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Exoenzyme activities as indicators of dissolved organic matter composition in the hyporheic zone of a floodplain river

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SUMMARY

1. We measured the hyporheic microbial exoenzyme activities in a floodplain river to determine whether dissolved organic matter (DOM) bioavailability varied with overlying riparian vegetation patch structure or position along flowpaths.

2. Particulate organic matter (POM), dissolved organic carbon (DOC), dissolved oxygen (DO), electrical conductivity and temperature were sampled from wells in a riparian terrace on the Queets River, Washington, U.S.A. on 25 March, 15 May, 20 July and 09 October 1999. Dissolved nitrate, ammonium and soluble reactive phosphorus were also collected on 20 July and 09 October 1999. Wells were characterised by their associated overlying vegetation: bare cobble/young alder, mid-aged alder (8-20 years) and old alder/old-growth conifer (25 to >100 years). POM was analysed for the ash-free dry mass and the activities of eight exoenzymes (x-glucosidase, B-glucosidase, B-N-acetylglucosaminidase, xylosidase, phosphatase, leucine aminopeptidase, esterase and endopeptidase) using fluorogenic substrates.

3. Exoenzyme activities in the Queets River hyporheic zone indicated the presence of an active microbial community metabolising a diverse array of organic molecules. Individual exoenzyme activity (mean ± standard error) ranged from 0.507 ± 0.1547 to 22.8 ± 5.69 umol MUF (g AFDM)$^{-1}$ h^{-1}, was highly variable among wells and varied seasonally, with the lowest rates occurring in March. Exoenzyme activities were weakly correlated with DO, DOC and inorganic nutrient concentrations.

4. Ratios of leucine aminopeptidase: B-glucosidase were low in March, May and October and high in July, potentially indicating a switch from polysaccharides to proteins as the dominant component of microbial metabolism.

5. Principal components analysis indicated that there were patch effects and that these effects were strongest in the summer.

6. DOM degradation patterns did not change systematically along hyporheic flowpaths but varied with overlying forest patch type in the Queets River hyporheic zone, suggesting that additional carbon inputs exist. We hypothesise that the most likely input is the downward movement of DOM from overlying riparian soils. Understanding this movement of DOM from soils to subsurface water is essential for understanding both the hyporheic metabolism and the carbon budget of streams and rivers.

Keywords: dissolved organic matter, exoenzyme activity, floodplain river, hyporheic, soil patches

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Introduction

An important component of many streams and rivers is the hyporheic zone, the region beneath and lateral to the wetted channel where surface water and groundwater mix (Triska *et al., 1*989; Edwards, 1998). This area is a dynamic habitat that influences both the amount and chemical composition of materials delivered from the catchment to the river (Triska, Duff & Avanzino, 1993; Valett *et al.,* 1996). Hyporheic sediments also harbour diverse biological communities (Williams & Hynes, 1974; Stanford & Ward, 1988; Boulton, Valett & Fisher, 1992) that can represent a significant proportion of ecosystem secondary productivity (Smock *et al., 1992).*

Hyporheic zones are heterotrophic and dependent on external sources of organic matter to support biological activity. In some rivers, buried particulate organic matter (POM) is the primary carbon source for microbial respiration in the hyporheic zone (Pusch & Schwoerbel, 1994; Fischer, Pusch & Schwoerbel, 1996; Naegeli & Uehlinger. 1997; Fischer, Wanner & Pusch, 2002). Most studies, however, consider labile dissolved organic matter (DOM) from surface water inputs to be the most important carbon source supporting hyporheic metabolism (Jones, Fisher & Grimm, 1995; Findlay & Sobczak, 1996; Holmes *et al.,* 1998). Where surface water penetrates the sediments, available DOM is rapidly immobilised by direct microbial uptake and physical sorption onto gravel surface epilithon. As water moves along subsurface flowpaths. DOM quantity and quality (bioavailability) decrease, leaving microorganisms at the downstream end of flowpaths potentially carbon limited (Findlay & Sobczak, 1996; Sobczak & Findlay, 2002). This pattern has been demonstrated in the hyporheic zone of many rivers where researchers have examined small-scale flowpaths (tens of metres) through gravel bars or processes centred at upwelling and downwelling sites (Jones *et al., 1995).*

In floodplain rivers, hyporheic flows are associated with former channel locations abandoned by channel meandering and can occur over hundreds of metres (Stanford & Ward, 1993; Edwards, 1998). If patterns of rapidly decreasing DOM bioavailability are consistent between small and large rivers, rapid DOM uptake at the flowpath head would leave downpath microorganisms carbon limited, resulting in most large river hyporheic zones being potentially unproductive.

Extensive riparian forests occur on large, intact floodplain rivers in the Pacific Northwest (PNW) ecoregion of the U.S.A. (Naiman & Bilby, 2001). These forests are a mosaic of successional stages ranging from recently deposited cobble, through alder of various ages, to mature old-growth mixed-conifer or hardwood stands. This diverse topography, coupled with the longer flowpaths of floodplain hyporheic zones, may allow for significant DOM inputs at points along the flowpath from overlying riparian soils. In the Queets River, Washington, previous research indicated that hyporheic DOC concentrations do not decrease monotonically as water flows beneath riparian terraces, suggesting that additional inputs occur along the flowpath, and the movement of material from overlying riparian soils is the possible source of additional carbon (Clinton, Edwards & Naiman, 2002). Soil carbon and nutrient composition vary systematically with age and forest patch composition such that soil leachates are predictably related to the overlying riparian patch structure (Bechtold, Edwards & Naiman, 2003). Therefore, we have hypothesised that labile DOM inputs from riparian soils are important in supporting microbial metabolism and that the bioavailability of these inputs varies with the overlying riparian vegetation structure.

