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Bioavailable phosphorus in humic headwater streams in boreal Sweden

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Abstract

Bioavailable phosphorus (BAP) concentrations were determined nine times between April and October 2010 in two humic boreal headwater streams draining forest- and mire-dominated catchments. BAP was analyzed in a bioassay in which natural P-limited bacterioplankton grew with natural P as the sole P source. In both streams, approximately 90% of the BAP occurred as dissolved species (passing a 0.2-μm filter), consisting partly of low-molecular-weight forms (passing a filter with nominal cutoff at 1 kDa) and partly of high-molecular-weight forms (passing a 0.2-μm filter but not a 1-kDa filter). Concentrations of total dissolved BAP varied between 1 μg L^{-1} and 14 μg L^{-1}, with the highest values in the middle of the summer. Compared to the forest stream, BAP concentrations were generally higher in the mire stream, where it occasionally amounted to nearly 50% of total P. Molybdate reactive phosphorus overestimated BAP considerably. Most of the BAP was in forms other than free orthophosphate. Temporal BAP variations showed no relationships with dissolved organic carbon (C) or iron but were positively related to air temperature and negatively related to the absorbance ratio (a254 : a365) of organic compounds in the water, indicating connections between terrestrial export of BAP and temperature-dependent terrestrial C metabolism. Concentrations of BAP can relieve stream bacteria from P limitation, and a significant share of BAP exported to streams can reach and be used in downstream lakes.

Phosphorus (P) has received attention for a long time in limnological research (Vollenweider 1968; Schindler 1977; Elser et al. 1990). Much of this interest was stimulated by observations of massive phytoplankton growth following excessive P loading (eutrophication) to lakes via sewage water or other anthropogenic sources (Vollenweider 1968). A major result of limnological P research has been that eutrophication can be successfully controlled by construction of sewage treatment plants and other measures for reducing P input to lakes.

However, some of the basic problems in aquatic P research have remained unsolved. The fundamental question about the quantity and quality of the P loading to streams and lakes that is available for incorporation in stream and lake biota has not received a satisfactory answer. Phosphorus enters aquatic food webs mainly via incorporation of orthophosphate into phytoplankton or bacterioplankton (Cembella et al. 1984; Karl 2000; Vadstein 2000). A problem here is that no reliable standard method exists for determination of orthophosphate concentrations in natural waters. Molybdate reactive phosphorus (MRP) is often taken as an approximate measure of orthophosphate and/or bioavailable P. However, many studies have shown that MRP overestimates true concentrations of orthophosphate, especially in unproductive lakes (Rigler 1966; Hudson et al. 2000; Nowlin et al. 2007). Another problem is that orthophosphate is reactive and combines with different inorganic and organic compounds (Tipping 1981; De Haan and De Boer 1986). Bioavailable P (BAP) is, by definition, also incorporated in, and regenerated from, living biomass. Consequently, the biological uptake of P depends on how rapidly microorganisms or abiotic processes can mobilize orthophosphate from combined phosphates, and "bioavailability" cannot be defined by orthophosphate concentrations alone. For example, if the biological orthophosphate uptake capacity exceeds or equals the supply rate, the orthophosphate concentration can be very low although the access to orthophosphate is high.

Efforts to determine BAP in streams and lakes have followed different routes. A common approach, often used in eutrophication research, is to use bioassays in which phytoplankton grow with natural particulate or dissolved P as the single source of P (*see* review by Boström et al. 1988). Another approach is to use the orthophosphate turnover rate as a measure of supply rate of available P (Hudson et al. 2000; Nowlin et al. 2007). This method (steady-state bioassay method) assumes that regeneration of P in P-limited systems approximately equals the uptake rate. The method also allows estimates of orthophosphate concentrations (Hudson et al. 2000).

Most studies on BAP have been carried out in eutrophic systems and, to some extent, in unproductive clear-water lakes. Humic streams and lakes with high concentrations of colored organic substances imported from terrestrial systems are less studied in this respect, which is a shortcoming for several reasons. A large share of the world's streams and lakes are substantially affected by colored humic substances of terrestrial origin (Sobek et al. 2007). Humic streams and lakes have relatively high concentrations of total P (TP), presumably due to formation of colloidal humus–metal–P complexes (Tipping 1981; De Haan and De Boer 1986). Concentrations in the range of 20–30 μg P L^{-1} with a very high share of MRP ($>$ 50%) are not uncommon (Meili 1992). In spite of these high P concentrations, humic lakes are often extremely unproductive in terms of primary

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production (Jones 1992; Karlsson et al. 2009). Consequently, humic lakes are a paradox; many of them can be classified as mesotrophic or eutrophic by their P concentrations but as dystrophic (extremely unproductive; Thienemann 1921) by their primary production. This discrepancy has led to suggestions that P in humic systems, in spite of high concentrations of MRP, is less available than in other types of lakes (Jackson and Hecky 1980; Heyman and Lundgren 1988; Jones 1998).

The BAP component in humic streams and lakes can be expected to be primarily taken up by bacterioplankton. Bacteria are more efficient scavengers of low orthophosphate concentrations than are phytoplankton (Currie and Kalff 1984; Jansson 1993; Vadstein 2000). Bacteria are often P limited in humic and clear-water lakes (Jansson 1998; Vadstein 2000; Karlsson et al. 2001), and they generally have lower cellular carbon (C) : P ratios than do phytoplankton (Vadstein 2000). Consequently, access to bioavailable P in humic lakes should control bacterioplankton production. Heterotrophic bacterial production (BP) is often much higher than phytoplankton production in humic systems (Jansson et al. 2000; Karlsson et al. 2002), and bacteria are used in aquatic food webs via flagellate and zooplankton grazing (Jones 1992; Hessen 1998; Berggren et al. 2010*b*). Therefore, BAP can be a driver of aquatic food webs via its incorporation in bacteria. Another aspect is that P-limited bacterial respiration of terrestrial C in humic streams and lakes contributes to CO_2 supersaturation and subsequent emission of CO_2 to the atmosphere, stressing the role of freshwater systems in global C cycling (Cole et al. 2007). Hence, BAP has a potential role in different aspects of aquatic C metabolism.

