MICROBIAL UTILIZATION OF DISSOLVED ORGANIC MATTER LEACHED 
FROM RIPARIAN TREE SPECIES OF DIFFERENT SERAL STAGES

by

MICHAEL DAVID McARTHUR

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The required standard 

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Department of Forest Sciences

The University of British Columbia
Vancouver, Canada

Date Oct. 14, 1999
Dissolved organic matter (DOM) leached from five coastal forest litterfall types. Red alder, vine maple, western red cedar, western hemlock and Douglas-fir, were studied to assess their DOM chemistry and relative ability to support growth of heterotrophic, stream bacteria. Bacterial growth was measured using [3H] leucine incorporated into protein over 24 hours of exposure to nutrient-amended leachates. Bacterial growth was greatest in deciduous and western red cedar leachates when controlling for dissolved organic carbon (DOC) concentration. The bacterial growth rates on most leachate types were greatest after one hour, then declined in a negative exponential pattern. The DOM less than 10 000 nmw supported lower bacterial growth rates than DOM from whole leachates on a per mg DOC basis. The DOM carbon to nitrogen atomic ratio was the best predictor of bacterial growth ($r^2 = 0.84$). A seven day leaching experiment revealed that DOC release from western hemlock needles increased linearly while the majority of red alder and western red cedar DOC was released after one or two days, respectively. The patterns recorded in stream DOM quantity and quality indicated that riparian vegetation type may directly influence stream DOM chemistry. Through successional changes in tree species composition, riparian forests can influence the stream microbial productivity based on the changes in dissolved organic matter.
# TABLE OF CONTENTS

ABSTRACT ................................................................. ii

LIST OF TABLES .......................................................... iv

LIST OF FIGURES ......................................................... v

ACKNOWLEDGEMENTS .................................................... vi

INTRODUCTION ............................................................ 1
  How to measure DOM? .................................................. 3
  Detecting microbial response ....................................... 4

METHODS ................................................................. 9
  Site description and litterfall collection ......................... 9
  Leachate preparation and analysis ................................ 10
  Bacterial production experiment ................................... 11
  Measuring bacterial productivity .................................. 12
  Longer-term leaching experiment .................................. 14

RESULTS ................................................................. 15

DISCUSSION ............................................................. 26
  Leachate chemistry .................................................. 26
  One week leaching of litterfall ..................................... 30
  Stream DOM monitoring .............................................. 35
  Implications for forest managers .................................. 36
  Summary ............................................................... 38

REFERENCES ............................................................. 39

APPENDIX ............................................................... 44
LIST OF TABLES

Table                                                                                      Page
1. Concentrations of low (LMW) and high (HMW) molecular weight dissolved organic carbon (DOC) and polyphenolics from dissolved organic matter (DOM) in forest litterfall leachates ........................................... 18
2. The bacterial carbon production (BCP) rates for seven dissolved organic matter (DOM) sources over the 24 hour experiment .................................................. 18
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>The mean + 1 SE (n = 3) DOC, C : N, C : H and polyphenolic content of high (HMW) and low (LMW) molecular weight forest litterfall leachates after 24 hours of leaching.</td>
<td>16</td>
</tr>
<tr>
<td>2.</td>
<td>The cumulative heterotrophic bacterial incorporation of $[^{3}\text{H}]$ leucine after 24 hours of exposure to various leachate types.</td>
<td>19</td>
</tr>
<tr>
<td>3.</td>
<td>The heterotrophic bacterial incorporation rates of $[^{3}\text{H}]$ leucine during 24 hours of exposure to various leachates types.</td>
<td>20</td>
</tr>
<tr>
<td>4.</td>
<td>The heterotrophic bacterial incorporation of $[^{3}\text{H}]$ leucine after 24 hours plotted against the C : N of five leachate DOM types.</td>
<td>22</td>
</tr>
<tr>
<td>5.</td>
<td>The cumulative DOC, C : N and C : H content of DOM leached from three litterfall types over a one week period.</td>
<td>23</td>
</tr>
<tr>
<td>6.</td>
<td>The DOC, C : N and C : H for DOM in two stream in the MKRF.</td>
<td>25</td>
</tr>
</tbody>
</table>
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INTRODUCTION

A basic requirement of all ecosystems is a continuous input of energy. Plants produce organic matter via photosynthesis and then this energy is passed onto consumers in the form of detritus. In most ecosystems, 70 to 90% of all primary production is eventually converted to detritus (O'Neill and Reichle 1980). The description of detritus, its sources and pathways represents a fundamental part of our understanding of aquatic ecosystem function. In forested streams, the dropping of leaves and needles from the riparian vegetation into the stream can link the terrestrial and aquatic food webs. Studies have shown that terrestrial detrital inputs are important to stream ecosystem productivity (Richardson 1991; Wallace et al. 1997). The rates of decomposition of various forms of detritus visibly differ in forests and streams. For example, leaves that fall to the ground in forests can disappear in one or two years while a fallen log can last a century or longer (Aber and Melillo 1991). The mechanisms behind these differences in decay rates and subsequent nutrient release are complex, but often involve the nutrient requirements of the microbes involved in the decomposition process. The ‘quality’ of detritus can be measured in terms of its relative availability to microbes as a substrate for growth and energy.

Detritus that falls to the soil is often consumed by terrestrial microbes before it reaches the water, but litterfall that goes directly into the stream is likely to provide a richer source of energy and nutrients to lotic microbes. The first step in the release of organic matter from litterfall is physical leaching of water soluble substances. The majority of leaching occurs during the first 24 h after immersion in water (Nykvist 1962;
Saunders 1976). Depending on litterfall types, leaching may cause a 20 - 40% loss of biomass (Saunders 1976). Beyond leaching there are stages of ‘conditioning’ by fungi followed by detritivorous invertebrate larvae. The water soluble substances leached from riparian litterfall, their chemistry and bioavailability to microbes, are the focus of this study.

In British Columbia’s coastal streams, the composition of the litterfall can change through land use such as forestry (Richardson 1992). The cutting of trees can increase light and nutrient availability, resulting in a shift from mature conifers to more early seral, deciduous tree species. Since deciduous leaves and conifer needles contrast in their surface area, nutrient retention, and several other factors, the detrital inputs to their surrounding streams may change in quantity and quality during succession from deciduous to coniferous tree species. To date, the effects of these changes in detrital inputs on stream microbial productivity in Pacific coastal streams have not been studied. Quantifying microbial use of detritus is an important step towards modelling changes in the food webs of these changing watersheds.

