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RESEARCH ARTICLE

Experimental assessment of a possible microbial priming effect in a humic boreal lake

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Abstract Global change models normally do not include interaction effects between different pools of recalcitrant humic organic carbon which can alter carbon cycling via their influence on biological activities. This issue is especially important in northern regions where lakes receive high inputs of allochthonous dissolved organic carbon (DOC) from the extensive surrounding peatlands. We investigated the threshold of added labile DOC necessary to promote a *priming effect* (PE); i.e. stimulation of bacterial metabolism with a subsequent increase in the mineralization of recalcitrant DOC and the accompanying changes in microbial community structure and function. Our study was carried out in a small highly humic lake (Mekkojärvi, southern Finland), physically divided by a plastic curtain into two experimental basins, one where fish were present (+FISH) and one that was fishless (−FISH). In each basin, we performed a factorial mesocosm experiment in which different amounts of labile DOC were supplied as cane sugar (control +6, +9, +12 mg C L^{-1}). Our results showed no priming effect in any carbon treatment, either in +FISH or in −FISH basins, despite a decreasing trend in total DOC concentration. Bacterial abundance and production did not increase as a response to carbon additions, while mixotrophic algae increased their abundance over time. In our experiments, the organisms that benefitted most after addition of labile DOC were mixotrophic algae, which can transform carbon into biomass by obtaining inorganic nutrients through phagotrophy. This appears most likely due to strong bacterial N limitation and dependence on resource availability and stoichiometry.

Keywords Bacterioplankton · Phytoplankton · Priming effect · DOC · Humic lakes

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Introduction

Inland aquatic ecosystems of northern regions receive high inputs of allochthonous dissolved organic carbon (DOC) from the extensive surrounding peatlands (Kortelainen 1993; Kortelainen et al. 2006; Roulet and Moore 2006). This DOC is dominated by dissolved humic substances (HS), which frequently comprise between 50 and 80 % of the total dissolved organic matter in aquatic ecosystems (Farjalla et al. 2009a; Rocker et al. 2012). HS are considered complex and recalcitrant (Anesio et al. 2005; Farjalla et al. 2009a) containing high proportions of high molecular weight compounds (Rocker et al. 2012), such as derivatives of lignin (Lehtonen et al. 2000). Due to their refractory nature, HS are less readily metabolized by bacterioplankton than other sources of carbon such as algal exudates (Farjalla et al. 2006; Kritzberg et al. 2005), amino acids and carbohydrates (Rosenstock and Simon 2003). However, HS can be decomposed by microorganisms (Farjalla et al. 2009b; Rocker et al. 2012) and/or by photochemical degradation (Granéli et al. 1996; Reche et al. 2001) that increases the concentration of lower molecular weight

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compounds, which can then be used more efficiently by planktonic communities than the original HS (Lindell et al. 1995; Farjalla et al. 2009a).

Bacterial breakdown of high molecular weight HS involves extracellular enzymatic degradation and can be promoted by co-metabolism between labile carbon molecules and HS (Münster and Tranvik 1998; Farjalla et al. 2009a). This phenomenon, termed "priming effect" (PE), was first defined by Bingemann et al. (1953) and has been reviewed by Kuzyakov et al. (2000) in the context of terrestrial ecosystems. PE consists of a modification in the mineralization rate of recalcitrant matter altering carbon and nutrient content after an input of labile organic matter (e.g. glucose, sucrose, which stimulates bacterioplankton (Guenet et al. 2010). PE mechanisms are not well understood (Fontaine and Barot 2005; Guenet et al. 2010; Bianchi 2011), but three possible explanations of this phenomenon have been proposed (Guenet et al. 2010): (1) co-metabolism between decomposers of labile organic matter (LOM) and decomposers of recalcitrant organic matter (ROM); (2) net mutualism of two different bacterial communities; and (3) a single decomposer community which produces enzymes capable of degrading both LOM and ROM.

Although PE has been well studied and is a widely accepted phenomenon in terrestrial ecosystems (Kuzyakov 2010), PE in aquatic ecosystems is not as well understood and has been scarcely studied. Aquatic studies of PE can be classified into three different groups according to the environment where experiments have been performed: (1) waterlogged saturated terrestrial soils, (2) oceanic sediments and surface waters and (3) inland waters. Turnewitsch et al. (2007) observed that fresh substrates from algal sources primed marine sediments. Nugteren et al. (2009) reported for marine sediments that algal organic carbon additions enhanced background remineralization by up to 31 % (i.e. PE). Carlson et al. (2002) incubated marine water with added organic and inorganic nutrients (i.e. glucose, nitrogen and phosphorus) and at the end of their experiments found less organic carbon in the added treatments than in the control, along with an enhanced bacterial production and utilization of recalcitrant DOC. This strongly suggests that PE occurred, as also indicated by Guenet et al. (2010); moreover, Guenet et al. (2014) reported that soils of different ecosystems had been more remineralized under waterlogged conditions with glucose addition compared to the same saturated soils without glucose. Each of these studies demonstrates that enhanced DOC metabolism was actively occurring after the addition of labile carbon, although confirmation that LOM directly leads to increased ROM metabolism in humic ecosystems is still lacking. Studies carried out in inland waters are scarce (e.g. De Haan 1977; Farjalla et al. 2009a) and often inconclusive because they were not specifically designed to study PE. De Haan (1977) reported that degradation of fulvic acids by a specific genus of bacteria (*Arthrobacter*) was stimulated by addition of benzoic acid. Farjalla et al. (2009b) concluded that LOM could enhance the turnover rates of ROM through a synergistic effect of LOM and ROM on bacterial production and growth efficiency, but they could not distinguish between pools of carbon (i.e. autochthonous vs. allochthonous) because carbon sources were not labelled. Hence the possible role of exogenous carbon sources in aquatic ecosystems remains controversial. Some recent studies focused on PE have reported contradicting results. Some authors have observed an increase in the degradation of leaf litter in streams when diatoms (Danger et al. 2013) or algae (Rier et al. 2007; Kuehn et al. 2014) were present and others detected an increase in the degradation of terrestrially derived dissolved organic matter after addition of algal organic carbon or a simple disaccharide (Bianchi et al. 2015). In contrast, others found no evidence of PE in a stream hyporheic zone within microcosms (Bengtsson et al. 2014), or for organic matter in NE Atlantic slope sediments (Gontikaki et al. 2013), or after pulse additions of labile carbon sources in lakes with different trophic states (Catalán et al. 2015).