Quantifying DOM, bioavailability is a challenge, however, because only a fraction of DOM is available for microbial growth (Thurman, 1985). Most DOM is composed of high molecular weight material that requires enzymatic degradation prior to microbial uptake (Chrost, 1991). Microorganisms express a suite of exoenzymes that reflect both microbial nutritional demand and DOM composition. Exoenzyme activities have been used to follow DOM catabolism in a variety of lake (Chrost, 1989; Munster *et al., 1*992), river (Sinsabaugh *et al., 1*997; Rulik & Spacil. 2004) and marine (Arnosti, 2002) ecosystems. By analysing a set of exoenzymes that acquire carbon (C), nitrogen (N) or phosphorus (P) acquiring exoenzymes, we can describe a 'microbial exoenzyme fingerprint' (MEF) that represents DOM undergoing degradation. Although this MEF does not provide information on DOM chemical composition or uptake rates, comparisons across time and space do tell us whether microbial communities are using similar or different forms of DOM. Analysis of individual exoenzyme rates with environmental parameters can also provide

insight into certain microbial processes (e.g. nutrient limitation).

In this article, we use microbial exoenzyme activities as a bioassay to infer differences in DOM composition in a floodplain river hyporheic zone. We measured eight exoenzyme activities in the hyporheic zone of a riparian terrace to determine seasonal DOM bioavailability, qualitative differences in DOM at different locations within the hyporheic zone and whether these differences relate more strongly to the overlying riparian vegetation or distance along a flowpath.

Methods

Study site

The study was conducted on the lower alluvial section of the Queets River (25 km from the ocean), Olympic National Park, Washington (Fig. 1). The Queets River is an intact coastal floodplain river draining 1153 km^2. The headwaters of the Queets River are on Mount Olympus and the catchment receives about 3 m $year^{-1}$ of precipitation, mostly as rain during autumn and winter.

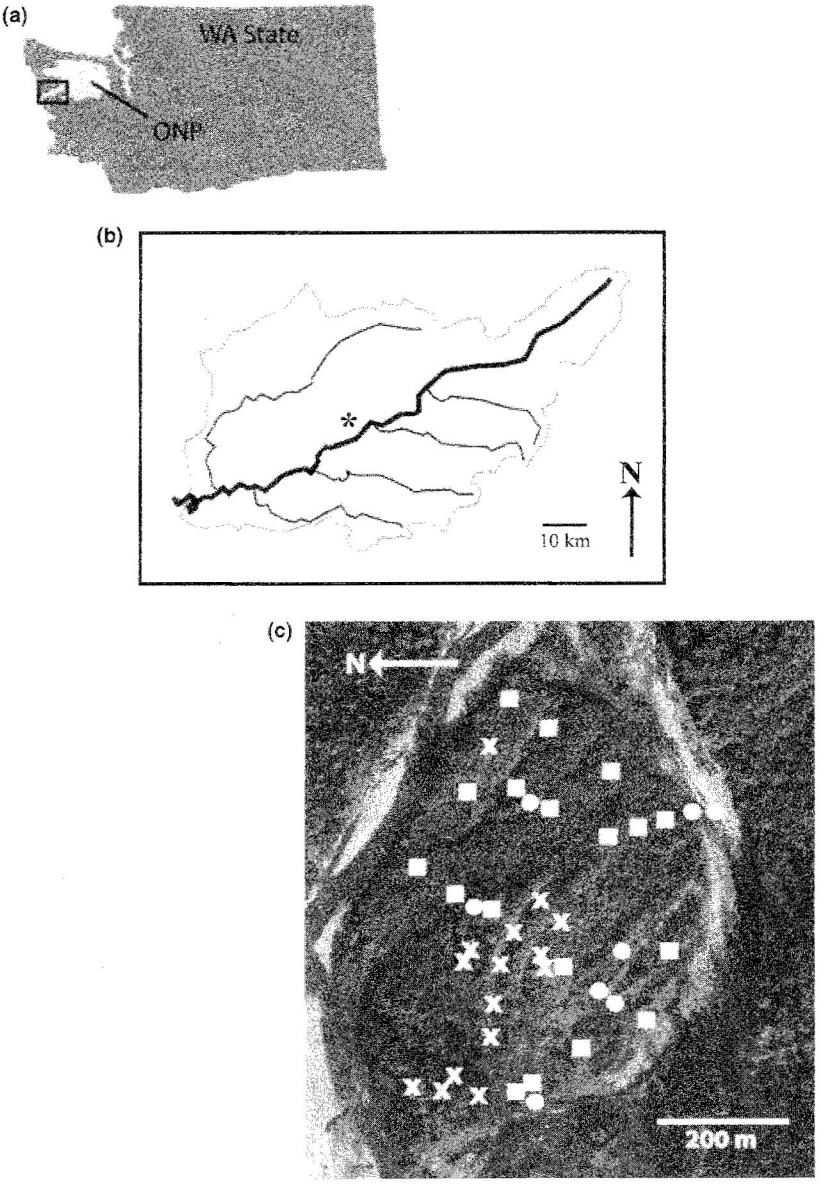

Fig. 1 Location of the riparian terrace (*) on the Queets River in Olympic National Park (ONP), Washington (WA), U.S.A. The aerial photograph of the terrace study site shows the sample well locations and the vegetation type designations where circles represent wells in gravel, squares represent wells in young/mid-aged alder and crosses represent wells in old alder/old growth (see Methods for further descriptions). The Queets Rivers flows east to west on the north side of the terrace. Not all wells were sampled on each date.

