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Elemental Stoichiometry of Freshwater Hyporheic Microorganisms

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ELEMENTAL STOICHIOMETRY OF FRESHWATER HYPORHEIC MICROORGANISMS

By

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ABSTRACT

The work presented herein is part of the Microbial Observatory Project in the Nyack Floodplain. The Nyack Valley Floodplain is a glacier-carved floodplain in the Middle Fork of the Flathead River on the south west border of Glacier National Park and north of the Great Bear Wilderness in northwest Montana.

The study focuses on the bacterial diversity including elemental stoichiometry along a hyporheic flowpath underlying the Nyack floodplain. We isolated and characterized 44 bacterial strains, sampled microbial biofilm communities across the floodplain, characterized the bacterial isolates and determined elemental ratios (C:N:P).

We tested the hypothesis that while the elemental stoichiometry of freshwater benthic bacteria is mostly homeostatic (doesn't change significantly when nutrient feed ratios change), the range can vary and is dependent on the taxa.

We were able to show homeostasis with respect to C:N ratios (3.7:1 – 8.1:1) and these did not depend on taxa, however, with respect to N:P (0.8:1 – 77.3:1) and C:P (5.1:1 – 323:1) ratios, these bacteria were not homeostatic and there was a difference with respect to taxa.

In a floodplain context and from a "flow path" perspective, we hypothesized that the residence time of the hyporheic water in the aquifer, the distance of the well from the main channel and the water quality (DOC, STN and SP) influence the elemental content of sediment biofilm. This elemental content would vary in part by influencing microbial community structure as well as entrainment of nutrients of varying carbon, nitrogen and phosphorous content.

The only significant correlation found was between the DOC content of the hyporheic water and the carbon content of the biofilm ($p=0.02$). Across seasons DOC, STN and SP were not correlated with distance from the main channel or residence time. The C:N ratio of the microbial biofilm might be driven by the dominant species of microbial community rather than by the water quality, distance from the river or the residence time of the water.

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1. INTRODUCTION

The Middle Fork of the Flathead River begins in the Bob Marshall Wilderness and the southern part of Glacier National Park and flows through narrow canyons until it reaches the glacier-carved Nyack Valley floodplain. As the river enters the upstream end of the valley, approximately 30 percent of the flow enters a shallow aquifer (hyporheic) before eventually re-entering the river channel 8 km downstream essentially creating a "flowpath" (Figure 1). The hyporheic zone is defined by Findlay (1995) as the "sediment hydrologically linked to the open stream channel". It is also considered as the area where ground water and surface water mix and is an important component of lotic systems because it includes the region of saturated sediments beneath the stream channel and often extends laterally into the floodplain (Brunke & Gonser 1997). It has been suggested that the heterotrophic microbial community in this area can provide an important food source for meiofauna (Storey *et al*. 1999, Hayashi & Rosenberry 2002 and Wagner & Beisser 2005). Importantly, in the Nyack floodplain, an abundance of large-bodied insects with life cycle stages uniquely tied to the hyporheic habitat have been discovered here (Stanford & Ward 1988; Stanford & Ward 1993; Ward *et al*. 1994). This terrestrial to aquatic interface can be visualized as a food web where nutrients washed from the forest floor are "re-packaged" into microorganisms and then transferred to the bacteriovores as they graze on the microbial biofilms present on the substrata surface. We have begun to characterize the bacterial diversity including elemental stoichiometry along a hyporheic flowpath underlying the Nyack floodplain. We have isolated and characterized 42 bacterial strains, sampled microbial biofilm communities across the floodplain, characterized the bacterial isolates and determined elemental ratios (C:N:P). Our primary goals were 1) to expand the knowledge base relative to the elemental stoichiometry of different bacterial groups, 2) assess the strictness of homeostasis with respect to elemental stoichiometry of subsurface bacteria, and 3) explore trends in the microbial communities' elemental stoichiometry relative to their resource composition and other environmental parameters.

Bacteriovore fitness has been linked to resource elemental composition (bacterial C:N:P) (Sterner 1990; Tezuka 1990; Nakano 1994). Here, we attempt to understand specific links between bacteriovores and the shallow aquifer microbial community using an ecological stoichiometric approach. A stoichiometric approach was chosen because it has been shown to be a useful tool for examining ecosystem function and food web linkages as related to nutrients, organisms and ecological processes (Sterner & Elser 2002; Frost *et al*. 2005a and 2005b; Liess 2006).

Although several studies have analyzed biofilm stoichiometry with respect to variations in environmental nutrients (e.g. Fagerbakke *et al*. 1996; Cross *et al*. 2005; Bowman *et al*. 2005), none have examined the response of hyporheic biofilms.

1.1. ELEMENTAL STOICHIOMETRY AND BACTERIOVORES – Previous stoichiometric studies of interactions between algae and zooplankton suggest that food quality can affect life processes such as growth, reproduction and maintenance in both autotrophs and heterotrophs (Vadstein *et al*. 1988 and 1995; Elser *et al*. 2000a; Frost *et al*. 2005a; Acharya *et al*. 2005). For example, the cellular nutrient content (C:N:P) of algae has been used as an indicator of food quality for primary consumers. Foods that are considered to be higher in quality have lower carbon to nutrient ratio (Sterner & Schulz 1998; Frost & Elser 2002; Stelzer & Lamberti 2002; Evans-White & Lamberti 2006). The elemental nutrient composition in food does not always match that required by consumers for life processes. When this occurs, there is an elemental imbalance (Sterner & Hessen 1994; Sterner 1997; Frost & Elser 2002; Frost *et al*. 2002) which may affect trophic interactions, community structure, population dynamics and nutrient recycling (Sterner *et al*. 1997; Andersen 1997; Elser *et al*. 1998; Elser & Urabe 1999; DeMott & Gulati 1999; Loladze *et al*. 2000; Makino *et al*. 2003). Ecological stoichiometry has also revealed that organisms tend to overcome elemental imbalances by preferentially selecting food rich in the limited nutrient required. It follows that the stoichiometry of the invertebrate consumer and their resources (microbial biofilms) affects the function and structure of food webs. For example, the growth rate of consumers will decrease when they are faced with microbes with a higher C:P or C:N ratio than their own; this decrease in growth rate

will eventually lead to low grazing pressures (Vrede *et al*. 2004). Mitra & Flynn (2005) suggest that prey quality is associated with its nutritional status and it varies with the bulk elemental stoichiometric relationship between predator and prey. Since the nutrient stoichiometry of bacterial consumers is higher than that of their prey (consumers tend to have low N and P content, therefore high C:N and C:P ratios), they are important nutrient recyclers (Cross *et al*. 2005). This occurs because the nutrients that are not assimilated will be excreted or egested back into the environment. Therefore, the nutrient levels available to heterotrophic microorganisms can be affected by consumers through the nutrient content of their excreted products. For example, organisms may select food with high N content (low C:N) because they are C limited, and N rich foods have the greatest quantities of readily assimilable C (Anderson *et al*. 2004).

In a floodplain context and from a "flow path" perspective, we hypothesize that invertebrates may localize in regions of the aquifer in which microbial communities, whose community structure is influenced by their resource allocation, have C:N:P ratio's more similar to their needs. An alternate hypothesis is that biofilm community elemental stoichiometry does not vary much along the flow path because microbial C:N, C:P, or N:P is more homeostatic than not and/or that the biofilm communities are diverse and exhibit a high degree of evenness, therefore the community elemental ratio's would have similar averages along the flowpath.

1.2. ELEMENTAL STOICHIOMETRY AND HOMEOSTASIS – Homeostasis, in ecological stoichiometry, is the resistance to change of the internal elemental composition of an organism in comparison to its external nutrient resources (Sterner & Elser 2002). In nature we can encounter cases of strict homeostasis, where the chemical composition of the consumer is independent from that of their resource. However, it does not mean that the consumer will exhibit zero variation in their nutrient content throughout their life cycle. Therefore we can say that there are two extremes with respect to homeostasis, the absence of homeostasis (Figure 2, panel A) and strict homeostasis (Figure 2, panel B). The degree of homeostasis will be the area in between these extremes (Figure 2, panel C).

The degree of homeostasis can be analyzed with equations where the resource stoichiometry (*x*) is plotted against the consumer stoichiometry (*y*) in a logarithmic scale (the consumer and resource stoichiometry have to be measured in the same scale; stoichiometry refers to any sort of ratio of substances or masses) to obtain the equation below.

$$\log(y) = \log(c) + \left[\frac{1}{H}\right] * \log(x)$$

where *c* is a constant and *H* is the regulation coefficient greater than one (Sterner & Elser 2002).

1.2.1. Invertebrate homeostasis – In herbivorous insects and zooplankton, C:P ratios vary more than C:N ratios due to the nutrient content variation in major biological structures and molecules like N-rich proteins versus N- and P-rich nucleic acids (Elser *et al*. 2000). It has been shown that the carbon, nitrogen and phosphorus ratio (C:N:P) of autotrophs varies widely among and within ecosystems (Frost *et al*. 2002). Other studies have shown that, across a broad range of environmental conditions and food qualities, benthic invertebrates are generally homeostatic (Stelzer & Lamberti 2002; Cross *et al*. 2003; Frost *et al*. 2003; Bowman *et al*. 2005; Evans-White *et al*. 2005). However, some invertebrate benthic taxa might be less so. For example, Cross *et al*. (2003) compared the nutrient ratio of stream insects (primarily Trichoptera) from a nutrient enriched stream to a reference stream and determined that there was a four-fold difference in their C:P and N:P ratios. Others have found that with increased P content in food resources, there is a significant increase of this nutrient in benthic insects (Frost & Elser 2002; Bowman *et al*. 2005) and snails (Elser *et al*. 2005). DeMott & Pape (2005) determined that there is a considerable difference in homeostasis with respect to phosphorous among zooplankton species. They determined this by feeding P-deficient and P-sufficient algae to different *Daphnia* taxa that represented different body sizes and habitat preferences. Makino *et al*. (2003) determined that, in a lake environment, autotrophs are not as strongly homeostatic as zooplankton or bacteria since there was no significant variation with respect to C:P and N:P ratio in each bacterium studied (*E. coli, P. fluorescence* and an environmental strain isolated from a lake community). The variability in the carbon to nutrient ratio of

autotrophs with respect to heterotrophic bacteria could be due to storage of elements found in excess in the environment (Frost *et al*. 2005b).

1.2.2. Bacterial homeostasis – In general, most bacteria, like animals, are thought to be homeostatic with respect to their elemental composition (Bratbak 1985; Nagata 1986; Goldman *et al*. 1987; Chrzanowski & Kyle 1996). Individual organisms must be able to adapt to low metabolic states and these adaptations will alter the size of the cell and macromolecular composition (Heldal *et al.* 1996; White 2000). It has been shown that the protein content of microorganisms is high under carbon limitation and low under nitrogen limitation, therefore, the growth conditions of microorganisms can be reflected in their elemental composition and may point to possible limiting factors (Egli 1991; Fagerbakke *et al*. 1996). Some bacteria are able to reduce the elemental imbalances between themselves and the environment due to differences in their internal nutrient content. Frost *et al.* (2002) determined that across a range of nutrient concentrations, bacterial C:N and C:P ratios varied, however, the C:N ratios did not vary as much as C:P ratios, which varied two-fold. It is thought that these bacteria (from a freshwater lake environment) can manipulate their elemental balance by selectively acquiring the elements needed for growth. Without stoichiometric adjustment, imbalances may affect physiological processes including growth, reproduction, and maintenance in both autotrophs and heterotrophs (Frost *et al*. 2005b).

In the literature there are elemental stoichiometry studies of only a few bacterial isolates. In these, the C:N:P ratio was determined under different growth conditions (Table 1). Chrzanowski & Kyle (1996) determined the elemental ratio for *Pseudomonas fluorescens* and found that the C:N ratio was 7.6:1 which is within range of values previously published (2.8:1-17.2:1) for a wide variety of bacteria (pure and mixed cultures) from freshwater and marine environments (Bratbak 1985; Nagata 1986; Lee & Fuhrman 1987; Goldman *et al*. 1987; Tezuka 1990; Kroer 1994). The N:P ratio of these bacterial species ranged between 10:1 and 27:1 and the C:P ratio was varied, below 50:1 (under C or mineral limitation) and 100:1 (under P limitation). In a review by Cross *et al*. (2005), the

C:N ratio of freshwater benthic bacteria from 49 literature sources spanned between 2.9:1 and 7.6:1 and the C:P ratio was between 5:1 and 370:1.

1.3. BIOFILMS – It is well documented that bacterial biofilms are an important source of nutrients for higher trophic levels in lotic environments since they are grazed on by a variety of river organisms such as protozoa and stream invertebrates (Bärlocher & Murdoch 1989; Mohamed *et al*. 1998). Hall & Meyer (1998) demonstrated this by using isotopic tracers to follow bacterial carbon through the food web where it was detected in invertebrate predators. They found that between 10 and 100% of invertebrate carbon was derived from bacteria. Wiegner & Seitzinger (2004) determined that carbon and nitrogen components of dissolved organic matter vary within bacterial communities thereby affecting the amount transferred to higher trophic levels. The elemental ratio of marine bacterial assemblages is relatively invariant (Goldman *et al*. 1987; Kirchman 1994; Fukuda *et al*. 1998). For example, Fukuda *et al*. (1998) determined that there was no significant difference among the C:N ratio of bacterial assemblages in coastal (6.8±1.2) and oceanic (5.9±1.1) environments. These values are similar to those obtained by Goldman *et al*. (1987) for an assemblage of marine bacteria (C:N=5:1) under different substrate C:N ratios. They determined that the C:N ratio of marine bacteria was homeostatically regulated since the substrate C:N ratio did not have an effect on the bacterial C:N ratio.

The stoichiometry of bacterial assemblages from one fresh water lake environment was not homeostatic in terms of C:P (31:1-464:1) and N:P (7:1-41:1) since the slope on a logarithmic plot of bacterial ratio against nutrient ratio supply was not statistically different from a slope value of one (Tezuka 1990). However, Makino & Cotner (2004) determined that the microbial community in a different lake was homeostatic because the slope values from the logarithmic plot were statistically different from a slope of one, the values in this environment, C:P=55:1-175:1 and N:P=11:1-31:1, were within the range determined by Tezuka (1990). It has been shown that the polysaccharide matrix confers a degree of inertia to the heterotrophic community in the epilithic biofilm with regards to water quality parameters (Romani & Sabater 2001). Romani & Sabater (2001) concluded

that epilithic biofilms are less sensitive to variations in physical and chemical parameters due to the high complexity of the biofilm. Therefore, we can suggest that a more diverse bacterial community would probably be more homeostatic to variations in nutrient supply whereas a less diverse community would be less homeostatic. A summary of the biofilm ratios in different environments is presented in table 2.

The biofilm variation in nutrient content could also be due to differences in species composition. For example, Battin *et al*. (2001) determined that the benthic biofilm in a lotic environment was composed of organisms that belonged to the subclass *Betaproteobacteria* and the domain *Archea*. However, Feris *et al*. (2003) determined that the microbial community in the hyporheic zone of a lotic system was dominated by *Alphaproteobacteria* rather than *Betaproteobacteria*. They determined that, even though there was little variability of the microbial community within a stream, the organisms they found belonged to a number of different genera and suggested that the composition and richness of the microbial community changes even though the bacterial density was relatively constant.

We hypothesized that the shallow aquifer bacteria are non-homeostatic and that there is a difference in the nutrient ratio depending on phylogeny. To determine this, we determined the C:N:P ratios of 44 bacterial isolates grown on two media (high and low C:N, N:P and C:P ratios) and show that the carbon, nitrogen and phosphorus ratios of 20 of these bacterial isolates vary with phylogeny. In a larger scale, we hypothesize that the C:N:P of sediment biofilm will be influenced by residence time of the hyporheic water in the aquifer, the distance of the well from the main channel and the water quality (DOC, STN and SP). To determine this we correlated all these variables.

Table 1: Elemental ratios of bacteria found in the literature

Environment	C:N	N:P	C:P	Study
P.putida (under C limitation)	4.5:1	3.5:1	16:1	Bratbak 1985
P.putida (under N limitation)	5.3:1	3.7:1	19.6:1	
P.putida (under P limitation)	5.6:1	90:1	500:1	
P.fluorescense (C:N:P medium 3:2:1)	2:1		40:1	Nakano 1994
E.coli (C:N:P medium 5:2:1)	3.8:1		65:1	
E.coli (C:N:P medium 27:12:1)	2.6:1		55:1	
E.coli (C:N:P medium 98:24:1)	3:1		54:1	
F.ferrugineum (C:N:P medium 3:2:1)	2.6:1		66:1	
P.fluorescens	7.6:1	16:1 – 24.5:1	111:1 – 200:1	Chrzanowski & Kyle 1996
Vibrio natriegens (growing)	3.8:1		20:1	Fagerbakke *et al*. 1996
Vibrio natriegens (stationary)	3.8:1		18.2:1	
E.coli (growing)	3.4:1		11.1:1	
E.coli (stationary)	4.5:1		16.1:1	
E.coli under different growth rates	4:1	9:1 – 18:1	40:1 – 75:1	Makino *et al*. 2003

Table 2: Elemental ratios of bacterial communities found in the literature

Environment	C:N	N:P	C:P	Study
Coastal Assemblage	5.0:1 – 7.7:1			Fukuda *et al*. 1998
Oceanic Assemblage	5.4:1 – 8.3:1			Fukuda *et al*. 1998
Marine	5:1	9:1	45:1	Goldman *et al*. 1987
Planktonic Lake Bacteria	4.5:1 – 17.2:1	7:1 – 42:1	31:1 - 515:1	Tezuka 1990
Planktonic Lake Bacteria	3.3 – 7.1			Nagata1986
Planktonic Lake Bacteria at different supply of C:P levels	5:1 – 5.5:1	10:1 – 30:1	55:1 – 180:1	Makino & Cotner 2004
Planktonic Lake bacteria (1m depth)			5.9:1 – 15.2:1	Vadstein *et al*. 1988
Planktonic Lake bacteria (3m depth)			2.8:1 –29:1	Vadstein *et al*. 1988
Brakish water under C limitation	4.8:1	1.6:1	7.7:1	Bratbak 1985
Brakish water under N limitation	6.7:1	3.1:1	20.4:1	
Brakish water under P limitation	6.3:1	8.9:1	55.6:1	
Brackish water Raunefjorde, June	3.8:1		17.5:1	Fagerbakke *et al*. 1996
Brackish water Raunefjorde, October	4.2:1		24.4:1	
Knebel Vig, Aarhus Bight, 1994	4.5:1		25:1	
Knebel Vig, Aarhus Bight, 1992	4.2:1		15.5:1	
Tvärminne (marine)	5.9:1		23.3:1	
Lake Kalandsvatnet	5:1		20.4:1	

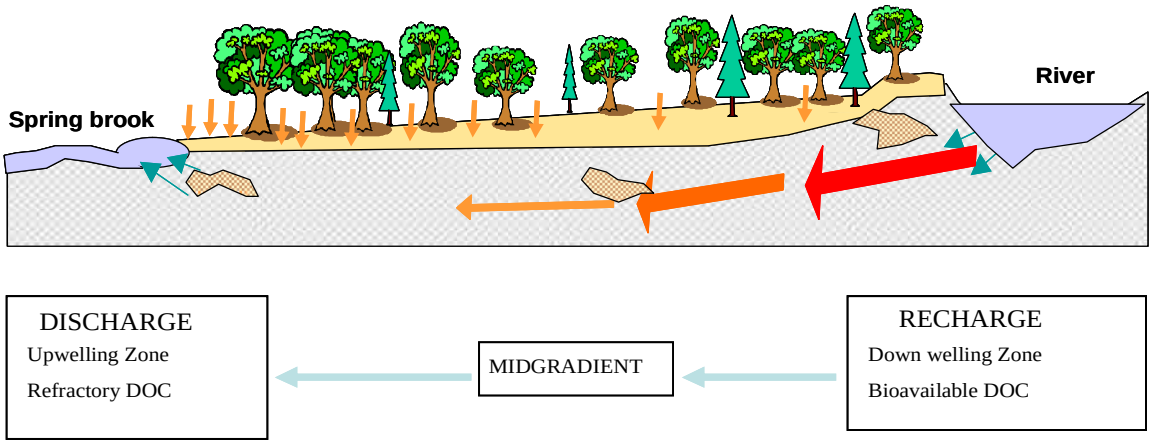

Figure 1: Explanation of a flow path (Stanford & Ward 1988)

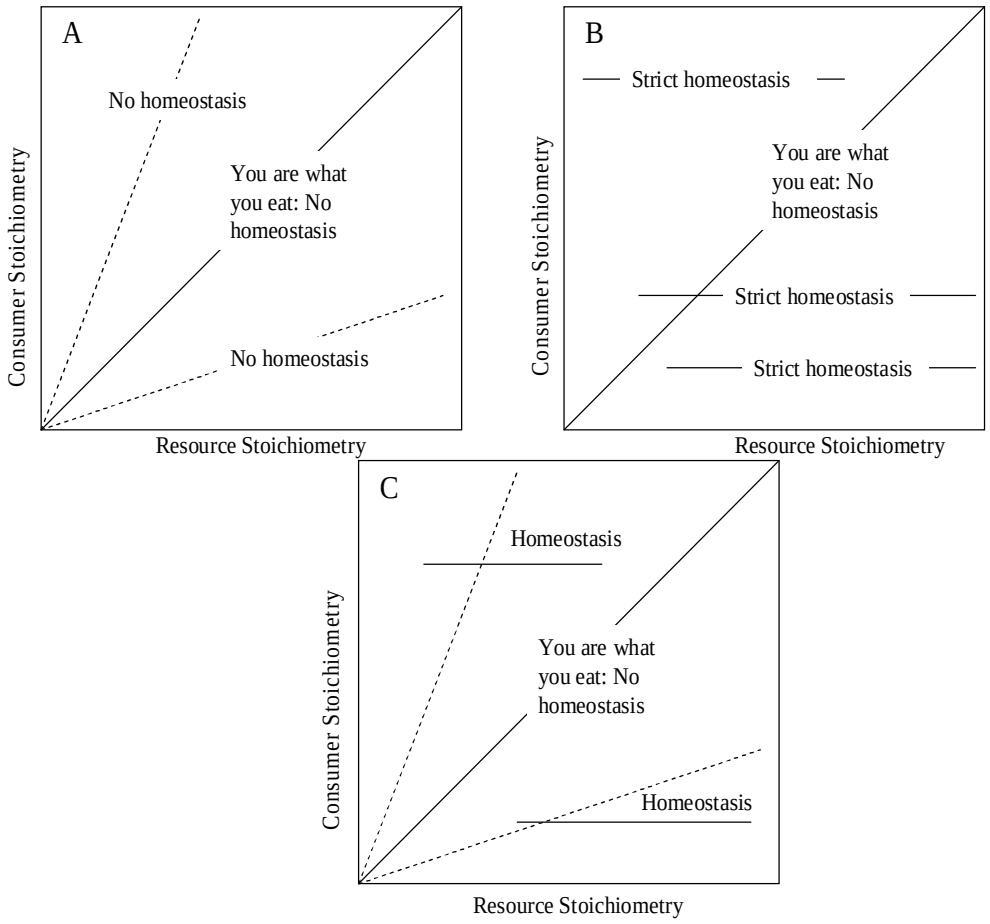

Figure 2: Graphic representation for homeostasis models. A: constant proportional model. B: consumers' stoichiometry is independent of the resource stoichiometry. C: expected ranges of homeostatic regulation (Sterner & Elser 2002).