Boreal humic lakes are mostly carbon rich but nutrient-poor ecosystems (Vuorenmaa et al. 2006; Karlsson et al. 2002; Kankaala et al. 2010a). Increased "brownification" linked to global change (Vuorenmaa et al. 2006) is expected to further alter the stoichiometric balance between DOC and inorganic nutrients in these waters. In this scenario, bacteria are thought to be the organisms that should benefit most due to their greater ability to take up inorganic nutrients at low concentrations relative to algae (e.g. Currie and Kalff 1984; Kritzberg et al. 2014) and their ability to convert ROM into LOM and amino acids for growth. However, various algae and cyanobacteria are also capable of exploiting organic carbon resources (Tittel et al. 2009; Fontes et al. 2013). Hence investigating the capability of bacterioplankton and algae to utilise complex DOC and degrade ROM–LOM (via priming) could improve understanding of the overall fate of dissolved organic matter (DOM) in humic lakes within the context of global change. In this study we sought evidence for PE in a humic lake, and whether any PE was dependent on a particular threshold concentration of labile DOC. To address these issues we performed a factorial experiment in which different levels of labile DOC addition (cane sugar) were supplied to mesocosms in a small oligotrophic highly humic lake (Mekkojärvi) in southern Finland. Mekkojärvi is naturally fishless due to prolonged water column anoxia

during winter ice cover. During our experiments the lake had been separated by a plastic curtain as part of a parallel research programme on food web interactions, to create two experimental basins. One basin remained fishless (−FISH) while the other had fish introduced (+FISH). We took advantage of this setup to assess whether differences in the structure of the planktonic community determined by the presence or absence of fish might also influence PE. This approach is based on trophic cascades theory such that in the absence of fish (−FISH basin), Mekkojärvi typically supports a very high biomass of the crustacean zooplankter *Daphnia longispina* during the open water season, which leads to low phytoplankton biomass and production (e.g. Taipale et al. 2008). However, the introduction of fish into the +FISH basin rapidly decimated the *Daphnia* biomass, allowing an increase in phytoplankton (J. Syväranta unpublished data). In both basins, the phytoplankton community composition was dominated by mixotrophic algae (mainly Chrysophyta, Dinophyta and Cryptophyta) able to ingest bacteria which preferably use organic carbon of algal origin (e.g. Kritzberg et al. 2005); strictly autotrophic algae (mainly Bacillariophyta and Chlorophyta, including Desmidiaceae) were present in lesser proportions in both basins.

White cane sugar (sucrose) was used in our experiments as a source of low molecular weight form of DOC (see also Blomqvist et al. 2001; Kankaala et al. 2010a; Peura et al. 2014) because sucrose (1) does not alter water colour and hence does not affect the light availability for primary producers, (2) is a first major product of plant photosynthesis and can reasonably represent the labile fraction (typically around 15 %) of natural DOM and, (3) its elemental composition, unlike that of natural DOM, does not introduce phosphorus and nitrogen which could stimulate microbial activity and confuse any true PE effect arising from added labile DOC. In addition, the use of cane sugar (originating from C_4 plants) allowed us to evaluate incorporation of LOM, because its stable carbon isotope value ($\partial\ ^{13}C$ −11 ‰) is higher than that of the natural organic carbon originating from local terrestrial C_3 plants ($\partial\ ^{13}C$ −26 to −28 ‰; Kankaala et al. 2010a; Peura et al. 2014). Our aims were to investigate experimentally (1) the existence of a threshold of added labile DOC able to promote PE and (2) whether the changes in the microbial community structure (algal and bacterial abundance) and function (primary and bacterial production) were induced by a gradient of increasing labile DOC. Based on previous studies of terrestrial ecosystems and wetland soils, we hypothesised that production and abundance of bacteria would be stimulated by the addition of labile carbon promoting a PE, while phytoplankton would benefit to a lesser extent from DOC additions.

Materials and methods

Study area

Mekkojärvi (61°13′N; 25°08′E) is a small highly humic lake (area 0.35 ha, mean depth 3 m) located in the Evo forest area in southern Finland. The lake has acidic water (pH 4.6–6.2) due to the coniferous forest catchment (Kankaala et al. 2010b) and high water colour (colour 300–700 mg Pt L^{-1}; Taipale et al. 2007). Mekkojärvi has winter ice cover for 4.5–5.5 months, and an ice-free period in which the water column is exceptionally strongly stratified, with anoxic conditions below ~0.5 m depth promoting sediment methane production and accumulation in the hypolimnion (Taipale et al. 2007) and that was also the case during the experimental period (Devlin et al. submitted). The lake usually mixes completely during autumn (Kankaala et al. 2010b). The epilimnetic concentration of DOC can reach 30 mg C L^{-1}, phosphates (PO_4^{3-}) up to 1 µg P L^{-1} and nitrates ($NO_3^- + NO_2$) up to 35 µg N L^{-1}. Further characteristics of Mekkojärvi can be found elsewhere (Kuuppo-Leinikki and Salonen 1992; Salonen et al. 2005). The lake is naturally fishless due to the regular and prolonged anoxia that develops throughout the shallow water column under winter ice cover. However, as part of a parallel research programme studying trophic cascade effects on lake ecosystem processes, Mekkojärvi had been divided into two basins by a plastic curtain during the ice-free periods of 2011 and 2012. Adult perch were introduced to one basin of the lake in early July 2012. An equivalent biomass of juvenile perch was introduced to the other basin but these quickly died so this basin can be considered as remaining fishless. Thus, the lake presented two contrasting environments, a fishless basin, simulating the natural conditions (−FISH), and a basin with fish introduced (+FISH).