The Queets River is hydrologically dynamic and rapid channel migration has created a complex pattern of side and abandoned river channels. The floodplain is composed of coarse glacial outwash sediments reworked by repeated flooding and channel movement. These coarse sediments and a meandering channel result in hyporheic flows of 100s of metres through cobble bars and vegetated riparian terraces.

A riparian terrace (Fig. 1) was instrumented with 55 piezometers during summer 1998 and 1999. Piezometers were 1" polyvinyl chloride (PVC) tubing open on both ends and placed 30 cm into the water table during summer low flow. Depending on well location, total wen depth varied from 1 to 4 m. Each piezometer was surveyed for elevation relative to an arbitrary datum using a stadia rod and level (TopCon AT-F6 autolevel, Tokyo, Japan), and lateral position using a Trimble (Sunnyvale, CA, U.S.A.) differentially corrected global positioning system (accurate to <1 m horizontally). Wells were capped when not in use to prevent surface contamination.

Each well was categorised based on the composition of the overlying riparian vegetation (Fig. 1). Overlying patches were classified as bare cobbles, various age classes of red alder *(Alnus rubra* Bong.), young conifer [mainly Sitka spruce *(Picea sitchensis* (Bong.) Carr.) with some Scoulers willow *(Salix scouleriana* Barr.)] and mixed old-growth forest. Red alder ranged in size from 2 to 40 cm (diameter breast height, DBH) and in age from 8 to 40 years old. The largest trees, which were primarily in the northwestern section of the terrace, were dominated by older Sitka spruce [Sitka spruce *(Picea sitchensis* (Bong.) Carr.] and black cottonwood *(Populus trichocarpa* Torr. & Gray). These trees ranged in size from 50 to 100 em (DBH) and are 50 to more than 100 years old. Three patch types were delineated: bare cobble/young alder, mid-aged alder (8-20 years) and old alder/old-growth conifer (25 to >100 years).

Hydraulic conductivity was measured once for each well in either 1998 or 1999 using the falling head test (Freeze & Cheery, 1979). In the Queets River hyporheic zone, sediments are highly conductive with measured conductivities ranging from 8.03×10^{-7} to 1.75×10^{-3} ms^{-1} (Clinton *et al.,* 2002).

Field and laboratory procedures

We sampled microbial biofilm using a plunger rod that consisted of fibreglass wands screwed together with leather pump washers mounted onto the bottom end. The washers made a watertight seal with the piezometer walls, so that when the rod was inserted to the bottom of the well and agitated, hyporheic water was rapidly forced in and out of the piezometer opening. The shearing action of this water movement suspended loosely attached biofilm from sediment surrounding the piezometer tip into the well water, which was sampled (25 ml.) into sterile Whirlpacks (NASCO, Fort Atkinson, Wisconsin, U.S.A.) using a peristaltic pump attached to a hand drill. Samples were kept on ice until return to the laboratory, within 24 h and then frozen until exoenzyme analysis. Biofilm was collected from a subset of wells representing the three patch types on 25 March, 15 May, 20 July and 9 October 1999. The number of samples analysed was 14, 27, 21 and 11, respectively.

Assayed exoenzymes and their associated fluorogenic substrates (substrate: abbreviation) included: x-glucosidase(4-MUF-x-D-glucoside:ALPHA), *B*-glucosidase (4-MUF-*B*-D-glucoside: BETA),*B* -N-acetyl-glucosaminidase (4-MUF-N-acetyl-*B*-glucosaminide: NAG), xylosidase (4-MUF-*B*-D-xyloside:XYL), phosphatase (4-MUF-phosphate: PHOS), leucine amino-peptidase (L-lucine-7-amido-4-methyl-coumarin: LEU), esterase (4-MUF-acetate: ACE) and endopeptidase (4-MUF-p-guanadinobenzoate: GUAN). All substrates were made up in distilled water except PHOS, ACE and GUAN, which were made up in a bicarbonate buffer solution. Two hundred micro litres of sample was incubated with 100 uL of each substrate in a Well plate (final substrate concentration 333 *u*M). Fluorescence was recorded over time at room temperature to develop a kinetic curve, and substrate degradation was calculated from the increase in fluorescence during the linear phase. Fluorescence values were corrected for quenching by adding a strong standard (10 nmol 4-methylumbelliferone) to each sample and comparing the fluorescence reading with the standard in buffer or water alone. Following exoenzyme assay, the samples were refrozen until further analyses for POM by mass loss on ignition. LEU and GUAN were classified as N-acquiring exoenzymes, PHOS as P-acquiring and the remaining five exoenzymes as C-acquiring.

Particulate organic matter was measured on pre-ashed Whatman *GF/F* filters (Maidstone, Kent, U.K.). Samples were initially dried for 48 h (60°C), weighed (dry mass), ashed in a muffle furnace for 3 h (500 °C) and reweighed (ashed mass). Organic matter was calculated as the dry mass minus the ashed mass. To standardise among piezometers, exoenzyme activity was expressed per g AFDM. Organic matter was analysed at the College of Forest Resources, University of Washington, Seattle, WA. All exoenzyme assays were conducted at the Institute of Ecosystem Studies, Millbrook, New York.

Head, temperature, dissolved oxygen (DO), electrical conductivity (EC) and dissolved organic carbon (DOC) were measured on each sampling date from each well. Head was measured by lowering a metal measuring tape down the well until interception with the subsurface water was indicated by a distinct sound. Temperature, DO and EC were measured using YSI (YellowSprings, OH, *U.S.A.)* metres. Dissolved organic carbon was collected by sampling water from each wen using a battery driven-peristaltic pump and polyethylene tubing. The water was immediately filtered through a Whatman *GF/F* filter (Maidstone, Kent, U.K.) into a 20-mL glass vial. Both the filter and the vial were pre-combusted at 450 and 500°C, respectively, prior to sampling. Water samples were collected in triplicate per well and preserved with mercuric chloride (l00 nM final concentration) in the field. Samples were analysed using a MQ 1001TOC analyzer (Quian & Mopper, 1996). These data are summarised in Clinton *et al.* (2002) for the complete well grid and in Table 1 for the subset of wells sampled for EEA.