This study aims to (1) determine BAP concentrations in humic surface waters, (2) assess the seasonal variation of BAP transfer from different boreal terrestrial systems to surface waters, and (3) test if temporal BAP variation is related to the variation of well-known P carriers like dissolved organic carbon (DOC) and iron (Fe). We measured bacterial uptake of P in water from two contrasting but adjacent boreal and humic first-order streams draining mires and forests, respectively. We selected small upland headwaters for characterization of P before it became modified by stream or lake metabolism, thus focusing on the potential support of aquatic P metabolism by material exported from terrestrial systems. To estimate BAP, we set up a bioassay in which we measured P uptake by natural P-limited bacterioplankton growing with natural P as the single source of P. We consider that we measured readily available P in the streams, which in this case also denotes the potential of different terrestrial systems to support freshwater bioproduction via export of BAP. We found unexpectedly high BAP concentrations (1–14 $\mu g\ L^{-1}$) in the streams, considerable temporal variation in BAP concentrations and characteristics, and differences in BAP concentrations between streams.

Methods

Study area—Two headwater streams in the Krycklan catchment at the Svartberget Long-term Ecological Research station (Svartberget LTER) in northern Sweden (64°14′N, 19°46′E) were selected for sampling during the summer of 2010. One stream, "Västrabäcken" (hereafter, the forest stream), drains a 0.13-km^2 forested catchment where 100% of the area consists of coniferous forest; and the other stream, "Kallkällsmyrbäcken" (hereafter, the mire stream), drains a 0.19-km^2 catchment where mires make up 50% of the area and the rest is forest (Buffam et al. 2007). The mires contribute the predominant part of the export of organic matter from this catchment during summer low-flow conditions (Laudon et al. 2011). The two streams were selected to study temporal variation in BAP concentrations because they have been included in 30 yr of mechanistic biogeochemical research and because their catchments represent the two most important and contrasting terrestrial environments in many boreal landscapes (Laudon et al. 2011). The vegetation of the forest component of the catchments is dominated by Norway spruce (*Picea abies*). The mire vegetation consists of peat-forming *Sphagnum*. Both streams are characterized by high concentrations of DOC of terrestrial origin (Ågren et al. 2008). The headwater character of both streams means that the time between water entrance into the stream and sampling always is less than 1 d, often considerably shorter (Laudon et al. 2007).

Sampling and bioassay—The forest stream and the mire stream were sampled on nine dates between late April and late October. Water was sampled from V-notch weirs by filling acid-washed 10-liter containers. Sampled water was immediately brought to the laboratory (within 1 to 5 h). Water was then filtered in two consecutive steps, first through Supor AcroPak 200 disposable filters (Pall Corporation) with a nominal cutoff of 0.2 μm (all sampling occasions) and thereafter (six sampling occasions from May until September) through an Ultrasett filter (Pall Corporation) with a nominal cutoff of 1 kDa. Each filtration was made in triplicate. Filtrations through the 0.2-μm membranes were made under low pressure ($< 2 \times 10^5$ Pa) using a peristaltic pump (Master Flex, Cole Parmer). The Ultrasett filter was washed with 0.1 mol L^{-1} NaOH before use on each occasion.

Filtrations with the 1-kDa filters were made using tangential flow filtration equipment (UltraLab, Pall Corporation). All glassware was acid-washed and sterilized (autoclaved) prior to filtrations. These filtration procedures enabled us to determine the BAP of three different fractions: BAP in 0.2-μm filtrates (hereafter, "dissolved BAP"); BAP in 0.2-μm filtrates that did not pass a 1-kDa filter (hereafter, "high-molecular-weight [HMW] BAP"); and BAP in 1-kDa filtrates (hereafter, "low-molecular-weight [LMW] BAP"). Dissolved BAP and LMW BAP were determined using 0.2-μm and 1-kDa filtrates in the assay described below, and HMW BAP was obtained from the difference between dissolved BAP and LMW BAP.

After filtration, 700 mL of water was immediately used for a bioassay according to the following procedure. We added (final concentrations within parentheses) glucose (100 mg C L^{-1}), ammonium nitrate (10 mg N L^{-1}), and L16 medium (Lindström 1991; 5% by volume) containing essential macro- and micronutrients (except P) and

vitamins. Finally, we added a bacterial inoculum (5% by volume) of unfiltered stream water to the incubation bottles. The final volume after these additions was 790 mL, which was incubated in 1-liter glass bottles sealed with a screw cap.

The bottles were placed on magnetic stirrers (with acid-washed and sterilized magnets) and incubated at 15°C in the dark for a period of 5 d. With this procedure we supplied all the necessary nutrients, except P, and thus were able to measure the available P in the natural waters as the amount of P incorporated in bacterial biomass. Each day we sampled 20 mL of the incubation water for measuring BP and bacterial numbers and biomass. Samples for bacterial biomass determination were preserved with formaldehyde (final concentration approximately 4%). We filtered water to collect particulate P at the end of the incubation. These filtrations (100 mL) were made using acid-washed 0.2-μm cellulose acetate filters. Since bacteria (in theory, *see* below) were the only particles on the filters, we used the difference in P content on the filter between the end and the start of the incubation as a measure of the amount of P taken up by bacteria, i.e., as a measure of BAP. BP increased rapidly during the first days of incubation and reached a stationary phase after 3–4 d in all experiments. We therefore always determined BAP by analyzing particulate P after 4 d of incubation. We made the following controls for errors and artifacts:

Stationary phase caused by P limitation: After BP reached the stationary phase (after 4 d), we added orthophosphate (plus 500 μg P L^{-1}) in two of the three incubations from each treatment (keeping one as a control). We then measured BP after 24 h. P additions always caused an increase in BP that was 5 to 54 times greater than the control. We therefore conclude that the stationary phase in our assay was caused by P limitation.