Experimental design and sampling

In summer of 2012 we performed a 4 × 3 factorial design mesocosm experiment that included four labile DOC (cane sugar) treatments: control [no addition]; +6 mg C L^{-1} [+6]; +9 mg C L^{-1} [+9] and +12 mg C L^{-1} [+12]. Each treatment included three experimental incubation times (24, 72 and 120 h after the addition) such that time was an independent variable potentially affecting the microbial community composition and function. Each DOC-time combination had three replicates, making 36 mesocosms in total for each basin of the lake. The experimental setup consisted of acid-washed 8 L mesocosms (transparent polyethylene terephthalate [PET] containers). Each mesocosm was incompletely filled (95 % of container volume)

with surface water that was filtered through a 45-µm mesh to remove zooplankton, then amended with DOC according to treatment, and submerged just below the surface of the lake for incubation. The incomplete filling of containers was intended to minimize potential development of hypoxic conditions during the course of the experiment, due to the impermeability of PET to gas exchange; the high primary-to-bacterial production ratio found (see below) indicates that oxic conditions were maintained throughout the experiment.

To explore a possible threshold for a PE with labile DOC, we assumed that 15 % of the total natural DOC (c. 20 mg C L^{-1}) was labile and increased this by two, three and four times in the treatments. The cane sugar (sucrose) used as source of labile DOC also served as an isotopic tracer to follow carbon transformations between pools, as the carbon stable isotopic signal ($\partial\ ^{13}C$) of cane sugar (C_4 plant) is ca. −11 ‰, which is appreciably higher than the signal of organic matter derived from the C_3 plants characteristic of boreal landscapes (between −26 and −28 ‰).

Chemical analyses

Analyses of chlorophyll-*a* and inorganic nutrient concentrations (P and N) were done using standard methods (http://www.sfs.fi/). Samples for nutrient analyses were kept on ice and frozen within 4 h after sampling pending analysis. For determination of DOC concentration, water was filtered through pre-combusted GF/F filters and frozen until analysis at Lammi Biological Station, University of Helsinki, using standard analytical methods (http://www.sfs.fi).

For $\partial\ ^{13}C$-DOM analysis, 500 mL of sample water was passed through pre-combusted GF/F filters (nominal retention size 0.7 µm), frozen at −20 °C and later freeze-dried with an Alpha 1–4 LD plus lyophilizer (Christ, Osterode, Germany; Kankaala et al. 2010a). For $\partial\ ^{13}C$-POM analysis, 3 L of water were filtered by tangential-flow filtration apparatus (Millipore, Durapore casette, pore size 0.22 µm) and ca. 100 mL of filtrate were then frozen at −20 °C and later freeze-dried as mentioned above. All samples were analysed with a Carlo-Erba Flash 1112 series Elemental Analyzer connected to a DELTA^{plus} Advantage IRMS (Thermo Finnigan, Bremen, Germany) and analysed using potato leaves as an internal laboratory working standard. The standard deviation between replicates was within 0.2 ‰ for carbon.

Functional variables

Bacterial production (BP) was measured as incorporation of ^{14}C-leucine following methods in Tulonen (1993). Briefly, 100 µl of ^{14}C-leucine (=30 nmol mL^{-1}) was added to vials containing 5 mL of water sample (three replicates and one blank for each experimental treatment). Vials were incubated at in situ temperatures for 60 min in darkness. The incubations were terminated by adding glutaraldehyde (GTA, 25 %) and samples were then filtered onto cellulose-acetate filters (25 mm diameter and 0.2-µm pore-size) after addition of 0.5 mL of 50 % trichloroacetic acid (TCA). The filtering apparatus was rinsed with 1 mL of 5 % TCA and the filters were placed into plastic scintillation vials with 0.25 mL of ethylenglygolmonomethylether and 9 mL of OptiPhase3 scintillation fluid solution. After 24 h the samples were analysed in a liquid scintillation counter (Packard Tri-Carb, PerkinElmer, USA). In all the calculations, data were corrected by blanks (bacteria were killed with GTA before addition of the radiotracer).

Primary production (PP) was measured as incorporation of ^{14}C-bicarbonate following the method of Schindler et al. (1972). Briefly, 20 mL of water sample were taken into glass vials (three replicates and one blank for each experimental treatment). Each replicate contained 20 µL of ^{14}C-bicarbonate with an activity of 10 µCi mL^{-1}. Samples were incubated in situ for 24 h and then fixed with GTA. After the incubation, 6 mL of sample water was added into plastic scintillation vials and phosphoric acid was added to remove the excess DI^{14}C. After 48 h, 9 mL of OptiPhase3 scintillation fluid solution was added and radioactivity measured with a Packard Tri-Carb liquid scintillation counter. The DIC concentration of each sample, required to calculate PP with the ^{14}C method, was obtained from alkalinity and pH measurements (APHA 1992).

Biological structure variables

Bacterial abundance (BA) was determined by flow cytometry (FACSCanto II, Becton–Dickinson Biosciences, Oxford, UK) by fixing 1.5 mL sample of natural water with particle-free 20 % w/v paraformaldehyde dissolved in Milli-Q water, 1 % final concentration. The fixed samples were stored at −80 °C. Before the analyses the samples were thawed and stained with SYBR Green I DNA (Sigma-Aldrich) 1:5000 final dilution of initial stock. Yellow-green 1-µm beads (Fluoresbrite Microparticles, Polysciences, Warrington, PA, USA) were added with known final concentration (10^5 beads mL^{-1}) to determine absolute cell concentrations (Zubkov and Burkill 2006).