2. BACTERIAL STOICHIOMETRY

ABSTRACT

The carbon to nutrient (C:N; C:P) and N:P ranges of bacteria isolated from the hyporheic zone of the Nyack Floodplain, in northwestern Montana, were examined to assess their range of variability under changing nutrient feed regimes. We tested the hypothesis that while the elemental stoichiometry of freshwater benthic bacteria is mostly homeostatic (doesn't change significantly when nutrient feed ratios change), the range can vary and depends on taxa. This difference in range could be important to the nutrient quality of bacteria within a trophic structure and perhaps influence feeding behavior. To determine this, we first selected 42 isolates and screened them for their elemental ratios on two media differing in C:N by 2-fold and then selected 18 of these for more detailed study on 5 media of varying C:N in order to assess elemental homeostasis using conventional procedures. These isolates represented members of different classes and genera of bacteria. We also included two type strains, *Escherichia coli* and *Aeromonas salmonicida*.

For the 42 isolates and two type strains grown on two media, the C:N ratio ranged between 3.7:1 and 7.8:1 on the low C:N medium and between 4.3:1 and 8.1:1 on the high C:N medium. In general, but not always, the C:N increased slightly (average 1.25X) with increasing media C:N. The greatest increase in biomass C:N was observed in *A. salmonicida*, *Arthrobacter* spp. and two *Flavobacterium* spp. where the C:N increased by a factor of about 1.5 suggesting that the homeostatic nature of some bacteria is not absolutely strict. In contrast to the C:N ratios, the N:P ratio varied considerably (average 6.7X) with increasing media N:P. The greatest increase was observed in *A. salmonicida*, *Rhodococcus* spp., three *Pseudomonas* spp., and one *Flavobacterium* spp. where the N:P increased by a factor greater than 10, suggesting that there is no homeostasis. Similar to the N:P ratios, the C:P ratios varied (average 5.3X) with increasing media C:P. The greatest increase was observed in *Rhodococcus* spp. (32X) and two *Pseudomonas* spp. (13X) suggesting that there is no homeostasis.

In order to take a more detailed look at the change in elemental stoichiometry, we chose 18 of the 42 hyporheic bacteria and the two type strains, grew them to late log phase on five media with increasing C:N ratio, and determined if the slope (1/H) in a logarithmic plot of cellular C:N vs. media C:N was significantly different from a slope value of one. We determined that the isolates selected were homeostatic with respect to their C:N ratio. However, we found that there was a non-homeostatic trend with respect to their N:P and C:P ratios.

2.1. INTRODUCTION

Bacteria, like animals, are generally thought to be homeostatic with respect to their elemental composition (Bratbak 1985; Nagata 1986; Goldman *et al*. 1987; Chrzanowski & Kyle 1996). Individual organisms must be able to adapt to low metabolic states that

alter both the size of the cell and macromolecular composition (Heldal *et al.* 1996; White 2000). The protein content of bacteria is high under carbon limitation and low under nitrogen limitation. Therefore, the growth conditions of microorganisms can be reflected in their elemental composition and may point to possible limiting factors (Egli 1991; Fagerbakke *et al*. 1996). Some bacteria are able to reduce the elemental imbalances between themselves and their environments due to differences in their internal nutrient content. Frost *et al.* (2002) determined that across a range of nutrient concentrations, bacterial C:N and C:P ratios varied. The C:N ratios did not vary as much as C:P ratios, which varied two-fold. It is thought that these bacteria (from a freshwater lake environment) can manipulate their elemental balance by selectively acquiring the elements needed for growth. Without stoichiometric adjustment, imbalances may affect physiological processes including growth, reproduction, and maintenance in both autotrophs and heterotrophs (Frost *et al*. 2005b).

In the literature there are elemental stoichiometry studies of only a few bacterial isolates. In these, the C:N:P ratio was determined under different growth conditions (Table 1) making finer scale comparisons difficult. Chrzanowski & Kyle (1996) found that the C:N ratio for *Pseudomonas fluorescens* was 7.6:1, which is within the broad range of values previously published (2.8:1-17.2:1) for a wide variety of bacteria (pure and mixed cultures) from freshwater and marine environments (Bratbak 1985; Nagata 1986; Lee & Fuhrman 1987; Goldman *et al*. 1987; Tezuka 1990; Kroer 1994). The N:P ratio of these bacterial species ranged between 10:1 and 27:1 and the C:P ratio varied from below 50:1 (under C or mineral limitation) to 100:1 (under P limitation). In a review by Cross *et al*. (2005), the C:N ratio of freshwater benthic bacterial sources spanned between 2.9:1 and 7.6:1 and the C:P ratio was between 5:1 and 370:1.

In this study, we first selected 42 isolates and screened them for their elemental ratios on two media differing in their C:N ratio by 2-fold and then selected 18 of these for more detailed study on 5 different media. These isolates represented members of different classes and genera of bacteria. We also included two type strains, *E. coli* and *A. salmonicida*. We were able to show homeostasis with respect to C:N ratios and these did

not depend on taxa, however, with respect to N:P and C:P, these bacteria were not homeostatic but there was a difference with respect to taxa.

2.2. MATERIALS AND METHODS

2.2.1. Source and description of isolates – The bacteria used for this study were isolated from the hyporheic zone of the Nyack Floodplain by conventional surface plating on a soil/river water agar. The phylogenetic relationship of the isolated bacteria was determined using the Ribosomal Database Project (RDPII) website (http://rdp.cme.msu.edu/) after subjecting the purified DNA to PCR amplification using the general primers 536f and 907r (Holben *et al.* 2002). The closest phylogenetic match for each isolate is presented with the S_{ab} score (Table 1). The S_{ab} score reflects how well the sequence being analyzed matched other sequences contained in the RDP II database. We considered a score equal or greater than 0.970 as the same species and scores between 0.900 and 0.960 as the same genera but not species (Holben *et al*. 2002).

Two type strains, *A. salmonicida* subsp *salmonicida* (ATCC®#33658, Manassas, VA) and *E. coli* (CSH Hfr62) were also included in this study as reference organisms.

2.2.2. Determination of elemental ratios – We determined the C:N ratio of 44 isolates on two different media [Luria Bertani (LB) and 0.2R2A] having different C:N ratios (8.2 and 4.1, respectively). In order to evaluate the non-homeostatic or homeostatic elemental ratios, we tested 18 isolates from these 42 and included *E. coli* and *A. salmonicida*. We selected isolates that represented different genera and that presented a high difference in their C:N ratio while growing on LB and 0.2R2A. We also selected pairs of species within each genus where available. The isolates chosen were II14 and II11 (*Pedobacter spp*.), I31, I33 and II46 (*Flavobacterium spp*.), II57, II5, II37, I73, II38 and I53 (*Pseudomonads spp*.), II6 (*Rhodoferax spp*.), II43 (*Janthinobacterium spp*.), II48 (*Rhizobium spp*.), I18 and II55 (*Brevundimonas spp*.), I89 (*Rhodococcus spp*.) and I46 (*Arthrobacter spp*.). The different classes analyzed were *Actinobacteria* (I46 and I89), *Alphaproteobacteria* (I18, II48 and II55), *Betaproteobacteria* (II43, II52 and II6), *Flavobacteria* (I31 and I33), *Gammaproteobacteria* (*A. salmonicida, E. coli*, I53, I73,

II38, II5 and II57) and *Sphingobacteria* (II11 and II14). The isolates were grown on five different media (LB, 0.2R2A, 0.2R2Am1, 0.2R2Am2 and 0.2R2Am3) with different C:N ratios (Table 2).

All isolates were grown until they reached late log phase. When this state was reached, the cells were pelleted by centrifugation (7000 rpm for 15 minutes), washed three times with sterile 0.85% NaCl and after the final wash, the pellet was resuspended in 10ml of 0.85% NaCl and freeze dried.

The carbon and nitrogen content of the bacterial isolates was determined by the analytical facilities at Flathead Lake Biological Station (University of Montana, Polson, MT.) by a modified Dumas (1831) method as described by Pella (1990) using a CHN Analyzer (Fisons NA1500, 1995). The values were determined in triplicate.

The phosphorus content was measured by the acid persulfate digestion method described by the American Public Health Association (APHA, 1998) where 100-200mg of dry sample was resuspended in 50ml of DI water. The liberated orthophosphate was measured by the ascorbic acid method as described in APHA (1998) and the absorbance was measured using HP 8453 UV-vis spectrophotometer.

2.2.3. Elemental homeostasis – The C:N ratios of the 44 isolates on two media (LB and 0.2R2A) were analyzed using one-way ANOVA (SPSS for Windows version 15.0) to determine if there was a significant difference ($P<0.05$) between the ratios in these two media.

The slope values from the stoichiometric model were analyzed by linear regression analysis to test whether the slopes were significantly different from a slope value of one and from each other (Tezuka 1990, Makino & Cotner 2004). This analysis was preformed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San Diego California USA, www.graphpad.com. This method is equivalent to an Analysis of Covariance (Motulsky & Christopoulos, 2003)

To determine if bacteria isolated from the freshwater shallow aquifer were homeostatic, we applied the stoichiometry model of Sterner & Elser (2002). In this model, the nutrient ratio of each medium was plotted against the nutrient ratio of the bacterial isolate in a logarithmic scale to obtain the equation below.

$$\log(y) = \log(c) + \left[\frac{1}{H}\right] * \log(x)$$

Where y is the nutrient ratio of the bacteria, x is the nutrient ratio of the media, c is a constant of integration and H is the regulation coefficient greater than one. The bacterial isolates were considered homeostatic when the slope [1/H] value obtained was significantly different from a slope value of one. The isolates were considered non-homeostatic when the slope value was not significantly different from one.

2.3. RESULTS

2.3.1. Differences in nutrient ratios – For the 44 isolates grown on two media, the C:N ratio ranged between 3.7:1 and 7.8:1 on the low C:N medium (4.1) and between 4.3:1 and 8.1:1 on the high C:N medium (8.2). The C:N values obtained on the two media were statistically different for all isolates except I89 (*Rhodococcus* spp. S_{ab}=0.957, P=0.31). With the exception of 4 isolates (I31, I46, II21 and *A. salmonicida*), the C:N content of bacteria increased modestly as the C:N content of the medium increased (Table 1). The majority of the bacteria had C:N ratios between 5 and 7 (on 0.2R2A medium) with the exception of three (I40, I89 and II56) that presented ratios greater than 7.

While the C:N ratio of the two media varied by a factor of 2, the average isolate C:N ratio difference on the two media was a 1.25 fold increase with a range of 1.0 to 1.6. The bacteria with the largest variations in C:N were *A. salmonicida* (1.56), *Arthrobacter* spp. (1.50), and two *Flavobacterium* spp. (1.5). Within the genus *Pseudomonas* (a total of 17 strains were analyzed) the variation in C:N ranged from a 0.89 to a 1.44 fold increase with an average of 1.25. We found no variability within the *Arthrobacter*, *Pedobacter* and *Brevundimonas* genera. The class with the largest variation in C:N was

Betaproteobacteria (1.4) with no difference in the C:N variation for the other classes: *Actinobacteria* (1.2), *Alphaproteobacteria* (1.1), *Gammaproteobacteria* (1.3), *Flavobacteria* (1.2) and *Sphingobacteria* (1.2).

The N:P ratio of the 44 bacteria studied with the lower N:P media (58.7) resulted in isolate N:P's ranging from 0.8 to 30.2 and the high N:P media (232.4) ranging from 7.4 to 77.3 . The N:P values, when grown on the two media, were statistically different for all isolates except for II55 (*Brevidomonas* spp. S_{ab}=0.906, p=0.30). The variation in the N:P content of bacteria appeared to be correlated with the N:P content of the growth medium. We observed that with an increase in N:P of the media, there was an increase in the N:P ratio of the bacteria (Table 1).

While the N:P ratio of the two media varied by a factor of 4, the average isolate N:P difference on the two media was a 6.5 fold increase with a range between 1.2 to 18.7 with I89 (*Rhodococcus* spp. S_{ab}=0.957) giving the highest ratio increase (33.1). The bacteria with the largest variations in N:P where the *Rhodococcus* spp. (33.1), three *Pseudomonas* spp. (18.7; 17.3 and 10.3), one *Flavobacterium* spp. (12.4) and *A. salmonicida* (10.3). There was a high variability within the genus *Pseudomonas* with an average 4.5 fold increase, where six of these isolates had ratio increases greater than 9. We found no ratio variability within the *Arthrobacter* genus. The class with the largest variation in N:P was *Betaproteobacteria* (7.2). Differences in the N:P variation for the other classes were as follows: *Actinobacteria* (4.9), *Gammaproteobacteria* (4.6), *Flavobacteria* (3.1), *Sphingobacteria* (2.7) and *Alphaproteobacteria* (1.6).

The class with the largest fold variation in N:P was *Betaproteobacteria* (7.2). This could be due to the fact that three of the six bacteria were not able to grow in the high N:P medium. If we consider only the three that grow on both media, the average fold difference for this class is 5.5, which is closely related to other classes like *Actinobacteria* (4.9) and *Gammaproteobacteria* (4.6). Four of the seven bacteria that are part of the *Flavobacteria* class were not able to grow in the high N:P media, so if we consider only the three that do, the fold difference of this class would be 5.9. Considering only the

bacteria that grow in both media, the fold difference for *Sphingobacteria* was 2.9 and for *Alphaproteobacteria* was 2.4.

The C:P ratio of the 44 bacteria studied varied between 5.1:1 and 141.4:1 on the low C:P medium (350.4) and between 36.4:1 and 323.3:1on the high C:P medium (477.5). The C:P values between the two media were statistically different for all bacteria except for II14 (*Pedobacter* spp. S_{ab}=0.878, p=0.05), II30 (*Pseudomonas* spp., p=0.44) and II55 (*Brevidomonas* spp. S_{ab}=0.906, p=0.495). II30 was 90% similar to II60 based on REP banding pattern and II60 had a S_{ab}=0.981 to *Pseudomonas poae*. The variation in the C:P content of bacteria appeared to be correlated with the C:P content of the nutrients available. We observed that with an increase in the C:P ratio of the media, there was an increase in the C:P ratio of the bacteria (Table 1).

The average isolate C:P difference on the two media was a 5.3 fold increase with a range of 1.0 to 13.7. The isolate I89 (*Rhodococcus* spp. S_{ab}=0.957) presented the highest ratio increase (32.1). The isolate with the highest variations in C:P where *Rhodococcus* spp. (32.1) and two *Pseudomonas* spp. (13.7; and 13.1). There was a large variability within the genus *Pseudomonas*, which had an average 3.7 fold difference with the majority having an increase close to the average (below a 5-fold difference). We found no ratio variability between the two *Arthrobacter* spp. and within the *Pedobacter* and *Brevundimonas* genera we found that one of the three isolates analyzed had a higher increase than the average. In both cases, this could be due to the fact that the one that had the high increase was classified based on REP fingerprinting patterns. The class with the largest variation in C:P was *Betaproteobacteria* (5.7) and the differences in the other classes were as follows: *Actinobacteria* (4.3), *Gammaproteobacteria* (3.8), *Sphingobacteria* (2.1) and *Alphaproteobacteria* (1.4).

2.3.2. Homeostasis experiments – In order to take a more detailed look at the change in elemental stoichiometry, we chose 18 of the 42 hyporheic bacteria, grew them to late log phase on five media with increasing C:N ratio, and determined if the slope (1/H) in a

logarithmic plot of cellular C:N vs. media C:N was significantly different from a slope value of one.

The C:N ratios of the 20 organisms selected ranged between 3.6:1 and 8.1:1 with I89 (*Rhodococcus* spp. S_{ab}=0.957) reaching a C:N ratio of 11.3:1 on 0.2R2Am3 medium (Table 3a). When analyzing the different genera, *Rhodococcus* presented the highest C:N ratio value of 11.3:1 on 0.2R2Am3 medium (Table 3b) and when they were analyzed by class, *Actinobacteria* presented the highest C:N ratio value of 8.5:1 on 0.2R2Am3 medium (Table 3c). We found that while the C:N ratio of two media (0.2R2Am3 and LB, largest C:N ratio difference) increased by a factor of 3, the average C:N ratio increase of the bacteria was of 1.3 (Figure 1). While the C:N ratio of two media (0.2R2Am1 and LB, lowest C:N ratio difference) increased by a factor of 1.8, the average C:N ratio increase of the bacteria was of 1.1 (Figure 2). This suggests homeostasis with respect to the C:N ratio since the elemental ratios of the organisms was maintained as the elemental ratio of the media varied.

We found that the slope for each isolate was significantly different (Table 6) from a slope of one and that there was no significant difference (p=0.99) between the 20 slopes, suggesting an overall homeostatic behavior with respect to their C:N ratio. We found the same results for genus and class. The difference between the slopes of each genus was not significant (p=0.99) and the difference between the slopes of each taxonomic class was also not significant (p=0.87).

The N:P ratio of the 20 organisms varied between 1.2:1 and 48:1 with II43 (*Janthinobacterium* spp. S_{ab}=0.985) reaching a value of 77.3:1 on LB (Table 4a). When the organisms were analyzed by genus, *Janthinobacterium* presented the highest N:P ratio of 77.3:1 on LB medium (Table 4b) and when they were analyzed by class, *Betaproteobacteria* presented the highest N:P ratio of 51.9:1 on LB medium (Table 4c). II14, II55 and II6 were the only organisms that maintained a relatively constant N:P with increasing fold difference of the media suggesting that these microorganisms are homeostatic with respect to N:P ratios. We found that while the N:P ratio of two media

(0.2R2A and LB) that present the largest C:N ratio difference increased by a factor of 4, the average N:P ratio increase of the bacteria was of 4 (Figure 3). While the N:P ratio of two media (0.2R2A and 0.2R2Am1) that presented the lowest N:P ratio difference increased by a factor of 1.4, the average N:P ratio increase of the bacteria was of 1.5 (Figure 4). This suggests non-homeostasis with respect to the N:P ratio since the fold difference of the organisms varied as the fold difference of the media varied.

We found that only II14, II55 and II6 presented slopes significantly different (Table 6) from a slope of one, which suggests that these isolates are homeostatic with respect to their N:P ratio, which corroborates what was mentioned previously. However, the overall difference between the slopes of the 20 organisms was not significant ($p=0.17$) which suggests a non-homeostatic trend. The slope obtained by genus was not significantly different from a slope of one with the exception of genera *Pedobacter* and *Rhodoferax* (Table 6), however, the difference between the slopes was not significant ($p=0.19$) which suggests a trend of non-homeostasis. The slopes for the taxonomic classes *Alphaproteobacteria* and *Sphingobacteria* were significantly different (Table 6) from a slope of one, however, the difference between the slopes was not significant ($p=0.09$), which suggests that the trend presented by these bacteria is to be non-homeostatic.

The C:P ratio of the 20 organisms varied between 9.8:1 and 204.4:1 with I89 and II43 reaching values of 314.6 and 323.2:1 on LB, respectively (Table 5a). When they were analyzed by genus, *Janthinobacterium* presented the highest C:P ratio of 323:1 on LB medium (Table 5b) and when they were analyzed by taxonomic class, *Betaproteobacteria* and *Actinobacteria* presented the highest C:P values of 218.1:1 and 217.9:1 on LB medium (Table 5c). I46, II14 and II55 were the only organisms that maintained a relatively constant C:P with increasing fold difference of the media suggesting that these microorganisms are homeostatic with respect to C:P ratios.

When we analyzed the C:P homeostasis, only the isolates I46, II14 and II55 presented a slope significantly different (Table 6) from a slope of one, which suggests that these isolates are homeostatic with respect to C:P ratios corroborating what was mentioned

above. However, the difference between the slopes of the 20 microorganisms was not significant (p=0.62) which suggests a non-homeostatic trend (overall). The genera *Brevundimonas*, *Arthrobacter* and *Pedobacter* presented a slope significantly different (Table 6) from a slope of one, as did the slopes of the taxonomic classes *Alphaproteobacteria* and *Sphingobacteria* suggesting that these genera and taxonomic classes have a homeostatic behavior with respect to the C:P ratios. However, the difference between the slopes of each genus was not significant (p=0.54) and the difference between the slopes of each taxonomic class was also not significant (p=0.73). This suggests that the trend presented by the 20 organisms is to be non-homeostatic.

2.4. DISCUSSION

The isolate I89 (*Rhodococcus* spp. S_{ab}=0.957) was the only one that presented no significant difference (p=0.31) in the C:N ratio between the high and low C:N ratio media. It is known that *Rhodococcus* spp. have high lipid content (Yoon *et al*. 2000), which may attribute to high carbon content. This could explain why there is no variation in the C:N ratio of this isolate.