Inorganic nutrients were collected on 20 July and 9 October 1999by sampling water from each well with a battery-driven peristaltic pump and polyethylene tubing prior to sampling biofilm for exoenzyme analysis. Samples were immediately filtered **in** the field through Whatman *GF/F* filters (Maidstone, Kent, U.K.) into acid-washed (10% HCl) 60-mL polyethylene bottles. Samples were kept on ice and immediately frozen on return to the laboratory. All nutrients were analysed using protocols from the Joint Global Ocean Flux Study (Knap *et al.,* 1994).Nitrate-nitrogen was analysed using a modification of Wood, Armstrong & Richards *et al.* (1967) procedure and orthophosphate using a modification of the Bernhardt and Wilhelms (1967) method. All nutrient analyses were conducted at the School of Oceanography analytical chemistry laboratory, University of Washington, Seattle, Washington.

Data analyses

Seasonal and patch differences among exoenzymes were examined using two-way analysis of variance (ANOVA). There were no strong interaction effects between date and patch; therefore, main effects are reported separately. Significant differences among dates were tested using the Games-Howell test. The Games-Howell was chosen over other multiple comparison tests because of the uneven sample sizes and the non-homogeneity of variance among factors for a given exoenzyme. Linear regression was used to investigate relationships at the whole terrace level between exoenzyme activities and hydraulic head, DO, DOC and inorganic nutrient (for N- and P-acquiring exoenzymes) concentrations. Hydraulic head served as a surrogate for position along the flowpath for all wells in the terrace (Clinton *et al., 2002).* Exoenzyme activity data were log transformed as necessary to meet assumptions of normality for both the ANOVA and linear regression analysis.

In addition to individual activities, exoenzymes were grouped to represent the relative importance of various organic molecules to microbial metabolism: sum of C-acquiring, sum of N-acquiring, P-acquiring, mean nitrogen availability and mean phosphorus availability. Mean nitrogen availability (N_{avail}) was summarised as the mean of the ratio of each carbon acquiring enzyme to the sum of the nitrogen acquiring enzymes (E_N) (Sinsabaugh & Moorhead, 1994).

$$E_N = \Sigma LEU + GUAN \tag{1}$$

$$\begin{aligned}\text{Total } N_{avail} &= \Sigma E_C/E_N \\ &= ALPHA/E_N + BETA/E_N + NAG/E_N \\ &\quad + XYL/E_N + ACE/E_N\end{aligned} \tag{2}$$

$$N_{avail} = \frac{\text{Total } N_{avail}}{5} \tag{3}$$

Mean phosphorus availability (P_{avail}) was summarised as the mean of the ratio of each carbon acquiring enzyme (EC) to the phosphorus acquiring enzyme (E_P) (Sinsabaugh & Moorhead, 1994).

$$E_P = \Sigma PHOS \tag{4}$$

Table 1 Mean ± standard deviation and range of physical and chemical parameters measured on each sample date from all wells in the hyporheic zone of the Queets River, Washington

	Head (m)	Temperature (degrees Celsius)	Dissolved oxygen (mg L^{-1})	Electrical conductivity (ohms cm^{-1})	Dissolved organic carbon (mg L^{-1})
25 Mar 99 (14)	97.7 ± 0.70 (96.5–98.5)	6.56 ± 0.97 (4.30–8.20)	5.79 ± 2.13 (1.80–8.94)	63 ± 8 (44–70)	1.26 ± 1.05 (0.47–4.33)
15 May 99 (20)	97.4 ± 0.62 (96.4–98.3)	9.95 ± 1.13 (8.30–12.60)	4.74 ± 2.11 (1.75–9.19)	67 ± 7 (44–76)	0.73 ± 0.252* (0.36–1.38)
20 Jul 99 (21)	97.5 ± 0.67 (96.4–98.5)	12.01 ± 1.58 (10.20–16.70)	3.82 ± 2.33 (0.57–7.64)	66 ± 7 (57–80)	1.89 ± 1.31** (0.54–4.55)
09 Oct 99 (10)	97.7 ± 0.59 (96.5–98.4)	11.63 ± 0.89 (10.00–13.00)	4.60 ± 1.56 (2.03–7.02)	72 ± 6 (62–81)	0.47 ± 0.114*** (0.31–0.58)

	Ammonium (mg L^{-1})	Nitrate (mg L^{-1})	Soluble reactive phosphorus (mg L^{-1})
25 Mar 99 (14)			
15 May 99 (20)			
20 Jul 99 (21)	3.60 ± 4.34 (0.27–17.4)	42.0 ± 30.4 (2.87–114)	2.76 ± 1.47 (0.90–6.86)
09 Oct 99 (10)	1.10 ± 0.840 (0.38–3.14)	107 ± 85.6 (1.73–305)	3.82 ± 1.71 (1.86–7.31)

Sample sizes for each date are indicated in parentheses. *sample size = 19 wells; **sample size = 18 wells; ***sample size = 8 wells. Physical and chemical parameters for the complete well grid are available in Clinton, 2001 and Clinton *et al.*, 2002.

$$\text{Total } P_{avail} = \Sigma E_C/E_P = ALPHA/E_P + BETA/E_P + NAG/E_P + XYL/E_P + ACE/E_P \quad (5)$$

$$P_{avail} = \frac{\text{Total } P_{avail}}{5} \quad (6)$$

Mean nitrogen and phosphorus availability are an indication of the microbial investment in nutrient acquisition. For example, a low N_{avail} would indicate a relatively higher microbial investment in N-acquisition that reflects the lower N availability in the environment.

