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Variations in bioavailability of dissolved organic matter during a spring flood episode in northern Sweden

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ABSTRACT

This study of spring flood episodes was performed within the catchment of Krycklan, in Svartberget, northern Sweden. The purpose was to better understand changes in total organic carbon (TOC) concentrations and the bioavailability of dissolved organic matter (DOM) during the spring flood and how they differ between stream sites, ranging in size and characteristics. DOM bioavailability was estimated from CO_2 production rates (PrRs) and total CO_2 production (TPrs), derived by applying standardized bacterial bioassays during approximately 90 days.

TOC concentrations in most of the forested streams increased during spring flood. Furthermore, the PrRs per unit of TOC increased by up to 95% when comparing discharge before and after spring flood. These patterns can be explained by the activation of new superficial flow paths in the riparian zone, increasing not only the DOM concentration per se, but also its susceptibility to biological degradation. At a mire-influenced headwater stream, TOC concentrations decreased during peak flow and no differences in DOM bioavailability per unit TOC was observed before and during spring flood. This suggests that the hydrological flow paths in the mire are superficial during most of the year and do not provide any new sources of DOM to the stream during spring flood. Instead, existing sources are diluted due to higher discharge.

PrRs and TPrs ranged from 0.10% to 0.43 % of total C pool per day and from 6% to 20% of total C pool over 80 days, respectively. PrRs and TPrs were low in headwaters and increased further downstream. This downstream change was observed during all three bioassays, but was largest before and during spring flood. PrRs were up to four times higher in the outlet of the catchment of Krycklan than in the forest and mire headwaters.

The residence time of water in streams within the catchment is small and water temperatures during spring flood are low. Therefore, it is likely that most of the bioavailable DOM will be utilized outside the catchment of Krycklan and spring-flood contributions can, consequently, play an important role for carbon and nutrient cycling in rivers, lakes and the ocean further downstream.

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INTRODUCTION

Dissolved organic carbon (DOM) has an important role influencing chemical and physical characteristics of water ecosystems. DOM has been shown to control the buffering capacity in many boreal streams and thereby also the pH (Lydersen, 1998; Laudon, 2000(b)). It affects light attenuation (Jones, 1998) and complexation of trace metals by humic and fulvic acids (McKnight et al, 1998). DOM is also the dominating carbon pool in most streams and rivers (Allan, 1995), and functions as an energy source (Moran and Hodson, 1990; Kaplan and Newbold, 1993; Munster and De Haan, 1998), and a nutrient source (Stepanauskas, 2000; Berman and Bronk, 2003) for heterotrophic organisms.

The degradation of DOM by microorganisms is one of the most important processes for DOM turnover in streams (Allan, 1995). In addition to physical factors such as temperature and pH, the potential microbial utilization also depends on the quality of the DOM (Sun et al., 1997). DOM dynamics are usually related to primary production and hydrological events (Allan, 1995). The main hydrological event in northern boreal regions is the spring flood, when up to 50% of the annual water discharge may occur, and many streams experiences elevated DOM concentrations and increased downstream transport of DOM during this period of time (Bishop and Pettersson, 1996). Although the basic patterns of DOM concentration fluctuations associated with spring-flood events in boreal regions are well known, less is known about the qualitative aspects of DOM and how this affects microbial degradation.

This study was a part of a major survey performed during the spring 2003 in the Krycklan catchment, in northern Sweden. The main objectives were to investigate the changes in DOM bioavailability during spring flood and to study spatial differences with respect to bioavailability. The hypothesis of this study was that spring-flood water would affect the DOM bioavailability, and that the effects differed between streams of different sizes and characteristics. Variations in DOM bioavailability during spring flood were studied by employing long-term bacterial bioassays based on bacterial carbon dioxide production. Water was examined before, during and after spring flood, and comparisons were made between seven stream sites ranging in size and catchment characteristics. Particular focus was put on three stream sites: One head-water site within a forested catchment, one mire-influenced headwater site and one site which represents the whole 68 km^2 catchment of this study. Additionally, this study has examined possible limiting factors for bacterial production by employing nutrient additions to samples.

THEORETICAL BACKGROUND

The role of organic matter in stream ecosystems

In streams, organic matter (OM) exists in various stores and moves among these stores by various processes. The sizes of OM stores and processes can differ as a function of stream size, depth and velocity, upstream inputs, the surrounding vegetation and geology, and season

(Allan, 1995). Nonetheless, OM dynamics in streams can be generalized according to the conceptual model displayed in figure 1. Organic inputs to a stream can either arise from allocthonous material, produced outside the stream, or from autochthonous material produced within the stream. Since autotrophic production often is limited due to shading of vegetation and turbid water, streams are generally characterized by a lotic food web driven by heterotrophic microorganisms. Therefore, the contribution of allochtonous OM to the streams is highly important in stream ecology. The allochtonous organic matter that reaches a defined stream ecosystem originates either from terrestrial litter such as leaves and branches from surrounding vegetation, or from soil particulates or solvents, transported by soil and ground water.

The dominating carbon pool in boreal streams is dissolved organic matter (DOM), in this study defined as organic matter <1 μm. Dissolved organic carbon (DOC) comprises approximately 40-50% of DOM and is often used as a surrogate in analyses of DOM (Allan, 1995; Kaplan and Newbold, 1998). Proportions of DOC can in boreal streams represent more than 95% of the total organic carbon pool (TOC; Bishop and Pettersson, 1996). Most of the DOM originates from soil organic matter, but leachates or degradation products from particulate organic matter (POM) can also be of importance, especially during fall and early winter when large amounts of litter can reach the stream (Allan, 1995).

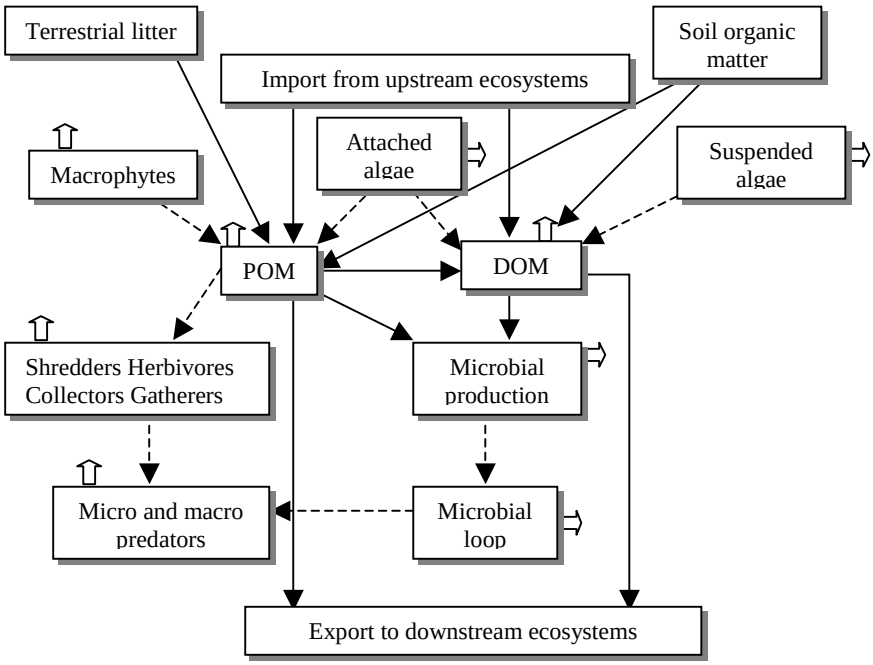


Figure 1*. A model of the principal carbon fluxes and pools in a stream ecosystem. Dominant fluxes are illustrated by solid arrows and less important fluxes are illustrated by dashed arrows.* ⇧ *represents the flux of carbon dioxide from respiration or photochemical degradation. Modified from Allan (1995).*

DOM that enters a defined stream area can experience two possible fates. It can be exported to downstream ecosystems, or lost as carbon dioxide due to biotic oxidation or abiotic photochemical processes (Figure1; Allan, 1995). The utilization of microorganisms mainly occurs on biofilms on particle surfaces, such as stones, leaves and branches. These biofilms can have a tight internal cycling with a high turnover within the microbial loop (Meyer, 1994; Allan, 1995). However, water column DOM supports bacterial growth to various extents depending on turbulence and DOM concentrations (Allan, 1995).