This study compliments past studies of bacterial isolate homeostasis. The C:N ratios determined in the present study (ranging between 3.6:1 and 11.9:1) are within the range previously reported by several authors ranging between 2.8:1 and 17.2:1 (Bratbak 1985; Chrzanowski & Kyle 1996; Fagerbakke *et al*. 1996; Sterner & Elser 2002; Makino *et al*. 2003). Our data indicated that freshwater hyporheic bacteria are homeostatic with respect to their C:N ratio and that the ratio does not change with that of the environment. The lack of statistical difference (p=0.99) between the slopes of our 20 bacterial isolates suggests that bacteria from freshwater hyporheic environments do not differ in their C:N ratios.

The majority of the bacteria had C:N ratios between 5 and 7 (on 0.2R2A medium) with the exception of three (I40, I89 and II56) that presented ratios greater than 7. Even though three isolates present a higher C:N ratio than the rest of the bacteria studied, these values are still within range of C:N ratios previously described (Bratbak 1985;

Chrzanowski & Kyle 1996; Fagerbakke *et al*. 1996; Sterner & Elser 2002; Makino *et al*. 2003).

The isolate II55 (*Brevidomonas* spp. S_{ab}=0.906) was the only isolate that presented no significant difference (p=0.30) in the N:P ratio between the high and low N:P ratio media. In the homeostasis experiment, the bacteria II14, II55 and II6 were the only ones that maintained a relatively constant N:P with increasing fold difference of the media and they presented a slope value significantly different (p=0.03; p=0.01 and p=0.002 respectively) than a slope value of one. These results combined suggest that these isolates are strictly homeostatic with respect to its N:P ratio.

There was a large variation in the N:P ratio of the 44 bacteria analyzed. Since proteins and DNA content are associated with nitrogen content (Fagerbakke *et al*. 1996), differences in the content of these molecules in each bacteria could explain the large variations we found in the N:P ratio of the bacteria.

The N:P ratios of environmental bacteria reported in the literature are between 6:1 and 27:1 (Nagata 1986; Lee & Fuhrman 1987; Goldman *et al*. 1987; Tezuka 1990; Kroer 1994) which is a smaller range than obtained in the present study (0.8:1 and 77:1). The variation with respect to the literature could be due to environmental differences. Based on the N:P ratio homeostatic study, we can say that not all freshwater hyporheic bacteria are homeostatic with respect to their N:P ratio. Different species of bacteria behave differently with respect to their N:P ratio. This can alter the cycling of these nutrients in the environment. We found no statistical difference between slopes of the different genera studied in the logarithmic plot however, only two showed homeostasis. When grouped by genus, bacteria have a tendency to be non-homeostatic with respect to their N:P ratio.

The isolates II14 (*Pedobacter* spp. S_{ab}=0.878), II30 (*Pseudomonas* spp.) and II55 (*Brevundimonas* spp. S_{ab}=0.906) were the only ones that presented no significant difference (p=0.05, p=0.44 and p=0.49 respectively) in their C:P ratio between the high

and low C:P ratio media. II14 and II55 presented a homeostatic behavior since the slope was significantly different from a slope value of one. These results suggest that these organisms are strictly homeostatic with respect to C:P ratios. Although II30 was characterized as a *Pseudomonas* spp. based on REP fingerprint patterns, it is possible that the isolate was not a *Pseudomonas* spp. and therefore had a different nutrient ratio than other *Pseudomonas* spp.

The C:P ratio of environmental bacteria reported in the literature varies between 31:1 and 515:1 when the C:P ratio of the media they were grown in varied between 50:1 and 1200:1 (Tezuka 1990). Tezuka (1990) raises the point that it is not known how bacteria will behave beyond the range of the nutrient substrate studied. In the present study, the lowest C:P ratio was within the range of other studies and the highest C:P ratio was beyond the highest ratio studied before now (Tezuka 1990; Makino *et al*. 2003). It is for this reason that it had not been seen before the C:P ratio behavior that we see in the present study.

The C:P values of the bacteria increased, reaching a maximum value to later decrease with increasing C:P ratio of the medium. This suggests that after a C:P substrate ratio of 1000, P becomes limiting for bacteria. Since the bacteria are encountering resources of lower quality (higher C:P ratio), they are not able to meet their nutrient needs (Hessen 1997; Vrede *et al*. 2004). It is for this reason that we see a decline in the bacterial C:P ratio when the substrate C:P ratio increases beyond this value.

We can conclude that bacteria are homeostatic with respect to their C:N ratio but not with respect to their N:P or C:P ratios. We can also say that the homeostatic behavior varies according to phylogeny. They can present a homeostatic behavior with respect to their C:P ratio in the different classes but it may not be the same between different genera and there are more difference among the different species.

Table 1: Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
MRA	II61	Actinobacteria; Actinomycetales; Microbacteriaceae; Agreia	*Agreia pratensis (0.887)*	4.81	4.2	<0.05	8.94	49.99	<0.05	43.03	209.85	<0.05
Wally E-Rep	II2	Actinobacteria; Actinomycetales; Micrococcaceae; Arthrobacter	*Arthrobacter oxydans (0.967)*	5.98	4.24	0.012	6.78	17.72	0.004	40.45	74.13	0.005
Wally B	I46	Actinobacteria; Actinomycetales; Micrococcaceae; Arthrobacter	*Arthrobacter ramosus (0.963)*	6.84	4.56	0.006	10.76	26.59	<0.05	73.46	121.13	0.007
HA 20	I18	Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	*Brevundimonas intermedia (0.977)*	6.07	4.56	<0.05	9.89	29.24	<0.05	60.06	133.31	<0.05
MRA	II49	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium limicola (0.891)*	4.28	NG	SNA	9.37	NG	SNA	111.31	NG	SNA
MRI	II46	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium limicola (0.926)*	5.71	NG	SNA	11.59	NG	SNA	66.16	NG	SNA
MRA	II51	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium omnivovorum (0.919)*	5.46	7.1	<0.05	18.16			99.05		

NG = no growth in that medium SNA = statistics not available

Table 1(cont.): Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
MRE	II69	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium omnivorum (0.922)*	5.21	NG	SNA	14.31	NG	SNA	74.58	NG	SNA
Wally B	I31	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium micromati (0.912)*	5.51	3.65	<0.05	3.71	45.92	0.001	20.46	167.41	0.001
Wally B	I33	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium saccharophilum (0.956)*	5.5	3.99	0.004	7.6	26.51	0.004	41.78	104.23	0.004
Wally G	II21	Flavobacteria; Flavobacteriales; Flavobacteriaceae; Flavobacterium	*Flavobacterium saccharophilum (0.918)*	5.68	3.8	<0.05	4.45	20.76	<0.05	25.22	79.04	<0.05
MRI	II43	Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Janthinobacterium	*Janthinobacterium lividum (0.985)*	5.31	4.18	0.024	9.24	77.32	<0.05	48.92	323.16	<0.05
MRA	II52	Betaproteobacteria; Burkholderiales; Oxalobacteraceae; Janthinobacterium	*Janthinobacterium lividum (0.930)*	4.78	4.02	<0.05	13.15	67.07	<0.05	62.91	270.04	<0.05
MRA	II55	Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	*Brevidomonas subvibrioides (0.906)*	4.93	4.69	0.005	15.06	17.25	0.301	74.28	80.9	0.495

NG = no growth in that medium SNA = statistics not available

Table 1(cont.): Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
Wally E-Rep	II11	Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Pedobacter	*Pedobacter piscium (0.804)*	5.25	4.47	<0.05	10.74	32.19	<0.05	56.38	143.77	<0.05
Wally G	II14	Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Pedobacter	*Pedobacter africanus (0.878)*	6.49	4.85	<0.05	11.11	21.89	0.008	72.12	106.53	0.052
MRA	II53	Betaproteobacteria; Burkholderiales; Comamonadaceae; Polaromonas	*Polaromonas naphthalenivorans (0.888)*	4.96	NG	SNA	12.96	NG	SNA	64.2	NG	SNA
Wally B	I40	Betaproteobacteria; Burkholderiales; Comamonadaceae; Polaromonas	*Polaromonas vacuolata (0.871)*	7.35	NG	SNA	2.91	NG	SNA	21.37	NG	SNA
Wally E-Rep	I96	Betaproteobacteria; Burkholderiales; Comamonadaceae; Polaromonas	*Polaromonas vacuolata (0.871)*	6.37	NG	SNA	0.8	NG	SNA	5.1	NG	SNA
MRA	II64	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas congelans (0.959)*	4.78	4.99	0.017	12.16	55.53	<0.05	58.15	277.06	<0.05
Wally B	I62	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas grimontii (0.976)*	5.22	3.82	<0.05	1.49	27.78	0.001	7.74	106.2	0.001

NG = no growth in that medium SNA = statistics not available

Table 1(cont.): Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
Wally E-Rep	I72	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas grimontii (0.981)*	5.05	3.74	<0.05	4.93	35.51	<0.05	24.77	133.06	<0.05
Wally E-Rep	II5	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas grimontii (0.983)*	5.62	4.24	0.001	2.77	48.01	0.019	15.57	204.43	0.021
Wally G	II37	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas grimontii (0.983)*	5.59	3.89	<0.05	2.36	21.26	<0.05	13.22	82.87	0.001
MRE	II68	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas kilonensis (0.998)*	4.42	4.96	0.001	16.47	33.2	0.007	72.78	164.37	0.003
Wally B	I53	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas mandelii (0.975)*	5.23	3.78	<0.05	6.19	28.29	<0.05	32.37	106.94	<0.05
Wally G	II38	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas mandelii (0.976)*	5.27	3.68	<0.05	3.44	17.85	0.001	18.1	65.72	0.001
MRA	II60	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas poae (0.981)*	4.79	4.11	0.001	10.81	37.17	0.001	51.73	152.88	0.001

NG = no growth in that medium SNA = statistics not available

Table 1(cont.): Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
Wally G	II35	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas rhodesiae (0.984)*	5.09	3.73	<0.05	3.58	37.13	0.001	18.23	138.46	0.001
MRA	II57	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas veronii (0. 924)*	5.59	4.49	0.002	2.59	24.34	<0.05	14.47	109.19	<0.05
Wally E-Rep	I73	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas antarctica (0. 975)*	5.84	4.26	<0.05	7.79	41.45	0.006	45.43	177.74	0.011
MRI	II48	Alphaproteobacteria; Rhizobiales; Rhizobiaceae; Rhizobium	*Rhizobium giardinii (0.927)*	4.68	5.6	<0.05	30.18			141.35		
Wally E-Rep	I89	Actionbacteria; Actinomycetales; Nocardiaceae; Rhodococcus	*Rhodococcus koreensis (0.957)*	8.08	7.82	0.308	1.22	40.29	<0.05	9.82	314.63	<0.05
Wally E-Rep	II6	Betaproteobacteria; Burkholderiales; Comamonadaceae; Rhodoferax	*Rhodoferax ferrireducens (0.894)*	5.25	4.25	<0.05	8.52	26.61	<0.05	44.73	113.07	<0.05
type strain	Aerom	Gammaproteobacteria; Aeromonadales; Aeromonadaceae; Aeromonas	*Aeromonas salmonicida* subsp *salmonicida* (ATCC®#33658, Manassas, VA)	6.94	4.45	<0.005	2.5	25.87	0.014	17.34	116.38	0.022

NG = no growth in that medium SNA = statistics not available

Table 1(cont.): Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
type strain	Ecoli	Gammaproteobacteria; Enterobacteriales; Enterobacteriaceae; Escherichia	*Escherichia coli*	5.77	4.21	0.003	3.74	21.04	<0.05	21.59	88.24	<0.05
MRA	II56	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas* $^{\tau}$	7.78	NG	SNA	7.59	NG	SNA	59.05	NG	SNA
MRA	II62	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas* $^{\tau}$	4.53	4.11	<0.05	20.18	28.64	0.002	91.42	117.74	0.003
MRE	II66	Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Pedobacter	*Pedobacter* $^{\tau}$	5.45	NG	SNA	12.17	NG	SNA	66.26	NG	SNA
Wally E-Rep	II12	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas* $^{\tau}$	5.33	5.58	0.001	5.11	7.38	0.001	27.26	41.18	<0.05

NG = no growth in that medium SNA = statistics not available $^{\tau}$ = based on Rep fingerprint pattern

Table 1(cont): Phylogenetic classification of Nyack Isolates and statistical analysis on two different media.

Origin Site[a]	Sample Name	Classification	Best Match to type strain in RDP (Sab score)	Average C:N (0.2R2A=8.16)	Average C:N (LB=4.10)	ANOVA	Average N:P (0.2R2A=58.73)	Average N:P (LB=232.37)	ANOVA	Average C:P (0.2R2A=477.53)	Average C:P (LB=350.40)	ANOVA
Wally E-Rep	II13	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas* [τ]	5.41	4.57	<0.05	5.52	52.28	0.008	29.83	239.03	0.008
Wally G	II30	Gammaproteobacteria; Pseudomonadales; Pseudomonadaceae; Pseudomonas	*Pseudomonas* [τ]	5.27	4.2	0.003	6.8	8.7	0.018	35.86	36.36	0.443
Wally G	II33	Alphaproteobacteria; Caulobacterales; Caulobacteraceae; Brevundimonas	*Brevundimonas* [τ]	5.13	3.83	<0.05	3.71	24.93	<0.05	19.05	95.37	<0.05
Wally G	II34	Sphingobacteria; Sphingobacteriales; Sphingobacteriaceae; Pedobacter	*Pedobacter* [τ]	4.95	4.11	<0.05	4.2	21.76	0.001	20.81	89.37	0.001

NG = no growth in that medium SNA = statistics not available *T* = based on Rep fingerprint pattern

Table 2: Composition per liter of the media used for isolate growth and their C:N ratio

Component	LB	0.2R2A	0.2R2Am1	0.2R2Am2	0.2R2Am3
Tryptone	10gr				
Sodium Chloride	10gr				
Yeast extract	5gr	0.1gr	0.1gr	0.1gr	0.1gr
Proteose peptone		0.1gr	0.1gr	0.1gr	0.1gr
Casamino acids		0.1gr	0.1gr	0.1gr	0.1gr
Dextrose		0.1gr	0.05gr	0.2gr	0.5gr
Soluble starch		0.1gr	0.1gr	0.1gr	0.1gr
Sodium pyruvate		0.06gr	0.06gr	0.06gr	0.06gr
Potassium phosphate (dibasic)		0.06gr	0.06gr	0.06gr	0.06gr
Magnesium sulfate		0.01gr	0.01gr	0.01gr	0.01gr
C:N ratio	4.10	8.16	7.31	9.30	13.33

Table 3a: C:N ratio of the isolates growing in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	C:N ratio				
Isolate	LB	0.2R2Am1	0.2R2A	0.2R2Am2	0.2R2Am3
A.salmonicida	4.45	5.83	6.94	6.68	5.87
E. coli	4.21	4.39	5.77	4.56	5.03
I18	4.56	4.49	6.07	4.52	5.37
I31	3.65	4.68	5.51	4.53	5.79
I33	3.99	4.69	5.50	4.75	5.07
I46	4.56	5.68	6.84	5.53	5.80
I53	3.78	4.52	5.23	4.53	4.07
I73	4.26	4.56	5.84	4.71	6.37
I89	7.82	7.47	8.08	6.98	11.25
II11	4.47	4.91	5.25	5.14	6.60
II14	4.85	4.77	6.49	6.25	5.79
II37	3.89	4.55	5.59	4.51	5.78
II38	3.68	4.45	5.27	4.45	4.09
II43	4.18	4.32	5.31	4.21	5.55
II46	NG	5.39	5.71	5.17	5.57
II48	5.60	4.73	4.68	5.73	6.69
II5	4.24	4.73	5.62	4.53	5.78
II55	4.69	4.70	4.93	4.80	5.61
II57	4.49	4.83	5.59	4.61	5.16
II6	4.25	4.67	5.25	4.56	5.89

NG: no growth

Table 3b: C:N ratio of the isolates grouped by genera growing in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	C:N ratio				
Genus	LB	0.2R2Am1	0.2R2A	0.2R2Am2	0.2R2Am3
Aeromonas	4.45	5.83	6.94	6.68	5.87
Arthrobacter	4.56	5.68	6.84	5.53	5.80
Brevundimonas	4.62	4.60	5.50	4.66	5.49
Escherichia	4.21	4.39	5.77	4.56	5.03
Flavobacterium	3.82	4.92	5.57	4.82	5.48
Janthinobacterium	4.18	4.32	5.31	4.21	5.55
Pedobacter	4.66	4.84	5.87	5.70	6.20
Pseudomonas	4.06	4.61	5.52	4.56	5.21
Rhizobium	5.60	4.73	4.68	5.73	6.69
Rhodococcus	7.82	7.47	8.08	6.98	11.25
Rhodoferax	4.25	4.67	5.25	4.56	5.89

Table 3c: C:N ratio of the isolates grouped by class growing in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	C:N ratio				
Class	LB	0.2R2Am1	0.2R2A	0.2R2Am2	0.2R2Am3
Actinobacteria	6.19	6.58	7.46	6.26	8.52
Alphaproteobacteria	4.95	4.64	5.23	5.02	5.89
Betaproteobacteria	4.21	4.49	5.28	4.39	5.72
Flavobacteria	3.82	4.92	5.57	4.82	5.48
Gammaproteobacteria	4.12	5.02	6.00	5.32	6.16
Sphingobacteria	4.66	4.84	5.87	5.70	6.20

Table 4a: N:P ratio of the isolates growing in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	N:P ratio				
Isolate	0.2R2A	0.2R2Am1	0.2R2Am3	0.2R2Am2	LB
A.salmonicida	2.50	14.94	4.76	5.58	25.87
E. coli	3.74	13.90	8.74	13.63	21.04
I18	9.89	14.20	58.11	14.44	29.24
I31	3.71	9.99	66.64	11.46	45.92
I33	7.60	13.61	16.28	19.38	26.51
I46	10.76	13.25	8.84	17.82	26.59
I53	6.19	11.83	9.70	16.43	28.29
I73	7.79	12.83	11.08	15.75	41.45
I89	1.22	12.64	17.88	19.79	40.29
II11	10.74	14.36	19.94	17.41	32.19
II14	11.11	14.39	21.21	22.55	21.89
II37	2.36	8.31	15.42	17.85	21.26
II38	3.44	9.69	12.59	16.96	17.85
II43	9.24	9.29	16.65	21.59	77.32
II46	11.59	8.32	15.44	13.88	NG
II48	30.18	12.10	12.09	35.56	NG
II5	2.77	11.63	12.00	13.58	48.01
II55	15.06	17.45	23.43	20.55	17.25
II57	2.59	13.53	8.20	19.62	24.34
II6	8.52	11.28	18.17	18.43	26.61

NG: no growth

Table 4b: N:P ratio of the isolates grouped by genus in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	N:P ratio				
Genus	0.2R2A	0.2R2Am1	0.2R2Am3	0.2R2Am2	LB
Aeromonas	2.50	14.94	4.76	5.58	25.87
Arthrobacter	10.76	13.25	8.84	17.82	26.59
Brevundimonas	12.48	15.83	40.77	17.50	23.25
Escherichia	3.74	13.90	8.74	13.63	21.04
Flavobacterium	7.64	10.64	32.79	14.91	36.21
Janthinobacterium	9.24	9.29	16.65	21.59	77.32
Pedobacter	10.93	14.37	20.58	19.98	27.04
Pseudomonas	4.19	11.30	11.50	16.70	30.20
Rhizobium	30.18	12.10	12.09	35.56	NG
Rhodococcus	1.22	12.64	17.88	19.79	40.29
Rhodoferax	8.52	11.28	18.17	18.43	26.61

NG: no growth

Table 4c: N:P ratio of the isolates grouped by class in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	N:P ratio				
Class	0.2R2A	0.2R2Am1	0.2R2Am3	0.2R2Am2	LB
Actinobacteria	5.99	12.94	13.36	18.80	33.44
Alphaproteobacteria	18.38	14.58	31.21	23.52	23.25
Betaproteobacteria	8.88	10.29	17.41	20.01	51.97
Flavobacteria	7.64	10.64	32.79	14.91	36.21
Gammaproteobacteria	3.92	12.08	10.31	14.92	28.51
Sphingobacteria	10.93	14.37	20.58	19.98	27.04

Table 5a: C:P ratio of the isolates growing in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	C:P ratio				
Isolate	0.2R2A	0.2R2Am1	LB	0.2R2Am2	0.2R2Am3
A.salmonicida	17.34	87.16	116.38	37.26	27.95
E. coli	21.59	60.96	88.24	62.17	43.95
I18	60.06	63.77	133.31	65.33	10.81
I31	20.46	46.78	167.41	51.88	11.50
I33	41.78	63.84	104.23	92.01	82.56
I46	73.46	75.31	121.13	98.54	51.30
I53	32.37	53.43	106.94	74.43	39.43
I73	45.43	58.54	177.74	74.20	70.58
I89	9.82	94.38	314.63	138.17	201.07
II11	56.38	70.52	143.77	89.55	131.69
II14	72.12	68.57	106.53	140.94	122.91
II37	13.22	37.77	82.87	80.51	89.11
II38	18.10	43.14	65.72	75.40	51.46
II43	48.92	40.12	323.16	90.92	92.42
II46	66.16	44.87	NG	71.81	86.03
II48	141.35	57.25	NG	203.92	80.83
II5	15.57	55.05	204.43	61.50	69.37
II55	74.28	82.06	80.90	98.66	131.36
II57	14.47	65.28	109.19	90.41	42.34
II6	44.73	52.65	113.07	84.07	106.96

NG: no growth

Table 5b: C:P ratio of the isolates grouped by genera in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	C:P ratio				
Genus	0.2R2A	0.2R2Am1	LB	0.2R2Am2	0.2R2Am3
Aeromonas	17.34	87.16	116.38	37.26	27.95
Arthrobacter	73.46	75.31	121.13	98.54	51.30
Brevundimonas	67.17	72.92	107.10	82.00	71.09
Escherichia	21.59	60.96	88.24	62.17	43.95
Flavobacterium	42.80	51.83	135.82	71.90	60.03
Janthinobacterium	48.92	40.12	323.16	90.92	92.42
Pedobacter	64.25	69.55	125.15	115.24	127.30
Pseudomonas	23.19	52.20	124.48	76.07	60.38
Rhizobium	141.35	57.25	NG	203.92	80.83
Rhodococcus	9.82	94.38	314.63	138.17	201.07
Rhodoferax	44.73	52.65	113.07	84.07	106.96