Limnologists and oceanographers have become increasingly aware of the importance of detritus from streams and rivers as a source of energy and nutrients. In general, the concentration of dissolved organic matter (i.e., DOM < 0.45 μm) is approximately an order of magnitude greater than the particulate organic matter (POM) in streams, lakes and upper oceans (Saunders 1976). The majority of DOM is carbon - based and has been the subject of many stream studies. For example, it has been
estimated that river runoff transports about $2.0 \times 10^{14}$ g of dissolved organic carbon (DOC) into the oceans annually (Deuser 1988) and that leaf litter contributes about 30% of the daily DOC export from some small, forested streams (Meyer et al. 1998).

Heterotrophic bacteria consume DOM and convert it to living biomass. The introduction of fluorescent microscopy and more recently, radioisotope uptake techniques has greatly improved ecologists’ ability to estimate bacterial production rates (Azam et al. 1983; Ward and Johnson 1996). From these rates it has been estimated that bacteria consume 40% to 80% of the fixed carbon in seawater (Azam et al. 1983). The rate at which DOM is consumed and assimilated by microbes has also been shown to vary depending on its source (Kaplan and Bott 1983). The quality of DOM is often measured in terms of its relative availability or recalcitrance for heterotrophic microbes.

**How to measure DOM?**

Many techniques have been used to characterize DOM and rate its bioavailability. One common method is the use of chromatography, e.g. XAD-8 resin, to isolate acidic functional groups based upon their hydrophobic character (Thurman 1985). Specific compounds in DOM such as polyphenols or carbohydrates may be targeted by assays using chromatography, e.g. HPLC, or spectroscopy when there is prior information on their biological significance (Gremm 1997). Another popular method is to separate organics based on molecular size using ultrafiltration or reverse osmosis (Freeman et al. 1990; Serkiz and Perdue 1990). The ratio of stable isotopes such as $^{13}$C : $^{12}$C has been
used to help identify the source(s) and pathway(s) of DOM by comparing them to known isotopic ‘signatures’ (Schiff et al. 1990; Hullar et al. 1996; Kelly et al. 1998). In recent years, high resolution techniques have been used to describe the atomic and molecular characters of DOM constituents. One such method is $^{13}$C nuclear magnetic resonance (NMR) spectroscopy which can provide structural information about complex mixtures of organic compounds (Norden and Berg 1990; Benner et al. 1992; Cook and Langford 1998). Another fine scale method, atomic elemental analysis, has been used to characterize DOM while comparing its availability to bacteria (Goldman et al. 1987; Sun et al. 1997).

**Detecting microbial response**

Because the quality of DOM is often measured in terms of microbial bioavailability, many microbial assays have been employed to measure their response to various DOM sources. Some studies have measured the loss of DOM or oxygen to infer the uptake and metabolism of microbes (Lock and Hynes 1975; Findlay et al. 1995). A more direct approach is to count samples of bacteria that have been stained with a fluorescent dye (Porter and Feig 1980) before and after they have been exposed to DOM. To consume DOM, bacteria use extracellular enzymes to break down the organics, hence scientists have used the production of these enzymes as an index of bacterial consumption (Findlay et al. 1998). Heterotrophic bacteria vary in size and growth efficiency such that it is difficult to estimate the amount of bacterial carbon that is generated from a DOM source using the methods listed above. Once organic matter is consumed by bacteria, it will be respired as carbon dioxide, released as energy, excreted
as waste or assimilated into new biomass. The relative amount of carbon in each of these pathways can vary depending on environmental variables, e.g. temperature, the taxa involved, time of day or season, and a multitude of other factors, hence confounding indices developed for indirect methods of microbial measurement. By measuring the incorporation of a radioisotope, e.g. $[^3]$H leucine, into bacterial protein along with a DOM source, scientists can measure the relative transfer rates of detrital carbon to living biomass. Many studies have used this powerful tool in marine and freshwater systems (Fuhrman and Azam 1982; Kirchman et al. 1985; Thomaz and Wetzel 1995).

Using the above methods, scientists have learned much about microbial use of DOM. In aquatic ecosystems, DOM is the largest pool of organics in the water column, of which 40-60% is typically fulvic acid (McKnight and Aitken 1998). DOM can influence the physical characters of aquatic systems by trace metal complexation (McKnight and Bencala 1990) or by absorbing visible and UV radiation (Schindler and Curtis 1997). Evidence from stream studies suggests that bacterial assemblages acclimate to local DOM sources and that foreign sources can be less labile (Kaplan and Bott 1985; McArthur and Marzolf 1986).

The influence the molecular weight of DOM has on microbial growth remains unclear. An early hypothesis predicted that smaller molecules are less complex and require less processing than macromolecules before they can be utilized by bacteria, hence are more labile (Saunders 1976). Others have noted an inhibitory effect of organic matter $>$1000 nmw on enzyme activity (Freeman et al. 1990). However, some reports
have shown that larger molecular weight compounds possess similar or higher availability to bacteria. Meyer et al. (1987) found the <1000 nmw fraction to be most readily used by bacteria while the >10 000 nmw fraction was a better substrate for bacterial growth than the 1000 - 10 000 nmw DOM. Another study describing bacterial growth on humics in lakes has shown greater bacterial growth on DOC >10 000 nmw compared to that of <10 000 nmw (Tranvik 1990). It has been suggested that small molecules may have undergone substantial diagenic change while larger DOM molecules are less transformed hence potentially hydrolyzed and consumed by bacteria (Tranvik 1998). Furthermore, it may be the complexes attached to a particular DOM size fraction, such as metals, that are ultimately important to bacterial growth.