The exoenzyme ratios ALPHA : BETA and XYL : BETA were used to indicate the potential importance of polysaccharide degradation in microbial metabolism. Higher values of ALPHA indicate the dominance of starch relative to cellulose (BETA) whereas a higher value of XYL indicates the dominance of hemicellulose relative to cellulose (BETA). Starch is a component of algae and plant seeds, whereas cellulose forms plant cell walls and is a component of algae. Hemicellulose, while also forming cell walls, has a molecular structure that includes other sugars besides glucose such as xylose and mannose, Finally, the LEU: BETA ratio was used to indicate the potential importance of protein to carbohydrates in microbial metabolism.

Principal component analysis (PCA) was used to investigate whether microbial communities within the terrace expressed similar allocations of enzyme activities. By analysing for the complete set of C-, N- and P- acquiring exoenzymes, we can describe a 'microbial exoenzyme fingerprint' (MEF) that describes DOM undergoing degradation for a specific location and time. We divided each exoenzyme activity by its highest value to standardise the rates so that absolute activity did not influence the PCA. Statistical procedures were implemented using SPSS v.16 statistical software (SAS Institute Inc.. Cary, NC, U.S.A.).

Results

Patterns of individual exoenzymes were inconsistent and difficult to interpret. Absolute exoenzyme activity was highly variable among wells, regardless of sample date (Table 2). Surprisingly, only a few exoenzyme activity metrics (NAG, LEU and N_{avail}) showed significant differences among sample dates (Table 2). NAG was lowest in March compared to the other 3 months, whereas LEU and Navail varied among the 4 seasonal sampling points. Individual exoenzymes did not vary by patch (two-way ANOVA; $P > 0.05$). ALPHA: BETA in wells overlain by old alder and old-growth trees was lower than sites without vegetation but was not significantly different from wells overlain by mid-aged alder (two-way ANOVA $P = 0.02$).

Although LEU: BETA was not significantly different among seasons (Table 2, $P = 0.064$), there were interesting relationships between the two exoenzymes (Fig. 2). In March ($r^2 = 0.64$), May ($r^2 = 0.80$) and

Table 2 Exoenzyme activity across all wells in the hyporheic zone of the Queets River, Washington. Activities [μmol MUF (g AFDM)$^{-1}$ h^{-1}] are mean ± SE and range

Enzyme	March 1999 (n_{wells} = 14)	May 1999 (n_{wells} = 27)	July 1999 (n_{wells} = 21)	October 1999 (n_{wells} = 11)
ALPHA	0.902 ± 0.186 (0.221–2.37)	3.09 ± 0.961 (0.053–22.6)	1.75 ± 0.581 (0.071–12.1)	3.19 ± 0.775 (0.215–7.57)
BETA	0.890 ± 0.239 (0.058–2.88)	3.41 ± 0.814 (0.099–17.4)	1.72 ± 0.556 (0.079–10.9)	2.80 ± 0.707 (0.601–7.78)
NAG	0.192 ± 0.033^{a} (0.039–0.471)	0.685 ± 0.175^{b} (0.033–3.97)	0.725 ± 0.192^{b} (0.034–3.78)	1.26 ± 0.265^{b} (0.398–2.76)
XYL	0.171 ± 0.047 (0.021–0.653)	0.760 ± 0.168 (0.037–3.62)	0.507 ± 0.154 (0.021–3.00)	0.579 ± 0.121 (0.178–1.52)
PHOS	2.34 ± 0.554 (0.250–9.09)	8.95 ± 3.29 (0.224–82.8)	10.9 ± 3.04 (0.218–55.4)	9.22 ± 2.12 (1.00–20.1)
LEU	0.745 ± 0.144^{a} (0.134–2.11)	2.95 ± 0.744^{b} (0.010–14.5)	1.04 ± 0.325^{ac} (0.024–4.21)	3.14 ± 0.665^{b} (0.591–8.12)
ACE	3.60 ± 1.26 (0.250–14.1)	22.2 ± 7.14 (0.125–156)	19.5 ± 7.11 (0.336–149)	22.8 ± 5.69 (1.00–52.8)
GUAN	4.16 ± 1.74 (0.037–17.6)	17.3 ± 5.47 (0.005–96.0)	18.0 ± 8.73 (0.037–184)	18.8 ± 5.02 (0.119–50.5)
P-avail	0.564 ± 0.128 (0.085–1.89)	0.691 ± 0.064 (0.029–1.40)	0.518 ± 0.056 (0.172–1.08)	0.691 ± 0.070 (0.452–1.10)
N-avail	0.445 ± 0.067^{ab} (0.152–0.917)	0.502 ± 0.051^{a} (0.155–1.11)	0.281 ± 0.041^{b} (0.050–0.903)	0.350 ± 0.056^{ab} (0.178–0.825)
LEU : BETA	1.17 ± 0.209 (0.359–3.46)	0.944 ± 0.129 (0.065–3.20)	6.0 ± 2.85 (0.002–46.2)	1.28 ± 0.148 (0.521–2.00)
ALPHA : BETA	1.52 ± 0.344 (0.034–4.29)	0.976 ± 0.211 (0.152–4.43)	1.24 ± 0.177 (0.085–3.07)	1.41 ± 0.337 (0.189–3.24)
XYL : BETA	0.273 ± 0.085 (0.053–1.24)	0.343 ± 0.065 (0.055–1.20)	0.367 ± 0.40 (0.097–0.820)	0.251 ± 0.037 (0.117–0.477)

n_{wells} = number of wells sampled. Means with different letters are significantly different for that individual exoenzyme parameter (ANOVA, $P < 0.05$).