Particulate P dominated by bacteria: We tested the assumption that the P collected on the 0.2-μm filters at the end of the incubation was dominated by bacterial P by analyzing parallel incubations of 0.2-μm filtered water without a bacterial inoculum. Particulate P yield in these test systems included filter content of P, absorption of dissolved P species from the test water, and abiotic formation of particles during the incubation. The sum of filter backgrounds, absorption, and abiotic particle formation averaged 0.9 μg P L^{-1} (range: 0.1–1.5 μg P L^{-1}).

We performed this test on three occasions (07 July, 23 August, and 23 September). These occasions represent situations with the lowest BAP values (September), moderately high BAP values (August), and the highest BAP values (July), i.e., the controls covered the whole range of BAP variation recorded in our study. Since variation among control values was at a low P concentration level and not systematic, we consider that our controls were adequate for correction of abiotic contributions to particle P yield during all incubations. Consequently, we subtracted 0.9 μg L^{-1} from all BAP values to adjust for the possible influence of abiotic processes and consider that our corrected particulate-P analyses reflected biological P uptake.

Effects of different bacterial communities in the different streams: To check for the possibility that differences in bacterial uptake of P between streams were caused by different bacterial characteristics, rather than characteristics of the P sources, we ''cross-incubated'' bacteria. We inoculated bacteria from the mire stream into water from the forest stream and vice versa. We did not find any differences between these cross-inoculated systems and the original systems (paired *t*-test, $t = -0.291$, df = 4, $p = 0.786$ in the forest stream and $t = 0.612$, df = 4, $p = 0.573$ in the mire stream). We therefore consider that the differences in BAP between streams were caused by access to bioavailable P.

Incubation effects on pH: Experiments were carried out at in situ pH (varied between 4.0 and 5.7). Since pH can affect the P-binding capacity of metal oxides or hydroxides (Boström et al. 1982), we tested the pH effects by addition of C, N, and trace elements and by pH variation during incubation. We included the mire stream and the forest stream plus six other small streams in the Krycklan catchment in this test. Sampling was made in March 2011, and pH varied between 4.4 and 6.3 in the streams; this covers 95% of all streams in the region (Buffam et al. 2008). We measured pH in 0.2-μm filtered water before and after addition of the trace element plus C and N solutions and then after 4 d of incubation (in the dark at 15°C). Moreover, pH was measured in unfiltered water before and after 4 d of incubation in the dark at 15°C. The pH value increased 0.1 (SD: 0.1) unit following C, N, and trace element addition to filtered water and by 0.4 (SD: 0.3) unit during 4 d of incubation in filtered water. pH increased by 0.1 unit during 4 d of incubation in unfiltered water. We consider that the pH variations within the pH range (4.4 and 6.3) observed in our study did not significantly affect bacterial BAP exploitation in our bioassay. Insignificant influences of pH on the BAP assay were also indicated in that seasonal BAP and pH variation in the mire and forest stream were not correlated ($r^2 = 0.11$, $p = 0.17$, $n = 18$).

The bioassay could not be used for determination of BAP in unfiltered water. To be able to compare dissolved BAP with particle-bound BAP we, therefore, first plotted BAP vs. the integrated (over time) BP during incubations in 0.2-μm filtrates. These relationships were: Log[BAP] = 0.448[LogBP] − 0.403, $r^2 = 0.78$, $p < 001$, $n = 23$ in the mire stream and Log[BAP] = 0.313[LogBP] − 0.2753, $r^2 = 0.62$, $p < 001$, $n = 23$ in the forest stream. We then used these relationships and BP values measured during incubation in unfiltered water to estimate BAP in the unfiltered water (the unfiltered water was enriched before incubation in the same way as the filtered water). Then we subtracted the BAP in the 0.2-μm filtrates from estimated BAP in unfiltered water to obtain particulate-bound BAP.

Analyses—Chemical analyses were made on water before the bioassay and included DOC, total nitrogen (TN), TP, and MRP. These analyses were made on all three fractions (unfiltered water, 0.2-μm filtrates, and 1-kDa filtrates). Air temperature and total Fe concentrations were obtained from the regular analytical program in the Krycklan Experimental Forest. Air temperature was measured as

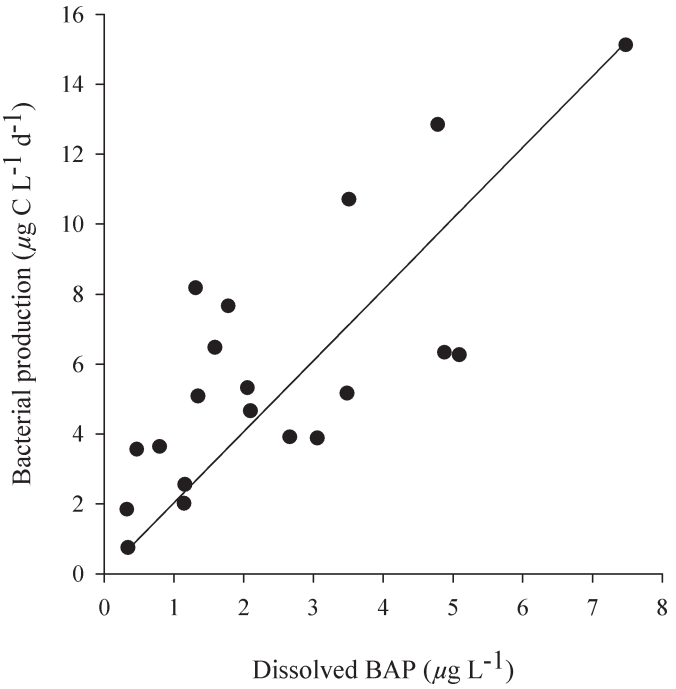

Fig. 1. Relationship between dissolved BAP and bacterial production (BP; stationary phase). BP = 2.03 BAP ($r^2 = 0.45$, $p < 0.01$).