Phytoplankton were preserved in amber glass bottles (200 mL) with acid Lugol's reagent (approximately 1 % vol/vol). An aliquot of 50 mL from each sample was settled in an Utermöhl chamber of 2.6 cm diameter for 24 h to ensure complete sedimentation of the smallest algal species (Utermöhl 1958). Cells were counted in 100 randomly selected fields of view at 1000× magnification (oil immersion) under an inverted microscope (Olympus IM2)

Table 1 Mean values of the main physical, chemical and biological variables of the two basins of Mekkojärvi

Variable	+FISH basin	−FISH basin
Chl *a* (μg L^{-1})	3.5 ± 0.1	1.5 ± 0.0
DOC (mg C L^{-1})	32.6 ± 0.3	35.9 ± 0.2
PO_4^{3-} (μg P L^{-1})	1.0 ± 0.0	2.0 ± 0.0
$NO_2^- + NO_3^-$ (μg N L^{-1})	38.3 ± 2.9	54.0 ± 1.0
C:N:P (molar)	84,217:85:1	46,371:60:1
BP (μg C L^{-1} h^{-1})	0.010 ± 0.001	0.008 ± 0.000
PP (μg C L^{-1} h^{-1})	2.5 ± 0.3	1.4 ± 0.6
PB (μg C L^{-1})	66 ± 20	18 ± 3
BA (cells mL^{-1})	$2.3 \times 10^5 \pm 9.9 \times 10^4$	$2.2 \times 10^5 \pm 2.0 \times 10^4$

and identified based on Tikkanen (1986). For each sample, at least 400 cells of the more abundant phytoplanktonic species were counted, and 20–30 cells of each species were measured for each date using a micrometer to estimate cell volume according to a corresponding geometrical shape. The biovolume density (μm^3 mL^{-1}) for each taxon was determined by multiplying mean cell volume by abundance. Cell volume was converted to carbon using specific conversion factors reported by Rocha and Duncan (1985).

Phytoplankton community composition was quantified at genus level, and organisms were grouped into strict autotrophs and mixotrophs based on criteria reported by Jansson et al. (1996) and Isaksson et al. (1999). Thus, Bacillariophyta and Chlorophyta (including Desmidiaceae) were considered strictly autotrophic, and Chrysophyta, Dinophyta, and Cryptophyta were classified as potentially mixotrophic.

Data analysis

For both basins of the lake (+FISH, −FISH), the effects of carbon addition, time and their interaction were tested using two-way ANOVA. When the interaction term was significant, a conservative Tukey's post hoc test was applied to check statistical significance between treatments. Normality (by Kolmogorov–Smirnov and Shapiro–Wilks' W-test) and homoscedasticity (Levene's test) were tested to confirm ANOVA assumptions. STATISTICA 7 software (StatSoft Inc 2005) was used for all statistical tests, and we took $p < 0.05$ as a threshold level for rejection of H0.

Results

Initial conditions

Chemical variables in the two experimental basins of the lake showed significant differences (Student-*t* in all cases $p < 0.01$). Inorganic nutrients and DOC were generally slightly higher in −FISH than in +FISH (PO_4^{3-} 2 times, $[NO_3^- + NO_2^-]$ 1.4 times, DOC 1.1 times; Table 1). The biological structure and function variables also showed differences between the lake basins. Bacterial abundance and production were higher in +FISH than in −FISH basin (p value <0.05; Table 1). Also, phytoplankton variables were higher in the +FISH basin than in −FISH basin (Student-*t* in all cases p value <0.05; Chl *a* 2.3 times, algal biomass 3.6 times, primary production [PP] ca. 1.8 times). In both basins of the lake, bacterial production (BP) was very low (i.e. below 0.06 μg C L^{-1} h^{-1}) in comparison to that of PP (i.e. ca. 2 μg C L^{-1} h^{-1}).

The phytoplankton community in both basins of the lake was dominated (up to 60 % of the algal biomass) by potentially mixotrophic algae, mainly Cryptophyta, with ca. 35 % of strictly autotrophic algae (mainly Chlorophyta) biomass.

Chemical changes during the experiments

Phosphate (PO_4^{3-}) showed a generalized increasing trend (two-way ANOVA: time main effect, p value <0.05). In the +FISH basin mesocosms PO_4^{3-} varied from 1.0 μg P L^{-1} [24 h] to 2.3 μg P L^{-1} [120 h], except in the highest carbon treatment where PO_4^{3-}decreased by the final time (from 2.1 μg P L^{-1} [72 h] to 1.6 μg P L^{-1} [120 h]; Fig. 1). In contrast, the PO_4^{3-} values varied little over time in the −FISH basin mesocosms. Nitrogen ($NO_2^- + NO_3^-$) showed an increasing trend in both basins of the lake (two-way ANOVA: time main effect, p value <0.05), more markedly in the −FISH basin mesocosms (from 58.5 μg N L^{-1} [24 h] to 65.1 μg N L^{-1} [120 h]).

DOC concentration increased immediately after carbon addition as expected (Fig. 1). However, DOC concentration decreased significantly over time for all C-addition treatments in the +FISH basin mesocosms (two-way ANOVA: time and carbon main effects, p value <0.01; Fig. 1), without significant interaction (two-way ANOVA: carbon × time interaction effect, p value >0.1). In

