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Bacterial utilization of imported organic material in three small nested humic lakes

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Introduction

Lakes import organic carbon in the form of dissolved humic substances and other terrigenous compounds. This allochthonous organic carbon is consumed by heterotrophic bacteria, with significant consequences for the biostructure and energy pathways of freshwater ecosystems (Jansson et al. 2007). Laboratory studies generally show that a minor share of terrigenous material (~10 %) is available to bacterial consumption on short term scales (weeks) and that the degradation thereafter proceeds at slow rates (Tranvik 1998). Although natural exposure to solar UV radiation may increase the availability (Lindell et al. 1995, Tranvik 1998), it is not certain that this can compensate the loss of bioavailable carbon from bacterial consumption. We hypothesized that as allochthonous organic carbon is processed and aged during its residence time in lakes, its potential to support bacterial growth, both in terms of rates and efficiency, decreases successively. We employed a short term (12 day) bioassay approach to study 3 small nested lakes in Northern Sweden, almost entirely dominated by allochthonous organic carbon. The first 2 lakes drained almost directly into the third, making it possible to examine a downstream gradient from inlet to outlet and from lake to lake.

Key words: allochthonous organic carbon, bacterial production, bioassay, respiration

Materials and methods

The 3 study lakes (Fig. 1) were sampled on 29 June 2006 after a 3-week period of stable base flow conditions preceded by the annual spring flood (Apr–May). Theoretical water renewal time is about 1 year for L. Lilla Björntjärnen and 60–70 days for the 2 other lakes. See Jansson et al. (2001) for lake descriptions.

Water (2 L) from all inlets and outlets, plus surface water from the middle of the lakes (mix from 0–2.5 m depth), was filtered in the field through 50-µm filters and collected in HDPE bottles. The samples were equilibrated with standard air (78 % N_2, 21 % O_2, and 0.03 % CO_2) to remove supersaturation of CO_2 and then subsampled into 22-ml gas tight glass bottles, leaving a 12-ml headspace flushed with standard air. All bottles were incubated in the dark at 20 °C. Samples for chemical analyses were frozen directly after arrival to the laboratory.

Unique triplicates of the 22-ml incubation bottles were analyzed for bacterial production (BP) and bacterial respiration (BR) every second and fourth day, respectively, during a period of 12 days starting approximately 24 hr after sealing the bottles. Bacterial respiration was measured as dissolved inorganic carbon (DIC) production on a Perkin-Elmer GC-FID, with a headspace auto-sampler that operated directly on the incubation bottles. Before analysis, the samples were acidified to pH 2.5 and shaken. Bacterial production was measured in 1.2-ml portions with the leucine incorporation method (Smith & Azam 1992, Karlsson et al. 2002). Average rates of BP and BR during the 12-day bioassays were used to calculate total amounts of carbon used for growth and respiration.

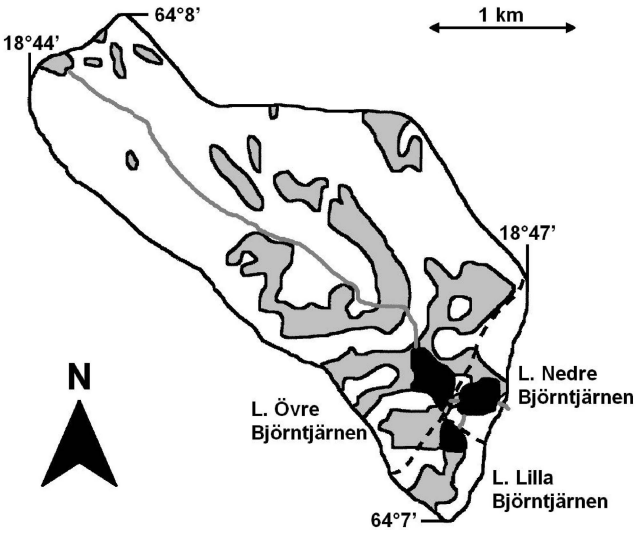

Fig. 1. Study catchment area. Mires and streams are in grey, forests in white, and lakes in black. Dotted lines represent sub-catchment borders.