part of the Svartberget climate long-term monitoring program, 1.5 km from the study streams following the Water Management Council protocol. Absorbance at 254 nm and 365 nm was measured on 0.45-μm filtered water in 1-cm quartz cuvettes with a Japanese Spectroscope Corporation (JASCO) V560 spectrophotometer. DOC and TN were analyzed on an IL-550 total organic C analyzer (Hach-Lange), and MRP with the standard molybdenum blue method, using a spectrophotometer V560 (JASCO). TP of unfiltered water, filtered water, and material collected on filters was measured with the molybdenum blue method after digestion with $K_2S_2O_8$ for 60 min in an autoclave. We measured pH in unfiltered water with an Orion pH meter (Cole Parmer). We analyzed for Fe in unfiltered water using an inductively coupled plasma–optical emission spectroscopy (Varian Vista Pro Ax) instrument. In order to ensure the accuracy of the analysis an externally certified multi-element aqueous standard solution, SPS-SW1 reference material for measurements of elements in surface waters (Spectrapure Standards) was included in the protocol at regular intervals. The uncertainty of the analyses was less than 2%.

We measured BP with the ^{3}H-leucine (Perkin-Elmer) incorporation method as described by Karlsson et al. (2002). Counting of the ^{3}H isotope activity was made on a Beckman LS-6500 scintillation counter. The amount of isotope was selected from a dose–response curve, in which incorporation was saturated at a concentration of 46 nmol L^{-1} ^{3}H-leucine. BP was measured in triplicate samples from each incubation bottle. The biomass of bacteria was estimated by staining the bacteria with acridine orange (Wetzel and Likens 1991) before collecting the bacteria onto black 0.2-μm polycarbonate filters (Sorbent AB). Stained bacteria were photographed using a Nikon inverted microscope (Eclipse T*i* U) and a SPOT (R T3) digital camera, using the SPOT 4.6 software (Diagnostic Instruments). Bacterial numbers were manually counted on a computer screen from nine photos from each filter. The length and width of bacteria were analyzed from three photos from each filter, using the software CellProfiler (Broad Institute). Cell volumes were determined from the length and width (Bratbak 1985) of between 45 and 151 cells on each filter. C biomass was estimated using a cell-specific C content value of 0.308 pg C μm^{-3} (Fry 1988).

Results

Our estimates of BAP were well correlated with the BP measured at the stationary phase (Fig. 1). The assay procedure was designed so that BP and biomass yield would be controlled by BAP and that BAP would not be dependent on BP or bacterial biomass. The relationship in Fig. 1, in combination with the fact that BP always increased considerably by addition of orthophosphate after the BP had reached the stationary phase (*see* Methods), shows that BP was controlled by BAP in our assay. These results also validate that our assay provides a correct estimation of BAP. The BP development during the incubations was clearly related with bacterial biomass calculated from bacterial cell counting (analyzed on water from the August sampling), indicating that both BP and cell yield were dependent on BAP (forest stream, slope = 0.84, $p < 0.01$, $r^2 = 0.99$, $n = 4$; mire stream, slope = 0.59, $p < 0.01$, $r^2 = 0.89$, $n = 4$).

Chemical characteristics, including dissolved BAP of the investigated streams, are shown in Table 1. The mean estimated BAP in unfiltered water in the mire stream was 4.1 μg L^{-1} compared to dissolved BAP values of 3.6 μg L^{-1}. The corresponding figures in the forest stream were 2.3 μg L^{-1} and 2.1 μg L^{-1}. Thus, nearly all (ca. 90%) of the BAP occurred in dissolved form in both streams. Both streams also showed a similar temporal variation of dissolved BAP concentrations, with a clear increase in the middle of the summer (most pronounced in the mire stream; Fig. 2). The summer BAP peak was connected to

Table 1. Characteristics of 0.2-μm filtered water (except pH, which was measured on unfiltered water) in the forest (Västrabäcken) and the mire (Kallkällsmyrbäcken) streams. Seasonal means (May–October; $n = 9$ [except MRP, $n = 7$]) and, in parentheses, standard deviations.

	Area (km^2)	pH	DOC (mg L^{-1})	TN (μg L^{-1})	TP (μg L^{-1})	MRP (μg L^{-1})	BAP (μg L^{-1})
Forest	0.14	4.9(0.3)	20.5(5.2)	348(69)	21(5)	10(3)	2.5(1.2)
Mire	0.19	4.3(0.5)	34.7(10.3)	512(112)	22(7)	17(5)	5.7(4.0)

