generally seen after DOC addition (Kankaala et al. 2010, Faithfull et al. 2012).

Glucose or sucrose, here in the form of cane sugar, are commonly used to simulate labile DOC in experiments (e.g., Blomquist et al. 2001, Smith and Prairie 2004, Faithfull et al. 2012) and could represent fresh biodegradable detritus of both autochthonous and allochthonous origin (cf. Søndergaard et al. 2000, Kiikkilä et al. 2011). In addition to carbohydrates (including sugars), low-molecular-weight terrestrial DOC contain other compounds such as amino acids and carboxylic acids, which also are easily degraded by microbes (Jonsson et al. 2007, Berggren et al. 2010, Kiikkilä et al. 2011). However, more than half of terrestrial DOC consists of high-molecular-weight humic compounds, which are recalcitrant to immediate degradation by aquatic microbes (e.g., Jonsson et al. 2007, Räsänen et al. 2018). Thus, the impacts of colored humic substances are negative rather than positive on lake food webs by reducing light availability for primary producers (Thrane et al. 2013, Kelly et al. 2018), thus impairing food resource availability for the higher trophic levels (Karlsson et al. 2015, Taipale et al. 2016). Moreover, colored substances promote steeper temperature and oxygen stratification in summer, which narrow potential habitats for fish survival and worsen foraging opportunities for visually feeding fish (Estlander et al. 2010, Williamson et al. 2015).

Although DOC enhances bacterial production, growth efficiency of bacteria utilizing both low-molecular-weight and high-molecular-weight DOC is strongly related to availability of inorganic nutrients (N, P; Smith and Prairie 2004, Jansson et al. 2006, Räsänen et al. 2018), which also impacts the magnitude of DOC-origin carbon available for consumers and/or respired and finally released as CO₂ to the atmosphere. Although available for grazers, bacteria are poor-quality diets for zooplankton and zoobenthos because they lack essential polyunsaturated fatty acids and sterols (Martin-Creuzburg et al. 2011, Taipale et al. 2012). Even when the experimental addition of labile DOC to AM (22 g m⁻² yr⁻¹) was of the same order of magnitude as annual PP (AM 18–20 g m⁻² yr⁻¹; Peura et al. 2014), only <20% of top consumer (perch) carbon originated from labile DOC (Jones et al. 2018). This finding

indicates the inefficiency of DOC-based food chains and consumer diet requirements to
495 include high-quality food resources produced by algae (Taipale et al. 2016).

The $\delta^{13}\text{C}$ label of DOC addition could be detected in AM consumers (crustacean
zooplankton, benthic invertebrates, fish) but not in the components of seston $<100\ \mu\text{m}$
(phytoplankton, bacteria, detritus, protozoans, rotifers), which could not be separated and
analyzed, so that the actual microbial food chain routes could not be followed. In addition to
500 pelagic and benthic microbial food chains, flocculation of cane-sugar DOC to particulate
organic carbon (von Wachenfeldt and Tranvik 2008) could have been another route for
benthic consumers, contributing to their $\delta^{13}\text{C}$ enrichment. Although the flocculation of cane
sugar in AM is speculative, the results from a whole-lake scale experiment with maize leaf
addition to 2 German lakes showed that benthic invertebrates could play a significant role in
505 transferring terrestrial POC to fish (Scharnweber et al. 2014). However, the contribution of
maize leaves, corresponding to the autumnal alder leave input, was only 1–3% of omnivorous
fish carbon, and the benthic microbial food chain in these lakes proved to be inefficient
(Lischke et al. 2017). More investigations are needed to clarify how DOC increase and
browning influence benthic–pelagic coupling in lakes.

510 In conclusion, long-term monitoring of a boreal headwater lake confirmed the general
trend of increasing DOC concentration and browning in northern lakes and associated
changes in pelagic community structure as well as in productivity. The decrease in lake PP
was also reflected in higher trophic levels, here represented by the growth of young perch.
Both long-term monitoring and whole-lake DOC manipulation showed an increase in CO_2
515 efflux to the atmosphere and changes in pelagic community structure, followed by an increase
in DOC concentration. Long-term integrated monitoring of aquatic ecosystems is without
question important in revealing responses of lake parameters to environmental change.
However, given normal high between-lake variability, data obtained from an individual lake
and catchment will not necessarily support trends based on correlation or regression analyses

of parameters measured on large regional and/or time scales. The whole-lake manipulation approach has the advantage of detecting ecosystem-scale responses to one major driver, here to labile DOC, but not simultaneously affecting other factors, like browning, that also suppress light availability for primary producers. This experimental manipulation, applying $\delta^{13}\text{C}$ differences in cane-sugar and catchment-origin DOC, revealed the importance of benthic pathways from DOC to perch (Jones et al. 2018). Benthic DOC pathways were not detected in the long-term monitoring of VK and in the mesocosm experiments, although both these approaches detected increased pelagic heterotrophy after DOC increase (Table 1; Kankaala et al. 2010). Short-term whole-lake manipulation experiments can provide insights into the processes behind the patterns and trends demonstrated in long-term studies.