NG: no growth

Table 5c: C:P ratio of the isolates grouped by class in LB, 0.R2Am1, 0.2R2A, 0.2R2Am2, and 0.2R2Am3 media

	C:P ratio				
Class	0.2R2A	0.2R2Am1	LB	0.2R2Am2	0.2R2Am3
Actinobacteria	41.64	84.85	217.88	118.36	126.18
Alphaproteobacteria	91.89	67.69	107.10	122.64	74.33
Betaproteobacteria	46.83	46.38	218.11	87.50	99.69
Flavobacteria	42.80	51.83	135.82	71.90	60.03
Gammaproteobacteria	22.26	57.67	118.94	69.48	54.27
Sphingobacteria	64.25	69.55	125.15	115.24	127.30

Table 6: Slope value for each isolate selected for the stoichiometry model

	C:N ratio			N:P ratio			C:P ratio		
Isolate	1/H	r2	p*	1/H	r2	p*	1/H	r2	p*
A. salmonicida	0.28	0.48	0.02	0.97	0.32	0.97	-0.06	0.002	0.27
E. coli	0.16	0.28	0.01	0.92	0.59	0.87	0.35	0.15	0.27
I18	0.13	0.17	0.01	0.84	0.42	0.79	-0.89	0.31	0.09
I31	0.37	0.77	0.01	1.75	0.66	0.38	-0.28	0.03	0.29
I33	0.21	0.58	0.01	0.81	0.92	0.27	0.46	0.55	0.11
I46	0.21	0.4	0.01	0.51	0.41	0.25	-0.1	0.03	0.04
I53	0.09	0.1	0.01	0.9	0.75	0.76	0.2	0.06	0.18
I73	0.32	0.63	0.02	0.96	0.7	0.93	0.31	0.12	0.24
I89	0.24	0.31	0.03	2.16	0.79	0.17	1.65	0.51	0.54
II11	0.31	0.84	0.003	0.71	0.91	0.11	0.52	0.58	0.16
II14	0.2	0.36	0.01	0.54	0.88	0.03	0.51	0.85	0.03
II37	0.32	0.68	0.01	1.51	0.86	0.24	1.21	0.75	0.64
II38	0.11	0.14	0.01	1.1	0.81	0.76	0.7	0.53	0.49
II43	0.21	0.44	0.01	1.45	0.83	0.32	0.62	0.19	0.63
II46	0.02	0.02	0.01	0.4	0.46	0.19	0.3	0.54	0.07
II48	0.14	0.16	0.02	-0.07	0.003	0.35	0.13	0.02	0.28
II5	0.24	0.57	0.01	1.67	0.82	0.24	0.8	0.26	0.81
II55	0.14	0.61	0.001	0.16	0.26	0.01	0.35	0.81	0.01
II57	0.11	0.28	0.003	1.29	0.63	0.64	0.62	0.2	0.63
II6	0.25	0.69	0.004	0.82	1	0.002	0.61	0.71	0.17

p*: Deviation from slope = 1

Table 6(cont.): Slope value for each genera and class selected for the stoichiometry model

	C:N ratio			N:P ratio			C:P ratio		
Genus	1/H	r2	p*	1/H	r2	p*	1/H	r2	p*
Aeromonas	0.28	0.48	0.02	0.97	0.32	0.97	-0.06	0.002	0.27
Escherichia	0.16	0.28	0.01	0.92	0.59	0.87	0.35	0.15	0.27
Brevundimonas	0.13	0.38	0.003	0.5	0.42	0.23	-0.27	0.17	0.03
Flavobacterium	0.3	0.74	0.01	1.12	0.88	0.64	0.16	0.04	0.18
Arthrobacter	0.21	0.4	0.01	0.51	0.41	0.25	-0.1	0.03	0.04
Pseudomonas	0.2	0.51	0.01	1.24	0.84	0.5	0.64	0.35	0.53
Rhodococcus	0.24	0.31	0.03	2.16	0.79	0.17	1.65	0.51	0.54
Pedobacter	0.25	0.78	0.002	0.62	0.99	0.003	0.51	0.82	0.04
Janthinobacterium	0.21	0.44	0.01	1.45	0.83	0.32	0.62	0.19	0.63
Rhizobium	0.14	0.16	0.02	-0.07	0.003	0.35	0.13	0.02	0.28
Rhodoferax	0.25	0.69	0.004	0.82	1	0.002	0.61	0.71	0.17
Class									
Actinobacteria	0.23	0.59	0.006	1.33	0.82	0.43	0.77	0.38	0.72
Alphaproteobacteria	0.14	0.45	0.002	0.34	0.63	0.02	-0.13	0.06	0.04
Betaproteobacteria	0.23	0.57	0.007	1.13	0.93	0.52	0.61	0.36	0.47
Flavobacteria	0.3	0.74	0.007	1.12	0.88	0.64	0.16	0.04	0.18
Gammaproteobacteria	0.29	0.73	0.006	1.17	0.76	0.69	0.51	0.24	0.43
Sphingobacteria	0.25	0.78	0.002	0.62	0.99	0.003	0.51	0.82	0.04

p*: Deviation from slope = 1

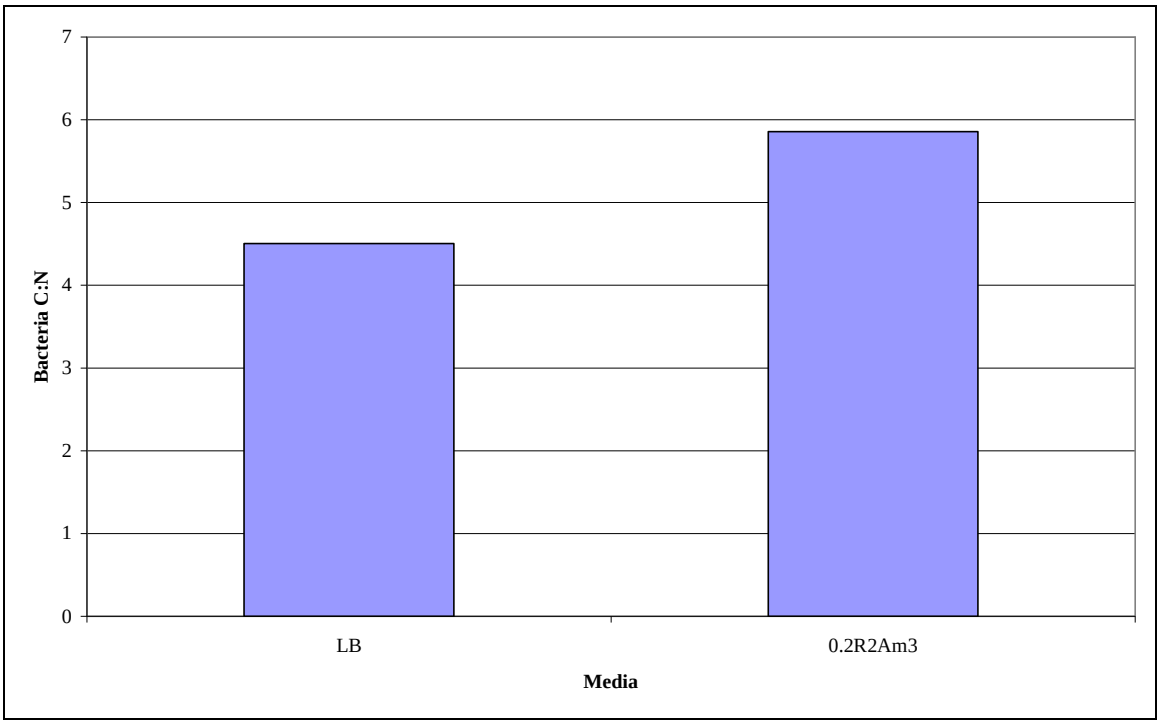

Figure 1: Average C:N increase of bacteria on two media that present a C:N increase factor of three.

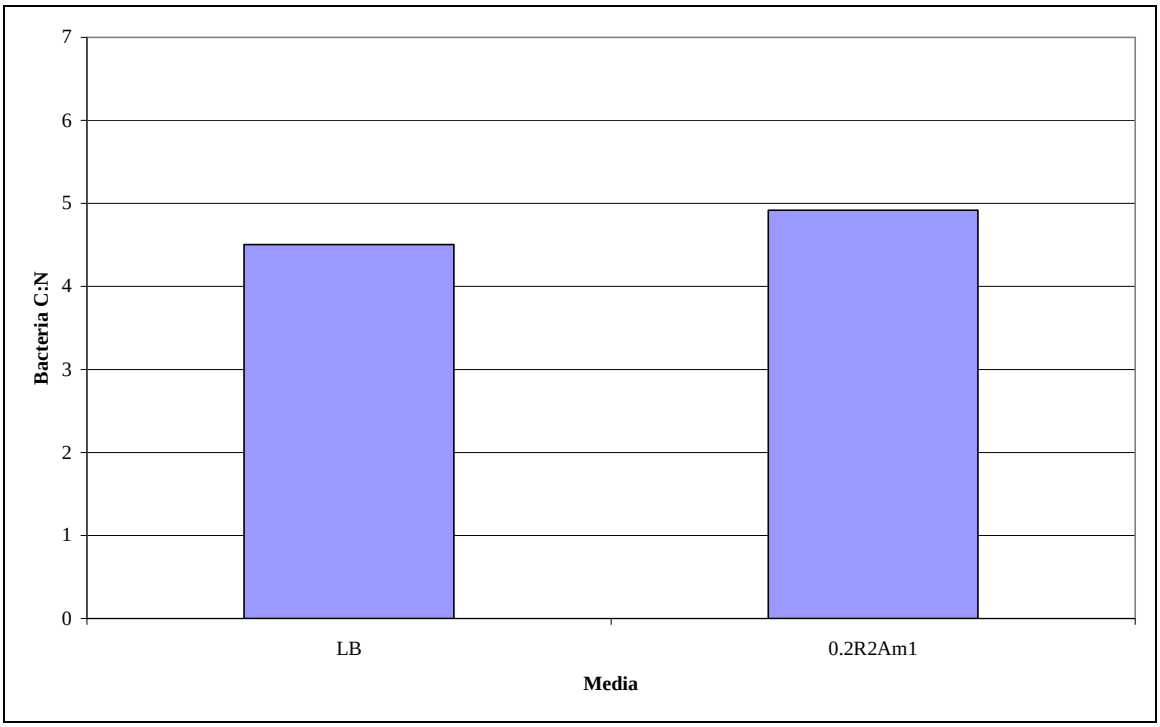

Figure 2: Average C:N increase of bacteria on two media that present a C:N increase factor of 1.8.

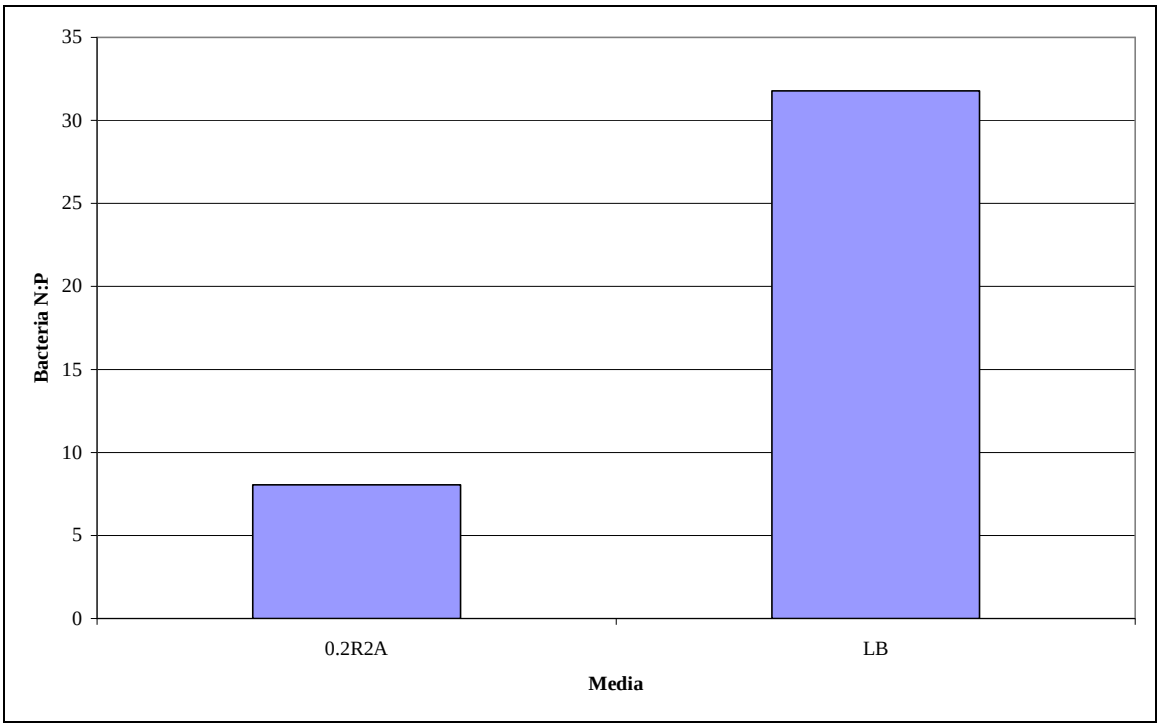

Figure 3: Average N:P increase of bacteria on two media that present a N:P increase factor of four.

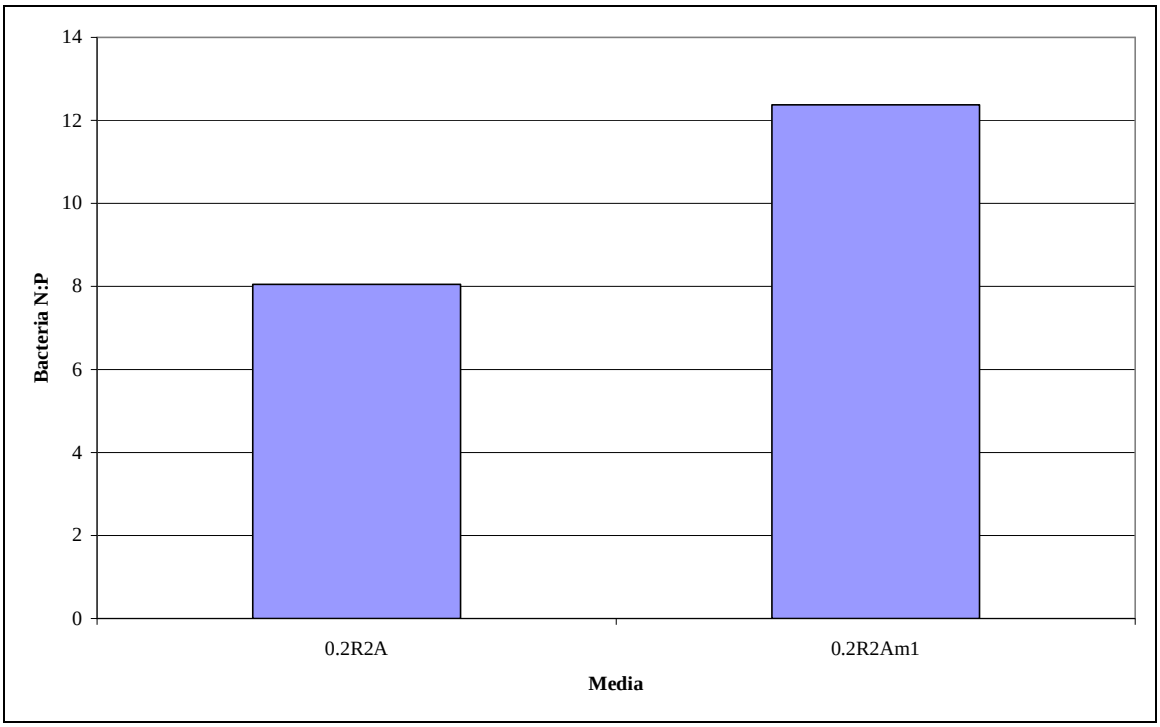

Figure 4: Average N:P increase of bacteria on two media that present a N:P increase factor of 1.4.

3. BIOFILM STOICHIOMETRY

ABSTRACT

Bacterial biofilms are an important source of nutrients for higher trophic levels in lotic environments since they are grazed by a variety of river micro- and macro-fauna. We hypothesized that the residence time of the hyporheic water in the aquifer, the distance of the well from the main channel and the water quality (Dissolved Organic Carbon, Soluble Total Nitrogen and Soluble Phosphorous) influence the elemental content of sediment biofilm. The biofilm elemental content would vary in part by a changing microbial community structure as well as entrainment of nutrients of varying carbon, nitrogen and phosphorous content. To determine if there was an influence of these variables on the carbon and nitrogen content as well as on the carbon to nitrogen ratio (C:N) of the biofilm, these variables were analyzed using a correlation analysis. The only significant correlation found was between the dissolved organic carbon content of the hyporheic water and the carbon content of the biofilm ($p=0.02$). Across seasons the dissolved organic carbon, soluble total nitrogen and soluble phosphorous (DOC, STN and SP) were not correlated with distance from the main channel or residence time. The C:N ratio of the microbial biofilm might be driven by the dominant species of microbial community rather than by the water quality, or distance from the river, or the residence time of the water.

3.1. INTRODUCTION

Bacterial biofilms are an important source of nutrients for higher trophic levels in lotic environments since they are grazed on by a variety of river organisms such as protozoa and stream invertebrates (Bärlocher & Murdoch 1989; Mohamed *et al*. 1998). Using isotopic tracers Hall & Meyer (1998) followed bacterial carbon through the food web and found that between 10 and 100% of invertebrate carbon was derived from bacteria. Wiegner & Seitzinger (2004) showed that carbon and nitrogen components of dissolved organic matter vary within bacterial communities thereby affecting the amount transferred to higher trophic levels. In some marine microbial communities, researchers have found that the elemental nutrient ratio in bacterial assemblages is mostly invariant (Goldman *et al*. 1987; Kirchman 1994; Fukuda *et al*. 1998). For example, Fukuda *et al*. (1998) determined that there was no significant difference in C:N ratio of bacterial assemblages in coastal (6.8±1.2) and oceanic (5.9±1.1) environments. These values are similar to those obtained by Goldman *et al*. (1987) for an assemblage of marine bacteria (C:N=5:1) under varying substrate C:N ratios. They determined that the C:N ratio of

marine bacteria was homeostatically regulated since a changing resource substrate C:N did not have an effect on the bacterial C:N ratio. In a freshwater environment, the C:N ratio of biofilms has been found to be slightly more variable (3.3:1 – 7.1:1 by Nagata 1986; 4.5:1 – 17.2:1 by Tezuka 1990) but still confined to a fairly narrow range.

The elemental stoichiometry of bacterial assemblages from a freshwater lake environment was found be non-homeostatic in terms of C:P (31:1-464:1) and N:P (7:1-41:1) (Tezuka 1990). On the other hand, Makino & Cotner (2004) found that the microbial community in a moderately productive Minnesota lake was more narrow with respect to C:P than other reported lake microbial communities (C:P=55:1-175:1 and N:P=11:1-31:1) but were within the range determined by Tezuka (1990).

In addition to changes conferred by the resource chemistry, biofilm elemental variation could also be due to differences in species composition. For example, Battin *et al*. (2001) determined that the benthic biofilm in a lotic environment was composed of organisms that belonged mostly to the subclass *Betaproteobacteria* and the domain *Archea*. However, Feris *et al*. (2003) determined that the microbial community in the hyporheic zone of a lotic system was dominated by *Alphaproteobacteria* rather than *Betaproteobacteria*. These studies suggest that the species composition of the biofilm within lotic environments varies geographically.

In previous work, we analyzed the nutrient ratio of 42 bacterial isolates from the hyporheic zone of the Nyack floodplain, and found that 40.5% of the isolates belong to the subclass *Gammaproteobacteria*, 16.7% belong to *Flavobacteria*, 14.3% belong to *Betaproteobacteria*, and *Actinobacteria, Alphaproteobacteria* and *Sphingobacteria* were present in the same proportions (9.5%).

Herein, we studied sediment biofilms from a hyporheic habitat on the southern border of Glacier National Park. We hypothesize that the C:N ratio of sediment biofilm would be influenced by the residence time of the hyporheic water in the aquifer, the distance of the well from the main river channel and the water quality (DOC, STN and SP) since these

variables may influence the quality of the nutrients that are in contact with the biofilms and, to some extent may dictate the microbial community structure. To determine if there was an influence of these variables on the carbon and nitrogen content as well as on the C:N ratio of the biofilm, these variables were analyzed using a correlation analysis.

3.2. MATERIALS AND METHODS

3.2.1. Site description – The Nyack Floodplain is located on the Middle fork of the Flathead River between the southwest border of Glacier National Park and north of the Great Bear Wilderness in northwest Montana. This floodplain is approximately 8km long and 1km wide where approximately 30 percent of the flow downwells into the shallow aquifer at the upstream end of the floodplain creating a vast hyporheic zone (Stanford & Ward 1988). The hyporheic zone of the Nyack Floodplain is a complex component of this ecosystem, where surface-waters and ground-waters mix (Stanford & Ward 1988, 1993).

3.2.2. Well selection – The hyporheic water was collected from the hyporheic zone of the Nyack Floodplain through hollow auger (HA) wells installed throughout the floodplain (Figure 1). Of the twenty HA wells placed on the floodplain, only fifteen were selected for this study. HA3 was excluded due to its location on private property. Wells HA1, HA8, HA15 and HA16 were not included because they are not part of the seasonal sampling of biofilm.

3.2.3. Sediment collection – We extracted a core sediment sample in July 2006 (summer) to obtain a base line of the C:N ratio of the biofilm in each HA well. The core sediment sample was extracted from within a one meter radius of the well. To extract sediment from below the water table, a geoprobe (Model 5400 Soil Probe, Geoprobe Systems, Kejr Engineering Inc. Kansas) was used.