Another area of confusion surrounding the quality of DOM are compounds based on the 6 - carbon phenolic ring. There are generally two classes of these compounds that are recognized: 1) ‘tannins’ are smaller phenol polymers (polyphenols) comprised of several phenolic acids, and 2) ‘lignins’ which are larger, amorphous and very complex (Aber and Melillo 1991). The compounds released by leaching are largely those extractable in water, hence are typically carbohydrates and phenolics instead of fats, waxes, cellulose or lignin. Because these compound classes have no precise chemical definition they may vary in recalcitrance based on their molecular size, adsorbed or bound groups (i.e., metals or proteins), hydrogen saturation and several other factors. In order to predict bioavailability a description of the DOM at the sub-molecular level may be required in addition to traditional assays.
A recent study by Sun et al. (1997) examined the relationship between bioavailability and the atomic element composition of dissolved organic matter leached from litterfall collected near the Ogeechee River, Georgia, USA. The bacterial response over 3 days was well predicted ($r^2 = 0.933$) using parameters based on the relative magnitudes of $H:\text{C}$, $O:\text{C}$, and $N:\text{C}$ atomic ratios. Trends in their data suggest that bacterial growth was positively correlated with $H:\text{C}$ and $N:\text{C}$ while negatively correlated with $O:\text{C}$. From this study the authors concluded that aliphatic carbon was more labile than aromatic forms in the Ogeechee River. The $C:\text{N}$ atomic ratio of DOM can provide some indication of its molecular type. For example, proteins are relatively rich in nitrogen and hence have a low $C:\text{N}$. Similarly, the $C:\text{H}$ ratio can give insight into the structure of organic molecules. With increasing hydrogen atoms per carbon atom, there are more single bonds and less aromatic structures.

Bringing together techniques such as atomic element analysis and radioisotope uptake may bring scientists closer to being able to predict microbial responses from DOM sources in a variety of ecosystems. Furthermore, our understanding of processes like forest succession and its influence on the aquatic food web may improve with representative measures of the DOM and microbial assemblages.

In this study, bacterial response to different types of riparian litterfall was examined. Litterfall types representative of forest seral stages were selected to emulate the variety of DOM sources a stream microbial community would be exposed to during succession. This experiment focused on the primary stage of litterfall decomposition and
trophic transfer, i.e., the leaching of litterfall in water and its subsequent assimilation into bacterial protein. By measuring the incorporation rate of $[^3H]$ leucine into native, heterotrophic bacterial protein along with the DOM of riparian litterfall leachates the effects of DOM quality were examined. Several chemical parameters of the leachates were measured to characterize the DOM and to rate their relative importance to bacterial production. The chemical description of leachate DOM was based on atomic elemental composition, molecular size fractionation and polyphenolic content. Through these measurements this study aimed to better predict the influence of changes to forest litterfall types on the heterotrophic bacterial production in temperate, coastal streams.
METHODS

Site description and litterfall collection

Forest litter-fall samples were collected from the University of British Columbia’s Malcolm Knapp Research Forest (MKRF) in Maple Ridge, B.C. (49° 15' - 49° 22’ N and 122° 31’ - 122° 36’ W). Fires and clearcutting in the last 80 years have led this coastal forest to various stages of succession (Klinka and Krajina 1986). Because much of the MKRF is at an early seral stage, several tree and plant species are well represented. While conifers dominate the forest cover, there are patches of deciduous species throughout the forest, particularly in riparian areas. In this study, litterfall was collected from suspended, mesh screens (1 mm mesh size, ~ 2x2 m) each week from June to November 1998. Screens were installed near three, first order streams, each in different watersheds (East, Mike and Spring Creek) to serve as replicate stands for sampling of forest litter. After collection the litter was air-dried for at least 48 hours and stored at 4° C in sealed, plastic bags.

Leaves and needles from five species of trees were used for this experiment: red alder (Alnus rubra (Bong.)), vine maple (Acer circinatum Pursh), western red cedar (Thuja plicata Donn), western hemlock (Tsuga heterophylla (Raf.) Sarg.) and Douglas-fir (Pseudotsuga menziesii (Mirb.) Franco). Litterfall collected was identified using a local field guide (Pojar and MacKinnon 1994) and then checked by a local botanical authority (J. Worrall, UBC, per. comm.).
Leachate preparation and analysis

Leachates for each of the five tree species were prepared by adding 2 g of dried litter to 500 mL of sterilized, deionized-distilled water. The leaf litter and water were then mixed in the dark with a magnetic stirrer for 24 hours at 4° C. The particulate matter in the leachate was removed using a pre-combusted Gelman A/E glass fibre filter (Gelman Sciences, Ann Arbor, MI). These filters were chosen because they exclude particulates greater than 0.45 µm though it should be noted that free bacteria can pass through these Gelman filters. The 0.2 µm polycarbonate filters that remove free bacteria add carbon to aquatic samples so leaching and storage of DOM samples at 4° C was the method chosen to control for bacteria prior to the experiment. All matter that passed through the Gelman filters was considered to be dissolved. Ultrafiltration of the leachates was done in a stirred cell using a 10 000 nmw cut-off membrane (YM 10, Amicon Inc., Beverly, MA) with N₂ gas at < 69 kPa (10 psi). Membranes were washed with a 0.5% Terg-A-Zyme (Alconox Inc., New York, NY) solution for 30 min, then thoroughly rinsed with distilled water. All leachates were stored for less than one week in autoclaved, acid washed, polypropylene bottles at 4° C.

The carbon, hydrogen and nitrogen atomic ratios of the leachate and stream water DOM were determined using flash combustion in a Carlo Erba NA-1500 Elemental Analyzer, which has estimated absolute and relative detection limits of 0.62 g and 0.01% carbon, respectively (Verardo et al. 1990). Stream samples were concentrated prior to elemental analysis. Organic carbon was determined by removing carbonate carbon using sulfurous acid within aluminum sample cups. The polyphenol content of the leachates
was measured with a UV/VIS spectrophotometer (Turner model #690 @750 nm) using a Folin-Ciocalteau phenol reagent (Box 1983) and a tannic acid standard.

**Bacterial production experiment**

Heterotrophic bacteria were grown on sterilized, 6 mm (dia.) glass beads that were wrapped in mesh (1 mm) and placed at the bottom of Spring Creek during October and November, 1998. Shade cloth (90%) was suspended approximately 30 cm above the stream surface to limit photosynthesis. The day prior to the experiment, the beads were transported in stream water and kept in a cooler until they were put in a dark incubator (Innova 4230, New Brunswick Scientific) set at stream temperature (12°C) 2 hours after collection. The bacteria were kept in the incubator for 24 hours before they were added to the culture tubes filled with the different leachates.

To test for the bacterial response to qualitative carbon differences, the experiment was designed to expose the bacteria to one DOM concentration for all leachate types. Solutions of deionized-distilled water and leachates were made to 50 mg DOC L⁻¹ based on the results of C : H : N analyses done prior to the bacterial production experiment. Also, leachates were amended with a standard micronutrient solution (NO₃, PO₄, Cu, Zn, Co, Mn, Fe) in the same concentrations and proportions as described by Caron (1993) to help limit differences in bacterial production to changes in DOM quality only.