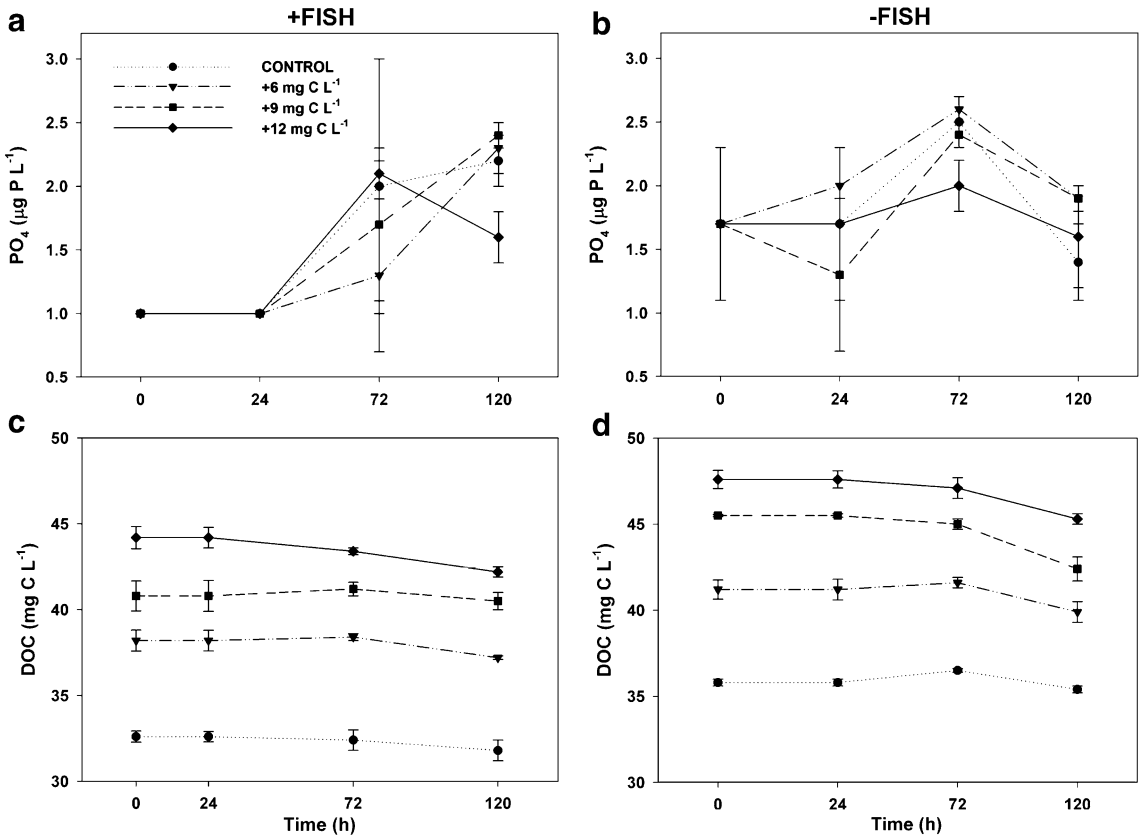

Fig. 1 Temporal responses of PO_4 and DOC in the mesocosms in the +FISH and −FISH basins of Mekkojärvi. *Symbols* represent mean values and *error bars* standard deviations

the −FISH basin mesocosms, DOC showed a decreasing trend in all the carbon-added treatments between 24 and 120 h (Tukey's post hoc p value <0.05) with a significant time × carbon interaction (two-way ANOVA p value <0.01).

Changes in carbon isotopic signal during the experiments

The ∂ ^{13}C-DOM after addition of cane sugar reflected the expected values according to the C-treatments, i.e. ∂ ^{13}C-DOM values were less negative with higher C-addition (Fig. 2). However, the isotopic signal of the dissolved fraction barely varied over time in any treatments.

Values of ∂ ^{13}C-POM showed a pattern in the mesocosms in both lake basins that matched the differences in ∂ ^{13}C-DOM according to the C-treatments (i.e. ∂^{13}C-POM values were less negative with higher C-addition), and this pattern was mostly maintained over time with some exceptions (Fig. 2). The [+6] treatment showed a notable increase in ∂^{13}C-POM between 24 and 120 h in the +FISH basin mesocosms, but a decrease relative to control values in the −FISH basin mesocosms (Tukey's post hoc p value <0.05). These patterns reveal that added-C was indeed incorporated into the biological fraction in both basins of the lake, although the incorporation varied between the +FISH and −FISH basin mesocosms, depending on carbon treatment.

Response of bacterial and primary production to carbon additions

Mesocosms in both basins of the lake showed a significant interaction effect for BP (two-way ANOVA: experimental time × carbon interaction effect; p value <0.01), with very low values (from <0.06 to <0.006 µg C L^{-1} h^{-1}) that varied over time depending on basin, carbon treatments and time (Fig. 3). In the +FISH basin mesocosms, BP increased only in the control and [+6] treatments after 24 h (Tukey's post hoc p value <0.05) without significant differences between them (Tukey's post hoc p value >0.05), and decreased by 72 h; BP did not significantly respond to the other C-treatments at any time. In the −FISH basin mesocosms, BP increased in [+6] treatment after 24 h (Tukey's post hoc p value <0.05) but decreased thereafter. In the other carbon treatments, BP did not change significantly (Tukey's post hoc p value >0.05). Noticeably, BP showed low values (below 0.014 µg C L^{-1} h^{-1}) in all