The distance from the soil surface to the water table at each well (Table 1) was determined by measuring the distance from a set surface point to the water. Knowing this

distance, we drilled through the ground to the water table with the geoprobe. Once this opening was established, a hollow PVC tube was then hammered past the water table to extract the sediment. The samples were placed in sterile plastic bags, stored on ice and brought to the lab for processing. At the lab, the sediment was sieved to a size fraction of 2.36 – 1.7 mm. We were unable to obtain the size fraction for sediment from well HA12, which was mainly silt and clay.

3.2.4. Biofilm extraction – For removing biofilm from the sediment size fraction we modified the method described by Khoshmanesh *et al*. (2001) as follows. Five grams of sediment were mixed with 15ml of 0.85% NaCl in a 50ml centrifuge tube and vortexed at maximum speed for 1 min. The vortexed sediment was sonicated for 60 minutes in a sonication bath (Branson 3210). During the sonication, the temperature of the suspension was maintained below 15°C. After sonication, the sediment was vortexed at maximum speed for 15 seconds and left to settle for 30 minutes; the supernatant was extracted and placed in a 40ml centrifuge tube. The sediment was washed by adding 5ml of 0.85% NaCl solution after which it was vortexed at maximum speed for 15 seconds and left to settle for 30 minutes after which the supernatant was extracted and pooled with the previous supernatant fraction. This was repeated three more times. The pooled supernatant was centrifuged (10,000 rpm for 10 min) to settle any bacteria. The residual pellet was resuspended in 10ml of 0.85% NaCl and freeze dried for further analysis.

To remove the inorganic carbon from the biofilm sample, 1.3 ml of 0.01N HCl (0.8333ml of concentrated HCl to 1L of di-water) was added to 160 mg of lyophilized sample and adjusted the volume of HCl according to the weight of each sample. The samples were left at room temperature for one hour, with the caps loosened before returning them to the freeze dryer.

3.2.5. Nutrient analysis – The carbon and nitrogen content of the biofilm was determined by the analytical facilities at Flathead Lake Biological Station (Polson, MT) using a modified Dumas method as described by Pella (1990) using a CHN Analyzer (Fisons NA1500, 1995).

3.2.6. Water collection and analysis – DOC, STN and SP content of the well water of the HA wells for summer and fall 2003 as well as for winter, spring, summer and fall 2004 time points were acquired from the Flathead Lake Biological Station Biocomplexity Project database. Water samples taken from the HA wells were collected during the spring 2006, fall 2006 and winter 2007 time points. The water samples were processed to determine the DOC, STN and SP content as described in Ellis *et al*. (1998). With these values we calculated C:N, N:P and C:P ratios of the hyporheic water. The DOC, STN and SP content of the hyporheic water for each season, as well as the ratios, were correlated with the distance of the well from the main channel and the residence time of the water in the aquifer. The total number of bacteria present in the biofilm was determined by indirect count as described in Ellis *et al*. (1998).

3.2.7. Data analysis – The data resulting from the biofilm carbon and nitrogen analyses was in percent carbon and percent nitrogen. These values were transformed to μmolar units per mg of dry sample and the ratio of carbon to nitrogen (C:N) was determined. The carbon and nitrogen contents as well as the C:N ratio of the biofilm were correlated with the DOC, STN and C:N ratio of the hyporheic water from spring 2006. To be able to do this, the DOC and STN units (mg/l and μg/l) had to be transformed to the same units as the C and N content of the biofilm (μmol/mg).

3.2.8. Physical characteristics – The distance of the well to main channel was measured as the shortest distance from the center of the well to the main channel. The residence time of the hyporheic water in each well was determined by radon analysis as described by Haggerty *et al*. (2004).

3.2.9. Correlation analysis – To test whether there was a correlation between the variables, we did a correlation analysis using SPSS for Windows (version 15.0). Linear regression analysis was used to determine significant differences between residence time and distance with the water quality among the different seasons. This analysis was preformed using GraphPad Prism version 5.00 for Windows, GraphPad Software, San

Diego California USA, www.graphpad.com. This method is equivalent to an analysis of covariance (Motulsky & Christopoulos 2003).

3.3. RESULTS

3.3.1. Biofilm elemental stoichiometry – The harvested biofilm from the sediments of the HA wells showed carbon content that ranged between 0.03 and 0.09 (µmol/mg) with a high of 0.13 (µmol/mg) in HA10 (Figure 2) and we found a significant difference ($p<0.01$, $f=219.4$, $n=32$) between the wells. Nitrogen content in the biofilm ranged between 0.002 and 0.007 (µmol/mg) with a high of 0.009 in HA10 (Figure 3). There was a significant difference ($p<0.01$, $f=30.77$, $n=32$) between the wells. With these levels of carbon and nitrogen, we found that the C:N ratios ranged between 9:1 and 16:1 with a high of 21:1 in HA17 (Figure 4) and there was a significant difference ($p<0.01$, $f=25.16$, $n=32$) between the wells.

It was not possible to determine the phosphorous content of the biofilm because the biofilm extracted from the sediment of each well was not sufficient enough to perform the analysis. In order to have the amount of biofilm necessary, the samples had to be pooled to have enough material to detect the phosphorous content and, in order to pool the biofilm from the different HA wells it is necessary to know how these wells are connected with each other.

3.3.2. Biofilm correlations – The carbon content of the biofilm was not significantly correlated to the distance from the main channel or with the residence time of the hyporheic water in the aquifer (Table 2). The nitrogen content was also not significantly correlated to the distance from the main channel or with the residence time of the hyporheic water in the aquifer. The C:N ratio, therefore, also did not significantly correlate with the distance of the well from the main channel or with the residence time of the hyporheic water.

The DOC content presented a significant correlation with the carbon content of the biofilm (Table 2). The STN content did not significantly correlated with the nitrogen content of the biofilm and the C:N ratio of the well water was not significantly correlated with the C:N ratio of the biofilm.

3.3.3. Water nutrient content – The hyporheic water showed average DOC values that ranged between 0.43 and 0.98mg C/l with the highest value in spring (Figure 5). There was no significant difference ($p=0.11$, $f=2.04$, $n=132$) between seasons. We found a slight significant difference ($p=0.06$, $f=1.95$, $n=132$) between time points (Figure 6, Table 3a).

The hyporheic water showed average STN values that ranged between 132.37 and 321.22µg/l with the highest value in spring (Figure 7) and there was a significant difference ($p=0.01$, $f=3.99$, $n=132$) between seasons. The difference was found between spring and summer ($p=0.01$, $n=60$) as well as between spring and fall ($p=0.05$, $n=75$). We also found a significant difference ($p=0.001$, $f=3.50$, $n=132$) between time points (Figure 8, Table 3b).

The hyporheic water showed average SP values that ranged between 4.95 and 24.38µg/l with the highest value in fall (Figure 9) and there was no significant difference ($p=0.41$, $f=0.97$, $n=132$) between seasons. The fall sample presented the highest variation. We also found no significant difference ($p=0.52$, $f=0.89$, $n=132$) between time points (Figure 10).

The water from the HA wells showed C:N ratio values that ranged between 4.33 and 16.98, with a mean of 7.87 and with the highest value in summer (Figure 11) and there was a significant difference between seasons ($p=0.02$, $f=3.31$, $n=4$) where summer was significantly different from fall ($p=0.03$, $n=75$). We also found a significant difference ($p=0.02$, $f=2.51$, $n=132$) between time points (Figure 12) where summer 2003 was significantly different from spring 2006 ($p=0.03$, $f=5.36$, $n=30$) and fall 2006 ($p=0.04$, $f=4.65$, $n=30$). In summer 2003 the highest C:N was found in the wells HA2, HA11,

HA12 and HA17. In spring 2006, wells HA12 and HA17 presented the highest C:N ratio and in fall 2006 it was found in wells HA2 and HA12.

The water from the HA wells showed N:P ratio values that ranged between 35.79 and 137.60, with a mean of 74.02 and with the highest value in spring (Figure 13) and there was a significant difference between seasons ($p<0.01$, $f=7.04$, $n=132$) where winter was significantly different from spring ($p=0.001$, $n=57$) and spring was significantly different from summer ($p=0.003$, $n=60$) and fall ($p=0.001$, $n=75$). We also found a significant difference ($p<0.01$, $f=4.72$, $n=132$) between time points (Figure 14, Table 3c).

The water from the HA wells showed C:P ratio values that ranged between 166.40 and 541.86, with a mean of 368.39 and with the highest value in spring (Figure 12) and there was a significant difference ($p=0.02$, $f=3.42$, $n=132$) between seasons where spring was significantly different than fall ($p=0.02$, $n=75$). We also found a significant difference ($p=0.04$, $f=2.14$, $n=132$) between time points (Figure 13, Table 3d).

3.3.4. Water nutrient correlations – We correlated the distance of the well from the main channel and the residence time of the hyporheic water with the DOC, STN and SP content, C:N, N:P and C:P ratio of the hyporheic water for each HA well in each season.

At each time point there was no significant correlation between the DOC content and the distance from the main channel (Table 4a) and there was no significant difference between time points ($p=0.99$). At each time point there was no significant correlation to the residence time of the hyporheic water and there was no significant difference between time points ($p=0.41$). However, the DOC content of the hyporheic water in spring 2006 significantly correlated with the carbon content of the biofilm (Figure 17, $r^2=0.57$, $p=0.002$, $n=14$).

We determined that there was a significant positive correlation between the STN content and the distance from the main channel at each time point (Table 4b) and there was no significant difference ($p=0.15$) between the time points. The significant correlations were

positive, indicating that the STN content of the water increases as it flows away from the main channel. We also determined that at each time point there was no significant correlation to the residence time of the hyporheic water in the aquifer and there was no significant difference (p=0.99) between time points. The STN content of the hyporheic water in spring 2006 did not significantly correlate with the nitrogen content of the biofilm (Figure 18, r^2=0.16, p=0.16, n=14).

We determined that the SP content of the water was significantly correlated to the distance from the main channel in fall 2003 (p=0.02) and summer 2004 (p=0.05) with no significant correlation found for other time points (Table 4c) and there was no significant difference (p=0.65) between the time points. The significant correlations were positive, indicating that the SP content of the water increases as it flows away from the main channel. We also found that, at each time point, there was no significant correlation with the residence time of the hyporheic water in the aquifer and there was no significant difference (p=1) between time points.

We determined that in only four time points, the C:N ratio was significantly correlated to the distance from the main channel (Table 5a) with no significant correlation found for the other time points, however, we did find a significant difference (p=0.05) between the time points. The significant correlations found were negative, the C:N ratio decreases as the water moves away from the main channel. We also found that, at each time point, there was no significant correlation to the residence time and there was no significant difference (p=0.26) between time points. The C:N ratio of the hyporheic water in spring 2006 did not significantly correlate with the C:N ratio of the biofilm (Figure 19, r^2=0.16, p=0.15, n=14).

The N:P ratio was significantly correlated to the distance from the main channel in four time points (Table 5b) with no significant correlation for the other time points, however, there was no significant difference (p=0.06) between the time points. The significant correlation found were positive, the N:P ratio of the water increases as the water flows away from the main channel. We also found that at each time point there was no

significant correlation to the residence time and there was no significant difference (p=0.91) between time points.

The C:P ratio was significantly correlated to the distance from the main channel in two time points (Table 5c) with no significant correlations found for the other time points and there was no significant difference (p=0.96) between them. The significant correlations found were negative, the C:P ratio decreases as the water moves away from the main channel. We also found no significant correlation to the residence time of the water in the aquifer and there was no significant difference (p=0.22) between time points.

3.4. DISCUSSION

We found that there was a significant moderate correlation between the C content of the biofilm and the DOC content of the hyporheic water, suggesting that the DOC content of the water has an influence over the biofilm C content. This correlation could be due to an increase in the amount of the exopolysaccharide matrix, by an accumulation of DOC on the exopolysaccharide matrix of the biofilm, by an increase in the relative abundance of bacteria that are able to store carbon in their cells, by variations in the DOC quality or by the influence of invertebrate consumers of biofilm.

An increase in the amount of polysaccharide could explain the correlation found between DOC concentration of the water and C content of the biofilm since it has been shown that the polysaccharide matrix can comprise up to 90% of the biofilm biomass and it is composed mainly of carbon (Kaplan *et al*. 2004). The other possibility is that DOC accumulates in the polysaccharide matrix. Freeman & Lock (1995) were able to determine that the polysaccharide matrix acts as a reserve of carbon during starvation conditions suggesting that this matrix is able to accumulate environmental carbon such as DOC. They also suggest that the matrix has the ability to act as a buffer against changing organic substrate supplies.

In Movie Road, a gravel bar in the Nyack Floodplain, it was determined that DOC ($4.5*10^{-5}$ – $9*10^{-5}$ μmol/mg) did not influence the microbial diversity of the biofilm (Lowell, J., personal communications). This could be occurring throughout the floodplain; therefore it is possible that there might be an increase in the relative abundance of microorganisms that are able to accumulate C within the community which could explain the correlation between DOC and biofilm C content. Since total DOC does not vary spatially, it could be possible that the quality of DOC is influencing relative abundance of microorganisms in the biofilm as shown by Sobczak & Findlay (2002), that in aquatic ecosystems, sediment bound bacteria are often C limited and variations in DOC quality can be an important control on their metabolisms.

The DOC content was significantly correlated with the carbon content of the biofilm but the STN was not correlated with the nitrogen content of the biofilm suggesting that the N content of the biofilm may be influenced by other variables. There was no significant correlation between the C:N ratio of the water and the C:N ratio of the biofilm which could indicate that nitrogen has a more important role in influencing the C:N ratio.

Another possibility for not observing a significant correlation between the nutrients in the water and the biofilm could be due to the consumers present in the environment. It has been shown that consumers are important nutrient recyclers and because of this they are able to modify the nutrients available to heterotrophic microbes (Elser & Urabe 1999; Vrede *et al*. 2004; Cross *et al*. 2005). However, in this study, consumers have a lower C:N ratio than their food (biofilm) which will lead the invertebrates to consume more C than necessary to acquire sufficient amount of N, therefore invertebrates will be excreting C back to the environment where it becomes available to microorganisms.

As suggested by several studies (Ingendahl *et al*. 2002; Battin *et al*. 2004; Findlay & Sinsabaugh 2006) variations in biofilm productivity, metabolic activity or community composition could be due to differences in nutrients that may be caused by differences in watershed characteristics, changes in hydraulic flow paths and point source inputs. The

variations in biofilm productivity could explain why we see a relation between the DOC content and the C content of the biofilm and no relation between the STN and N content.

Though the DOC and the SP content of the hyporheic water were not significantly correlated to the distance from the main channel, the STN content was. This suggests that the STN behaves differently than the other nutrients which could be due to the fact that we did not consider the vertical input of nutrients through leaching of surface water from the soil into the water table.

Fukuda *et al.* (1998) determined that total bacterial assemblage C:N ratio in marine environments is relatively invariant (between 5:1 and 8.3:1), suggesting that bacteria from aquatic environments are homeostatic with respect to their C:N ratio. In this study, however, the C:N ratio range of the microbial biofilm was higher and broader, it varied between 9:1 and 21:1. It seems that the C:N ratio of biofilm from marine and coastal environments is lower than that of freshwater hyporheic environments. This difference is likely due to the environment from where the biofilm was harvested, suggesting that the C:N ratio of microbial biofilms will vary depending on the environment they are collected from since in different environments the biofilms are exposed to nutrient differences.

The C:N ratio of the water was correlated to the distance from the main channel in summer 2003 ($p=0.03$), summer 2004 ($p=0.01$), spring 2006 ($p=0.04$) and fall 2006 ($p=0.03$) and the N:P ratio was significantly correlated to the distance from the main channel in summer 2003 ($p=0.0004$), winter 2004 ($p=0.02$), summer 2004 ($p=0.006$) and fall 2006 ($p=0.007$). This could be explained by the high flow of the water in these seasons, by the vertical input of nutrients that were not considered in this study or a combination of both.

The variability in C:P and N:P ratios in bacterial communities could be due to differences in the diversity of the community (Makino & Cotner 2004) therefore we can speculate that the difference in the C:N ratio of the biofilms from different environments could be

due to the microorganisms present in the biofilm of each environment. Since the microbial population of the biofilms might differ, it could explain the variation of the C:N ratio through the environments. The C:N ratio of the microbial biofilm might be driven by the dominant species of microbial community rather than by the water quality, distance from the river and the residence time of the water.

The C:N ratio of the biofilm from the well HA10 has the same value as the C:N ratio of the biofilm from HA13 which was 15:1. Although both of these wells are located in forest type soil, they are located in different parts of the floodplain and distance from the main channel. HA10 is located at the end of the floodplain, 575m away from the main channel while HA13 is located in the central area of the floodplain, 75m away from the main channel.

The well HA17 was the one that presented the highest C:N ratio, this could be due to a decrease in microbial activity. It has been suggested by Caron & Goldman (1990) that the C:N and C:P ratios of the organic carbon substrates could be lowered by the increase in mineral excretion caused by bacterivorous protozoa. Also, Cross *et al*. (2005) suggested that a decline in C:N ratio reflects increase in microbial activity and potentially result in higher food quality for benthic organisms. According to this, we would expect to find a lower amount of benthic consumers in well HA17 compared to HA14 which has the lowest C:N ratio.

There was no significant correlation between the distance of the well from the main channel and the residence time of the water in the aquifer. For example, HA19 had the shortest distance to the main channel but the highest residence time. This could be due to soil compaction or that in this area the water of the main channel is not flowing towards the floodplain. It could be a preferential flow path with hyporheic water from further up stream. This compliments what has been shown in other studies (Stanford & Ward 1993, Stanford 1998, Ellis *et al*. 1998) that this environment is very complex, with preferential flow paths and different sediment sizes.

Bärlocher & Murdoch (1989) determined that the DOC content did not affect the number of bacteria present in the biofilm when it was measured at the same time point or four weeks prior to the biofilm sampling point. We found that the number of bacteria present in the biofilm did not correlate with the DOC content of the hyporheic water in fall 2006 and in winter 2007. We also found no correlation among these variables when we looked at the DOC water content from fall 2006 and the bacteria present in winter 2007 (after 16 weeks). The DOC of the water was low and relatively constant and we expect bacterial numbers to be related to flux rather than bulk water content. However, Rubin & Leff (2007) found that the total bacterial numbers were related to DOC concentrations. These different observations could be due to differences between streams or carbon quality present in the environments from where the biofilms were extracted.

Table 1: Depth to the water table in May 2006, distance to the main channel (m) and residence time (days) for each well.

Well	surface to water table (m)	Distance (m)	Residence Time (days)
HA 2	1.10	150	1.98
HA 4	0.91	1000	6.54
HA 5	0.75	1100	N.A.*
HA 6	1.16	1350	7.31
HA 7	1.13	1350	N.A.
HA 8	1.97	1250	N.A.
HA 9	1.55	850	N.A.
HA 10	2	600	7.77
HA 11	1.28	450	N.A.
HA 12	1.08	300	3.28
HA 13	2.30	100	4.67
HA 14	1.14	350	5.38
HA 15	1.04	400	6.13
HA 16	N.A.	250	6.06
HA 17	1.18	100	7.42
HA 18	0.70	400	12.33
HA 19	1.64	150	26.95
HA 20	1.27	210	8.75

* N.A.: data not available

Table 2: Correlation between biofilm nutrient content and distance to the main channel, water residence time and water nutrients.

Correlation	slope	r^2	p	n
Biofilm C – distance	$1x10^{-5}$	0.05	0.44	15
Biofilm C – residence time	$-6x10^{-4}$	0.02	0.71	11
Biofilm C – Water DOC	669.09	0.57	0.002	14
Biofilm N – distance	$1x10^{-6}$	0.09	0.27	15
Biofilm N – residence time	$1x10^{-6}$	$2x10^{-4}$	0.97	11
Biofilm N – Water STN	52.16	0.16	0.16	14
Biofilm C:N – distance	-0.002	0.08	0.31	15
Biofilm C:N – residence time	-0.13	0.05	0.51	11
Biofilm C:N – Water C:N	0.42	0.16	0.15	14

Table 3a: Significant differences between time points for water DOC content

	Fall 2003			Winter 2004			Summer 2004		
	p	f	n	p	f	n	p	f	n
Spring 2004	0.04	4.84	30	0.006	8.95	29	0.02	6.49	30
Spring 2006	0.03	5.07	30	0.001	13.3	29	0.01	7.54	30
Fall 2006				0.005	9.26	29	0.03	5.22	30
Winter 2007				0.004	10.28	28	0.02	6.19	28

Table 3b: Significant differences between time points for STN water content

	Spring 2006			Fall 2006		
	p	f	n	p	f	n
Summer 2003	0.006	9.01	30	0.05	4.23	30
Fall 2003	0.002	12.13	30	0.02	6.72	30
Winter 2004	0.005	9.23	29	0.04	4.41	29
Spring 2004	0.03	5.17	30			
Summer 2004	0.01	7.83	30			
Fall 2004	0.004	9.81	30	0.04	4.59	30

Table 3c: Significant differences between time points for N:P water content

	Fall 2003			Spring 2006		
	p	f	n	p	f	n
Summer 2003				0.01	6.82	30
Winter 2004				0.002	12.31	29
Spring 2004	<0.01	26.33	30			
Summer 2004	0.04	4.49	30	0.01	7.14	30
Fall 2004	0.03	5.06	30	0.01	7.73	30
Spring 2006	<0.01	17.66	30			
Fall 2006	0.004	9.99	30			
Winter 2007	0.01	8.03	28	0.01	7.04	28

Table 3d: Significant differences between time points for C:P water content

	Fall 2003			Winter 2004		
	p	f	n	p	f	n
Summer 2003				0.04	4.85	28
Spring 2004	0.003	10.98	29	0.001	12.61	28
Spring 2006	0.05	4.18	29	0.04	4.79	28
Fall 2006	0.02	6.04	29	0.005	9.44	28
Winter 2007	0.03	5.39	27	0.02	6.59	26

Table 4a: Correlation between DOC and the distance from the main channel (dist) as well as between DOC and residence time (RT) for each season

	DOC-dist				DOC-RT			
Season	slope	r^2	p	n	slope	r^2	p	n
Summer03	-0.0004	0.05	0.43	15	-0.04	0.09	0.37	11
Fall03	-0.0006	0.01	0.74	15	-0.004	0.006	0.82	11
Winter04	-0.0001	0.09	0.31	14	-0.01	0.1	0.33	11
Spring04	-7E-06	2.10E-05	0.99	15	0.03	0.1	0.33	11
Summer04	-0.0003	0.16	0.14	15	0.03	0.32	0.07	11
Fall04	-0.0001	0.01	0.69	15	-0.03	0.11	0.32	11
Spring06	-0.0002	0.05	0.42	15	-0.01	0.04	0.56	11
Fall06	-0.0003	0.07	0.33	15	-0.04	0.26	0.11	11
Winter07	-0.0002	0.04	0.51	13	-0.02	0.08	0.47	9

Table 4b: Variables from the correlation between STN and the distance from the main channel (dist) as well as between STN and residence time (RT) for each season

	STN-dist				STN-RT			
Season	slope	r^2	p	n	slope	r^2	p	n
Summer03	0.25	0.74	<0.001	15	-1.29	0.005	0.83	11
Fall03	0.13	0.56	0.001	15	-1.35	0.019	0.68	11
Winter04	0.11	0.34	0.027	14	-1.01	0.005	0.83	11
Spring04	0.15	0.56	0.001	15	-3.11	0.051	0.5	11
Summer04	0.26	0.89	<0.001	15	-2.31	0.019	0.68	11
Fall04	0.11	0.51	0.003	15	-3.74	0.17	0.21	11
Spring06	0.27	0.39	0.01	15	-6.36	0.05	0.49	11
Fall06	0.25	0.59	0.001	15	-2.28	0.019	0.68	11
Winter07	0.21	0.32	0.04	13	-3.11	0.09	0.43	9

Table 4c: Variables from the correlation between SP and the distance from the main channel (dist) as well as between SP and residence time (RT) for each season

	SP-dist				SP-RT			
Season	slope	r^2	p	n	slope	r^2	p	n
Summer03	0.001	0.02	0.6	15	-0.07	0.019	0.68	11
Fall03	0.01	0.35	0.02	15	-0.06	0.015	0.71	11
Winter04	-0.0002	0.001	0.9	14	0.17	0.1	0.34	11
Spring04	0.002	0.25	0.05	15	-0.07	0.04	0.55	11
Summer04	0.003	0.26	0.05	15	-0.03	0.005	0.84	11
Fall04	-0.03	0.046	0.44	15	0.3	0.0006	0.94	11
Spring06	0.001	0.008	0.74	15	0.02	0.0002	0.96	11
Fall06	-0.001	0.01	0.71	15	-0.08	0.01	0.74	11
Winter07	0.006	0.16	0.17	13	-0.03	0.002	0.9	9

Table 5a: Variables from the correlation between C:N ratio and the distance from the main channel (dist) as well as between C:N ratio and residence time (RT) for each season.