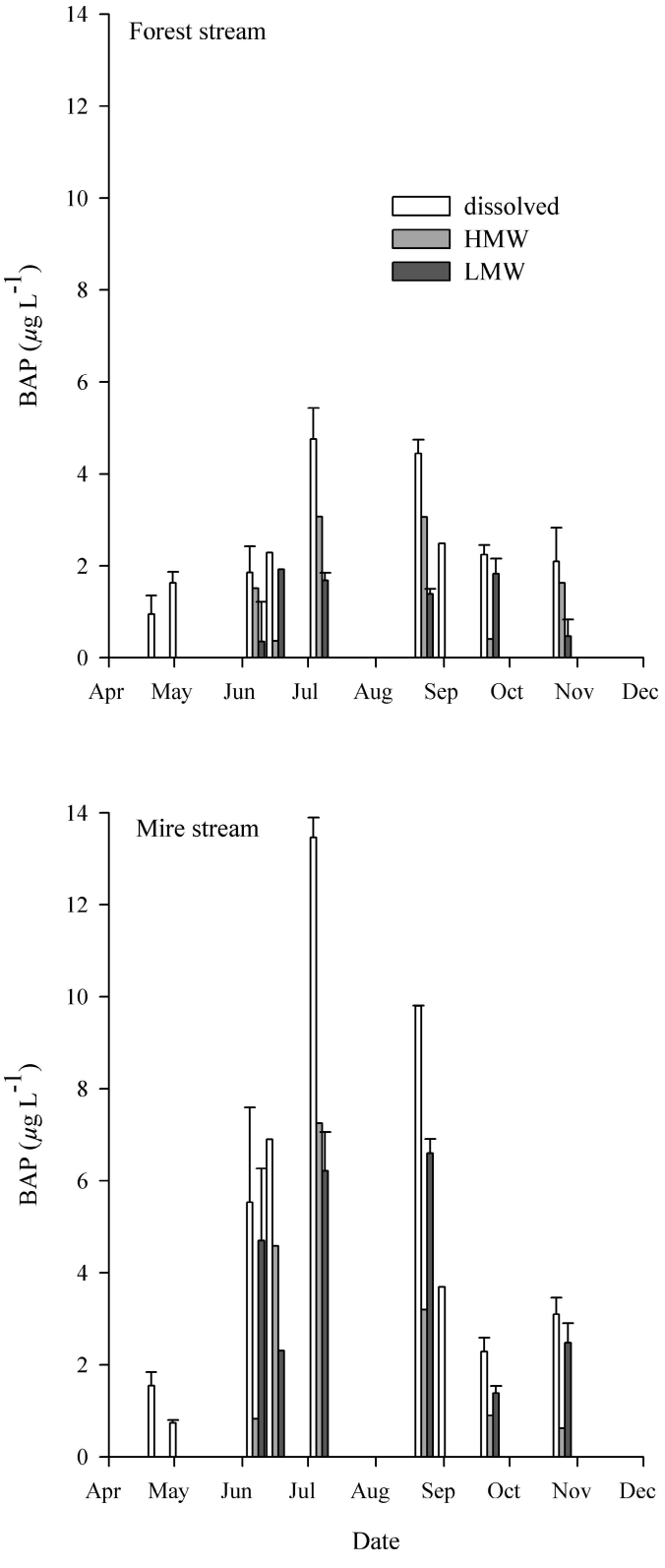

Fig. 2. Dissolved BAP, HMW BAP, and LMW BAP in the mire stream and the forest stream during the period April–October 2010.

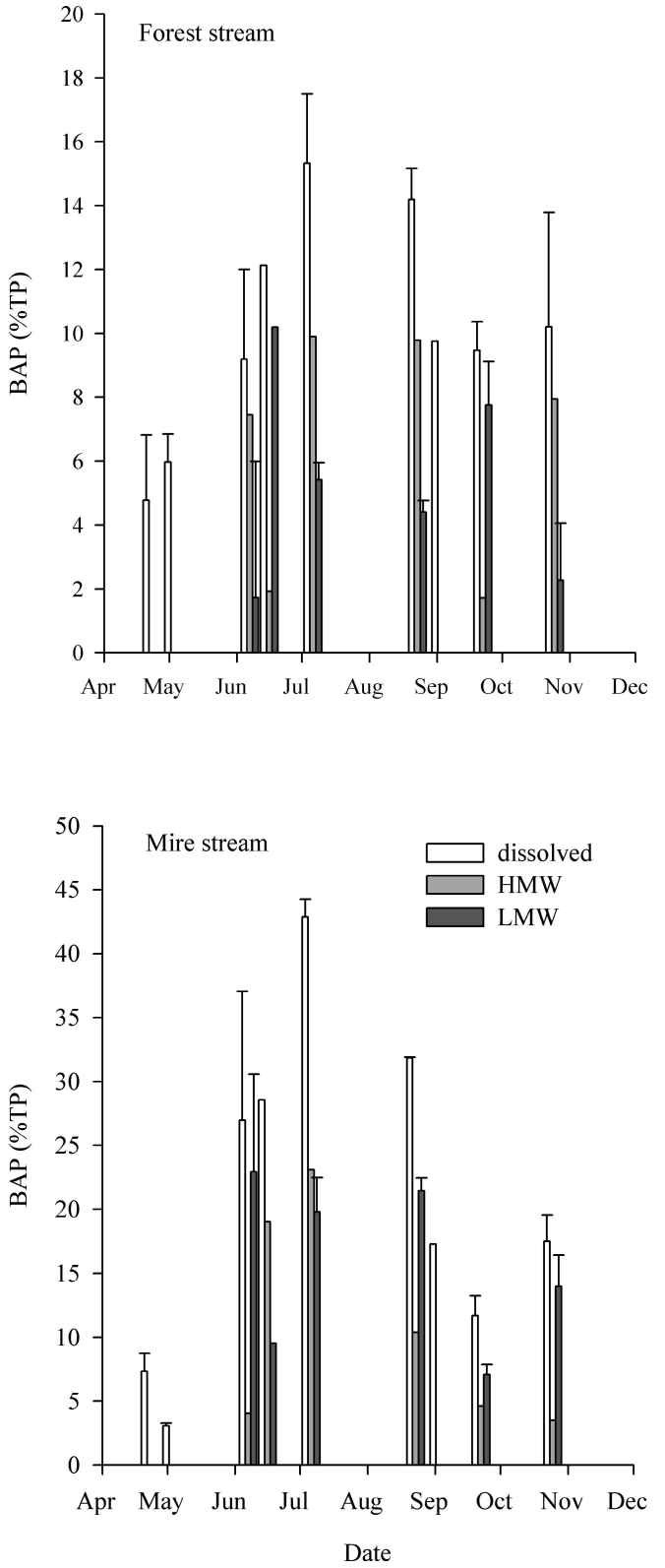

Fig. 3. Dissolved BAP, HMW BAP, and LMW BAP as the percentage of total P in the mire stream and forest stream during the period April–October 2010.