DOM bioavailability

The availability of DOM as an energy and carbon source for microorganisms is related to the chemical structure of the organic molecules (Sun et al., 1997). Bacteria prefer the labile fraction of DOM, since it is the potentially important heterotrophic energy pathway (Leff and Meyer, 1991; Allan, 1995). The bulk of the DOM in most freshwater systems consists of humic and fulvic compounds, which are recalcitrant to heterotrophic degradation (Münster et al, 1998). However, humic and fulvic compounds are not totally biounavailable. Since concentrations of such substances often are high in boreal regions, they can contribute to a significant proportion of the total decomposition. Biounavailable fractions of DOM can also be transformed, through photochemical reactions, into more bioavailable fractions (Tranvik, 1998; Köhler et al, 2002; Engelhaubt et al, 2003). The bioavailable carbon ranges between less than 1% to over 50% of the total carbon pool (Meyer, 1994).

DOM bioavailability can be estimated in many different ways: By chemical analysis of the material itself, e.g. molecular weight (Balogh et al., 2003), or elemental ratios such as H/C and N/C (Sun et al., 1997), or by looking directly at the degree of bacterial activity (e.g. changes in total DOC, loss of dissolved oxygen (Balogh et al., 2003), or bacterial bioassays (Buffam, 2001). In this study I have chosen a bioassay approach, looking at the development of bacterial carbon dioxide production. When performing bioassay-based investigations, DOM bioavailability can be derived in two different ways. Figure 2 describes the pattern of bacterial production over time in two different limited media. The log phase is the phase where bacterial production is largest and the derivative of the curve at this phase provides a production rate. This rate describes how quickly bacteria can utilize bioavailable DOM. Medium 1 in Figure 2 has a higher reproduction rate than Medium 2, which indicates that the quality of the substrate is better and can easier be utilized by bacteria. A second way of understanding heterotrophic bioavailability is to look at the total production, which focuses on the total bioavailable pool as opposed to the quality of the bioavailable pool. In limited mediums the bacterial production will finally stop, which is illustrated by the lag phase in Figure 2. Total production can be calculated by subtracting lag phase production with stationary phase production. In the example of Figure 2 the two media have the same total production, and therefore the same size of the bioavailable pool.

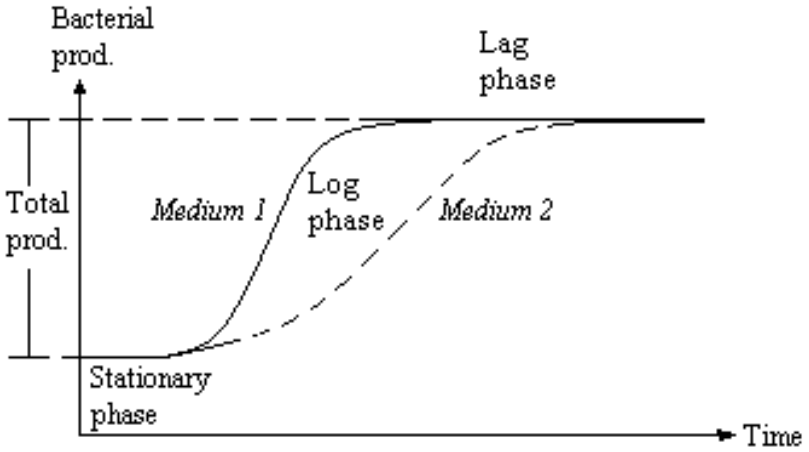


Figure 2. *An illustrative example of bacterial production over time in two media with different DOM quality, but with the same bioavailable pool.*

MATERIAL AND METHODS

Study sites

This study was performed in the area around Svartberget Science Park, located in Vindeln, Sweden (64˚, 14' N, 10˚ 46' E). The upper part of the catchment of Krycklan is well documented, because both climatic and hydrological studies have been performed in the area for over two decades (Anon., 1981-2003)

The climate of Svartberget Science Park is characterized by boreal climate conditions with short summers and long winters. Snow covers the ground from the end of October to the beginning of May. The growing season starts at the end of May and ends in late September. The annual mean precipitation is 600 mm, where about 35% falls as snow and the annual day mean temperature is 0˚C (Vindeln Science Parks, 2004). The upland parts of the catchment are mainly forested with Norway spruce (Picea abies) in low-lying areas and Scots pine (Pinus sylvestris) in upslope areas. There are also patches of mires, which have a large areal coverage in some sub catchments. A glacial till is covering the bedrock and the soils are mainly well-developed iron podzols. Overland flow is rare due to the high infiltration capacity of the till soils (Bishop, 1991). Further down, the catchment changes in some characteristics. Norway spruce and Scots pine is still the dominating vegetation, but deciduous trees and shrubs are more commonly found along the streams. The glacial till gives way to glacier fluvial sediments consisting mainly of sand, and strips of agricultural land are found further down in the valley.

Seven sites within the Krycklan catchment were studied. Three of them are located in 1st order streams of different catchment characteristics (forest, mire and lake). The other four sites represent 2nd, 3rd, 4th order streams (Figure 3 and Table 1). Here follows a short description of each site:

Västrabäcken (#2 SVV)
Västrabäcken is a small tributary with a catchment area of 13 ha. It is surrounded by 100-year-old Norway spruce (*Picea abies*) and forest floor covered with woodland horsetail (*Equisetum sylvaticum*), bilberry (*Vaccinium myrtillus*) and different types of mosses. The catchment is comprised entirely of till soils, with podzols covering most of the subcatchment except for organic rich soils along the stream channel. Västrabäcken has been ditched and straightened in the 1930s and is now incised down in the soil (Bishop, 1991). The sampling site is situated just upstream of its connection to Kallkällbäcken (#7SVW).

Kallkällmyren (#4 SVE)
This site is located 50 m downstream the outlet from an 8 ha mire and is the start of the stream Kallkällbäcken. The mire has peat deposits up to several meters in depth (Bishop, 1991). The stream has, as Västrabäcken, been ditched in the 1930th.

Stortjärnen (outlet) (#5 STO)
The stream Stortjärnbäcken starts as an outlet from the small, shallow, humic-rich forest lake Stortjärnen. Surrounding wetlands but also coniferous forest influences the forest lake water.

Kallkällbäcken (#7 SVW)
This site is located about 1 km downstream site #4SVE, where Västrabäcken connects to Kallkällbäcken. Between site #4SVE and site #7SVW, Kallkällbäcken flows through a similar landscape as Västrabäcken. A ridge with Scots pine is running parallel to the stream and marks the boundary of the subcatchment. Both streams cross a small forest road through culverts 300 meters upstream of the site. Kallkällbäcken is the main contributor to stream flow at site #7SVW.

Långbäcken (#13 LB)
This site is located just upstream of a main country road 4 km downstream from site #7SVV. Kallkällbäcken and Stortjärnbäcken are some of the contributors to stream flow. The stream has temporarily left the dense spruce forests and is surrounded by birch and deciduous shrubs at the site. Glacier fluvial sediments of mainly sand and silt build up the soil.

Övre Krycklan (#15 OKL)
The site Övre Krycklan is located upstream of the junction with Långbäcken. Consequently, this stream belongs to another subcatchment than earlier mentioned streams. Scots pine and Norway spruce are dominate the landscape, but the influence of mires and small lakes is significant in the upper parts of the subcatchment.

Krycklan (#16 KL)
This site marks the outlet of the Krycklan catchment, after what it continues to join the Vindeln River 8 km downstream. It flows through a rather deeply incised valley of coniferous forest, with a riparian zone of mainly birch and alder. The soil is mainly composed by sand/silt sediments in lower reaches and till soil in higher locations.