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Fluorescence was analyzed (Perkin Elmer LS 45) after McKnight et al. (2001), and an index was calculated as the emission intensity at a wavelength of 450 nm to that of 500 nm, obtained at an excitation of 370 nm. Ultra violet (UV) absorbance spectra were measured between 190 and 510 nm with 1-nm increments (Hewlett Packard 8452A) and analyzed using principle components analysis (PCA) in SPSS 13.0 (unrotated solutions and regression method for factor scores). Analyses of the nitrogen (N) and phosphorus (P) fractions (Table 1) were made with standardized methods at the Department of Ecology and Evolution, Uppsala University (Bergström & Jansson 2000). Total organic carbon (TOC) was measured using a Shimadzu TOC-V_{PCH} analyzer.

Results and discussion

The fluorescence index was 1.39–1.43 (Table 1), indicating a strong dominance of allochthonous organic carbon. Assuming the end members 1.40 for allochthonous carbon (C) and 1.90 for autochthonous C, (McKnight et al. 2001), 94–100 % of the organic carbon pool was allochthonous (Table 1).

Total bacterial carbon consumption during the 12-day incubations, calculated as the sum of BP and BR, were 8–11 % of TOC. In general, the fraction of TOC that supported BR was about 4 times larger than the fraction supporting BP. However, while BR did not show any clear systematic pattern, BP decreased successively from upstream to downstream samples (Table 2). Both carbon specific and absolute BP were significantly higher in the 3 mid-lake samples compared to the 3 outlet samples (paired t-tests; $t > 16$, $df = 2$, $p < 0.01$). In the lakes with distinct inlets, L. Övre Björntjärnen and L. Nedre Björntjärnen, BP TOC^{-1} decreased by 23–53 % from inflowing to outflowing water. Bacterial growth efficiency (BGE = BP/[BP + BR]) during the incubations showed patterns similar to those of BP (Table 2). From values around 0.25 in the 2 upper lakes, BGE decreased to 0.15–0.19 in the outlets, and finally to 0.13 for the outlet of the most downstream lake. The 3 lake samples showed significantly higher BGE than the 3 outlet samples (paired t-test; $t = 7.0$, $df = 2$, $p < 0.05$). Observed values of BGE fit the range of previous reports from unproductive lakes (Del Giorgio & Cole 1998).

The PCA extracted 2 significant components from the absorbance data. The PC1 component explained 97.7 % of the variation and loaded strongly on all wavelengths ($r > 0.95$). Its factor scores were positively correlated to TOC ($R^2 = 0.57$, $n = 7$, $p < 0.05$). We interpreted this component as a measure of organic carbon quantity. The PC2 component explained 2.3 % of the variation and had increasing negative factor loadings for decreasing wave-

Table 1. Fluorescence index (FI, see methods), total organic carbon (TOC), soluble reactive phosphorus (SRP), dissolved inorganic nitrogen (DIN), total phosphorus (TP) and total nitrogen (TN) for inlets, outlets, and surface waters of three humic lakes.

	FI	TOC mg L^{-1}	SRP µg L^{-1}	DIN µg L^{-1}	TP µg L^{-1}	TN mg L^{-1}
Övre Björntjärnen, inlet	1.39	19.8	7	7	32	0.68
Övre Björntjärnen, lake	1.42	16.0	2	18	22	0.48
Övre Björntjärnen, outlet	1.43	15.4	1	12	20	0.46
Lilla Björntjärnen, lake	1.42	16.4	1	10	16	0.38
Lilla Björntjärnen, outlet	1.42	15.2	4	11	18	0.40
Nedre Björntjärnen, lake	1.43	14.8	2	16	20	0.47
Nedre Björntjärnen, outlet	1.43	14.9	3	15	23	0.50

Table 2. Absolute and carbon specific bacterial metabolic measures from 12-day dark incubations. SD of three parallel incubation series in brackets.