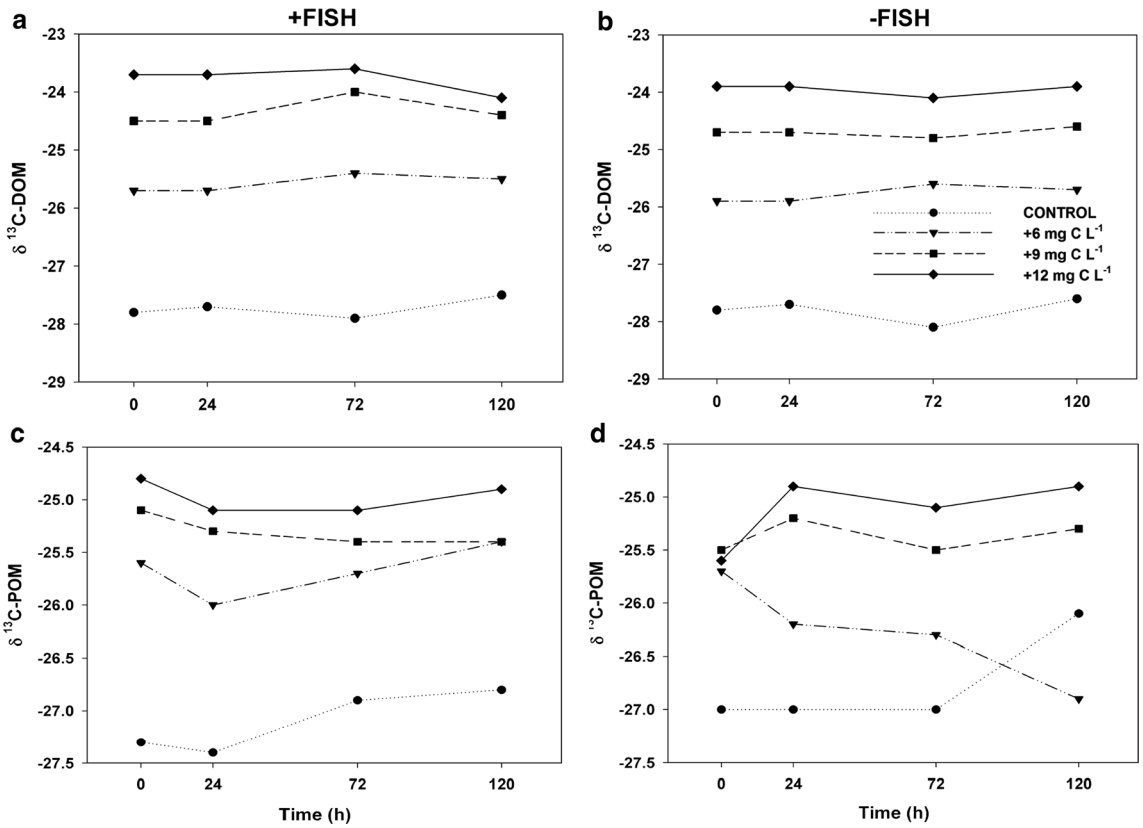

Fig. 2 Temporal responses of ∂ ^{13}C-DOM and ∂ ^{13}C-POM in the mesocosms in the +FISH and −FISH basins of Mekkojärvi. *Symbols* represent mean values and *error bars* standard deviations

treatments and experimental times of the −FISH basin mesocosms.

PP was affected by the interaction of both factors (two-way ANOVA: experimental time × carbon interaction effect; p value <0.001) in mesocosms in both basins of the lake (Fig. 3). In the +FISH basin mesocosms, PP increased in all treatments from initial conditions to final time, more markedly in the [+9] and [+12] treatments (1.7 and 1.9 times respectively relative to the control, p value <0.05). In the −FISH basin mesocosms, PP responded positively and strongly only in the control at 72 h (7.8 times with respect to 24 h control values), while C-addition treatments were lower than the control and with no significant differences among them (Tukey's post hoc p value <0.05). However, at the final time (120 h), PP had decreased in the control treatment to the values of the C-addition treatments, with no significant differences between treatments (Tukey's post hoc p value >0.05), but with higher values than those at initial conditions.

Response of PP:BP ratio to carbon additions

In the +FISH basin mesocosms, BP was negatively related to PP values ($R^2 = 0.22$; p value <0.05), while in the −FISH basin mesocosms the relationship was not significant ($R^2 = 0.009$; p value >0.1).

The PP:BP ratio did not show a significant relationship with DOC for either basin of the lake ($R^2 < 0.015$; $p > 0.05$). However, in the +FISH basin mesocosms the ratio was high (>8) in all C-added treatments, especially in those with higher DOC concentration (PP:BP ratio varied from 8 to up 190) while in the −FISH basin mesocosms the highest PP:BP values were found in the control and [+6] treatment (from 15 to 143).

Response of microbial plankton structure to carbon additions

BA was not affected by interaction between experimental time and carbon, nor by carbon addition (two-way ANOVA; p value >0.05), but was affected by time (two-ways ANOVA; p value <0.01) in mesocosms in both basins of the lake. Thus, BA increased in all treatments (control included) 24 h after the addition in both basins of the lake, then decreased over time, but much more markedly in the −FISH basin mesocosms (Fig. 3). There were no differences between control and carbon addition treatments at any experimental time (Tukey's post hoc p value