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Figure legends

Figure 1. (a) Median DOC concentration (as C: mg L⁻¹ with 25 and 75% quartiles and outliers, x indicates mean value) in Valkea-Kotinen during 1990–2009. (b) Median and mean
785 water color (mg Pt L⁻¹, symbols as in panel a) in the lake during the same period. Dash lines show regime shift time points calculated as regime shift index (RSI; DOC = 0.87, color = 0.93, $p < 0.001$ in both DOC and color) according to Rodionov (2004); see methods for details.

790 Figure 2. Non-metric multivariate scaling (NMS) figure visualizing the changes in phytoplankton community composition (group proportions) in Valkea-Kotinen (VK, reference lake) and Alinen Mustajärvi (AM) during the pre-manipulation (2007) and manipulation years (2008–2009). Markers represent monthly mean values, and the samples

within a given year are connected by a line. Variables are presented as vectors. The final

stress for the 2D solution was 0.05.

Figure 3. (a) First-year and (b) second-year growth of perch in lakes Alinen Mustajärvi (white) and Valkea-Kotinen (grey) given as annual length increments for 2005–2011. Box plots indicate median values, first and third quartiles, and range. Horizontal arrow indicates the seasons of cane sugar addition.

Table 1. Median values (with 25–75% range) of water chemical variables at 1 m depth (except DIC at 0–30 cm), CO₂ efflux from lake surface, phytoplankton primary production (PP), community respiration (CR, uppermost 1 m layer), phytoplankton biomass (PB), and zooplankton density in June–September (epilimnion) during the “low-DOC_{period}” (1990–2000) and “high-DOC_{period}” (2001–2009) as well as those of perch density and growth during the first and second year in Lake Valkea-Kotinen during the same periods. CO₂ efflux, PP and CR measured as C. Statistical differences between the periods were tested with Mann-Whitney *U* test (*p* denotes significance, ns = not significant).

Variable	Low-DOC _{period} (1990–2000)			High-DOC _{period} (2001–2009)			<i>p</i>
	median	range (25–75%)	<i>n</i>	median	range (25–75%)	<i>n</i>	
DOC, mg L ⁻¹	11.3	(10.3–12.1)	207	12.9	(12.4–13.6)	170	<0.001
Color, mg Pt L ⁻¹	135	(124–147)	192	162	(152–179)	170	<0.001
pH	5.2	(5.1–5.4)	192	5.3	(5.1–5.4)	162	0.025
Alkalinity, mmol L ⁻¹	0.005	(0.0009–0.0175)	177	0.087	(0.013–0.023)	146	0.006
O ₂ , mg L ⁻¹	8.9	(8.4–9.5)	190	8.1	(7.4–8.8)	219	<0.001
TN, µg L ⁻¹	460	(412–526)	298	461	(436–504)	242	ns
TP, µg L ⁻¹	15	(13–18)	298	15	(13–18)	242	ns
DIC, mg L ⁻¹	0.40	(0.27–0.61)	192	0.48	(0.30–0.78)	146	0.021
CO ₂ efflux, mg m ⁻² d ⁻¹	138	(58–288)	192	201	(74–393)	146	0.029
PP, mg m ⁻² d ⁻¹	116	(82–149)	190	75	(52–103)	132	<0.001