	CN-dist				CN-RT			
Season	slope	r^2	p	n	slope	r^2	p	N
Summer03	-0.03	0.31	0.03	15	-1.27	0.14	0.25	11
Fall03	-0.005	0.18	0.11	15	-0.05	0.003	0.87	11
Winter04	-0.007	0.16	0.15	14	-0.13	0.01	0.76	11
Spring04	-0.008	0.16	0.14	15	0.37	0.06	0.47	11
Summer04	-0.011	0.4	0.01	15	0.65	0.28	0.09	11
Fall04	-0.004	0.14	0.17	15	-0.18	0.05	0.49	11
Spring06	-0.004	0.27	0.04	15	0.016	0.0008	0.93	11
Fall06	-0.004	0.31	0.03	15	-0.21	0.15	0.24	11
Winter07	-0.006	0.28	0.06	13	0.025	0.001	0.93	9

Table 5b: Variables from the correlation between N:P ratio and the distance from the main channel (dist) as well as between N:P ratio and residence time (RT) for each season.

	N:P-dist				N:P-RT			
Season	slope	r^2	p	n	slope	r^2	p	n
Summer03	0.11	0.63	0.0004	15	-0.18	0.0007	0.94	11
Fall03	-0.004	0.008	0.76	15	-0.59	0.03	0.61	11
Winter04	0.03	0.38	0.02	14	-1.24	0.11	0.31	11
Spring04	0.027	0.13	0.19	15	-0.39	0.006	0.82	11
Summer04	0.07	0.45	0.006	15	-1.01	0.04	0.55	11
Fall04	0.05	0.23	0.07	15	-2.31	0.17	0.21	11
Spring06	0.06	0.08	0.32	15	-4.81	0.16	0.22	11
Fall06	0.09	0.44	0.007	15	-0.96	0.01	0.77	11
Winter07	0.02	0.06	0.41	13	-1.69	0.1	0.41	9

Table 5c: Variables from the correlation between C:P ratio and the distance from the main channel (dist) as well as between C:P ratio and residence time (RT) for each season.

	C:P-dist				C:P-RT			
Season	slope	r^2	p	n	slope	r^2	p	n
Summer03	-0.22	0.07	0.32	15	-16.23	0.07	0.42	11
Fall03	-0.13	0.26	0.05	15	-1.58	0.009	0.78	11
Winter04	-0.06	0.11	0.24	14	-7.83	0.34	0.06	11
Spring04	-0.32	0.14	0.17	15	35.95	0.33	0.06	11
Summer04	-0.28	0.29	0.04	15	20.52	0.31	0.08	11
Fall04	-0.13	0.02	0.6	15	-20.56	0.1	0.34	11
Spring06	-0.33	0.07	0.35	15	-28.13	0.08	0.39	11
Fall06	-0.08	0.03	0.49	15	-9.96	0.09	0.36	11
Winter07	-0.33	0.14	0.21	13	-16.64	0.07	0.49	9

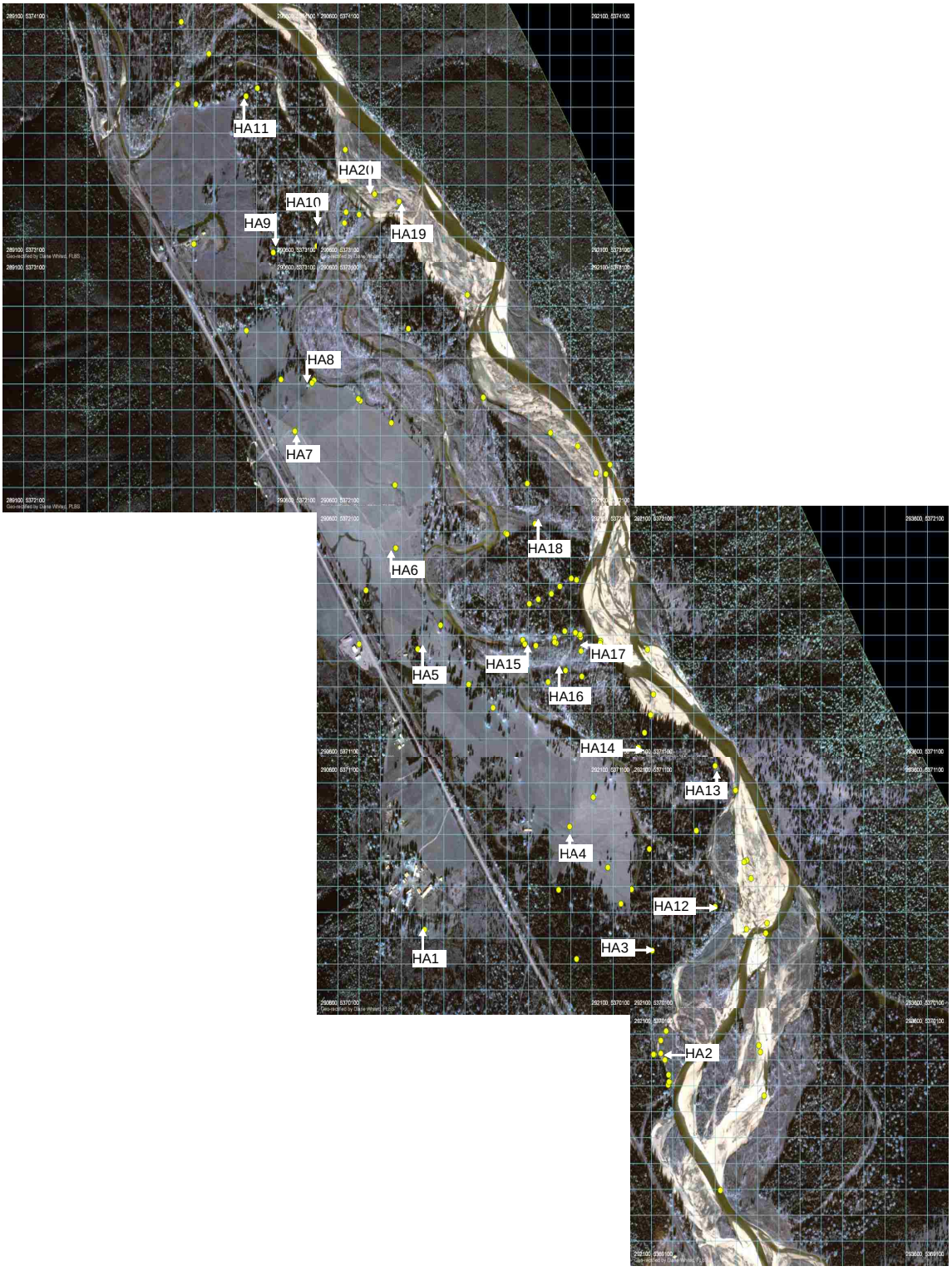

Figure 1: Location of the HA wells throughout the Nyack Floodplain

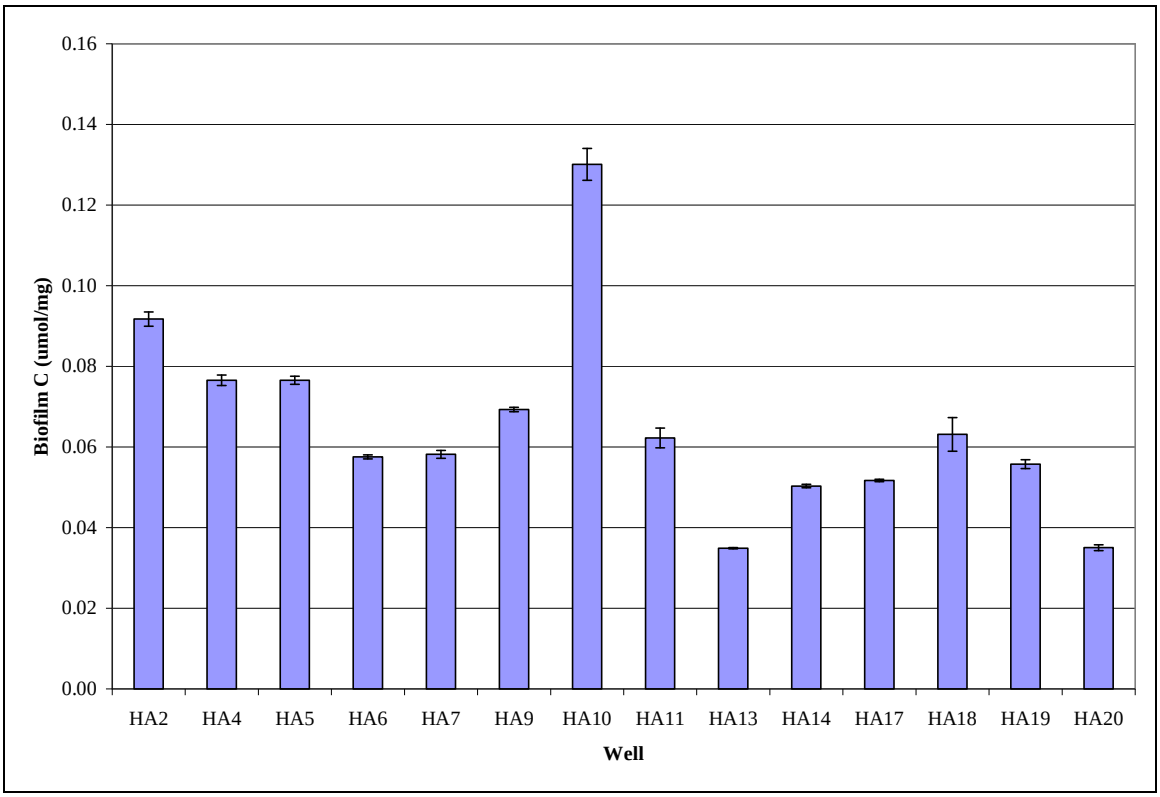

Figure 2: Carbon content of the biofilm extracted from each well sediment. There is a significant difference between the wells ($p<0.05$).

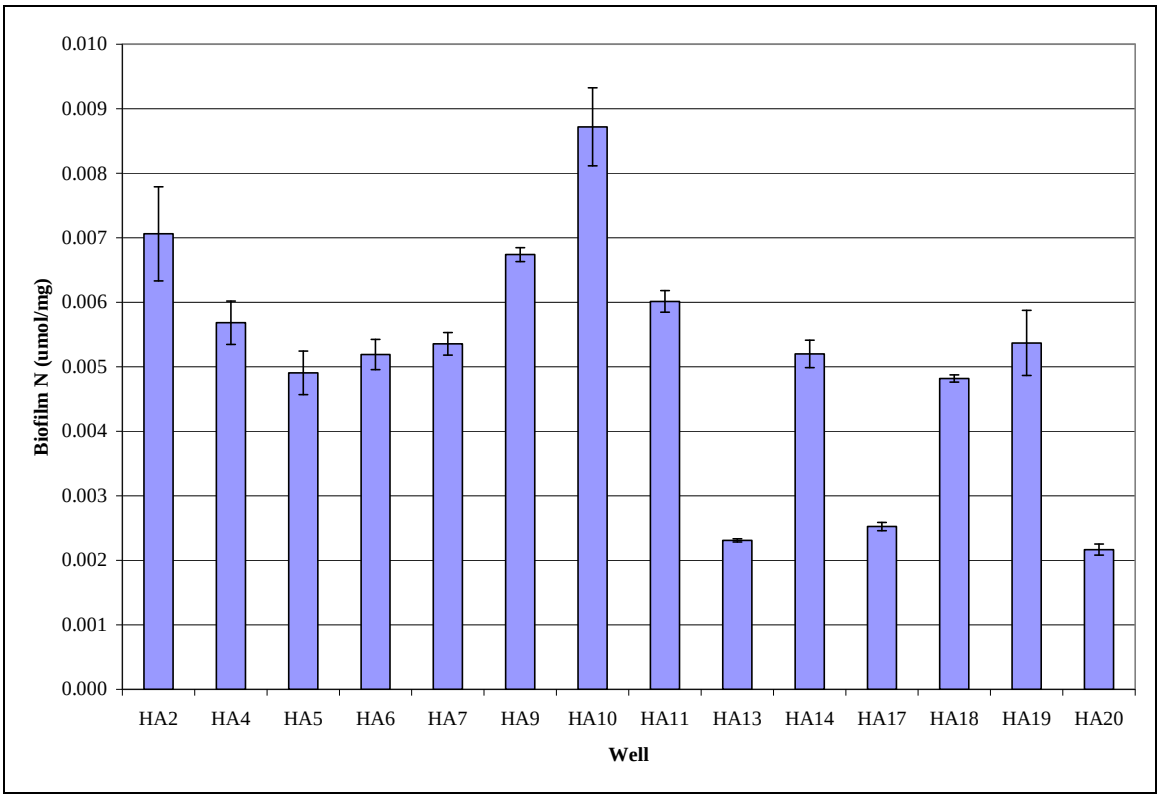

Figure 3: Nitrogen content of the biofilm extracted from each well sediment. There is a significant difference between the wells ($p<0.05$).

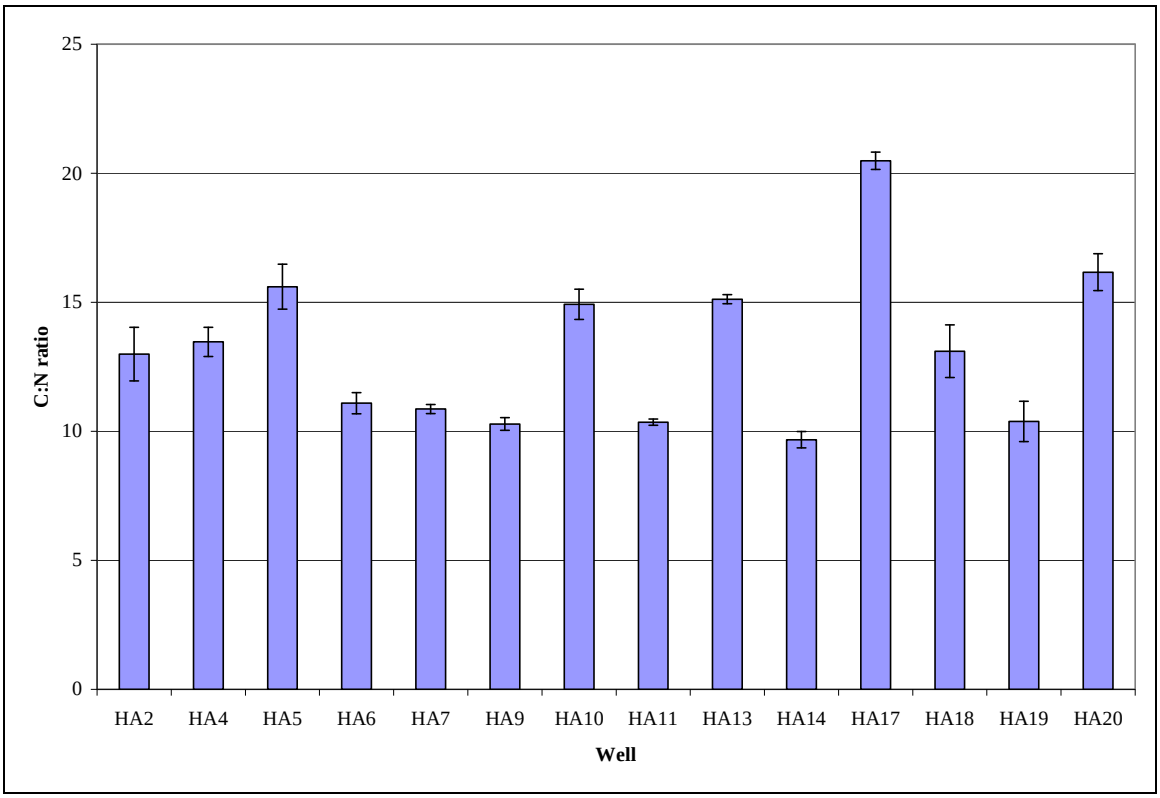

Figure 4: C:N ratio of the biofilm extracted from each well sediment. There is a significant difference between the wells ($p<0.01$).

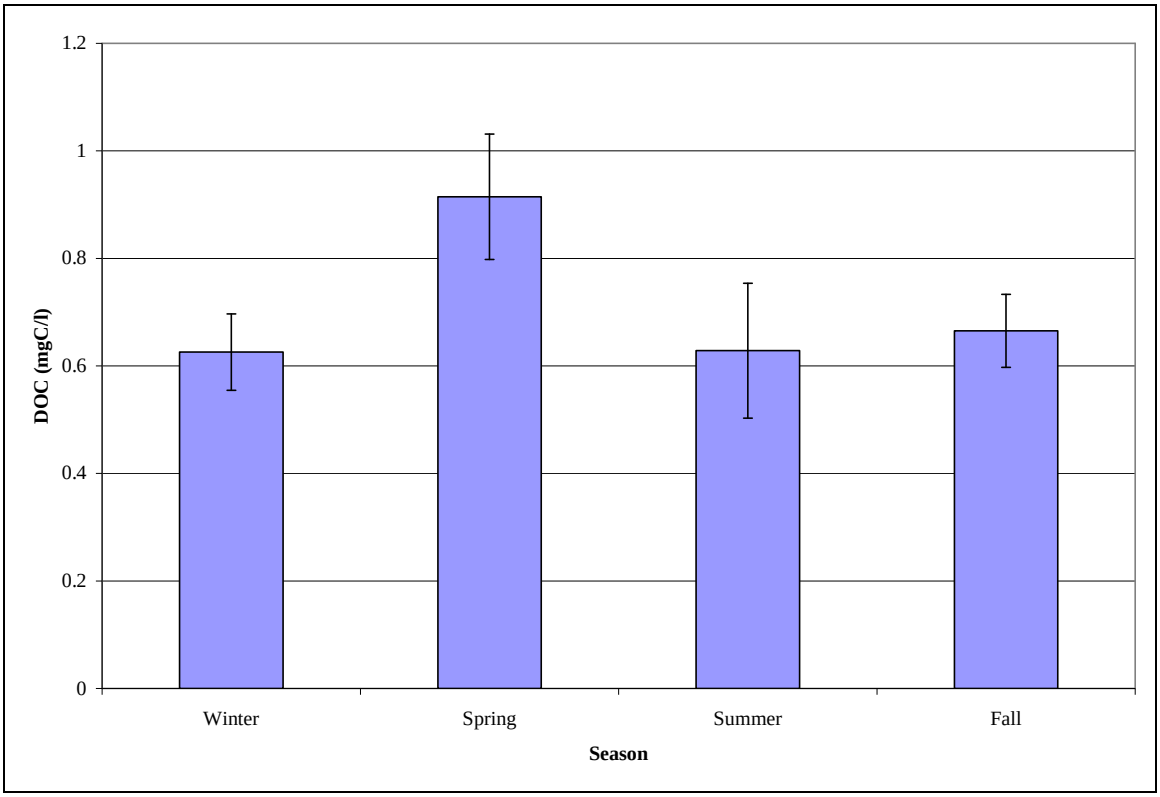

Figure 5: Variation of the DOC content of the hyporheic water through seasons. There is no significant difference between seasons (p=0.11)