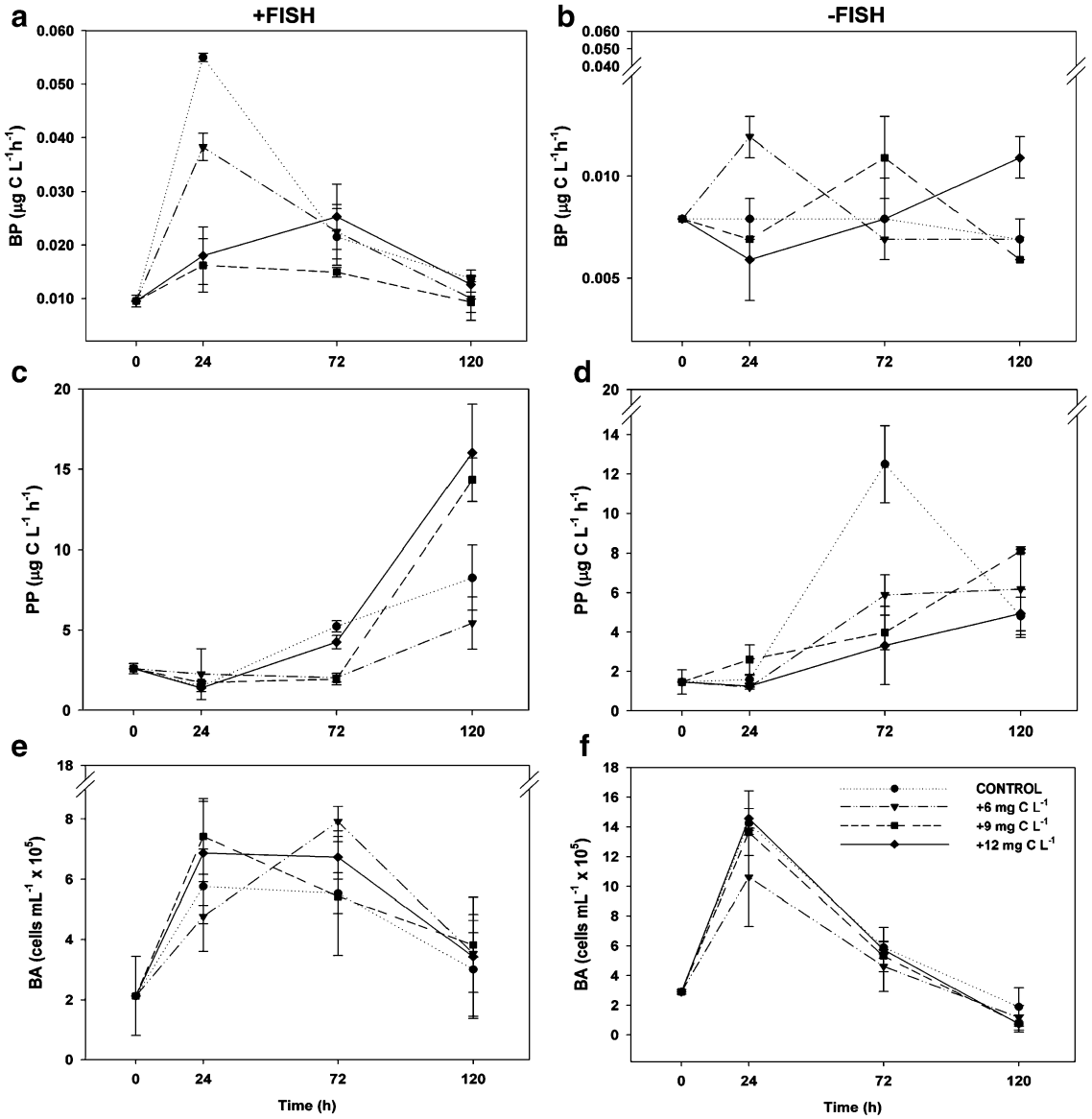

Fig. 3 Temporal responses of bacterial production (BP), primary production (PP) and bacterial abundance (BA) in the mesocosms in the +FISH and −FISH basins of Mekkojärvi. *Symbols* represent mean values and *error bars* standard deviations

>0.05) on either basin of the lake; therefore carbon addition did not modify BA.

In the +FISH basin mesocosms, phytoplankton biomass (PB) increased in the control and C-addition treatments over time, but the proportions of strict autotrophs and potentially mixotrophic algae (i.e. ~20 and ~80 % respectively) barely changed. However, in contrast to the control, PB increased in the C-addition treatments until 72 h, after which PB slightly decreased (Fig. 4a, c). Additionally, the C-addition treatments differed from controls in that cyanobacteria were up to 3.6 % of total PB at the final incubation time. In the −FISH basin mesocosms, PB increased in all treatments until 72 h, to decrease later, as occurred in the +FISH basin mesocosms (except in the control treatment). The proportion of algal functional groups changed in all treatments (control included) over time because potentially mixotrophic algal biomass increased relative to that of strict autotrophs, reaching values up to 90 % by the end of the experiment (Fig. 4b, d).

Discussion

Heterotrophic bacteria did not increase their production and/or abundance as a response to carbon additions, which suggests little influence of any priming effect on heterotrophic metabolism in Mekkojärvi, despite declining total

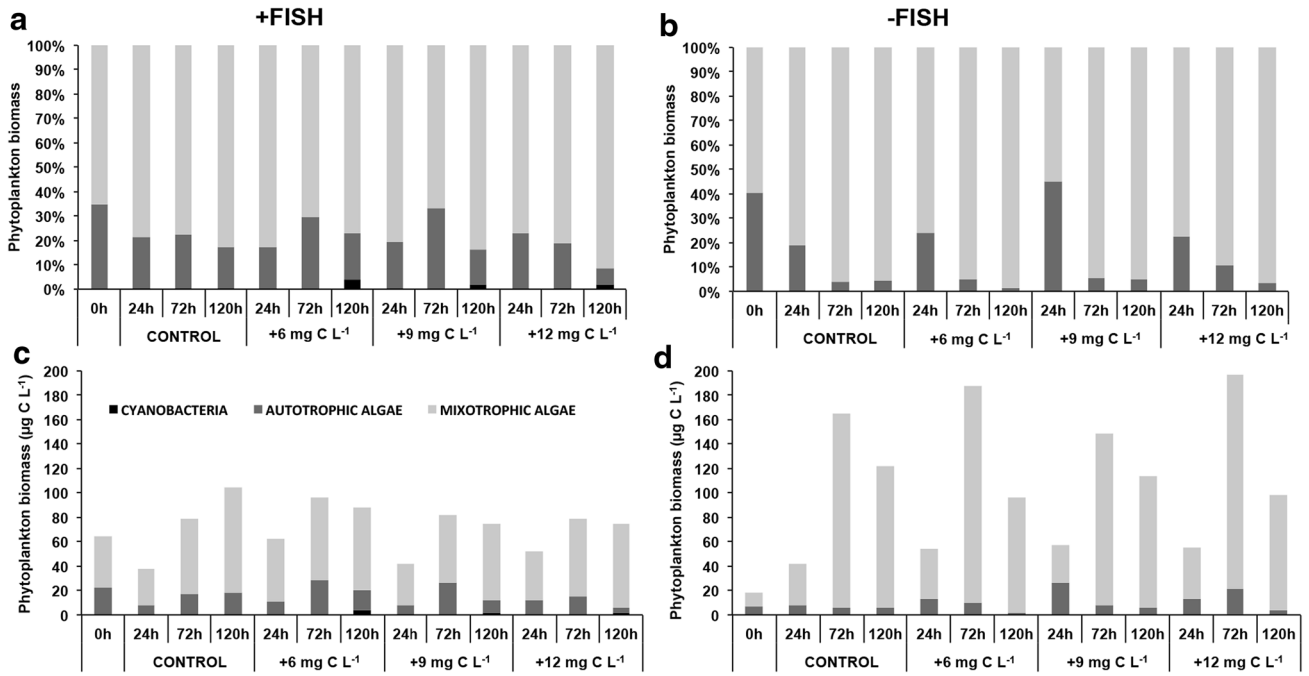

Fig. 4 Temporal responses of phytoplankton proportional biomass (**a**, **b**) and absolute biomass (**c**, **d**) in the mesocosms in the +FISH (**a**, **c**) and −FISH (**b**, **d**) basins of Mekkojärvi. *Columns* represent the mean values per mesocosm for three different phytoplankton functional groups

DOC concentrations during the course of the experiment in all treatments. Therefore, our results offer no evidence that bacteria in this humic lake, despite being fuelled by added LOM, were able to increase degradation of ROM significantly, even though bacteria are considered highly efficient in the uptake of inorganic nutrients from low concentrations (e.g. Currie and Kalff 1984). Moreover, the presence or absence of fish in the ecosystem and the accompanying changes in plankton community structure made no difference with respect to bacterial priming effect.