Table 2. Correlations (r^2 values) between different BAP fractions and chemical characteristics. Numbers marked in bold are significant at $p < 0.05$. Numbers in parentheses represent a different number of samples (n) than shown in the n column. All correlations are based on analyses within each BAP fraction.

BAP fraction	DOC	TN	TP	Fe	Abs ratio	n
Dissolved forest+mire*	0.19	**0.42**	0.19	**0.46**(16)	**−0.63**	18
Dissolved forest*	0.11	0.03	0.05	0.10(8)	**−0.57**	9
Dissolved mire	0.01	0.28	**0.58**	0.34(8)	**−0.78**	9
HMW forest+mire	0.15	0.08	**0.76**	—	**−0.47**(10)	12
LMW forest+mire*	−0.01	−0.03	0.10	—	**−0.67**(7)	12

* Log transformed.

increases in both HMW BAP and LMW BAP (Fig. 2). The dissolved BAP share of TP also varied during the investigation period, with the highest shares in the middle of the summer, showing that BAP was not a constant fraction of TP (Fig. 3).

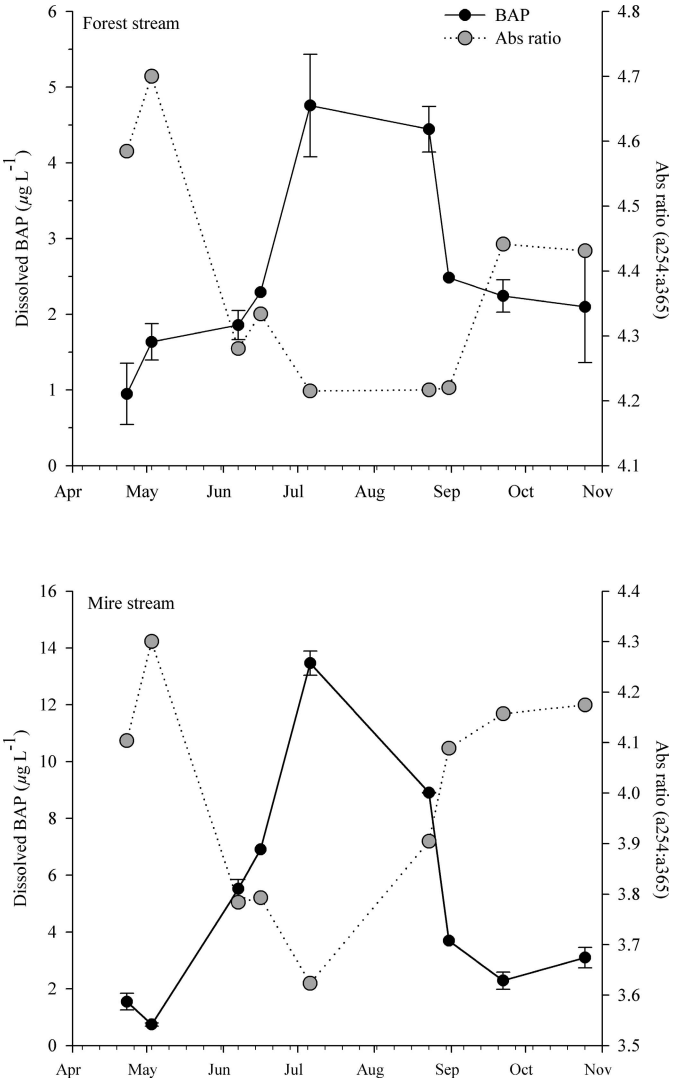

Fig. 4. Variation of dissolved BAP and absorbance ratios in the mire stream and forest stream during the period April–October 2010.

There was no relationship between BAP and DOC in any of the fractions (Table 2). Weak positive relationships between dissolved BAP and N and Fe were found only for the (total) dissolved BAP fraction and only when pooling data from the forest and the mire streams (Table 2). Dissolved BAP (mire stream) and HMW BAP (forest plus mire streams) were related to TP. Seasonal mean BAP values were approximately half of the MRP concentrations (Table 1).

We compared the BAP variation with that of the absorbance ratio (a254 : a365 nm). Previous studies in the forest and the mire streams have shown that the absorbance ratio is a proxy for labile organic C that supports effective bacterial growth (Berggren et al. 2007, 2010*a*). There were inverted temporal patterns of dissolved BAP and absorbance ratio in both streams (Fig. 4) and thus negative relationships between BAP and the absorbance ratio for all of the BAP fractions (Table 2). The strongest dependence was found for dissolved BAP in the mire stream. There were close parallel variations of dissolved BAP in both the mire and the forest streams and air temperature (Fig. 5). However, the BAP–temperature relationships were different in the mire and the forest streams (mire stream: BAP = 0.72 temp + 0.42, $r^2 = 0.77$, $p = 0.002$, $n = 9$; forest stream: BAP = 0.20 temp + 1.17, $r^2 = 0.68$, $p = 0.006$, $n = 9$), indicating that changes in temperature had larger effects on the BAP concentrations in the mire stream than in the forest stream.