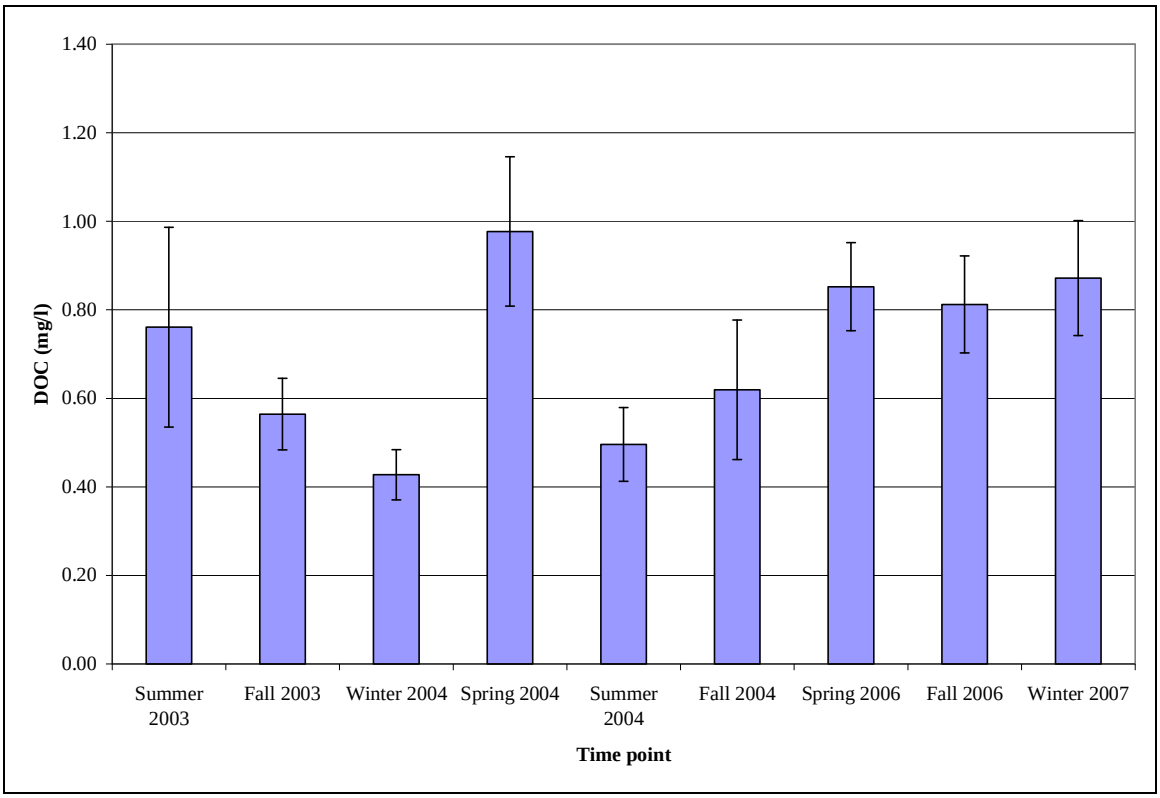

Figure 6: Variation of the DOC content of the hyporheic water through time points. There is no significant difference between time points (p=0.06)

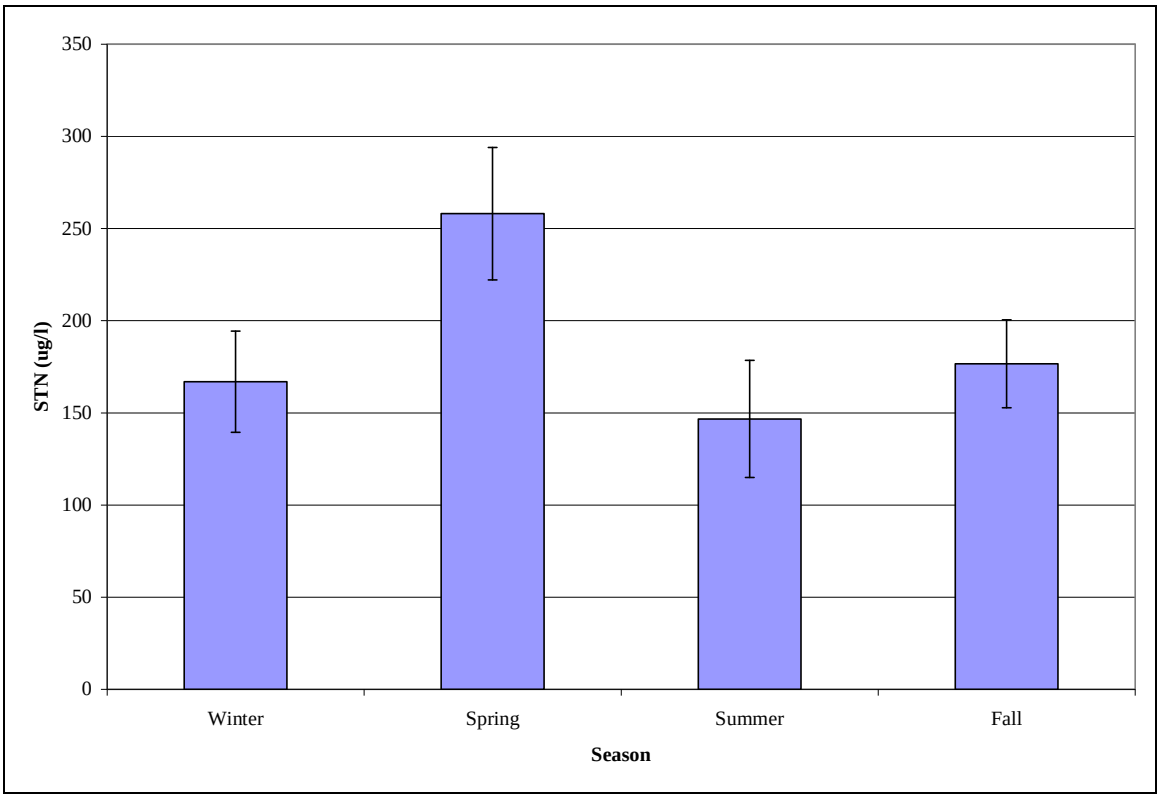

Figure 7: Variation of the STN content of the hyporheic water through seasons. There is a significant difference between seasons (p=0.01)

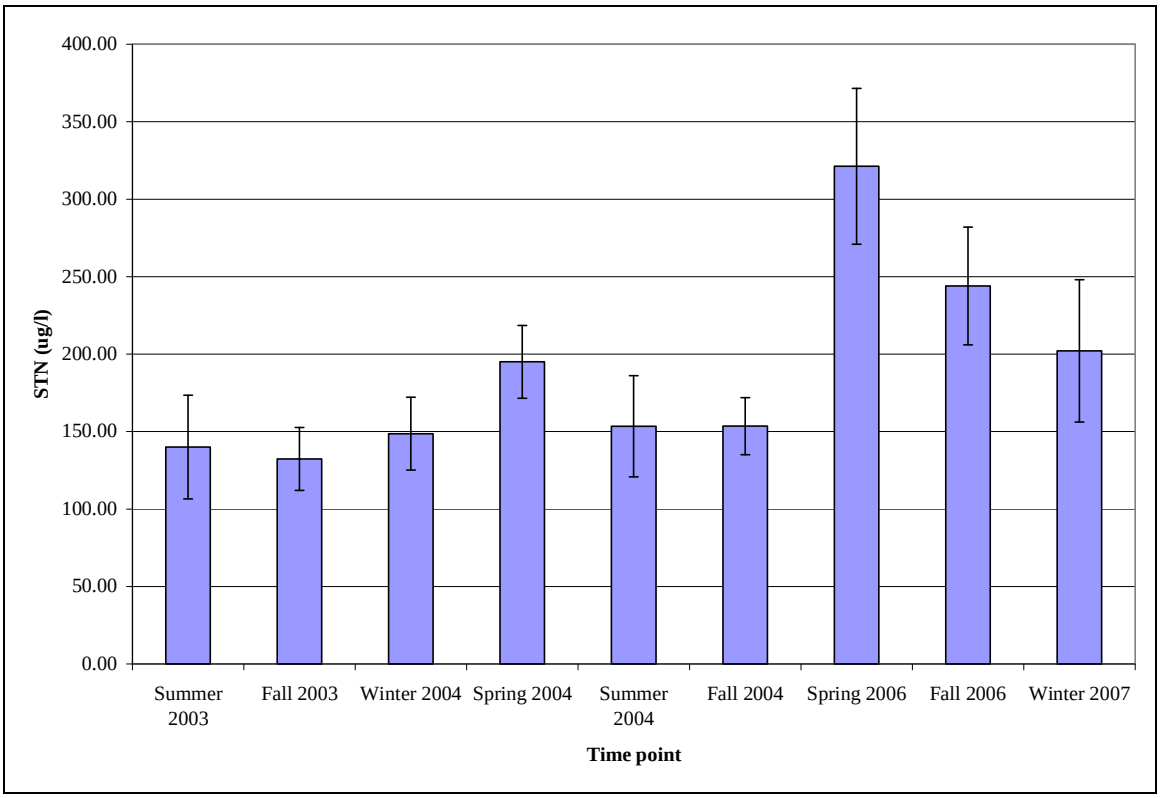

Figure 8: Variation of the STN content of the hyporheic water through time points. There is a significant difference between time points (p=0.001)

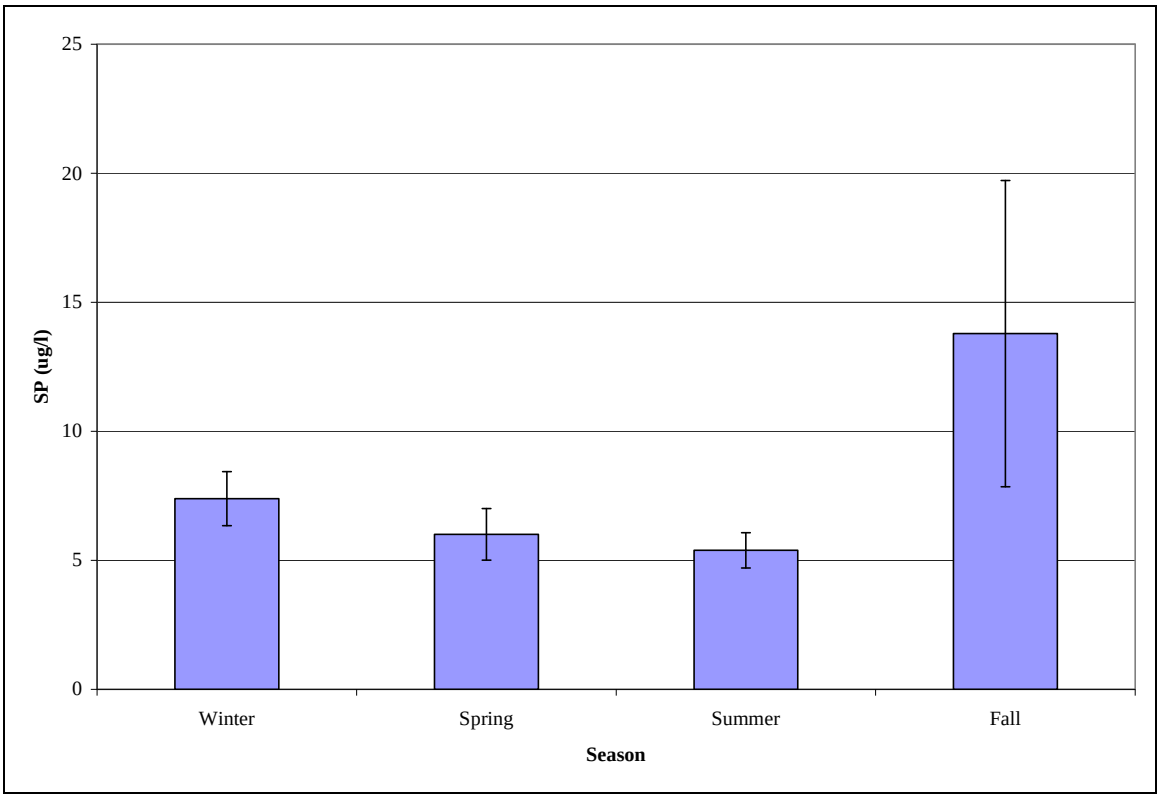

Figure 9: Variation of the SP content of the hyporheic water through seasons. There is no significant difference between seasons (p=0.41)

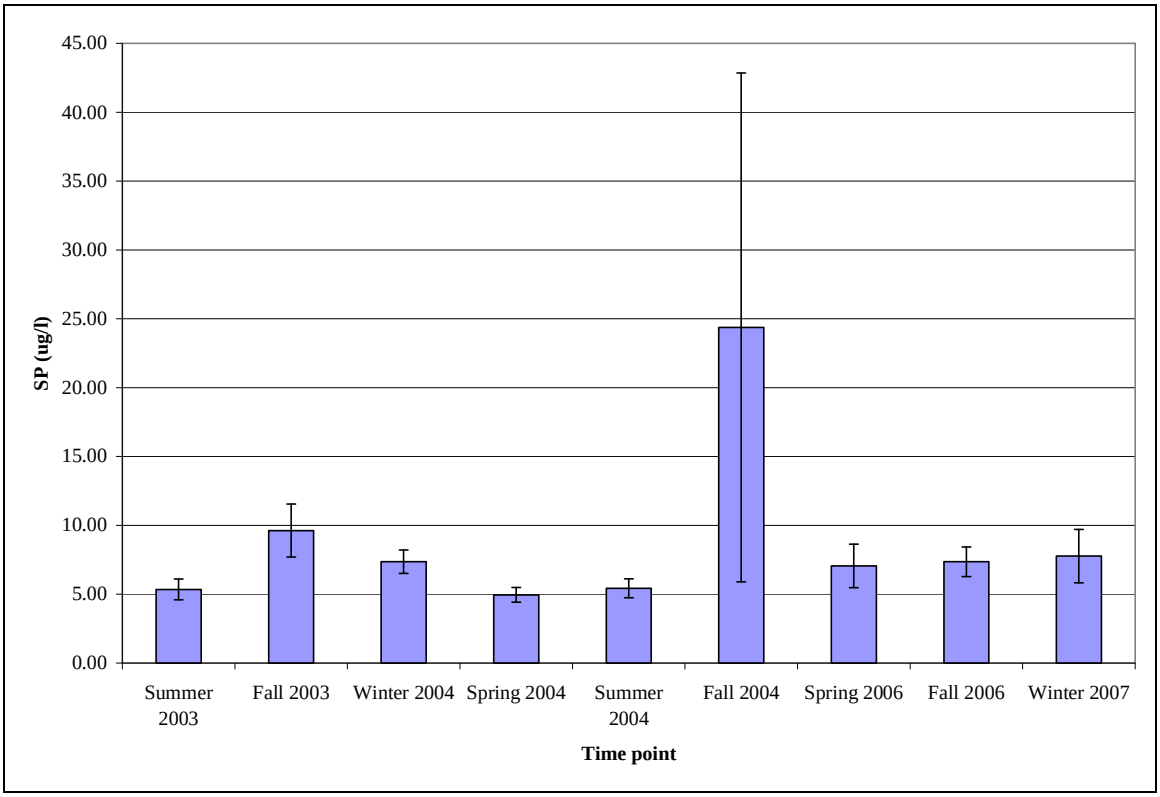

Figure 10: Variation of the SP content of the hyporheic water through time points. There is no significant difference between time points (p=0.52)

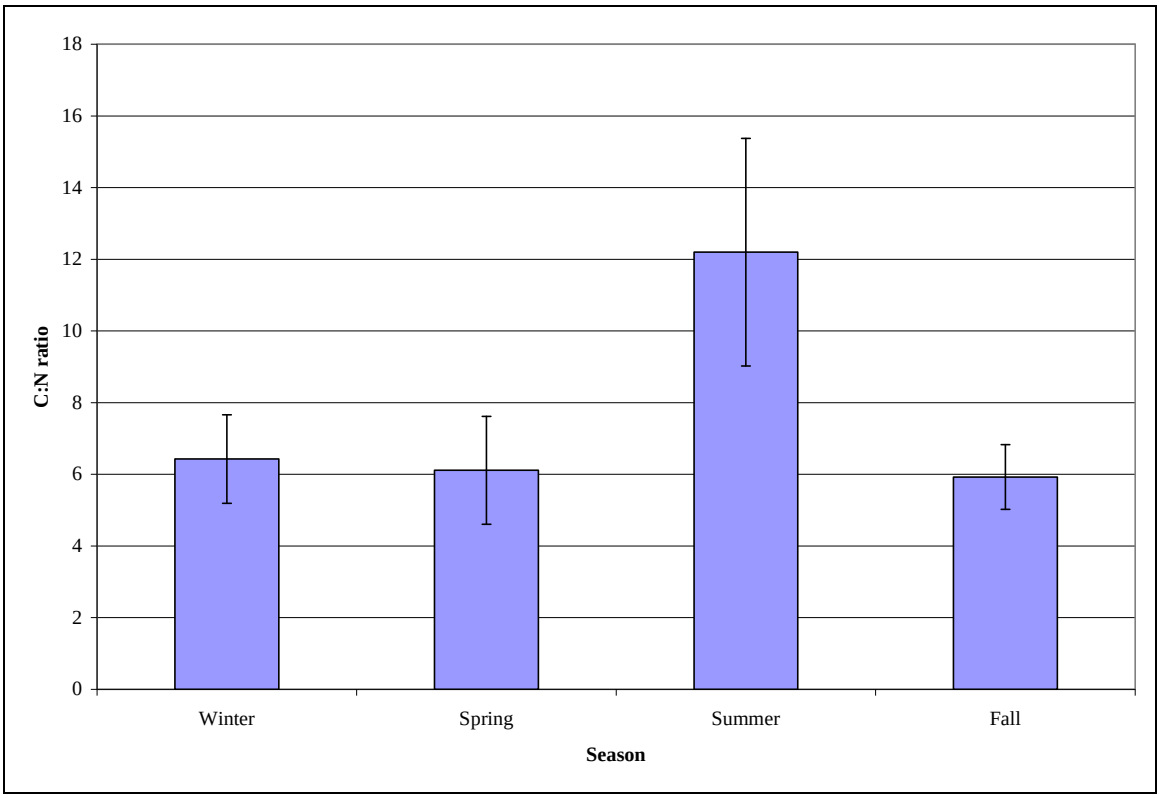

Figure 11: Variation of the C:N ratio of the hyporheic water through seasons. There is a significant difference between season (p=0.02)

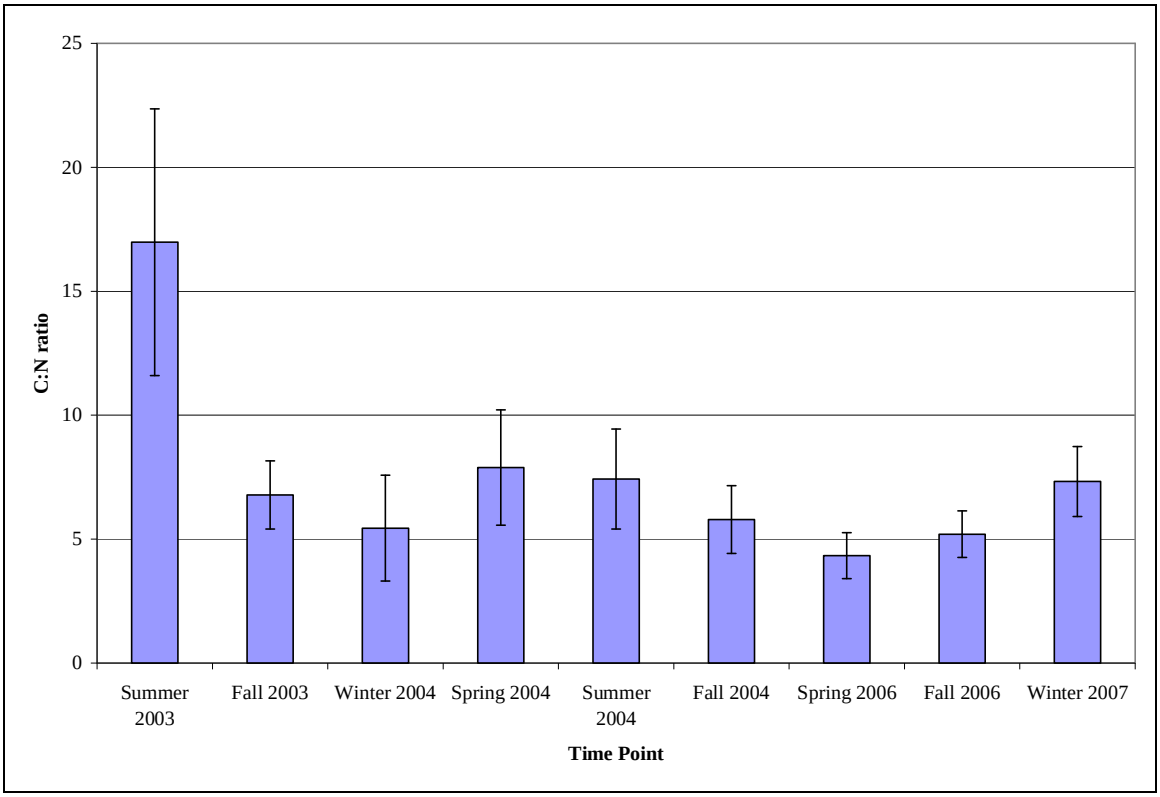

Figure 12: Variation of the C:N ratio of the hyporheic water at the different time points. There is a significant difference between time points (p=0.02)