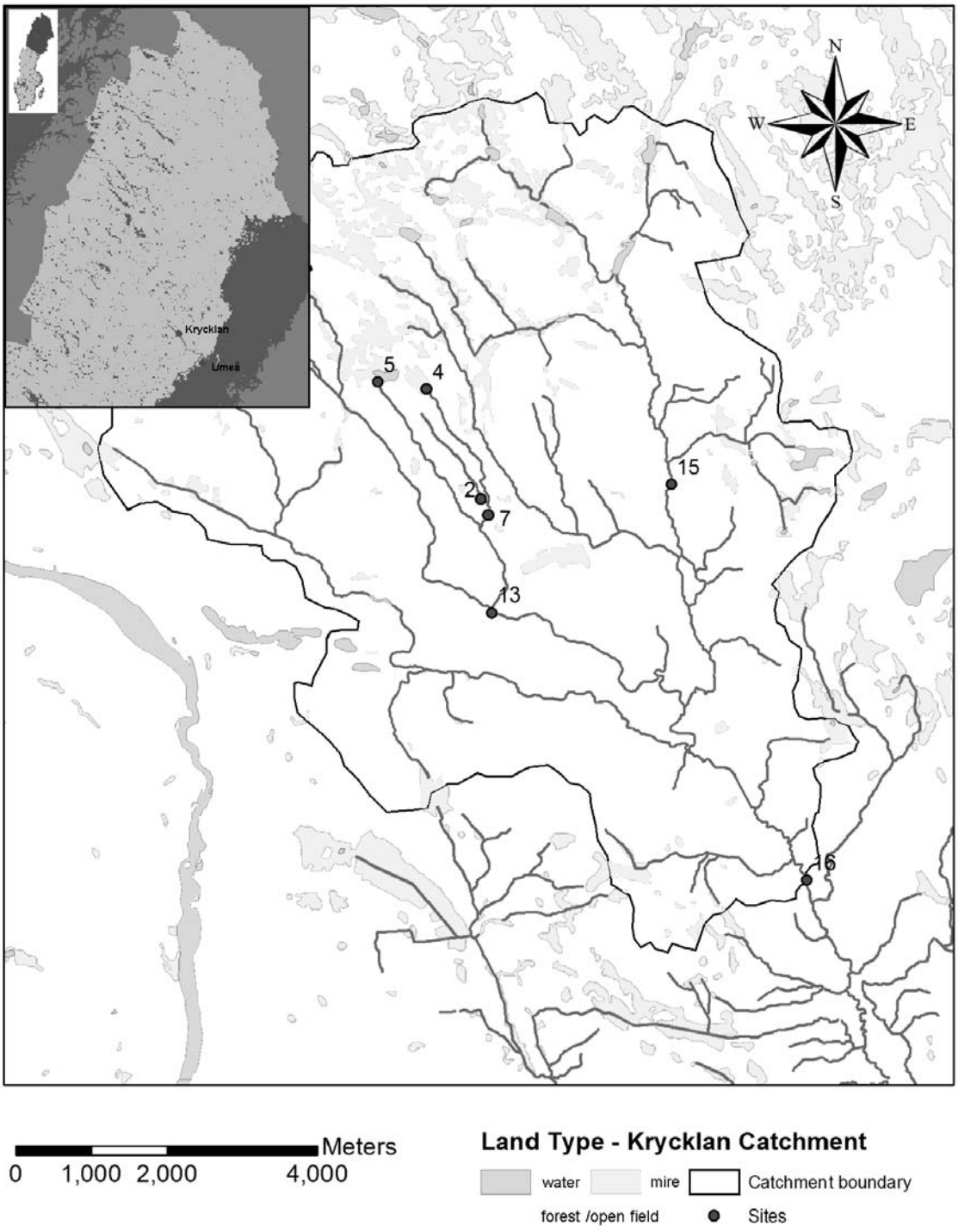


Figure 3. *Map of the catchment of Krycklan and the stream study sites.*

Table 1. *Physical characteristics of the seven study sites.*

Site ID	Site name	Stream order	Area (km2)	Mire (%)	Forest (%)	Lake (%)
#2SVV	Västrabäcken	1	0.13	0	100	0
#4SVE	Kallkällmyren	1	0.19	40	60	0
#5STO	Stortjärnen (outlet)	1	0.85	36	59	5
#7SVW	Kallkällbäcken	2	0.5	15	85	0
#13LB	Långbäcken	3	7.2	10	89	1
#15OKL	Övre Krycklan	4	20	14	83	2
#16KL	Krycklan	4	68	8	88	1

Sampling

Stream water was sampled in all seven sites at three different occasions. Water for the first bioassay was sampled just before the rising limb of the true spring flood, water for the second bioassay was sampled during the falling limb close to peak flow, and water for the last bioassay was sampled after the main spring flood in late May, (Figure 4). These three bioassays will from now on be denoted as “pre-flood”, “peak flood” and “post-flood”, respectively. At each site, the water was collected in two 2-litre, pre-washed and stream-water rinsed, HDPE bottles. The sampled water was stored at 4ºC and in the dark for maximum 24h until the bioassays were prepared.

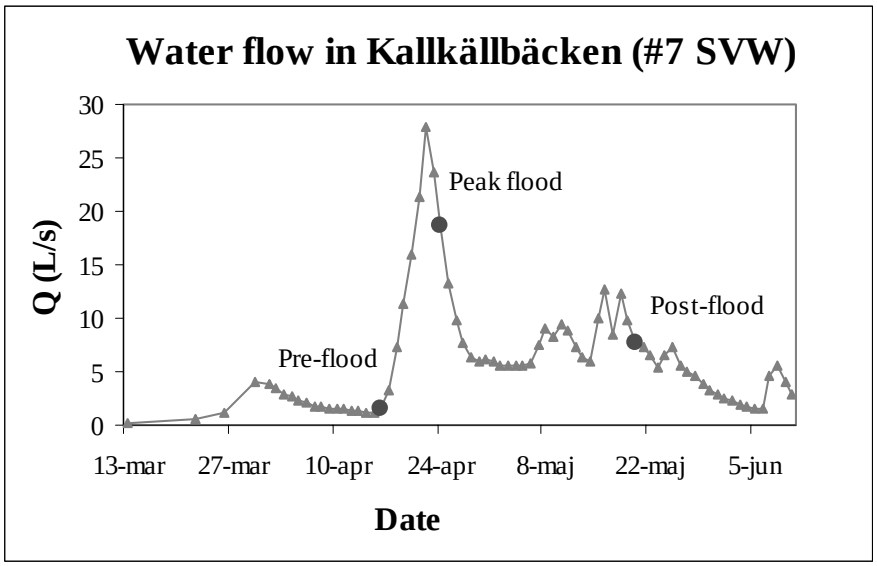


Figure 4. *Water hydrograph of Kallkällbäcken (#7 SVV). The red marks represent the three bioassays.*

Additional sampling

Water flow was measured continuously at all sites during the whole period of research. Periodic samples were taken for other important chemical and physical parameters, such as pH, aired pH, total organic carbon (TOC) concentrations, absorbance and major ions.

Sub sampling

The same sub sampling procedure was used for all three bioassays. Firstly, the water was filtered through 1µm glass-fiber filters (Whatman A/E) and poured into seven 4-litre glass bottles (one for each sample site). In this way, POM and macrofauna could be removed from the sample water. Thereafter, the water was stirred for several hours under reduced pressure, in order to get low CO_2 concentrations at the start of the bioassays. Finally, the water was sub sampled into washed and ashed 250ml glass bottles and sealed with gas tight caps equipped with septa. Bottles were weighed empty and with sample water. Maximum bottle mass, and cap mass were approximated from average values of bottle/ cap weights. Thereafter, sample volume and headspace volume in each bottle could be estimated. On average, initial water volumes were 200 ml with a 50 ml headspace.

Treatments

At each of the three bioassays, 70 bottles, 10 for each site, were used. The 10 bottles from each site consisted of 5 different treatments with two replicates for each treatment. The treatments were:

1) Control (C). No treatment, only addition of 2 ml Millipore water.
2) Nitrogen treatment (N). Addition of 1 ml NH_4NO_3- solution with 0.3 mg N ml^{-1} + 1ml Millipore water.
3) Phosphorous treatment (P). Addition of 1 ml K_3PO_4- solution with 0.03 mg P ml^{-1} + 1ml Millipore water.
4) Nitrogen and phosphorous treatments (NP). Addition of 1ml of each nutrient solution
5) Sterile filtration (S). Filtered through 0.22µm filters in order to get a bacteria-free solution. No other treatment, only addition of 2 ml Millipore water

The aim of the nutrient additions was to create a C:N:P ratio of 100:10:1, which are more than enough to satisfy bacteria in the water solution. Due to mistakes in the mixing of nutrient solutions, concentrations of only 0.15mg N ml^{-1} and 0.015mg P ml^{-1} were added in the pre-flood samples. However, these concentrations should still cover the potential need for the bacterial community and not limit mineralization rates. Nutrients were added, approximately six days from the start of each bioassay.

Between each analytical run the bottles were kept in sealed cardboard boxes in a dark and temperature controlled (20^oC) room, since both light and temperature are critical factors for CO_2 production. The bottles were shaken on a mechanical shaker during at least 12 hours before each analysis in order to equilibrate air and water within each sample. The samples were incubated for 89-93 days in total.