	BR mg C L^{-1}	BR TOC^{-1} %	BP mg C L^{-1}	BP TOC^{-1} %	BGE
Övre Björntjärnen, inlet	1.14 [0.08]	5.7 [0.4]	0.36 [0.02]	1.8 [0.1]	0.24 [0.03]
Övre Björntjärnen, lake	1.04 [0.08]	6.5 [0.5]	0.35 [0.04]	2.2 [0.2]	0.25 [0.01]
Övre Björntjärnen, outlet	1.18 [0.03]	7.7 [0.2]	0.21 [0.01]	1.4 [<0.1]	0.15 [0.01]
Lilla Björntjärnen, lake	1.36 [0.02]	8.3 [0.1]	0.46 [0.03]	2.8 [0.2]	0.25 [0.01]
Lilla Björntjärnen, outlet	1.36 [0.13]	8.9 [0.9]	0.33 [0.02]	2.2 [0.2]	0.19 [0.03]
Nedre Björntjärnen, lake	0.99 [0.03]	6.7 [0.2]	0.28 [0.01]	1.9 [0.1]	0.22 [<0.01]
Nedre Björntjärnen, outlet	1.01 [0.13]	6.8 [0.9]	0.15 [<0.01]	1.0 [<0.1]	0.13 [0.02]

lengths <375 nm and increasingly positive loadings for increasing wavelengths >375 nm. Factor scores on this component reflect the slopes of the absorbance spectra. Based on relationships between that feature and molecular size distribution (Strome & Miller 1978, Dehaan 1993, Dahlén et al. 1996), we interpret PC2 as a positive indicator of average molecular weight (i.e., a measure of organic carbon quality). The PC plot (Fig. 2) indicates that as we follow the major flow path through L. Övre Björntjärnen to L. Nedre Björntjärnen, the quantity of TOC decreased at the same time as the average molecular weight increased. Also in the third lake, L. Lilla Björntjärnen, representing a small side-catchment, the change from lake water to outlet was in the direction toward higher molecular weight (HMW) and lower TOC (Fig. 2).

Based on the monitored spring flood from the adjacent Krycklan catchment (Buffam et al. 2007) we calculated that the snow melt that peaked 2 months before sampling was sufficient for replacing all water in L. Övre Björntjärnen 1.9 times and in lakes Övre plus Nedre Björntjärnen 1.0 times. From what is known about allochthonous organic C degradation (Tranvik 1998), we assume that the highly bioavailable fractions of TOC that were imported during this flood had been depleted before our study sampling. Further, because the hydrological situation during the 3 weeks prior to sampling had been stable, we also assume that sampled inlet water at L. Övre Björntjärnen represented the post flood import of TOC to the system. The most plausible explanation for the observed downstream patterns is that the potential of imported TOC to support bacterial growth decreased with its residence time in the system. This agrees with a study from the nearby L. Örträsket, showing that both BP and BP TOC^{-1} can be regulated by the import of fresh terrestrial organic carbon (Bergström & Jansson 2000).

In conclusion, the organic carbon pool in the lakes was almost entirely allochthonous, and its potential to support BP and BGE decreased from upstream to downstream samples. We found no patterns in nutrients availability (Table 1) that could explain observed patterns in TOC bioavailability. Instead, both qualitative and quantitative changes in the organic carbon pool were identified along the flow paths. We suggest that decreases in BP and BGE were due to gradual exhaustion of imported low molecular weight (LMW) compounds that served as high quality substrates for bacterial growth. Our results indicate that allochthonous organic carbon can significantly stimulate BP and BGE in unproductive lakes, but that this support fades out as the material is processed and aged during its residence time in lacustrine systems.

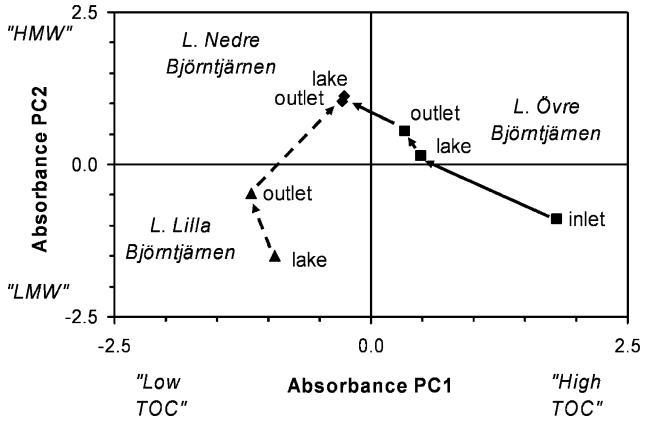

Fig. 2. Absorbance (190–510 nm) principle component scores for inlets, outlets, and surface waters of 3 closely connected humic lakes in northern Sweden. Arrows show flow paths.

Acknowledgements

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