October ($r^2 = 0.83$), LEU increased positively with BETA ($P < 0.001$, linear regression); however, LEU decreased with increasing BETA activity in July ($r^2 = 0.64, P < 0.001$, linear regression).

Exoenzyme activities did not show strong, consistent relationships with head gradient, DO or DOC concentrations (Table 3). Overall, DO and SRP decreased along the head gradient. ALPHA : BETA increased while LEU: BETA decreased with increasing oxygen. Few exoenzyme parameters correlated with DOC concentration; however, both nitrate and P_{avail} decreased with increasing DOC concentrations. P_{aveil} also decreased with increasing ammonium concentration. Neither E_N nor N_{avail} could be predicted by nitrate concentrations in either July or October. Similarly P_{avail} and Ep were not predicted by SRP concentrations in either July or October.

Although individual exoenzymes showed few relationships between patch and date, an analysis of all eight exoenzymes together via PCA produced interesting results. Patch differences were weakest during high flow (Fig. 3, March and October) compared to low flow (Fig. 3, May and July). The three patches segregated from each other in May but in July the gravel/young alder and mid-aged alder patches were similar while the old alder/old-growth patch was segregated from the other two patches. Also, individual patches moved through the PCA space over time indicating changing expression of the eight exoenzymes (Fig. 3).

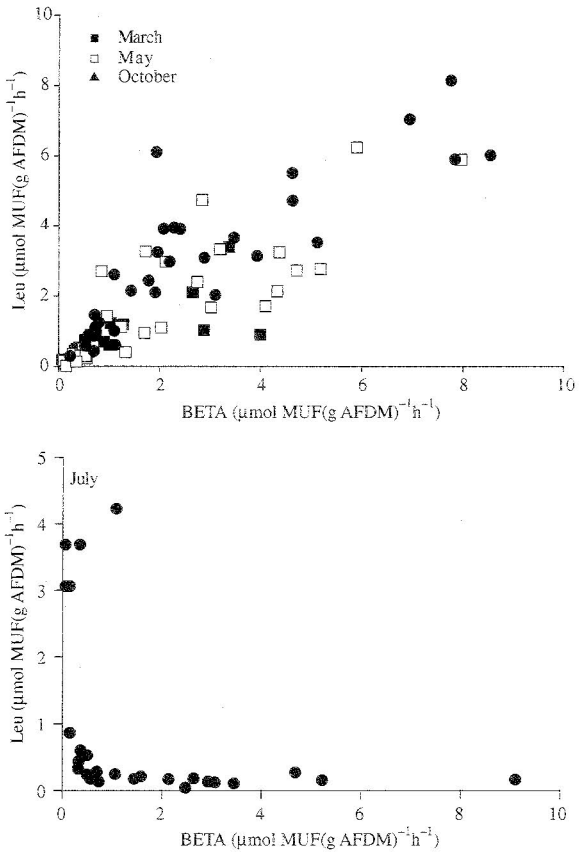

Fig. 2 The relationship between leucine aminopeptidase [LEU, μmol MUF (g AFDM)$^{-1}$ h^{-1}] and β-glucosidase [BETA, μmol MUF (g AFDM)$^{-1}$ h^{-1}] sampled on 25 March 1999, 15 May 1999 and 9 October 1999 (top). The relationship between LEU and BETA sampled on 20 July 1999 (bottom).

Table 3 Summary of Pearson's correlation coefficients between exoenzyme activities and physical and chemical parameters. Only significant correlations are shown. Correlations between individual exoenzymes (e.g. ALPHA and BETA) were always highly correlated and are not shown here (see text)

		Correlation coefficient
Head	DO	−0.316***
	SRP	−0.625***
DO	ALPHA : BETA	0.256*
	LEU : BETA	−0.243*
DOC	NO_3-N	−0.395*
	P_{avail}	−0.294*
NH_4-N	P_{avail}	−0.500*

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

The PCA loadings can be used to further describe patterns in exoenzyme expression. LEU loaded high on Axis 2 and was the main factor separating samples along that axis. In contrast, ALHPA, ACE, PHOS, GUAN and XYL loaded high on Axis 1 and were the main factors for separating samples along that axis. BETA and NAG loaded higher on Axis 1 than Axis 2; however, they only had a weak effect of separating samples on Axis 2.

Discussion

Exoenzyme activities in the Queets River hyporheic zone indicated the presence of an active microbial community metabolising a diverse array of organic molecules. As in other systems (Sinsabaugh & Linkins, 1988; Foreman, Franchini & Sinsabaugh, 1998; Rulik & Spacil, 2004), exoenzyme activity was variable since temperature, hydraulic conductivity, species composition and other factors might affect population size, rates of microbial exoenzyme kinetics and metabolism. Surprisingly, there was little seasonal variation compared to these other studies. It is not know whether this lack of seasonality is because of the small sample size for a comparatively large hyporheic zone or is a reflection of a certain measure of stability in large river hyporheic zones. Regardless, changes in the relative composition of all eight exoenzymes indicated that hyporheic microbial communities were responding to changing DOM sources.