The *in vitro* bacterial assimilation of DOM was tested for the five leachate types and two LMW weight leachates. Because of physical and time constraints on the number
samples that could be processed at the five time intervals, in triplicate, it was not possible to test the LMW DOM from all five leachate types so vine maple and western hemlock LMW leachates were chosen to test differences between a coniferous and a deciduous species.

Measuring bacterial productivity

The following methodology was adapted from the protocol outlined by Ward and Johnson (1996). To estimate bacterial productivity, a $[^3\text{H}]$ leucine incorporation method was chosen because the leucine content in bacterial protein is more constant than other amino acids (Ward and Johnson 1996). A stock leucine solution was prepared by adding 3 ampoules of $[4,5{}^3\text{H}]$ leucine (S.A. = 146 Ci/mmol, Amersham Corp.), each containing approximately 158.5 µCi, to 4.5 mL of distilled water. This solution was then added to 15 mL of unlabelled L-leucine (30 µM) for a final volume of 22.5 mL. A 150 µL aliquot of the leucine solution was added to each culture tube, filled with a 10 mL leachate solution such that the final $[^3\text{H}]$ leucine concentration was 20 nM. The 20 nM of $[^3\text{H}]$ leucine was expected to overwhelm the ambient leucine pool (about 1 nM) and maximize leucine uptake kinetics. To check the actual activity of the tritium added to each sample, 150 µL of stock leucine solution was also added to control tubes containing only distilled water (10 mL). Furthermore, controls for abiotic factors were addressed by adding formalin to a set of samples to stop any biological growth, then measuring the radioactivity as done with the other samples (see below). Another set of control samples consisted of bacteria
exposed to glucose solution (50 mg L\(^{-1}\)) such that bacterial growth from the addition of a readily available DOC could be tested.

The experiment began once four glass beads were added to each culture tube. Each treatment and time interval was represented by three replicates, one for each watershed. During the experiment the bacteria were kept in the dark, at 12° C. At five times during the experiment (1, 2, 4, 9, 24 h), 0.4 mL of formalin was added to one group of tubes to stop biomass production. After 24 h, all tubes were placed in a bath sonicator (Mettler Electronics) for 5 min to separate bacteria from the beads. Protein molecules are not soluble in trichloroacetic acid (TCA), hence all hydrolyzed, nonprotein molecules dissolved in TCA solution should pass through a 0.2 μm filter. To extract nonprotein molecules in the culture tubes, 2.44 mL of 20% v/v trichloroacetic acid (TCA) was added to each tube (4% v/v final conc.), mixed, then placed in a 95° C water bath for 30 min to hydrolyze all macromolecules except protein. After the tubes had cooled at room temperature for 20 min, a 0.5 mL aliquot was pipetted onto a 0.2 μm polycarbonate filter (Corning Nuclepore Track-Etch Membrane, 47 mm dia.) to collect the TCA-insoluble material. Each filter was flushed thoroughly with distilled water, before and after each sample was filtered. Filters were then placed in plastic scintillation vials (6 mL, Wheaton Scientific) filled with scintillation fluor (Aqueous Counting Scintillant, Amersham Corp.). Finally, the DPM (disintegrations per minute) of the tritium incorporated into bacterial protein was calculated using a Beckman LS 6500 liquid scintillation counter (Beckman Instruments Inc.) using an external standard to correct for quenching. Details of calculations for converting DPM to moles of leucine incorporated into bacteria,
bacterial protein production (g) and bacterial carbon production (g) are included in the Appendix.

**Longer-term leaching experiment**

To examine the litterfall leaching kinetics beyond 24 hours, a week-long experiment was conducted. After one and two days of leaf litter soaking, the leachates from red alder, western hemlock and western red cedar were replaced with distilled water and analyzed for their C : H : N and DOC content. Furthermore, after the leaf litter had been soaked for seven days it was analyzed as above. Again, samples from the three watersheds were used to achieve replication.

Data analyses, including analyses of variance and covariance, linear regression and tests for normality of data were done using SAS/STAT (SAS Institute Inc.) and Minitab (Minitab Inc.). A probability level of $\alpha = 0.05$ was used to determine statistical significance of the $ln$-transformed data.
RESULTS

Chemical analyses of litterfall leachates revealed large differences between deciduous and coniferous species. The highly-coloured leachates of alder and vine maple had significantly (ANOVA, p<0.05) higher DOC and polyphenolics than the conifer leachates (Fig.1) after 24 hours of leaching. The C : N atomic ratio for leachate DOM ranged from 25 to 85, and again the deciduous species were the highest. The least squares means (LSM) procedure was used to do pairwise comparisons, based on ln-transformed data. In the case of vine maple C : N, this meant that the ln-transformed mean was in fact higher in the low molecular weight (LMW) than the HMW fraction because of the data structure. Because the C : H data distribution was significantly different from a normal distribution, LSM pairwise comparisons were not done. The C : H atomic ratio for whole (i.e., HMW + LMW) leachate DOM varied from 0.36 (Douglas-fir) to 0.73 (vine maple) while the values for red alder, western red cedar and western hemlock were nearly identical.

Comparisons between the low molecular weight (<10 000 nmw) and high molecular weight (>10 000 nmw) size fractions showed wide variation amongst and within leachate type. With the exception of Douglas-fir, all leachates had a higher DOC content in the LMW fraction (Fig.1). Similarly, the polyphenolic content was also higher in the LMW leachates. The polyphenolic : DOC ratio (Table 1) was > 1 for all deciduous leachates while for samples from coniferous species, it was < 1, except for the HMW red cedar (1.4). There was no clear trend dividing the DOM size fraction and phenolic DOC ratio as three leachate types had a higher ratio for HMW, and two had a higher ratio in
Figure 1. The mean + 1 SE (n = 3) DOC, C : N, C : H and polyphenolic content of high (HMW) and low (LMW) molecular weight forest litterfall leachates after 24 h of leaching. * indicates the H for this leachate was not detectable. Letters denote significant differences (p<0.05) determined by Least Squares Means comparison of the natural log transformed data.
the LMW fraction (Table 1). The relatively high amount of LMW N in vine maple resulted in it being the only leachate type whereby the HMW fraction had a higher C : N ratio (Fig.1). Conversely, the low amount of N in the LMW cedar leachate led to a C : N that was higher than that of Douglas-fir and hemlock.