GC analysis

The samples were analyzed on a Perkin-Elmer Autosystem Gas chromatograph equipped with a flame ionization detector and a methanizer operating at 375ºC. Chromatography was carried out on a HaysepF column using He (70ml min^{-1}) as carrier gas. Headspace gas samples (0.3ml) were injected manually with 1ml plastic syringes (BD Plastipac) using disposable needles (Braun Stericon, 0.60*25 mm).

All samples were analyzed in duplicates. A deviation equal or less than 5% between the injections was accepted. CO_2 curves were detected by a GC recorder and integrated with Totalchrome 6.2 software. A standard gas mix with a CO_2 concentration of 5020 ppm was injected periodically during each analytical run. The ratio between peak area and known standard gas concentration was used to calculate the partial pressure of carbon dioxide for samples.

Calculations and statistics

Accounting for pH

The partitioning of carbon dioxide between bottle water and bottle headspace, are determined by the distribution of water and headspace volumes, but also by water pH, since carbon dioxide is buffered in water by the bicarbonate buffer system:

$$\left[H_2CO_3^*\right] = pCO_2K_H \qquad pK_H = 1.452 \qquad (1)$$

$$\left[H^+\right]\left[HCO_3^-\right] = \left[H_2CO_3^*\right] \qquad pK_1 = 6.352 \qquad (2)$$

$$\left[H^+\right]\left[CO_3^{2-}\right] = \left[HCO3^-\right] \qquad pK_2 = 10.33 \qquad (3)$$

where pCO_2 represents the partial pressure of carbon dioxide in headspace, and K_H, K_1 and K_2 are the equilibrium constants for equation 1, 2 and 3, respectively (Stumm and Morgan, 1996). If pH is known, total inorganic carbon (TIC) concentrations in a bottle can be calculated from the measured pCO_2 as follows:

-The amount of substance in headspace, is given by *the ideal gas law*:

$$n_H = \frac{pCO_2 * V_H}{R * T} \qquad (4)$$

where V_H is the headspace volume, R is the gas law constant and T is the temperature in Kelvin.

-The amount of substance in water is given by:

$$n_W = \left(\left[H_2CO_3^*\right] + \left[HCO_3^-\right] + \left[CO_3^{2-}\right]\right) * V_W \qquad (5)$$

where V_W is the volume of water in the bottle.

-Concentration of total inorganic carbon (moles/L) is given by:

$$[TIC] = \frac{(n_H + n_W)}{V_W} \qquad (6)$$

The calculations of total inorganic carbon concentrations are sensitive to changes in pH. The continuously increasing CO_2 concentrations in the bottles, also affects pH and, consequently, the input values in equations 1-6. Bottle pH was measured at the beginning and at the end of each bioassay. Sealed bottles made continuous pH measurements impossible. Therefore, the change in pH was estimated by modeled pH values, according to Laudon et al. (2000 (a)), using ANC, TOC, initial pH (aerated) and CO_2 as input data. This model provided a dynamic change in pH during the whole bioassays. The model is based on the assumption that: Changes in pH in the bottles are exclusively dependent on changes in CO_2 concentration, and everything else affecting pH remains constant. Since CO_2 concentrations were known from GC- analysis, they could all be used in the modeling process. Figure 5 presents the relation between measured and modeled pH values.

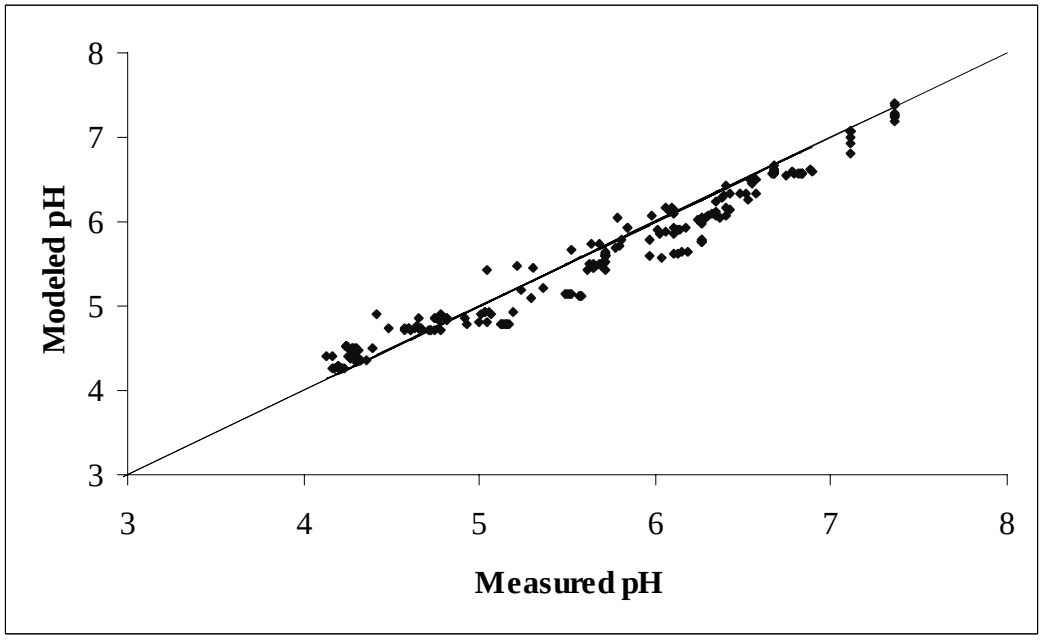


Figure 5. *The relation of measured pH and modeled pH. The line is a 1:1 line*

CO_2 production rates

The bioassay data sets, which describe the development of CO_2 production for all samples, were used to estimate CO_2 production rates (PrRs). No apparent logarithmic phase was detected in the data sets. Instead, PrRs were estimated by a linear regression of slopes, assuming zero-order kinetics, based on data from the day of nutrient addition and 15-16 days forward (Figure 6).

Total CO_2 production

The same data sets were used to estimate total CO_2 production (TPr). TPr is often defined as the difference between stationary phase and the lag phase (Figure 2). Because none of the samples reached a stationary phase during the 89-93 days of incubation, this definition was not applicable. Instead, TPr were defined in this work as "total production from the day of nutrient additions and 80 days forward". Since the number of days between the start point and end point were not exactly 80 days, the TPr were adjusted by using the rate between the last two measuring points (Figure 6).

PrRs and TPrs were normalized to total organic carbon (TOC) concentrations in order to compare the differences in bioavailability of the DOC pool despite differences in total concentrations. TOC were used as an approximation of DOC with based on the assumption that DOC/TOC ratios are high and remain constant in space and time (Laudon et al., 2000 (a)). Differences in PrRs and TPrs were explored through statistical analyzis (ANOVA Tukey´s test in Minitab 14; Bluman, 2001).

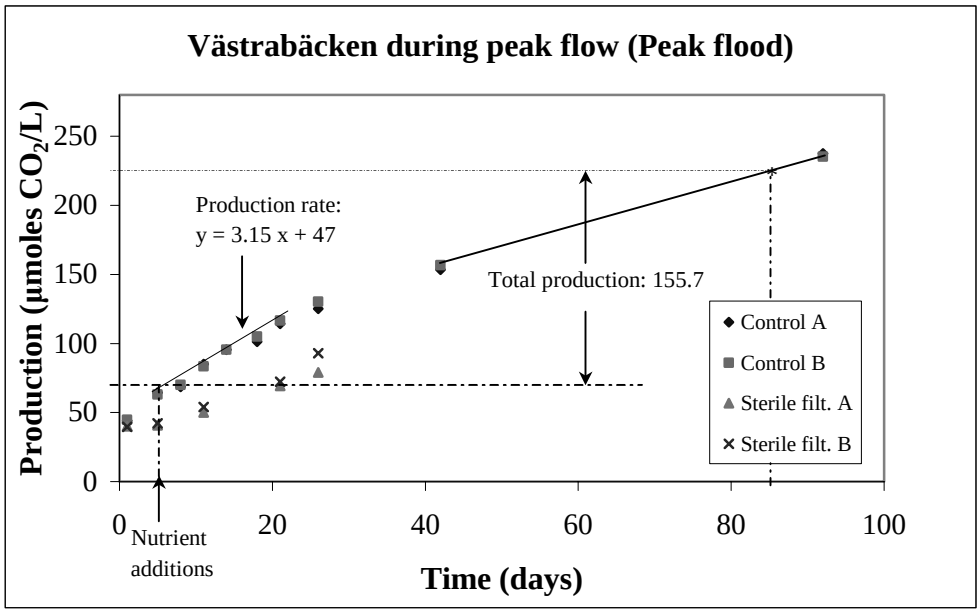


Figure 6. *Bacterial production in water from Västrabäcken (#2SVV) during peak flow (Peak flood) as an illustrative example. The figure includes two replicates of control bottles and two replicates of sterile filtered bottles. Linear regression has been used to estimate the CO_2 production rate, here represented by the slope of the line of Control bottle B. Total CO_2 production is represented by the production between the two dashed lines.*

RESULTS

The spring flood hydrograph

An early snowmelt started already in mid March, but was slowed down in the beginning of April due to a colder period. The main snowmelt started at 17th of April and peaked around 22nd of April. Thereafter, discharge declined, but not to the same low level as before the snowmelt. Recharged soils from snowmelt and rain kept the water flow relatively high until the end of May (Figure 7).