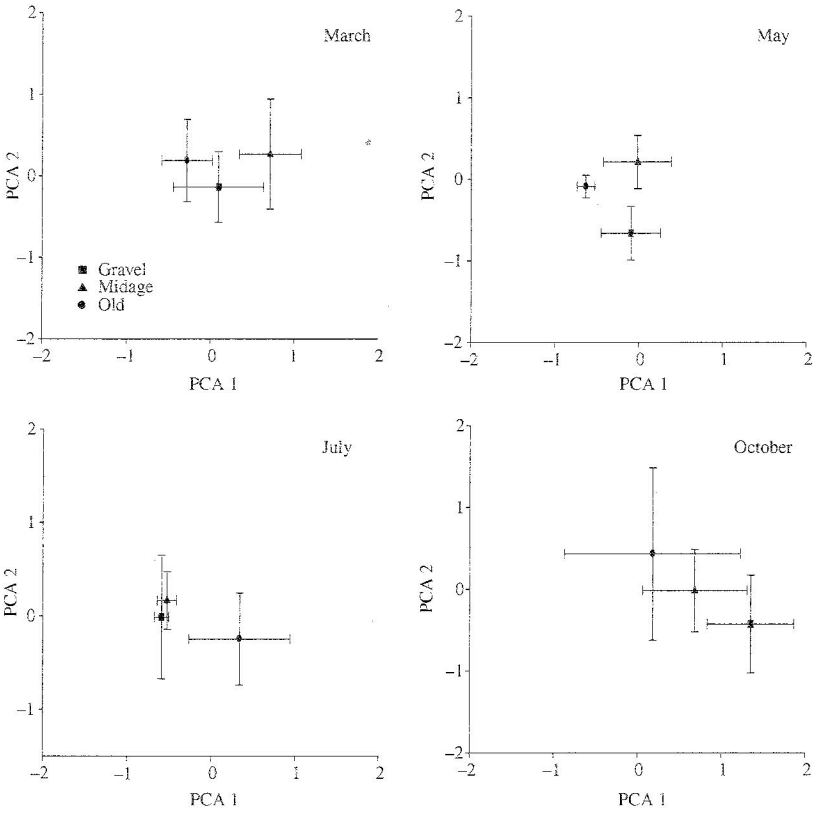

Fig. 3 Principal components analysis results for eight exoenzymes examining the effect of patch type. The first two principal components described 85% of the variation in the data. Values are mean ± SE for each patch sampled on 25 March 1999, 15 May 1999, 20 July 1999 and 9 October 1999.

Microorganisms degrade organic matter to acquire inorganic nitrogen and phosphorus as well as carbon. Nutrient availability is important because inorganic nutrients are used as building blocks for cell constituents and also influence uptake of small carbon compounds into bacterial cells (Skoog *et al., 2002)* and bacterial productivity (Pace & Cole, 1996). Red alder on the Queets River floodplain fix nitrogen at high rates, which results in large inorganic nitrogen accumulations in soils (Cole *et al.,* 1990; Bechtold, 2000; Compton & Cole, 2001) that are readily leached into subsurface water (Bechtold *et al.,* 2003). During wet periods, when large amounts of nitrate are leached into the hyporheic zone, alder-derived nitrate could potentially influence exoenzyme activation.

There was no relationship between LEU, PHOS and inorganic nutrients, and no differences between LEU activities in biofilm collected from wells located in mid-aged alder versus gravel/young alder and old alder/old growth-patches. This lack of pattern is surprising since LEU and PHOS are often negatively correlated with inorganic nutrient concentrations (Foreman *et al.,* 1998; Findlay *et al.,* 2001) and high concentrations of nitrogen and phosphorus are expected to depress the enzyme activation system, whereas low concentrations would induce it (Chrest. 1991). Nitrate and SRP concentrations may not have varied widely enough in this study to observe a relationship with exoenzyme activities. In contrast, we measured very high PHOS activities in biofilms grown at subsurface upwellings near alder terraces where there was no measurable SRP (Clinton, 2001). We did not measure dissolved organic nitrogen or dissolved organic phosphorus in this study, which may prove to be better predictors of LEU and PHOS activity.

In this study, N_{avail} changed seasonally but there was no significant difference among patches and no relationship between Navail and inorganic nitrogen concentrations. N_{avail} was lowest in the summer (July) when the floodplain soils are at their driest and little leaching occurs. Ammonium was higher in July compared to October (3.6 ugL^{-1} versus 1.1 ug L^{-1}, unpaired *t*-test, $N = 21$, $P = 0.006$) while nitrate was lower in July compared to October (42 ug L^{-1} versus 108 ug L^{-1}, unpaired t-test, $N = 21$, $P = 0.019$). Bacteria preferably take up ammonium as it is needed to make amino acids. Even though ammonium concentrations were higher in July, they may not have been adequate to fuel production which is also higher during the summer months (Clinton, 2001).

There was a striking seasonal relationship between LEU and BETA exoenzyme activities. LEU: BETA has been used as a potential indicator of the relative importance of protein to polysaccharide metabolism (Christian & Kart 1995; Fukuda *et al.,* 2000). Low LEU: BETA in the terrace hyporheic zone on 3 of 4 dates indicated that polysaccharides were the dominant component of metabolism. On those dates, LEU and BETA were positively correlated, implying that microbes were using both complex carbohydrates and proteins. In July, high LEU: BETA indicates a switch to protein metabolism, which may be because of a decrease in the availability of complex carbohydrates. This hypothesis is further supported by the negative correlation between LEU and BETA at this time and implies a change in DOM quality in the Queets River hyporheic zone. During the summer, DOM inputs from overlying soils would be decreased and DOM sources fuelling the microbial community could come from stored pOM associated with hyporheic biofilms, POM associated with buried wood and advecting DOM from surface water. Each of these sources would have varying bioavailablity. Thus, to fully understand the relative importance of DOM fractions to microbial production, we would need to know the ratio of respiration of components (e.g. carbohydrates or amino acids) to synthesis. In bacterioplankton, production is closely related to glucosidase activities; however, high production can also occur with high peptidase activity (Foreman *et al.,* 1998), indicating that both fractions are important to microbial communities. Also, since peptidase acquires both C and N, it is difficult to assign its role in microbial metabolism and differences in the carbohydrate and polysaccharide systems are probably tied to differences in cellular metabolism (Foreman *et al., 1998;* Findlay & Sinsabaugh, 2003).