The incorporation of leucine into heterotrophic bacterial protein was measured during exposure to the five leachate types and a LMW sample from a deciduous (vine maple) and coniferous (western hemlock) species. For most leachates, the greatest incorporation rates were at the one hour mark, then they declined in a negative, exponential pattern. The greatest leucine incorporation rates were associated with the two deciduous species and western red cedar during the 24 hour experiment (Fig. 2 and 3). The DOC concentrations in the leachates were made approximately equal prior to the experiment. Furthermore, leucine incorporation was expressed per unit DOC to adjust for fine scale differences in DOC concentration. An analysis of covariance (ANCOVA) with time as the covariate combined with Least Squares Means pairwise comparisons was performed using the ln-transformed incorporation rates over 24 hours. Essentially, this ANCOVA was a comparison of the lines in Figure 3. It was found that leucine incorporation rates for alder, vine maple and cedar (group 1) did not significantly differ from each other. The incorporation rates of Douglas-fir, total and LMW western hemlock, and LMW vine maple leachates (group 2) were significantly less than group 1 rates. The pairwise comparisons also showed that the leachates in group 2 did not significantly differ from each other in terms of leucine incorporation. Estimates of the mean bacterial
Table 1. Concentrations of low (lmw) and high (hmw) molecular weight dissolved organic carbon (DOC) and polyphenolics from DOM in forest litterfall leachate.

<table>
<thead>
<tr>
<th>Leachate type</th>
<th>DOC (mg L$^{-1}$)</th>
<th>Polyphenolics (mg L$^{-1}$)</th>
<th>Polyphenolic : DOC ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>hmw red alder</td>
<td>68</td>
<td>89</td>
<td>1.31</td>
</tr>
<tr>
<td>lmw red alder</td>
<td>82</td>
<td>111</td>
<td>1.34</td>
</tr>
<tr>
<td>hmw vine maple</td>
<td>23</td>
<td>37</td>
<td>1.60</td>
</tr>
<tr>
<td>lmw vine maple</td>
<td>65</td>
<td>98</td>
<td>1.49</td>
</tr>
<tr>
<td>hmw red cedar</td>
<td>3.0</td>
<td>4.2</td>
<td>1.40</td>
</tr>
<tr>
<td>hmw western red cedar</td>
<td>19</td>
<td>15</td>
<td>0.80</td>
</tr>
<tr>
<td>hmw Douglas-fir</td>
<td>21</td>
<td>0.91</td>
<td>0.04</td>
</tr>
<tr>
<td>lmw Douglas-fir</td>
<td>18</td>
<td>2.2</td>
<td>0.12</td>
</tr>
<tr>
<td>hmw western hemlock</td>
<td>4.5</td>
<td>1.9</td>
<td>0.42</td>
</tr>
<tr>
<td>lmw western hemlock</td>
<td>17</td>
<td>2.7</td>
<td>0.15</td>
</tr>
</tbody>
</table>

Table 2. The bacterial carbon production (BCP) rates for seven DOM sources over the 24 hour experiment. Rates are based on an intracellular isotope dilution of 50%.

<table>
<thead>
<tr>
<th>Leachate type</th>
<th>Mean BCP (μg C$_{\text{bacterial}}$ cm$^{-2}$ h$^{-1}$ mg DOC$^{-1}$)</th>
<th>BCP range (μg C$_{\text{bacterial}}$ cm$^{-2}$ h$^{-1}$ mg DOC$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>red alder</td>
<td>2.8</td>
<td>0.13 - 8.0</td>
</tr>
<tr>
<td>vine maple</td>
<td>2.5</td>
<td>0.37 - 8.3</td>
</tr>
<tr>
<td>western red cedar</td>
<td>2.2</td>
<td>0.26 - 3.8</td>
</tr>
<tr>
<td>western hemlock</td>
<td>0.19</td>
<td>0.01 - 0.77</td>
</tr>
<tr>
<td>Douglas-fir</td>
<td>1.6</td>
<td>0.14 - 3.3</td>
</tr>
<tr>
<td>lmw vine maple</td>
<td>0.24</td>
<td>0.18 - 0.82</td>
</tr>
<tr>
<td>lmw hemlock</td>
<td>0.27</td>
<td>0.01 - 1.3</td>
</tr>
</tbody>
</table>
Figure 2. The cumulative heterotrophic bacterial incorporation of [³H] leucine after 24 hours of exposure to various leachate types. Low molecular weight (LMW) fractions (<10 000 nmw) were only tested using vine maple and hemlock leachates. Data are means ± 1 SE (n = 3). Letters represent significant differences (p<0.05) determined by Least Squares Means comparisons of the ln-transformed data.
Figure 3. The rates of heterotrophic bacterial incorporation of $[^3]$H leucine during 24 hours of exposure to various leachate types. All incorporation rates are per mg leachate DOC. All data points are means ($n = 3$). Each point represents the hourly rate for the whole period from the preceding point. Lines are for visual aid but do not represent uptake rates between points.
growth in terms of bacterial carbon production range from 0.19 – 2.8 mg C_{bacteria} cm^{-2} h^{-1} mg DOC^{-1} (Table 2) and follow the same patterns as the leucine incorporation rates.

A stepwise regression was done to examine the amount of the variation in bacterial incorporation that could be explained by the C : N, C : H and polyphenol content of the five whole, leachate DOM types. The response variable here is the cumulative amount of leucine incorporated by bacteria after 24 hours of exposure to various leachate DOM types (μmol leu_{inc} cm^{-2} mg DOC^{-1}). The leachate C : N was found to explain most of the variation in bacterial incorporation (r^2 = 0.84, F_{1.4} = 15.66, p = 0.03) (Fig. 4). Other variables were found not to contribute significantly to the model.