CR, mg m ⁻² d ⁻¹	100	(74–131)	190	115	(81–149)	132	0.024
Chlorophyll <i>a</i> , µg L ⁻¹	13.0	(9.19–21.2)	190	13.7	(10.7–16.8)	132	ns
PB Tot, mm ³ L ⁻¹	2.15	(1.29–3.54)	220	1.70	(1.07–2.60)	171	0.002
Cryptophytes, mm ³ L ⁻¹	0.05	(0.02–0.11)	220	0.06	(0.03–0.11)	171	0.019
Dinophytes, mm ³ L ⁻¹	0.05	(0.01–0.21)	220	0.08	(0.02–0.25)	171	ns
Chrysophytes, mm ³ L ⁻¹	0.09	(0.02–0.23)	220	0.21	(0.11–0.35)	171	<0.001
Diatomophytes, mm ³ L ⁻¹	0.02	(0.01–0.05)	220	0.02	(0.01–0.07)	171	ns
Raphidophytes, mm ³ L ⁻¹	0.68	(0.11–1.74)	220	0.73	(0.07–1.61)	171	ns
Chlorophytes, mm ³ L ⁻¹	0.08	(0.02–0.19)	220	0.03	(0.02–0.06)	171	0.001
Cladocera, ind. L ⁻¹	2.1	(0.2–15.5)	190	3.5	(0.7–12.5)	113	ns
Copepoda, ind. L ⁻¹	26.6	(11.3–57.5)	190	30.0	(15.5–67.4)	113	ns
Perch density, ind ha ⁻¹	1107	(738–1738)	10	1214	(988–1298)	9	ns
Perch, 1st year growth, mm	62.0	(59.3–64.0)	995	50.8	(49.5–56.8)	459	<0.001
Perch, 2nd year growth, mm	38.1	(31.3–43.4)	967	31.8	(30.6–32.8)	405	<0.001

Table 2. Mean (SD) monthly values of June–September temperature and those of chemical variables (1 m depth, except DIC from 0 to 0.3 m depth) as well as carbon flux (CO₂ efflux from lake surface and primary production [PP] and community respiration [CR] in the 0–1 m layer, measured as C) in VK and AM before (2007) and during the cane sugar addition to AM (2008–2009). The differences were tested with paired *t*-test of monthly mean values based on 1–4 measurements per month (for *t*-test *n* = 4 in 2007, 8 in 2008–2009 in each lake). The results for the total phytoplankton biomass (BP) and cladoceran and copepod abundances in VK are only for epilimnion but in AM are averaged for epilimnion and metalimnion, and thus statistical differences were not tested. Differences in the first and second year growth of perch was tested with Mann-Whitney *U* test (*n* = 21 and 42 in VK and 44 and 66 in AM for 1- and 2-year perch in 2007; and 16 and 58 in VK and 17 and 39 in AM for 1- and 2-year perch in 2008–2009).

Paired <i>t</i> -test in SigmaPlot 13	2007 pre DOC add year Jun–Sep					2008–2009 during DOC add years Jun–Sep				
	VK mean	SD	AM mean	SD	<i>p</i>	VK mean	SD	AM mean	SD	<i>p</i>
Variable										
Temperature °C	16.7	2.5	15.6	3.5	0.136	15.6	2.6	15.7	2.3	0.960
DOC, mg L ⁻¹	12.2	0.5	10.4	0.4	0.008	12.4	0.3	12.0	0.8	0.256
Color, mg Pt L ⁻¹	162	7	98	10	<0.001	167	8	99	4	<0.001
pH	5.2	0.1	5.1	0.0	0.337	5.2	0.254	4.9	0.1	0.008
Alkalinity, mmol L ⁻¹	0.006	0.004	−0.012	0.003	0.010	0.005	0.002	−0.019	0.007	0.008
O ₂ , mg L ⁻¹	8.3	1.1	8.3	0.5	0.949	8.4	0.5	7.9	0.7	0.085
TN, µg L ⁻¹	515	77	378	8	0.036	462	21	377	26	<0.001
TP, µg L ⁻¹	20	5	10	2	0.026	20	2	12	2	<0.001
DIC, mg L ⁻¹	0.683	0.403	0.693	0.370	0.845	0.613	0.360	0.925	0.380	<0.001
Chlorophyll <i>a</i> , µg L ⁻¹	15.9	6.0	5.1	0.6	0.037	15.0	3.1	6.6	5.5	0.016
CO ₂ eff., mg m ⁻² d ⁻¹	267	202	268	143	0.979	240	190	484	196	<0.001
PP, mg m ⁻³ d ⁻¹	78	24	83	26	0.541	66	19	81	54	0.351
CR, mg m ⁻³ d ⁻¹	127	75	78	28	0.427	99	37	80	23	0.202
PB Tot., mm ³ L ⁻¹	2.4	0.7	1.0	0.7		2.7	0.6	1.1	0.6	

Cladocera, ind. L ⁻¹	6.6	3.3	18.4	13.7		8.5	7.4	19.5	16.5	
Copepoda, ind. L ⁻¹	66.3	47.1	8.3	9.0		69.2	53.7	16.9	16.6	
Perch density, ind. ha ⁻¹	775.0		n.d.			1238	352.0	1040.0	646.0	1.000
Perch, 1st year growth, mm	49.8	5.2	69.1	6.4	<0.001	52.4	4.6	63.7	6.9	<0.001
Perch, 2nd year growth, mm	31.1	4.1	31.1	11.3	0.767	31.7	5.7	34.4	8.1	<0.001