Patches versus flowpaths

Hyporheic processes are superimposed upon a strong physical template because of the organising structure of moving water. Where surface water enters sediments, and no other inputs are present, labile DOM derived from surface sources fuels hyporheic metabolism (Jones *et al.,* 1995; Findlay & Sobczak, 1996). As water moves along the flowpath, microbial respiration

and production decrease (Findlay & Sobczak, 1996; Sobczak & Findlay, *2002),* organic matter that entered the flowpath head becomes more refractory (Sobczak & Findlay, 2002) and water exiting the hyporheic zone is largely unavailable for metabolism (Sobczak & Findlay, 2002). However, hyporheic flowpaths are not independent and interact across various spatiotemporal scales to create a complex 3-dimensional flow system in stream sediments (Fraser & Williams, 1998; Ritzenthaler, 1998; Dent, Grimm & Fisher, 2001). Thus, hotspots of microbial production and biogeochemical activity can occur when multiple localised inputs of organic matter are superimposed upon, and override, the progressive processing of advected DOM along the flowpath.

In the Queets River floodplain hyporheic zone, lack of strong DOC patterns along flowpaths and large lateral variations in concentration support the interpretation that additional carbon inputs exist. Although other sources (buried OM, upwelling ground water) may exist, the most likely input is the downward movement or DOM from overlying riparian soils (Clinton, 2001; Clinton *et al.,* 2002). In this article, we hypothesised that DOM bioavailability varied with the age and type of overlying riparian vegetation and used microbial exoenzyme activities as a surrogate for DOM bioavailability to investigate this hypothesis.

Exoenzyme fingerprints indicated that patch effects were present and strongest during low flow conditions (May and July) compared to higher flow conditions (March and October). This pattern highlights the importance of the interaction between hydrology and patch structure for influencing subsurface DOM bioavailability. In a Rhone River gravel bar system, high subsurface velocities resulted in few dominant hyporheic flowpaths that create homogeneous rates of microbial processes. At slower flows local influences caused by vegetation and sediment characteristics can impose a signature on microbial activities to produce a patchwork of microbial processes (Vervier, Dobson & Pinay, 1993). Schade *et al.* (2001) also documented small-scale effects of vegetation on N dynamics in a southwestern Arizona, U.s.A. stream. The hyporheic flow net at our study site changes from focused to sheet flow with increasing discharge, affecting the interaction of hyporheic water beneath patches by altering subsurface residence times and linkages to surface water (Clinton *et al., 2002).*

The 3 patch types segregated from one another in May whereas in July wells overlain by old-growth vegetation were distinct from wells located in gravel and overlain by young alder. This suggests that microbes at these locations were degrading organic matter of different compositions. Wells beneath each patch type were arrayed across a range of head values, or positions along the flowpath, so the observed pattern suggests that inputs from overlying soils overrode advecting DOM to create this chemical variability. Furthermore, when all data were combined, no exoenzyme parameter responded to changes in head gradient, supporting our view that the forested terrace hyporheic zone receives the majority of its DOM from overlying riparian forest soils and not from the input of surface water from the Queets River.

The movement of DOM from overlying soils to hyporheic water is poorly understood but is a function of the metabolism, sorption and translocation processes in the soil profile. In temperate systems, sorption, rather than microbial uptake, is considered to be more important in regulating DOlv lretention in the soil column (Qualls, Haines & Swank, 1991; Qualls & Haines, 1992; Neff & Asner, 2001; Michalzik *et al.,* 2003). DOM from upper soil layers can support microbial metabolism in deeper layers (Neff & Asner, *2001).* In an old-growth Douglas fir forest, DOM leaving the soil column at 70 cm was composed or plant derived material, microbially synthesised material, and a mixture of free and combined amino acids (Yano *et al.,* 2004). Upon entering subsurface water, this material would then be available for microbial metabolism. Because leachates from conifer and alder leaves vary in DOM composition (McArthur & Richardson, 2002), it is reasonable to hypothesise that DOM moving from soils with different overlying vegetation would also differ in composition and bioavailability. Thus, DOM exiting the soil column would reflect this spatial heterogeneity and generate differences in microbial exoenzymatic signatures.

Regardless of position along the head gradient or overlying patch type, exoenzyme fingerprints for individual well locations varied seasonally, reflecting the heterogeneous composition of organic matter available for hyporheic microbes throughout the year. This dynamic nature of DOM quality influences nutritional requirements for subsurface microbes and suggests that either a single population of

microorganisms has the metabolic flexibility to access this DOM supply, or there is a constant turnover in hyporheic microbial community composition in response to resource variability. This subsurface complexity contrasts with patterns associated with movement of surface water through gravel bars in small streams where DOC quantity and quality decreased predictably along hyporheic flowpaths (Findlay & Sobczak, 1996; Holmes *et at.,* 1998) and water exiting the hyporheic flowpaths was largely unavailable for metabolism (Sobczak & Findlay, 2002). Within forested floodplain terraces of large rivers, hyporheic zones do not simply process river inputs but also contain a complex set of internal sources, transformations and sinks that interact with surface water in a manner that cannot be fully understood simply by monitoring input and output characteristics.

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