Variations in TOC concentrations

At most sites, the initially low TOC concentrations increased during the rising limb and slowly decreased after peak flow (Figure 7). There were two exceptions from this pattern: In Kallkällmyren (#4 SVE), the TOC concentration was initially very high. About 45mg TOC per liter water was found just before the rising limb. This TOC concentration drastically decreased to about 50 % at peak flow after which it slowly increased (Figure 7). Kallkällbäcken (#7 SVV), which is located just downstream the tributary of Kallkällbäcken and Västrabäcken, show a similar pattern as Kallkällmyren, but has a lower initial TOC concentration and smaller drop in TOC concentration during peak flow.

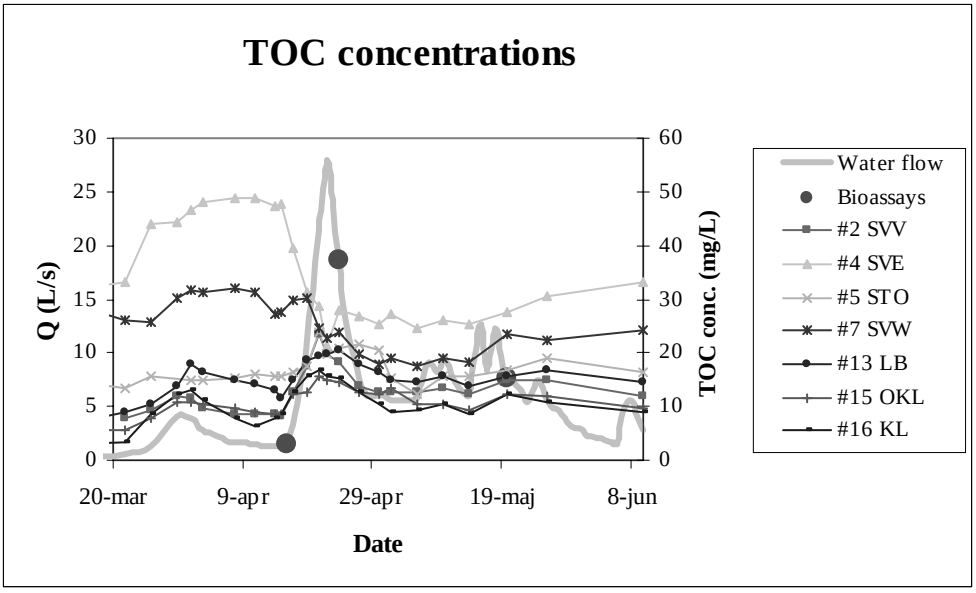


Figure 7. *Water flow and TOC concentrations during spring for the seven stream sites. Stream water flow is represented by the flow in Kallkällbäcken (#7 SVW). The red marks represent sampling dates of the three bioassays*

Variations in bioavailability over time

CO_2 production rates

Without normalizing the bioassay data to TOC concentrations, bacterial production rates (PrRs) were, in general, highest during peak flow. The only exception was the mire site, Kallkällmyren (#4 SVE), which had almost two times higher PrR during pre-flood than during peak flood (Figure 8a). After normalization to initial TOC concentrations the PrRs ranged from 0.10% to 0.43% of the initial carbon pool per day. Despite TOC normalization, there were still significant variations over time in bacterial CO_2 PrR. Almost all sites still had their highest PrRs during peak flow (Figure 8b). At Västrabäcken (#2 SVV), Kallkällbäcken (#7 SVW) and Övre Krycklan #15 OKL), peak-flood conditions enhanced PrRs with about 70%, 65% and 95%, respectively, in relation to pre-flood conditions. At Kallkällmyren (#4 SVE) and Krycklan (#16KL) the PrRs were approximately equal before and during peak flow. The lowest PrR at each site was found at post-flood conditions, except at Stortjärnen (#5 STO) (Figure 8b).

Total CO_2 production

The total CO_2 production over 80 days (TPr) normalized to TOC ranged from 6% to 20% of the initial TOC pool (Figure 9b). TPr revealed some differences in patterns compared to corresponding PrRs. The peaks that were observed for the PrRs at the forested sites during peak flow were less significant for the TPrs (Figure 9b). At Västrabäcken (#2 SVV), Kallkällbäcken (#7 SVV) and Övre Krycklan (#15 OKL), peak flow conditions enhanced TPrs with about 29%, 33% and 8%, respectively, in relation to pre-flood conditions. Stortjärnen (outlet) (#5 STO) revealed a unique pattern where TPr was highest after spring flood (Figure 9b).

Downstream comparison

In general, the PrR increased downstream in the catchment, with the lowest PrR in one of the 1st order stream sites and the highest PrR in Krycklan (#16 KL) or Övre Krycklan (#15 OKL) (Figure 8b). The increase in PrR at pre-flood conditions, when going from Västrabäcken (#2 SVV) to Krycklan, was 0.10% C day^{-1} to 0.43% C day^{-1}. The corresponding increase at peak flood and post-flood was 0.18% C day^{-1} to 0.42% C day^{-1} and 0.11% C day^{-1} to 0.18% C day^{-1}, respectively. This pattern is observed during the whole spring episode, with Stortjärnen (outlet) (#5 STO) as the only exception. Stortjärnen (outlet) had during post-flood, higher or almost equal PrR compared to sites further downstream. Similar downstream patterns as demonstrated by normalized PrRs, were found for the TOC normalized TPrs. The relative differences between sites were just slightly reduced (Figure 9b).

Comparison between different treatments

The additions of nutrients did not give a significant effect on CO_2 PrRs in any of the three selected stream sites (Figures 10a, 10c and 10e). In Västrabäcken (#2SVV) and Kallkällmyren (#4SVE), there were tendencies that additions of phosphorous, alone and in combination with nitrogen increased PrRs. These results indicated an up to 30% higher PrR compared to the

Control samples, at pre-flood and peak-flood conditions. At post-flood the pattern is less clear (Figures 10a and 10c). Krycklan (#16KL) demonstrates the reverse pattern. At pre-flood and at peak flood differences were irregular and indistinct, while the post-flood data indicated up to 50% higher PrRs after additions of nutrients (Figure 10e).