The week-long leaching of three litterfall types, western hemlock, western red cedar, and red alder, revealed more differences between the leachates. Roughly 74% of the total amount of DOC leached from alder was measured after 1 day while hemlock and cedar had only leached 14 and 30%, respectively, of their 7 day total (Fig. 5). After 7 days of leaching though, the hemlock and cedar leachates only had 40 and 20%, respectively, of the DOC in red alder leachate. Red alder also stood out in terms of C : N because it showed decreases from approximately 100 to 80 while the cedar and hemlock leachates increased in C : N over the 1 week period. The cedar leachate differed greatly from the other leachate types with an increase in C : H of nearly 40% over the week of leaching.
Figure 4. The heterotrophic bacterial incorporation of \(^3\)H leucine after 24 hours plotted against the C : N of the 5 leachate DOM types. Data points are means ± 1 SE (n = 3).
Figure 5. The cumulative DOC, C:N and C:H content of DOM leached from 3 litterfall types over a 1 week period. All data points are means ± 1 SE (n = 3). Errors bars are based cumulative totals at each time interval.
The DOC, C : N and C : H of two streams in the study area were monitored (Fig.6). Samples were collected in the summer and through the fall to capture changes in stream chemistry that could track litterfall inputs. Both streams showed similar changes in DOC concentration. The changes in stream DOC coincided with some litterfall events observed during collection. In July, the western hemlock needles were falling in greater quantity than other months while the stream DOC also showed an increase in DOC during this time. The C : N flux in Mike Cr. followed a similar pattern to its DOC changes with a peak in early August. In September and November the C : N for the two stream diverged such that East Cr. continued to increase while the Mike Cr. C : N decreased. In contrast, the C : H patterns of the two streams closely tracked each other throughout the months shown here. All three variables were shown to be capable of changing rapidly for all streams.
Figure 6. The DOC, C : N and C : H for DOM in two streams in the MKRF.
DISCUSSION

The goal of this study was to examine the heterotrophic bacterial use of various DOM sources. Specifically, the experiment set out to measure the differential transfer of organic matter from various riparian vegetation types to the biomass of benthic bacteria in streams. By measuring the incorporation of \([^3]H\)leucine, an amino acid known to be a requirement of bacterial protein, into bacteria this study provides estimates of the relative quality of various DOM types based on the bacterial biomass they support. To better understand why we may observe differences in bacterial growth, several chemical parameters were compared amongst the DOM types tested here. Leachate derived from the litterfall of riparian tree species of different seral stages was chosen as the DOM source because their inputs to the stream vary spatially, temporally and with land use related changes to forests. Also, the leaching of litterfall has been shown to be an important component of daily DOC exports from small forested streams (Meyer et al. 1998).

*Leachate Chemistry*

The leachate DOM chemistry varied greatly amongst the litterfall types. As expected, the leaves from the deciduous species, red alder and vine maple, produced highly-coloured leachates with DOC levels ranging from roughly 60 mg L\(^{-1}\) to 160 mg
L⁻¹ from 2 g of litter added to 500 mL of water. In contrast, needles from the coniferous species, western red cedar, Douglas-fir and western hemlock, produced lightly coloured or clear leachates with DOC less than 40 mg L⁻¹. To give some sense of scale in terms of DOC, the concentrations in the streams at the study sites were approximately 2 – 5 mg C L⁻¹ during the fall months in 1998. The red alder leaves were interesting because they would sometimes break apart during leaching, producing a more turbid leachate, or they would remain intact and produce a coloured, yet clearer leachate. Carbon analysis of these leachates later revealed that the more turbid leachate had higher DOC and phenolic concentrations. This variation in mechanical degradation of alder leaves seems to have contributed to the relatively wide variation in DOC and phenolics found in alder leachates. It would seem reasonable to expect the same breaking of alder leaves occurs during natural in-stream processes hence this aspect of the experiment did not add an artificial dimension to the results. Another leachate type to stand out was the Douglas-fir which had approximately double the DOC concentration of the other coniferous leachates types. This additional DOC comes from the high molecular weight fraction of the leachate.

The leaves and needles in this experiment were air-dried prior to leaching and this may have influenced leachate concentrations. In another study (Gessner 1991) it was found that dried litter can sometimes release DOM more readily than fresh litter. This could mean that longer periods of leaching may be required of fresh litter to achieve the DOM release reported here. The rationale of air-drying the litter here was standardize the
water content such that the litter could be weighed equitably amongst the different leachates.

More DOC was present in the LMW than HMW fraction of most leachates, which contrasted with studies done on first order streams in a watershed at the Coweeta Hydrologic Laboratory in North Carolina, Meyer et al. (1998) found HMW DOC to account for 67-78% of total DOC in a reference stream, and in a litter-excluded stream the contribution of HMW DOC decreased slightly. This opposite trend to the findings here may be a result of the different vegetation at Coweeta such as poplar, oak and other mixed hardwoods which may have different leaf chemistry than the tree species studied here. Also, if LMW DOC is preferred in the Coweeta streams, then the relatively high HMW concentrations may be a product of LMW loss.

The polyphenolic content of the leachates closely follows the trends of their DOC content. This is likely a result of the fact that polyphenolics contain organic carbon. In all leachates, the LMW fraction was found to have a higher proportion of polyphenolics than the HMW fraction. In a similar study, Freeman et al. (1990) found that when they measured colloidal and dissolved organic carbon in UK and German streams, the <1000 nmw fraction contained much less DOC and phenolics than samples >1000 nmw. The fraction of DOM which is chosen to be ‘low’ in molecular weight is somewhat arbitrarily chosen yet the <1000 and <10000 nmw DOM can have very different biogeochemical properties as shown by Meyer et al. (1998) and this may explain some of the difference between the results here and the study by Freeman et al. (1990).
The DOM from the leachates had C : N ratios ranging from 25 to 200, though nearly all were < 75. The LMW leachates consistently had the higher C : N suggesting a lower protein content and likely higher carbohydrate abundance than the HMW organic carbon. To put these C : N values into context, these leachates are comparable (mean approx. 56 for whole leachates) to the cypress and pine leachates (mean approx. 52) tested by Sun et al. (1997). Furthermore, the C : N measured during the fall in streams near the research sites were typically between 4 and 10, which may suggest a larger influence from conifers, which is consistent with the domination of conifers in the forest cover at these sites.

The leachate DOM tested here had similar C : H ratios to the stream samples in the MKRF yet lower C : H ratios than the purified blackwater river and leachate samples analyzed by Sun et al. (1997). It should be noted that the C : H ratios display some large variation in samples where the C : H is particularly high. This is a result of the especially low hydrogen concentration that is approaching the detection limit of the elemental analyzer so there may be considerable error in those samples.
One week leaching of litterfall

The week-long leaching of three litterfall types was a test of the dynamics of DOM release from litterfall. The linear increase of DOC release from hemlock could mean that its importance to bacterial production is higher than indicated by the relative amount of bacterial production it supported here based on 24 h leachates. Since the bacterial growth supported by hemlock leachate averaged 1.3 mg C cm⁻² h⁻¹ and all other leachates were 10 mg C cm⁻² h⁻¹ or higher, it is likely that hemlock will still be of relatively low importance to aquatic bacterial growth, on a per gram of litter basis. The 20% reduction in red alder C : N over 1 week was interesting since C : N was found to correlate well with bacterial growth. This could translate into a lower quality rating for DOM leached red alder in terms of its stimulation of bacterial growth. Finally, the C : H change over one week was particularly large for cedar (Fig. 4) as it went from 0.87 – 1.4. A possible reason for this increase may come from an increase in the representation of LMW organics in cedar, which were found to have a C : H that was approximately 100% greater in the LMW fraction.