Discussion

Our results demonstrate that boreal terrestrial systems can be a substantial source of BAP for surface waters. The concentration range for dissolved BAP (1–14 μg L^{-1}) recorded in this study represents values that are higher than those generally reported from unproductive lakes (Nowlin et al. 2007); the highest values compare with those found in eutrophic streams and lakes (Boström et al. 1988).

There was a considerable temporal variation of different BAP fractions during the period May–October, which was similar in the mire and the forest streams (Fig. 2). Both HMW BAP and LMW BAP and, therefore, also total dissolved BAP were higher in the middle of the summer than in spring and autumn. The temporal variation of BAP concentration and its quality characteristics, therefore, indicate that BAP export from mire- or forest-dominated catchments was controlled by similar mechanisms, al-

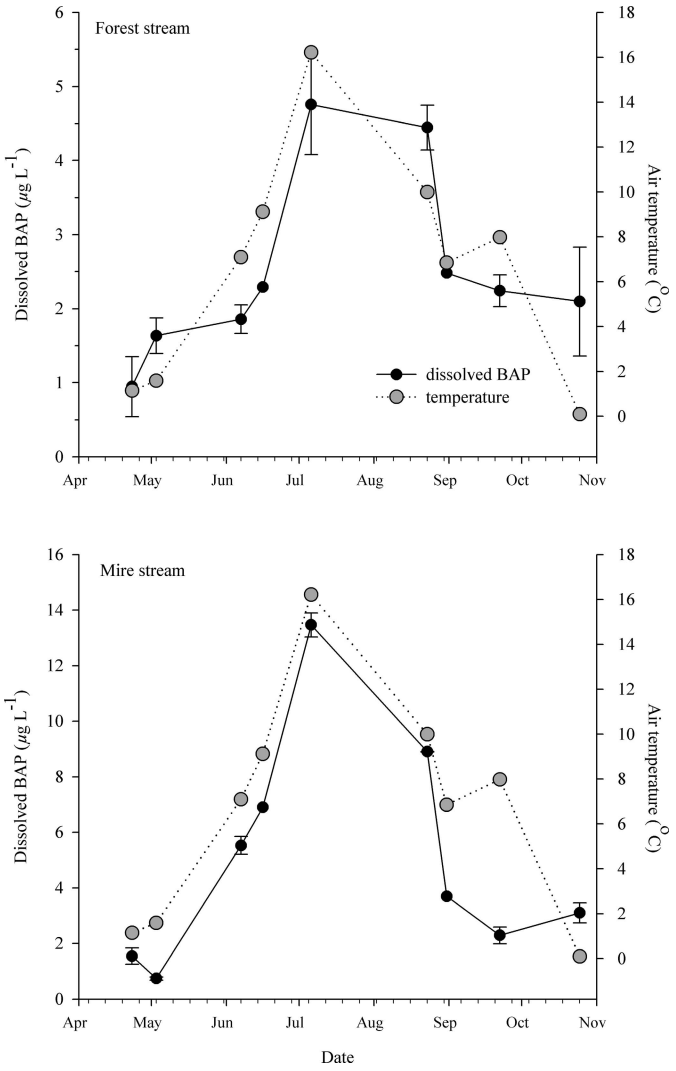

Fig. 5. Variation of dissolved BAP and air temperature in the mire stream and forest stream during the period April–October 2010.

though the concentrations of BAP were higher in water exported from the mire during summer (Fig. 2). We therefore suggest that the temporal variation and the quality of BAP may be similar to our results in a variety of boreal headwaters and that the proportions of mires and forests in the catchment may affect concentrations and quality of BAP in streams.

The separation of BAP into a HMW and a LMW component showed that much of the BAP occurred in forms other than orthophosphate. We found no clear co-variation between BAP and variables that represent potential P carriers. Several studies have shown that both TP and MRP in humic waters are linked to DOC and Fe (De Haan and De Boer 1986; Meili 1992) and that labile P in humic lakes may be associated with Fe–C complexes (Jones et al. 1988). However, the failure to connect DOC or Fe in different fractions to BAP (Table 2) shows that temporal variation of aquatic BAP (as defined in our study) could not be predicted or understood from simple quantitative relationships between BAP and terrestrial bulk export of DOC or Fe.

Dissolved BAP (which in our study dominated total BAP in the investigated streams; only 10% of BAP was in particulate form) was a highly variable share of the TP concentration in the streams (Fig. 3). Consequently, it appears as if BAP was exported from soils to streams via mechanisms that, to a large extent, are uncoupled from processes that determine not only the export of DOC and Fe but also other forms of TP export than BAP.