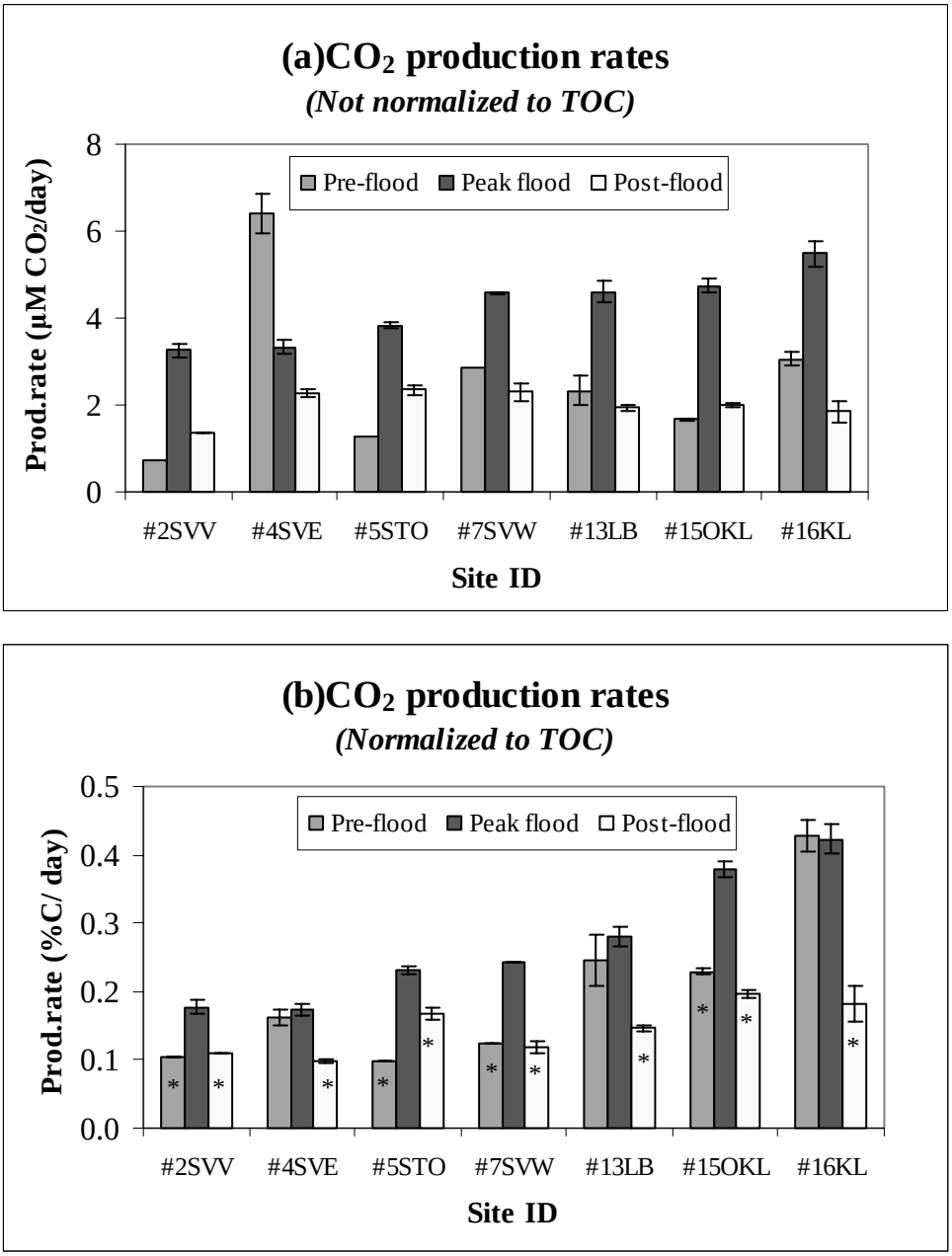
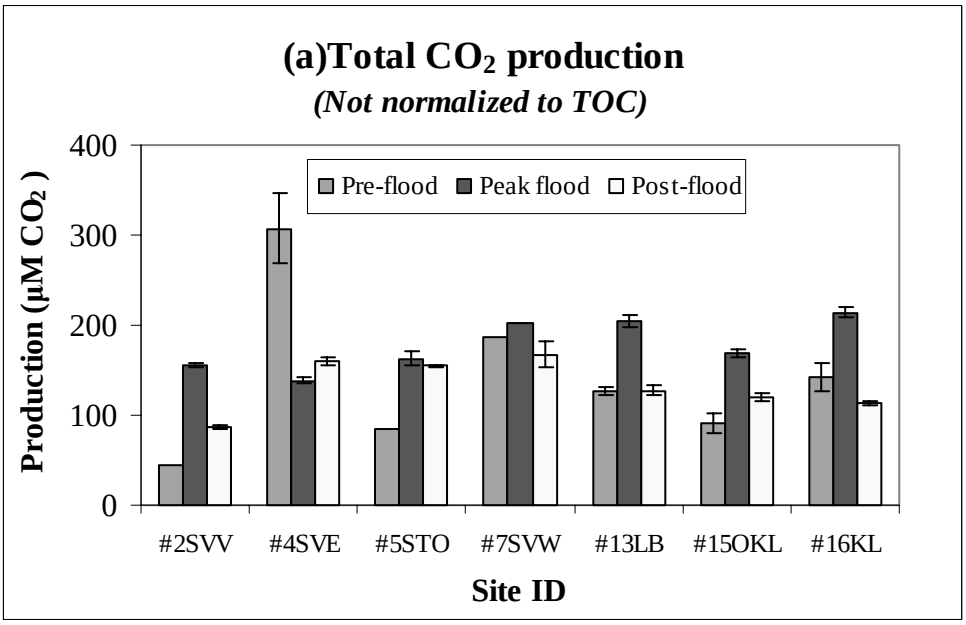


Figure 8a and 8b. *CO_2 production rates during spring 2003 for all seven sites. The boxes represents average PrRs from two replicates, except for #2 SVV, #5 STO and #7 SVV at the pre-flood bioassay, where only one replicate was used. The error bars represent +/- one standard deviation. PrRs are shown as "not normalized to TOC" (5a), given in µmoles per litre of water and day, and normalized to TOC (5b), given as percent of the total carbon pool that has been converted into CO_2 per day. * denotes that actual PrR is different from PrR at peak-flood conditions, with a statistical significance level of 0.05.*



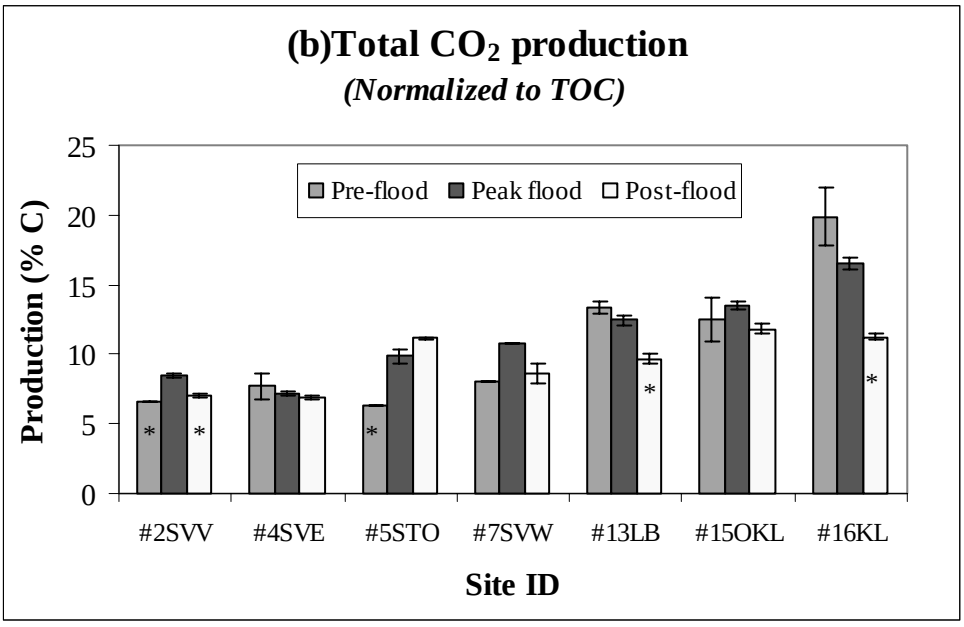


Figure 9a and 9b. *Total CO_2 production for all seven sites. The boxes represents average in TPrs from two replicates, except for #2 SVV, #5 STO and #7 SVV at the pre-flood bioassay, where only one replicate was used. The error bars represent +/- one standard deviation. TPrs are given in µmoles per litre of water (6a), and normalized TPrs are given in percent of the total carbon pool that have been produced as CO_2 (6b). * denotes that actual TC is different from TC at peak-flood conditions, with a statistical significance level of 0.05.*

Indications of increases in TPrs due to nutrient additions were found in all the three examined stream sites (Figures 10b, 10d and 10f). Phosphorous and especially phosphorous in combination with nitrogen had an effect at all sampling in both Västrabäcken (#2SVV) and Kallkällmyren (#4SVE). In Krycklan indications of nitrogen limitations were found prior to the spring flood (pre-flood) and phosphorous limitations post-flood. However, due to variability in replicates the differences were not significant.