However, PP generally increased in the experimental treatments, including the controls, perhaps reflecting reduced zooplankton grazing after the water had been prefiltered through a 45-µm mesh for the experiments. This increase was particularly evident in the highest carbon treatments of the +FISH basin (i.e. [+9] and [+12] treatments) at the final time [120 h], partly reflecting the higher initial phytoplankton biomass in the +FISH basin from which *Daphnia* had been absent prior to the start of the experiment. However, the particular response in the highest carbon addition treatments suggests that algae in this humic lake benefitted directly or indirectly (e.g. through bacterivory) from the addition of labile C, consistent with the reported ability of some algae to exploit organic carbon resources (Tittel et al. 2009; Fontes et al. 2013).

Our results from an in situ experiment offering no evidence of a priming effect agree with those of Bengtsson et al. (2014), but contrast with the findings of some other studies (Nugteren et al. 2009; Bianchi 2011; Guenet et al. 2014). The lack of any evident priming effect in Mekkojärvi during our experiment could have several possible explanations: (1) the particularly-refractory nature of ROM; (2) very low water transparency which significantly reduces water column photodegradation of DOC by ultraviolet radiation; (3) the use of added carbon by other organisms such as mixotrophic algae; (4) strong constraints on bacterioplankton by bacterivorous predation; and (5) mineral nutrient limitation of bacterial growth.

With regard to these possible explanations, we have no reason to suppose that ROM in Mekkojärvi should be particularly refractory. Although DOC concentrations in the lake are very high, the DOC originates from the same coniferous forest soils and *Sphagnum*-dominated peatlands that are widespread through the boreal zone. Low light penetration caused by the highly coloured water is offset by steep thermal stratification with an epilimnetic mixed layer of only 0.5 m, such that photodegradation effects are likely similar to those in more transparent water columns with deeper photic zones (Jones 1988). Although DOC concentrations in the treatments declined during the experiments, the concentrations were still high at the end of the experiment in all the C-addition treatments; thus, even if there was some uptake of the added labile organic carbon (LOC) by algae, this was clearly not sufficient to have prevented the use of LOC by bacteria. The absence of *Daphnia* in the +FISH basin of the lake could be expected

to promote populations of bacterivorous nanoflagellates which are otherwise consumed by *Daphnia*; consequently the +FISH treatments could experience higher predation pressure on bacteria. Despite this, rates of BP were broadly similar in +FISH and −FISH treatments throughout the experiment, suggesting that high loss of bacteria through predation does not account for the absence of a detectable priming effect. Therefore, we believe that nutrient limitation is the most likely explanation for the lack of a pronounced bacterial response to LOC additions. This conclusion is supported by the C:N:P molar ratio of the dissolved fraction, prior to carbon additions (Table 1), with values much greater than the stoichiometric ratios of actively growing bacteria (e.g. C:P ratio <40; Vrede et al. 2002). Similar findings were reported in a nearby lake by Peura et al. (2014) where nutrient limitation may have prevented a manifestation of any potential priming effect.

Recently, Guenet et al. (2014) reported a negative priming effect for forest soil organic matter (SOM) under waterlogged conditions, and suggested that the principal cause for this result was a high C:N ratio. They argued that bacteria preferentially used LOM as a substrate when the C:N ratio was high. Because microorganisms tend to be N limited, according to the N-mining phenomenon bacteria may demonstrate increased mineralization of C from SOM while scouring enough N for growth (Moorhead and Sinsabaugh 2006; Guenet et al. 2014). In our case, we found a very high C:N ratio (i.e. >770) in the dissolved fraction, as in the Guenet et al. (2014) experiment, coupled with a low P concentration, which could constrain bacterial growth (BP and BA), and even preventing a potential mining phenomenon, due to double nutrient limitation by nitrogen and phosphate. However, phytoplankton did increase their abundance and production, probably because the dominant potential mixotrophs could access mineral nutrients from bacteria through phagotrophy (e.g. Jones 2000) to help utilise the available LOC. Thus, LOC may have benefitted mixotrophs more than bacteria if the latter are well adapted to ROC metabolism.

The patterns of the isotopic signal of $\partial\ ^{13}$C-DOM and $\partial\ ^{13}$C-POM across the C-treatments confirm that the added cane sugar was labile and was consumed by plankton, as reported by Kankaala et al. (2010a) and Peura et al. (2012, 2014). However, those studies and ours suggest that the predicted increase in allochthonous DOC transport as a result of global change (IPCC 2013) and the predicted increased loading to Finnish lakes (Vuorenmaa et al. 2006; Einola et al. 2011), will not necessarily stimulate heterotrophic bacteria (BP and/or BA), if bacterial growth is constrained by the stoichiometry of DOC and by inorganic nutrients (cf. Peura et al. 2014).

In conclusion, bacterioplankton in Mekkojärvi did not increase production and abundance after addition of labile carbon substrate, which is most likely attributable to resource stoichiometry restrictions (i.e. high C:N:P ratio) that can impair PE. Thus our study provided no evidence for a priming effect in an oligotrophic humic lake underlining that PE is not a general phenomenon that affects all ecosystems. More experimental studies from a range of aquatic ecosystems are clearly required to evaluate how widespread a priming effect might be.

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