Bacterial Response to DOM sources

Previous studies have looked at bacterial carbon production in conjunction with leaf leachates and other DOM sources using a variety of microbial techniques (Cummins
et al. 1972; Dahm 1981; Weyers and Suberkropp 1996). The uptake rates of DOM can vary by nearly two orders of magnitude in field and lab experiments based on ambient chemical and physical conditions. Dahm (1981) found that the degree of initial DOC enrichment and length of study were key factors in determining uptake rates of DOC from red alder leachate. After 9 hours of growth on red alder leachate in a recirculating chamber, Dahm (1981) observed uptake of DOC at 43 mg C m$^{-2}$ h$^{-1}$ while in this study, biotic uptake after 9 hours was a comparable 60 mg C m$^{-2}$ h$^{-1}$ for the alder leachate. Kaplan & Bott (1983) reported DOC uptake rates ranging from 50 to 250 mg C m$^{-2}$ h$^{-1}$ for microbes exposed to jewel weed extract which is also comparable to the range of microbial uptake of <1 to 270 mg C m$^{-2}$ h$^{-1}$ found here. Direct comparison of above studies and this one are difficult though because the DOC uptake measured here was done with tritiated leucine incorporation, and the other studies measured removal of carbon from growth chambers. Abiotic factors and variations in bacterial growth efficiency mean that these comparisons serve more to assure ranges of growth rates are appropriate for this experiment. Meyer et al. (1987) did an interesting study that looked at the influence of DOC enrichment and found bacterial growth to change between 1.8 and 28 μg C$_{\text{bacterial}}$ mg DOC$^{-1}$ during a 72-hour experiment which may be comparable to the values here (Table 2) if they could account for growth per unit area.

Heterotrophic bacteria can vary greatly in growth efficiency in natural waters such that consumption and respiration rates do not reflect bacterial protein production (BPP). A bacterial assemblage may consume more DOM from one source than another, but if more of this carbon is respired as CO$_2$ rather than assimilated into living biomass,
then consumption or loss of DOC may not indicate trophic transfer up the food web. This experiment provides information on the initial response of bacteria in terms of their biomass accrual, a first step in trophic transfer in an aquatic food web.

The BPP is expressed in terms of $[^3]$Hleucine incorporated here because the intracellular, isotopic dilution factor for this particular bacterial assemblage is unknown and using a value from the literature may give false estimates of BPP. Similarly, a previous study (Meyer et al. 1987) expressed bacterial growth in terms of the incorporation of tritiated thymidine instead of grams of bacterial carbon when no correction for isotopic dilution was made. Since the transformation from $[^3]$Hleucine incorporation rates to BPP is a linear transformation, the relative differences between treatments in this study would not change. Hence $[^3]$Hleucine incorporation rates are a more robust way to express changes in bacterial biomass in this experiment. A conversion of leucine incorporation to bacterial carbon production was done however, to facilitate comparisons to previous studies and to add a biomass context to the values.

After 24 hours of exposure to seven DOM sources, the amount of $[^3]$Hleucine incorporated by bacteria ranged from <5 to >50 µmol cm$^{-2}$ mg DOC$^{-1}$. During the 24 hours of exposure to the leachates, the bacterial production rates monotonically declined after the 1 hour mark for most samples. These declines follow the general pattern for mass loss of soluble carbon during decomposition (Aber and Melillo 1991) hence support the expectation of carbon-limited growth during the experiment.
After a stepwise regression of C : N, C : H and polyphenolic concentration of the five whole leachates against bacterial leucine incorporation as the response variable, it was found that C : N was significant while no other variables added to the model explained significantly more of the variation in bacterial growth. The positive relationship between C : N and bacterial growth was unexpected. In a paper by Sun et al. (1997) the opposite trend was found for bacteria incubated for three days with purified DOM collected from a blackwater river and leachates from litterfall. The difference in trends could be a result of variation in incubation length or that the bacteria in this experiment preferred polysaccharides over proteinaceous moieties. Moreover, the DOM in this experiment was supplemented with nutrients to ensure carbon-limited growth, while Sun et al. (1997) did not, and this may have increased the carbon limitations on bacterial growth in this experiment.

The results of exposing the bacteria to different DOM size fraction show that the HMW fraction is important to bacterial growth. The LMW DOM (< 10 000 nmw) did not give rise to higher bacterial growth rates than the whole leachate, indicating that HMW DOM is important to bacterial growth. In a simple view of bacterial degradation, larger molecules are considered to be of lower nutritional quality because greater energy is require to break them down into constituents that bacteria can utilize. However, in more recent years the issue of molecular size and its influence on bacterial growth has become more complex. Studies have found that DOM greater than 1000 and 10 000 nmw can support consistently higher bacterial growth than their low molecular weight fractions (Meyer et al. 1987; Tranvik 1990; Amon and Benner 1997). One explanation
for HMW DOM being more labile is that LMW compounds are complexed with a recalcitrant, HWM core. Meyer et al. (1987) report that once the HMW DOM was hydrolyzed, bacterial thymidine incorporation rates declined while the LMW products of the hydrolysis stimulate bacterial growth.

Polyphenolics are a diverse group of molecules that correlated positively with bacterial growth in this experiment. As with the HMW DOM, polyphenolics are likely associated with smaller, less inhibitory compounds that may be ultimately responsible for supporting bacterial growth. Supporting this idea is the strong correlation between DOC and polyphenolics in the leachates. This positive relationship found here indicates that polyphenolic levels in bulk DOM samples should not be presumed to have a net inhibitory affect on bacterial assemblages. Furthermore, because polyphenolics are such a diverse groups of molecules, it should be noted that their quality in terms of bacterial inhibition can potentially change depending on DOM source.