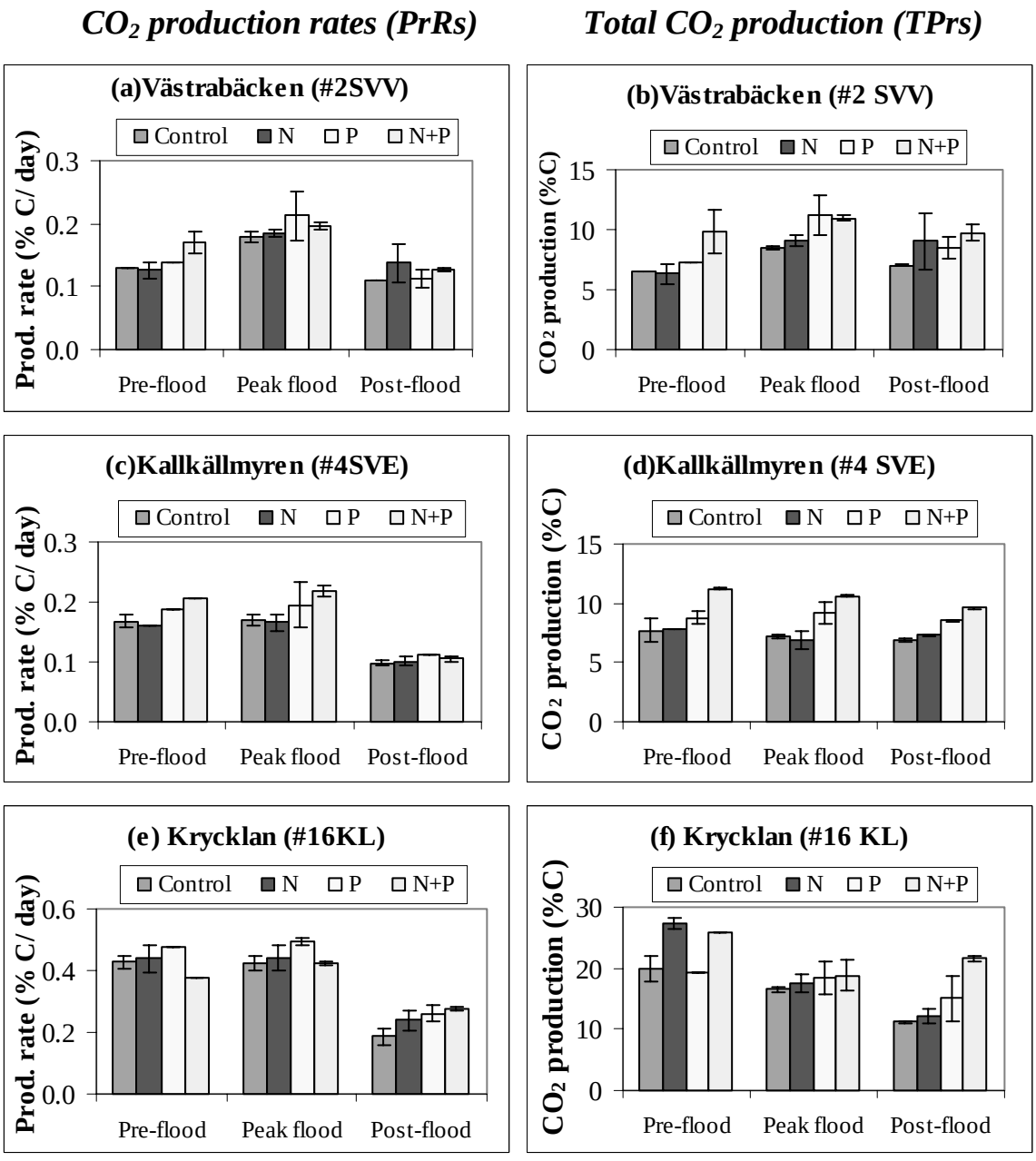


Figure 10a, b, c, d, e and f. *Nutrient effects on production rates and total production for Västrabäcken (a and b), Kallkällmyren (c and d) and Krycklan (e and f). The error bars represent +/- one standard deviation from the mean of two replicates. Where no error bars are found only one sample were correctly implemented.*

DISCUSSION

Variations in TOC concentrations

At most sites, low initial TOC concentrations increased rapidly during spring flood and slowly decreased after peak flow (Figure 7). This pattern agrees with patterns in earlier studies from the same area (Bishop and Pettersson, 1996; Hruska et al, 2001; Bishop et al, 2004), but also with other studies of major hydrological events (Hinton et al., 1998; Buffam et al., 2001). In the forested catchment Västrabäcken, the increasing TOC concentrations during peak flow are suggested to arise from changes in flow paths, as the ground water table reaches superficial, organic rich layers in the riparian zone (Bishop and Pettersson, 1996; Hruska, 2001). The mire-dominated catchment Kallkällmyren contradicts this pattern. That site had initially high pre-flood TOC concentration, which dramatically decreased during peak flow and, thereafter, slowly returned back to baseflow level. The high TOC concentration that was found before the spring flood had likely arisen from superficial flow paths through the organic rich soils in the mire. The quick drop in TOC concentrations during spring flood suggests that water from melting snow diluted stream TOC as no new flow paths were activated. In addition, impermeable soil frost could have induced overland flow, which would enhance the dilution (Bishop and Pettersson, 1996; Hruska, 2001). The similarity in pattern between Kallkällmyren and Kallkällbäcken illustrates the effect of mixing "mire-water" from Kallkällmyren, and "forest-water" from Västrabäcken and Kallkällmyren of downstream the mire (Figure 7).

Variations in bioavailability over time

TOC concentrations have large influences on the bacterial production rates and total production, since more DOM can be utilized per unit of water. The highest peaks found in the non-normalized data in figures 8a and 9a were to large extent induced by high TOC concentrations.

Even after data were normalized to TOC there were variations over time in bacterial PrR. Four of the sites still had, with more than 95% probability, their highest PrR during peak flow (Figure 8b). The results suggest that the relative concentrations of high quality DOM increased in forested systems during peak flow. A probable explanation for this pattern is that superficial flow in the riparian zone during spring flood, caused by raised ground water table, does more than enhance the TOC concentrations (Figure 7). The new flow paths also introduce more labile DOM in the streams, as the water passes through surface soils with relatively fresh detritus. An additional theory to explain the enhanced DOM bioavailability is that disrupted microbial cells due to freeze-thaw events can give a significant contribution to the bioavailable DOM pool (Christensen and Christensen, 1991; Stepanauskas et al, 2000). TPrs followed the same pattern as PrRs, but didn't vary as much as the PrRs. This indicates that spring flood didn't affect the size of the bioavailable pool to the same extent as it affected the DOM quality *per se* (Figure 8b and 9b). One exception from the above mentioned pattern was Kallkällmyren, where the PrRs were approximately equal at pre-flood and peak flood conditions. This pattern agrees with the conclusions of Bishop and Pettersson (1996)

concerning flow paths in Kallkällmyren. Since superficial flow probably is the main contributor to stream TOC even during winter, it is conceivable that the DOM quality is similar before and during spring flood.

Although both water flow and TOC concentrations were relatively high after spring flood, most stream sites had their lowest PrRs at this time (Figure 8b). This pattern suggests that high quality DOM, such as relatively fresh detritus and remains from disrupted bacteria, already had leached out to the stream during the spring flood and that the remaining DOM pool was more recalcitrant to bacterial consumption.

Similar patterns in DOM bioavailability during spring flood events have been observed in other Swedish studies. Wikner et al. (1999) found indications of elevated nutritional quality in estuarine waters during spring flood. In streams similar to the ones in this thesis, Stepanauskas et al. (2000) detected increased DOC concentrations and increased DON bioavailability during spring flood. On the other hand, in rivers from temperate and subtropical climates, no or negative effects on DOC bioavailability during high flows were reported (Leff and Meyer, 1991; Volk et al, 1997). Additionally, a study of an Appalachian catchment detected increasing DOC concentrations during stormflows due to changes in flow paths and DOC source pools, but no consistent changes in DOC bioavailability (Buffam, 2001). These contradicting results emphasis the importance of physical factors to stream DOM patterns during major hydrological events, such as surrounding vegetation, seasonality, temperature and flow paths.

An interesting note is the influences of mire and forest headwater on Kallkällbäcken. Mire water from Kallkällmyren seemed to have a large influence on the TOC concentration patterns in Kallkällbäcken (Figure 7). When instead comparing the patterns of PrRs, Kallkällbäcken seemed to be more influenced by forest water from Västrabäcken and Kallkällbäcken downstream Kallkällmyren (Figure 8b).

Downstream comparison

The data clearly suggests that bioavailability, both the quality (PrR) and the size of the bioavailable pool (TPr), increased further downstream in the catchment (Figure 8b and 9b). These results contradict results from other studies, where DOM bioavailability decreased downstream (Leff and Meyer, 1991; Sun et al., 1997). These downstream decreases in bioavailability are, according to Sun et al. (1997) mainly attributable to selective degradation of aliphatic carbon in riverine DOM. The degradation in the water of Krycklan during spring flood is probably rather small due to low water temperatures (close to 0ºC at peak flow), which reduces the relevance of comparing this study with the above-mentioned studies. However, this still does not explain the increasing pattern found in the Krycklan catchment. Some possible explanations of increasing bioavailability downstream the catchment are:

1) DOM bioavailability in the streams is improved due to photochemical degradation of refractory DOM.
2) The DOM contributions from riparian soils further downstream in the catchment are less recalcitrant.
3) Other chemical components (e.g. pH) and not only DOM bioavailability are affecting the bacterial production in the water.