The temporal dissolved BAP pattern was related to variables that affect soil metabolic rates (air temperature) and reflect soil C metabolism (absorbance ratio). There were almost identical patterns of air temperature and BAP variation in both streams (Fig. 5). All sampling was carried out at stable low water flow, implying that runoff variations (e.g., high-flow episodes) did not interfere with the soil export of BAP in this study. Consequently, we consider that both the mobilization of BAP in the soil systems and its subsequent export to surface waters were temperature dependent; this is reflected in the correlation between temperature and dissolved BAP (Table 2). The BAP increase per degree centigrade was higher in the mire stream than in the forest stream, which means that more BAP was mobilized in the mire at high temperatures or that the balance between formation and consumption of BAP was more in favor of formation in the mire soils than in the forest soils at high temperatures. It is therefore interesting to note the inverse variation (Fig. 4) and strong negative relationship (Table 2) between dissolved BAP and the absorbance ratio in both the mire and the forest streams. The absorbance ratio denotes the proportion between small and large C molecules (De Haan 1983); previous studies of streams in the Krycklan catchment showed that a high absorbance ratio was connected to high C availability for BP (Berggren et al. 2007, 2010*a*). C availability was higher in forest than in mire streams (Berggren et al. 2007). The patterns in Fig. 4, therefore, show that BAP export from soils to surface water was high when the export of available C was low and vice versa. Combining this result with the temperature dependence discussed above indicates that low concentrations of bioavailable C and high concentrations of BAP were exported from soils to streams during the warmest summer period, which was most evident in the mire stream. The results imply a possible connection between soil C and P metabolism. One possibility is that low production of labile organic C in the soil leads to lower consumption rates of soil BAP and, consequently, increased BAP concentrations in the water exported from soils to surface water. Another possibility is that increased BAP mobilization in P-limited boreal soils (Giesler et al. 2002, 2004) can deplete labile organic C with concomitant consequences for the export of labile C and P to surface-water recipients and subsequent aquatic C and P metabolism. Both possibilities open up for speculation that high BAP may be a reflection of soil accumulation and export of labile organic phosphates during summer. P diesters have, for example, been demonstrated to make up a large portion of soil P and to be only weakly correlated with Fe and Al in boreal soil systems (Vincent et al. 2012).

Considering that MRP is often used as a proxy for bioavailable P, it is interesting to compare our BAP values

with MRP. MRP concentrations were generally high (6–25 μg L^{-1}) and represented a large portion (40–90%) of TP. This portion was positively related to DOC ($r^2 = 0.66$, $p < 0.001$, $n = 14$). In all these respects our results are more or less identical to what was reported for lakes and streams in southern and central Sweden (Meili 1992). Thus, MRP is a large, often dominant, portion of TP in boreal humic streams. The high concentrations of MRP compared to BAP (Table 1) show that MRP generally overestimated BAP considerably. Thus, the general picture is that MRP to a large extent captured other fractions of P than BAP in the investigated streams and that MRP, therefore, cannot be used for predictions of readily available P in humic aquatic systems.

This study contributes to increased understanding of the nutrient dynamics in humic aquatic systems in several ways. We show that the input of BAP to boreal streams can be high compared with estimates of BAP in other unproductive aquatic systems. We sampled first-order streams in which the time period between entrance of water and dissolved species into the stream and the sampling was considerably less than 24 h on all sampling occasions in all streams. We, therefore, consider that our results denote the input of BAP to surface water and not BAP in systems in which BAP has been taken up and processed in aquatic organisms and food webs. In that respect, BAP in our study represents a pool of P that has the potential to readily sustain bioproduction at the basic trophic level (heterotrophic bacteria and phototrophs). The potential in this respect can be assessed if we assume a bacterial C : P ratio (by weight) of 25 (Caron et al. 1990) to estimate stream bacterial P uptake using previous analyses of bacterial C uptake for production (ca. 10–30 μg C L^{-1} d^{-1}) in the Krycklan streams (Berggren et al. 2007) and in larger tributaries to the humic Lake Örträsket (5–40 μg C L^{-1} d^{-1}; Bergström and Jansson 2000). The daily P uptake should then vary between 0.2 μg P L^{-1} d^{-1} and 1.6 μg P L^{-1} d^{-1}. The BAP values in the mire stream and the forest stream were between 1 μg L^{-1} and 14 μg L^{-1}. With a water renewal time of less than 24 h in these streams, this comparison indicates that BAP supply represents a surplus of P relative to the bacterial need of P for biomass production that can be used for production of several generations of bacteria in the natural stream environment. The results also indicate that P is not a limiting factor for BP in humic headwater streams, supporting previous results from nutrient enrichment experiments in similar settings (Jansson et al. 2001; Berggren et al. 2007).

Our bioassay demonstrated a more or less complete bacterial extraction of BAP within 3 to 4 d. This time frame should be considerably wider in natural systems, in which uptake and bacterial use of P can be expected to be hampered by low temperature and limited access to energy, N, and maybe also trace elements, which effects were eliminated in the bioassay. The mean summer transit time of water and dissolved substances from soil export to lake entrance in the nearby well-studied River Öre catchment was estimated by Berggren et al. (2010*b*) to range from days to weeks. Consequently, we can assume that considerable shares of BAP will not be consumed in streams before entering lakes but will reach the lakes in readily available forms, at least in aquatic systems with low hydrological turnover times. BAP consumption in lakes will allocate P from a readily available source to organism-bound P, with possible P limitation as a consequence. The observations in this study can in this way explain previous observations of a much higher BP in the tributaries to the humic Lake Örträsket than in the lake itself (Bergström and Jansson 2000). It is also interesting that the temporal variation of BP in these tributaries was strongly correlated to temperature (Bergström and Jansson 2000), similar to the BAP transfer from soils to streams in this study.

The occurrence of BAP in the humic systems of this study demonstrates previously unreported high concentrations of BAP with particular characteristics and stresses temporal variations in BAP and their probable dependence on soil processes. The soil export rates of BAP are likely higher than the uptake rates of BAP in receiving streams, suggesting that a considerable share of BAP exported from soils to streams will reach downstream lakes where BAP can support both the production of bacteria and phytoplankton and the bacterial respiration of terrestrial organic C. Considering the strong potential role of BAP in the performance of aquatic food webs and overall aquatic C cycling, this study calls for further investigations that can address, in more detail, the chemical characteristics of BAP and the uptake rates of different BAP forms. We also argue for the need for studies that can explore the fate and ecological importance of BAP in humic aquatic systems. The possible connection between BAP export and soil C metabolism indicated in this study stresses the need for studies of stoichiometric control of resources and terrestrial–aquatic cross-ecosystems interactions.

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