The biochemical trends reported here should be treated with caution for those looking to extrapolate to in-stream patterns of bacterial production. The leachate DOM in soil and water goes through many stages of chemical and biological decomposition such that its availability to bacteria may change. For example, the LMW DOM in natural waters may be relatively recalcitrant because it has been degraded by microbes or chemically weathered while larger molecular forms may represent more recently formed DOM, hence be more available to bacteria (McKnight and Aitken 1998). The above trend resembles the results found here but the underlying mechanisms are very different. This
experiment involves DOM concentrations approximately 10 times higher than those found in the study-area streams. Also, to ensure carbon-limited growth, leachates were amended with micronutrients which likely contributed to growth rates higher than should be expected in natural waters where heterotrophic growth is not always C-limited (Fagerbakke et al. 1996). Efforts were made to eliminate DOC concentration-dependent factors, such as diffusion gradients between leachate types by diluting all leachates to approximately equal DOC concentration prior to feeding them to the bacteria.

The underlying premise in this experiment is that the bacterial growth measurement gives insight into new biomass available for consumers. Some typical grazers of bacteria include protists, flagellates, and ciliates. During this experiment, attempts were not made to limit grazing on the heterotrophic bacteria, hence the results here may underestimate the actual BPP. Meyer et al. (1987) found protozoa assemblages present during their 72 hour bacterial growth experiment the used enriched stream water. The relatively short period of incubation (24 hours) and the fact bacteria were grown in an in vitro apparatus using leaf leachates prepared with distilled water, means that it is unlikely that grazing substantially affected the results.

Stream DOM monitoring

The DOC, C : N and C : H in the two stream studied here show rapid changes in magnitude. Western hemlock trees were dominant in the study sites so their inputs are likely to have an impact on stream DOM dynamics. The peak in hemlock needle-fall in July likely contributed to the concurrent rise in DOC found in both creeks. The C : N of
hemlock leachates was approximately 20 while in June the streams had <5, hence if the hemlock was contributing to the rise in DOC, it would be expected that a rise in C : N would also be observed. The C : N in Mike and East Cr. during late July / early August was their greatest value measured during the month reported here, thus giving support to the idea that hemlock needle inputs affect the stream DOM.

**Implications to forest managers**

The results presented here show that there are some clear biochemical differences between the litterfall of different tree species. The quality of DOM from deciduous litterfall is higher than that of conifers in terms of the aquatic bacterial growth supported. Changes to the riparian tree species composition will contribute to a change in DOM quality in streams and can lead to changes in the benthic bacterial biomass. Forest clearing and subsequent succession can lead to a shift from mature, coniferous trees like western hemlock and Douglas-fir to a more deciduous-based forest composed of earlier seral stage species like red alder. Hence, the new DOM inputs from forest litterfall may have a higher quality for the microbial food base.

This study is a primary step towards understanding the links between terrestrial and aquatic carbon. Forests will vary in their rates of litterfall input depending on tree density, age and the ecology of their site. The information of relative quality provided
here could be combined with estimates of the mass of litterfall inputs to better predict
effects on the stream bacteria.

Further experiments that would build on the information here would include a
study of the trophic transfer from increased bacterial protein to their grazers.
Furthermore, a study of bacterial dynamics beyond 24 hours is essential for
understanding the net effects of changes to DOM inputs on stream productivity. Also,
assemblages of heterotrophic bacteria vary spatially and temporarily in taxa diversity so a
study of changes in litterfall types would need to be tested on a variety of bacterial
sources. A study of bacterial response to change in DOM during all seasons would give
much needed insight into the longer-term meaning of the results shown here. Research in
the past (McArthur and Marzolf 1986) has reported that bacteria can ‘acclimate’ to local
DOM sources so the findings here may be less relevant to areas with different types of
vegetation and bacterial communities.
Summary

This study shows that dissolved organic matter leached from coastal forest litterfall can vary in quantity and quality. The deciduous litter DOM sampled here supported consistently higher aquatic, heterotrophic, bacterial growth rates than coniferous samples. Of the chemical variables measured, the carbon : nitrogen atomic ratio of the leachate’s dissolved, organic fraction was found to be the best predictor of bacterial leucine incorporation. Through changes in tree species, riparian forests can influence the stream microbial productivity based on the changes in dissolved organic matter.
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39


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APPENDIX

Calculation for [3H]leucine incorporation, bacterial protein production (BPP) and bacterial carbon production (BCP).

From Ward & Johnson (1996)

\[
\text{mol leucine}_{\text{inc}} \text{ cm}^{-2} \text{ h}^{-1} = \\
\frac{\text{dpm}_{\text{sample}} - \text{dpm}_{\text{killed}} \times \text{min/h}}{\text{sample} + \text{TCA, 13.25 mL} \times \text{formalin factor, 1.03}} \times (\text{dpm/Ci})(\text{specific activity in Ci/mmol})(\text{surface area, cm}^2)(\text{inc. time (min.)})(\text{mmol/mol})^{(\text{aliquot})}
\]

In the above, ‘aliquot’ = mL subsample filtered of 13.25 mL in the incubation tube, dpm/Ci = 2.2 x 10^{12} and mmol/mol = 1000.

To convert moles of leucine incorporated to grams of bacterial protein production, I followed the equation:

\[
\text{BPP (g)} = (\text{mol leucine}_{\text{inc}})(100/\text{mol\% leucine})(\text{leucine mw})(\text{ID})(\text{ED})
\]

where the mol % of leucine in protein is 7.3, and the mw weight of leucine is 131.2. The intracellular isotope dilution factor (ID) of leucine used here is 2. Simon and Azam (1989) found marine bacterioplankton to have a dilution factor of 50% while Jorgensen (1992) found freshwater bacteria to be more variable and exhibit a range of 30-90%, which would result in ID factors of 1.1 to 3.3. The external isotope dilution (ED) is entered into the calculations to account for the ratio of labeled to unlabeled leucine.

I calculate the extracellular dilution factor as follows:

The concentration of the unlabelled leucine in my stock solution = 450 nmol/22.5 mL = 20 \mu M. The concentration of [3H] leucine in the stock solution was 3.263 nmol/22.5 mL = 1.45 \mu M. Also, the ambient concentration of leucine found in stream water is often less than 1 nM but here it was treated as 1 nM.

\[
\text{ED} = (20+0.001/1.45) = 13.8
\]

Hence :

\[
\text{BPP (g)} = (\text{mol leucine}_{\text{inc}})(100/7.3 \text{ leucine})(131.1)(2)(13.8)
\]

or

\[
\text{BPP (g)} = (\text{mol leucine}_{\text{inc}})(49567)
\]

To convert bacterial protein (g) to bacterial carbon (g) I multiplied BPP by 0.86 (Ward and Johnson, 1996). The units for this final number are; g \text{C}_{\text{bacterial}} \text{ cm}^{-2} \text{ h}^{-1}. 

44