1) Photochemical degradation
Even though Wiegner and Seitzinger (2001) emphase the importance of microbial degradation rather than photochemical degradation, the transformation of DOM by solar radiation can be an important process for carbon turnover in surface waters, especially for humic waters (Tranvik and Bertilsson, 2001; Köhler et al., 2002). Köhler et al. (2002) have in studies at the area of Svartberget detected considerable changes in both TOC concentrations and TOC character due to photo degradation. However, the possibility that photo degradation improved DOM quality as spring-flood water moves downstream is not likely, since most of the water still was covered with ice during the sampling occasion and the residence time of water in the stream during peak flow is low (less than 24 hours).

2) Improved quality in DOM contributions further downstream
Further downstream in the catchment, more deciduous trees and shrubs are surrounding the streams. Litter from deciduous sources has, in general, higher nutritional quality than corresponding organic matter in coniferous or mire areas (Allan, 1995). Also cultured fields, which are found in some parts of the downstream areas, may have some influence on the DOM contributions. It is uncertain whether these contributions are large enough to enrich stream water DOM in competition with the poorer inputs from deep ground waters and upstream stream water.

3) pH as an controlling mechanism of bacterial production
The pH is, in general, an important controlling factor of bacterial production. Too low or too high pH will be an energy cost for the organisms, since more energy will be needed to maintain the pH within the cell at an appropriate level. Under highly acidic or alkaline conditions, cell components may be hydrolyzed or enzymes may be denatured. Additionally, extremely low pH (<4) can have secondary effects, such as increasing movement of aluminum, and decreasing availability of phosphate (Atlas and Bartha, 1987). Most of known bacterial species grow within the pH range of 4 to 9, or within smaller segments of the range (Paul and Clark, 1996).

pH varied from 4.2 in Kallkällmyren to 6.9 in Krycklan. Figure 11 illustrates the relation between PrRs and pH. pH generally increased moving downstream in the catchment (represented by the lines in figure 11). There were also changes in pH within the sites during the spring flood episode, since TOC concentrations affects the buffering capacity in streams (Lydersen, 1998; Laudon, 2000). Despite pH variations of more than one unit for some sites, no clear correlation with the PrRs could be found. At some sites the PrR was increased even as pH decreased. This suggests that changes in pH within sites during the spring flood are too small to affect the PrRs and that changes in PrRs within sites are dependent on other factors than pH, i.e. the DOM bioavailability. Overall, it is difficult to draw any certain conclusions concerning pH dependence based on the empirical material derived from this investigation, but it should not be ignored when evaluating the downstream patterns in DOM bioavailability.

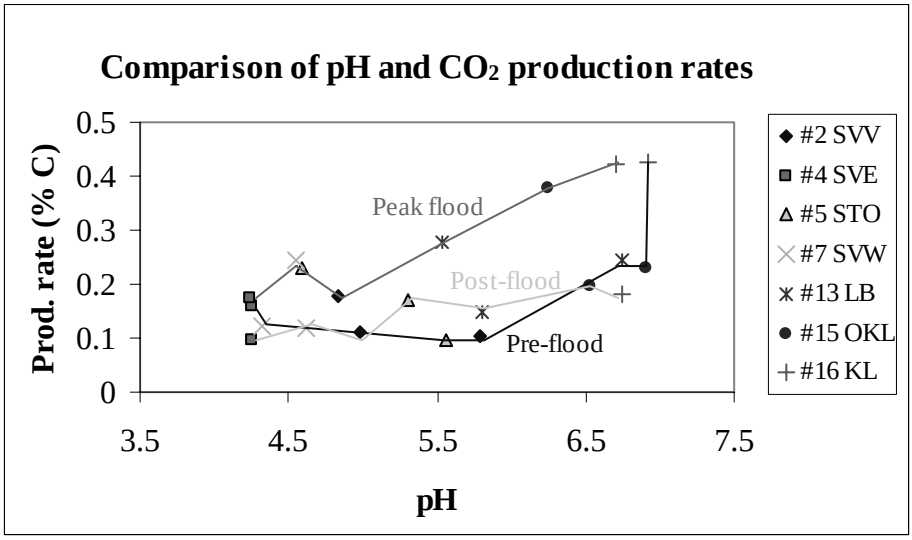


Figure 11. *Relations between pH and CO_2 production rates for all bio-assays and all sites. Each symbol represent a site and the symbols connected by a line represents a bioassay. Production rates are normalized to TOC.*

Comparison between different treatments

The nutrient additions did not give more than indications of increasing PrRs or increasing TPrs (Figure 10a-f). Stepanaukas (2000) detected increased potential DON bioavailability during peak flow in two streams in northern Sweden. This can explain why nitrogen additions, in general, had minor effects on PrRs and TPrs in this study. In most cases phosphorous alone or in combination with nitrogen yielded the largest PrRs and TPrs. The limited effects of nitrogen and phosphorous additions imply that carbon is the main limiting factor for bacterial production.

Consequences of DOM fluctuations to stream ecosystems

Both DOM concentrations and DOM bioavailability increased at most sites during spring flood. The question is how much this change in potential productivity will influence the stream ecosystems? Locally, the effects are likely to be small. Most of the microbial activity occurs in biofilms, which are fueled by OM. Studies of hydrological events have detected scouring of biofilms on rocks and sediments due to large velocity and turbulent water during peak flow (Gremm and Kaplan, 1998; Buffam, 2001). This implies that *in situ* productivity may have decreased as the production potential was removed to the water column.

The PrRs and TPrs in water column ranged between 0.10% and 0.43 % of initial TOC per day and between 6% and 20% of the initial TOC, respectively (Figure 7b and 8b). The residence time for headwater to reach the catchment outlet is at most a few days, which is a short period relative to the time scale of the TPrs (80 days). Obviously, the bacterial production in water

column cannot be high within the catchment. Furthermore, temperature is an important limiting factor or bacterial production (Atlas and Bartha, 1987). This study was performed at a constant temperature of 20ºC and water temperatures in situ ranged between 0 and 11ºC. The highest temperatures were found after spring flood in the lake outlet. During spring flood, when TOC concentrations and DOM bioavailability were highest, the temperatures were close to 0ºC in all streams. Consequently, before most of the bioavailable DOM is utilized by microorganisms it will probably be transported out of the catchment. This suggests that larger rivers, lakes and oceans will receive relatively high quality DOM that may be important for the energy and nutrient turnover.

CONCLUSIONS

In most streams the TOC concentration increased during the spring flood. These results supports conclusions from earlier studies of TOC patterns in the same catchment (Bishop and Pettersson, 1996; Hruska, 2001; Bishop et al., 2004), suggesting that ground water table reaches superficial organic rich soil layers in the riparian zone and enhances TOC concentrations in stream water.

In most streams the potential bacterial production increased during peak flood, but not only due to higher TOC concentrations in water. The potential bacterial bioavailability of DOM also increased. Both the total bioavailable DOM pool (TPr) and the quality of the bioavailable DOM pool (PrR) were higher in most forested streams during peak flood. Surface soil layers in the riparian zone, apparently, contain more bioavailable material with higher quality, such as relatively fresh detritus and, possibly, disrupted bacterial cells.

The mire outlet, Kallkällmyren, demonstrated the opposite TOC pattern, with high concentrations during winter and a dilution during spring from melting snow. DOM bioavailability of mire water stayed relatively constant before and during spring flood. This suggests that the flow paths are constantly superficial during most of the year and no new sources of DOM are transported to the stream.

PrRs and TPrs increase as we move downstream in the catchment of Krycklan. The downstream increase can possibly arise from improved litter quality in the riparian zone, but the positive correlation between pH and PrRs implies that pH may have an impact on bacterial mineralization rates. A follow-up study on this issue, augmented by analysis of pH-standardized samples between stream sites could further clarify the downstream patterns in DOM bioavailability.

The residence time of water within the catchment is small and water temperatures during spring flood are low. Therefore, it is likely that most of the bioavailable DOM will be utilized outside the catchment of Krycklan and spring-flood contributions can, consequently, play an important role for carbon and nutrient cycling in rivers, lakes and the ocean further downstream.

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