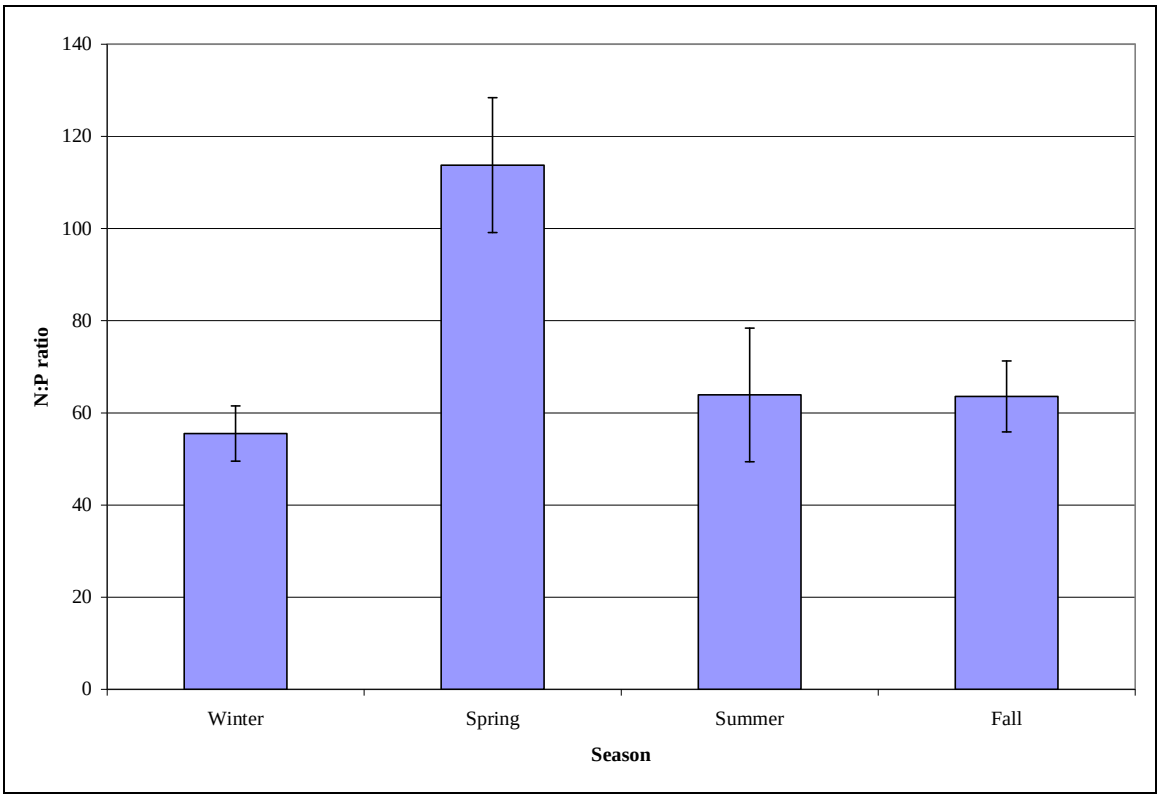

Figure 13: Variation of the N:P ratio of the hyporheic water through seasons. There is a significant difference between seasons ($p<0.01$)

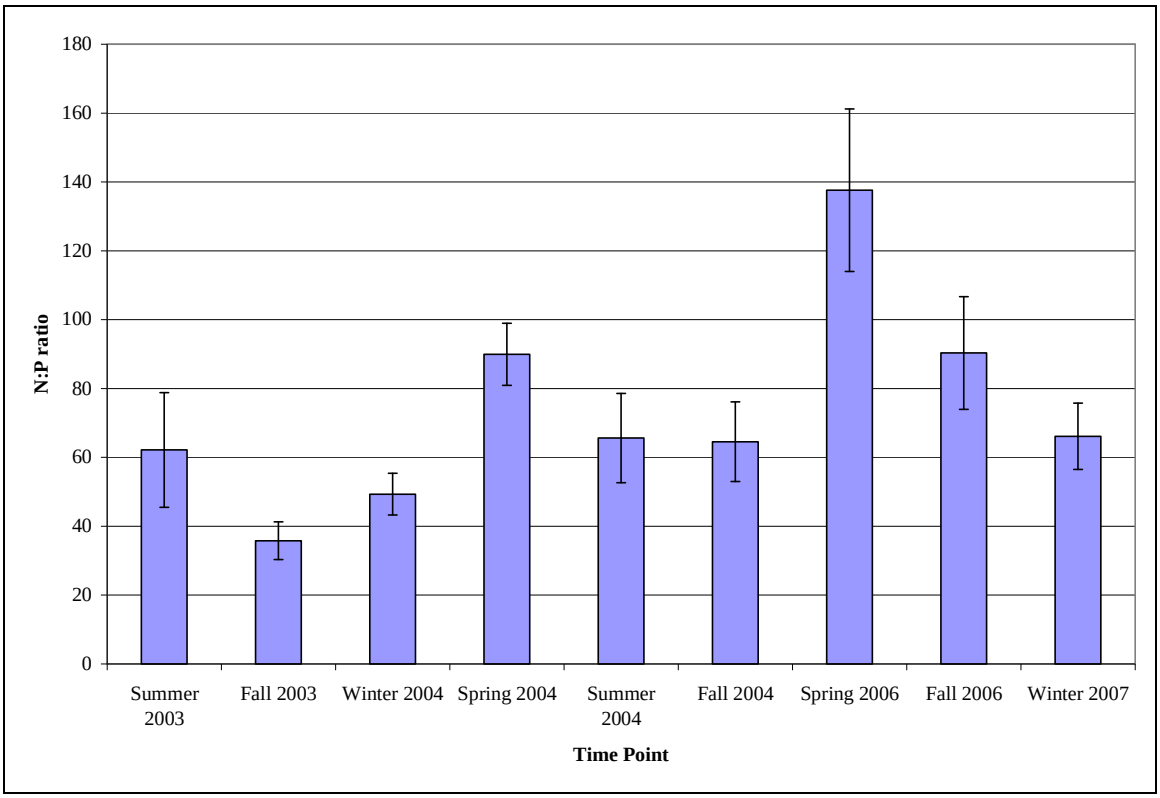

Figure 14: Variation of the N:P ratio of the hyporheic water at the different time points. There is a significant difference between time points ($p<0.01$)

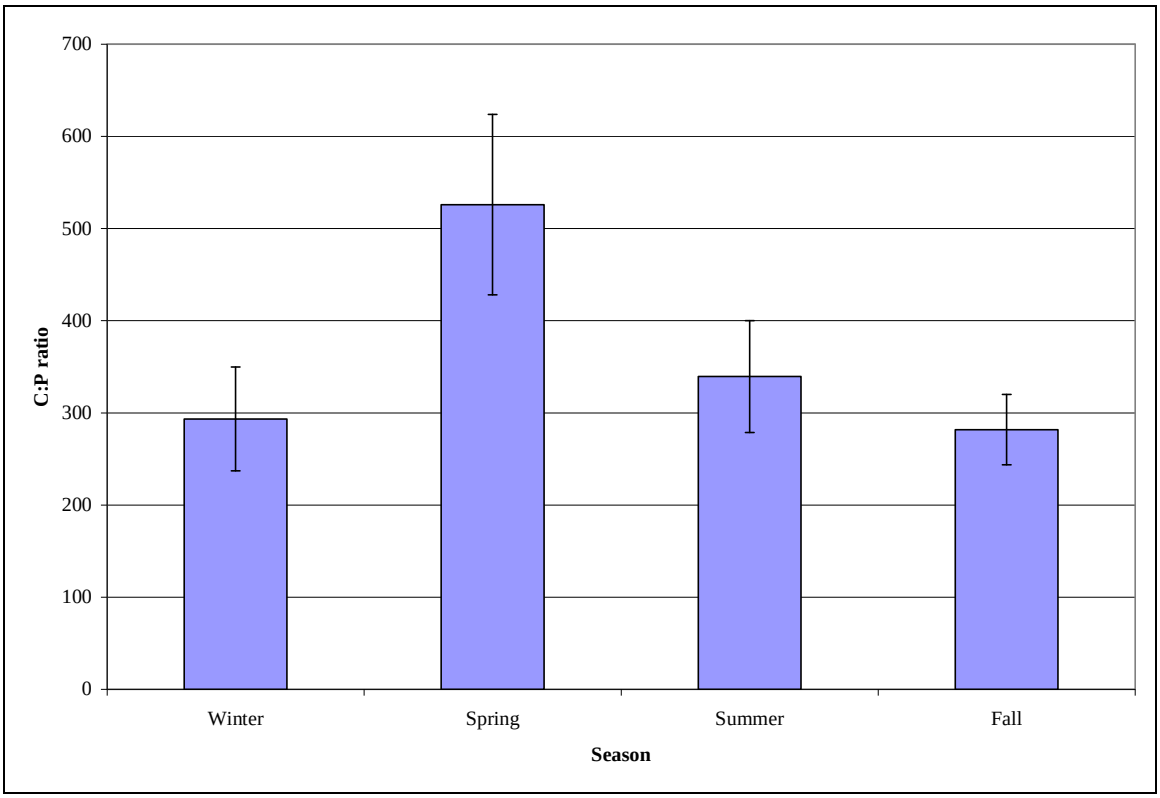

Figure 15: Variation of the C:P ratio of the hyporheic water through seasons. There is a significant difference between seasons (p=0.02)

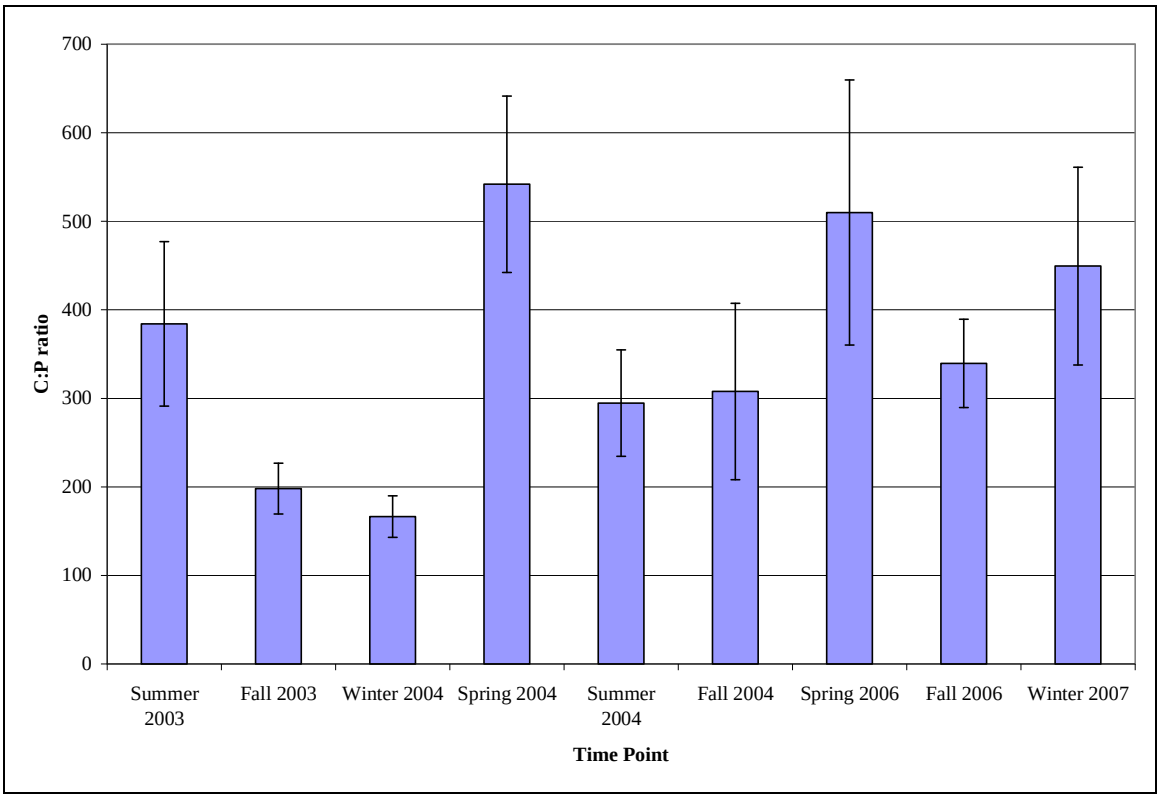

Figure 16: Variation of the C:P ratio of the hyporheic water at the different time points. There is a significant difference between time points (p=0.04)

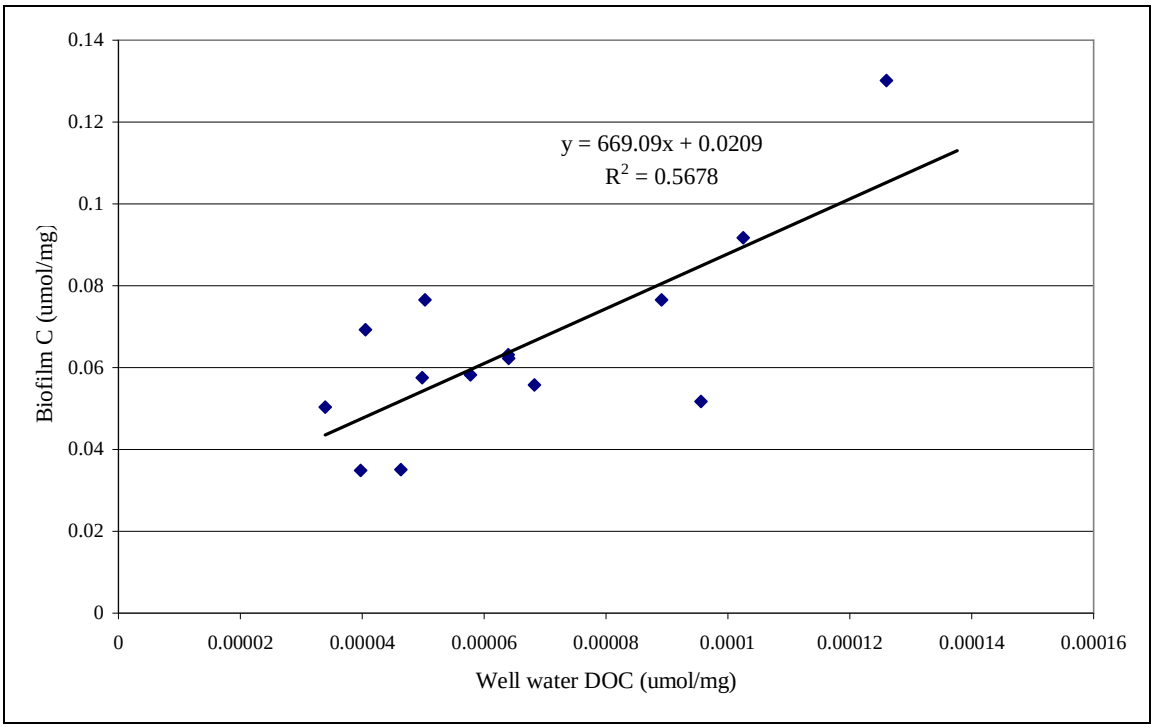

Figure 17: Correlation between the carbon content of the hyporheic water and the carbon content of the biofilm. There was a significant correlation (r^2=0.57, p=0.002, n=14) between these variables.

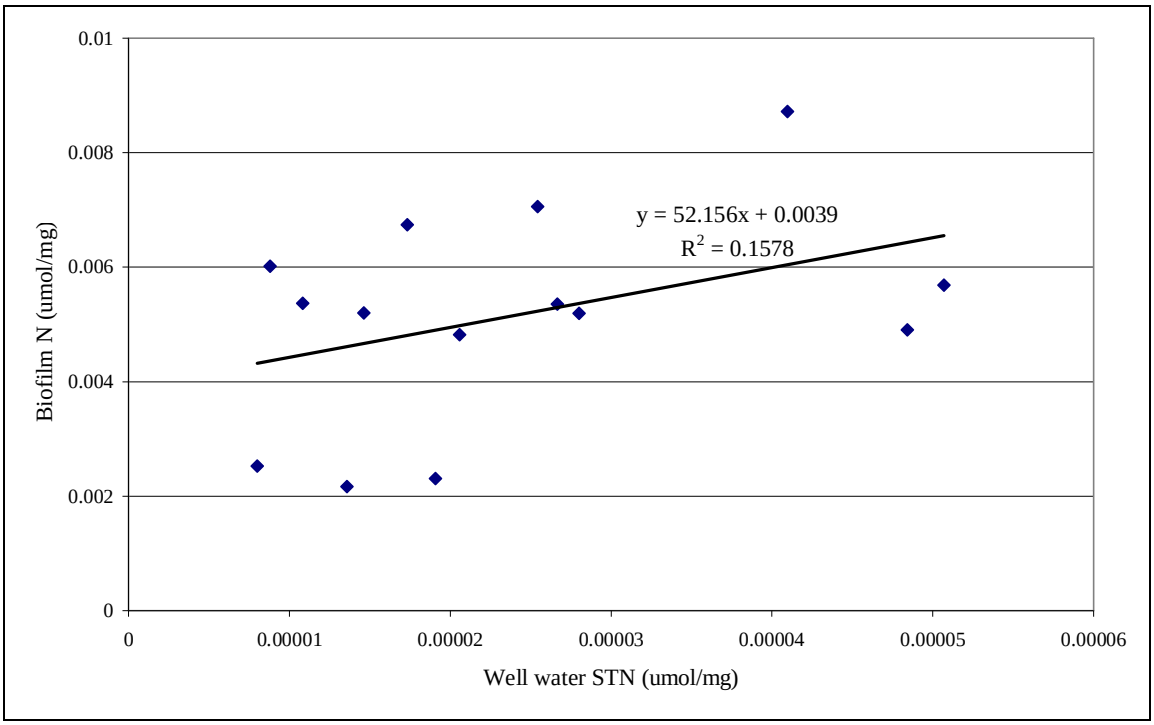

Figure 18: Correlation between the nitrogen content of the hyporheic water with the nitrogen content of the biofilm. There was no significant correlation (r^2=0.16, p=0.16, n=14) between these variables.

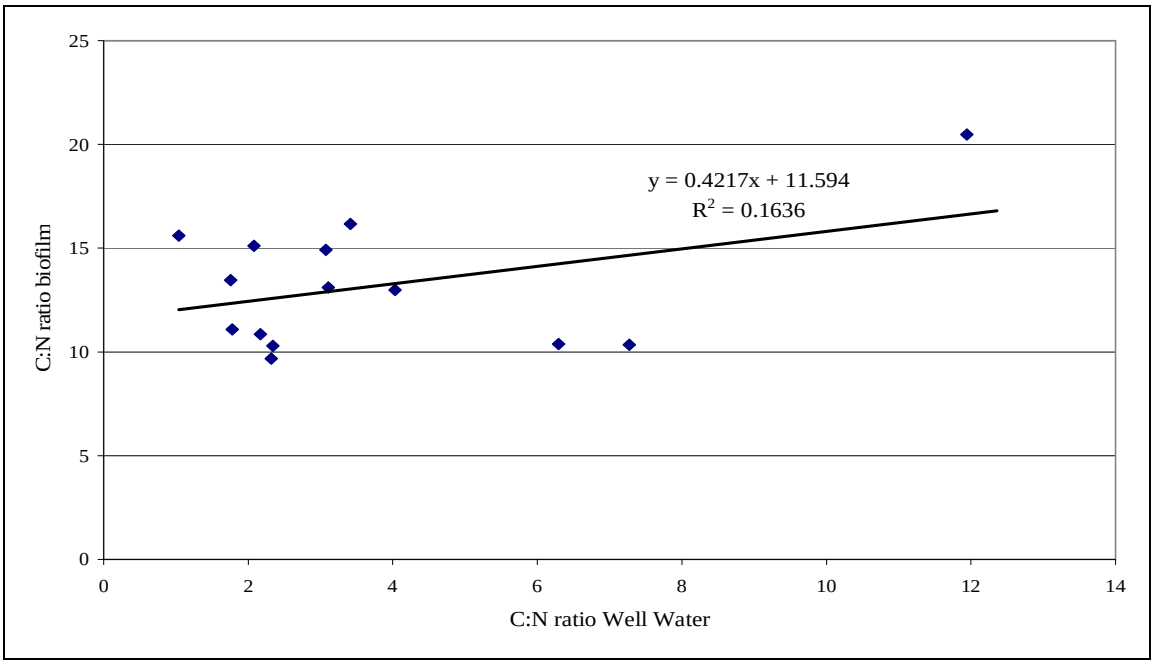

Figure 19: Correlation between the C:N ratio of the biofilm with the C:N ratio content of the hyporheic water. There was no significant correlation (r^2=0.16, p=0.15, n=14) between these variables.

4. CONCLUSIONS

4.1. BACTERIAL STOICHIOMETRY

This study compliments past studies of bacterial isolate homeostasis. Our data indicates that freshwater hyporheic bacteria are homeostatic with respect to their C:N ratio. That the overall trend of these bacteria is to be non homeostatic with respect to their N:P and C:P ratios.

The C:P values of the bacteria increased, reaching a maximum, to later decrease with increasing C:P of the medium. This decline in the C:P of bacteria could be due to P becoming limiting. Low food quality present high C:P, therefore we can say that resources with C:P greater than 1000 are of low quality and P becomes limiting for bacteria (Hessen 1997, Vrede *et al.* 2004).

We can also say that the homeostatic behavior with respect to C:N does not vary according to phylogeny. However, the N:P ratios vary according to phylogeny since we found that two (*Pedobacter* and *Rhodoferax*) of the eleven genera and two (*Alphaproteobacteria* and *Sphingobacteria*) of the six taxonomic classes studied presented homeostasis. And the C:P ratios also vary according to phylogeny since we found that three (*Brevundimonas*, *Arthrobacter* and *Pedobacter*) of the eleven genera and two (*Alphaproteobacteria* and *Sphingobacteria*) of the six taxonomic classes studied presented homeostasis.

4.2. BIOFILM STOICHIOMETRY

We determined that the harvested biofilm presented a significant difference ($p<0.01$) between the wells for their carbon (C) and nitrogen (N) content and C:N ratio. We also found that the DOC content of the water significantly correlated with the C content of the biofilm.

In this study, the C:N ratio range of the microbial biofilm was higher and broader (9:1 and 21:1) than in previous studies (5:1 and 8.3:1). It seems that the C:N ratio of biofilm from marine and coastal environments is lower than that of freshwater hyporheic environments. This difference is likely due to the environment from where the biofilm was harvested, suggesting that in the freshwater hyporheic environment, biofilms are limited by nitrogen.

There was a slight significant difference ($p=0.06$) between time points for the DOC content of the hyporheic water. There was a significant difference between seasons ($p=0.01$) and between time points ($p=0.001$) for the STN content and the nutrient ratios of the hyporheic water. However, we found no significant difference between seasons or time points for the SP content.

We found a significant correlation between the STN content of the hyporheic water and the distance of the well to the main channel. We also found that C:N of the water correlated with the distance to the main channel in four time points with a significant difference between time points. However, for the SP content, N:P and C:P ratios, we found significant correlations for a few time points but no significant difference between time points.

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