LINKING RIVER SEDIMENT PHYSICAL PROPERTIES TO BIOFILM BIOMASS AND ACTIVITY

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Dipòsit legal: Gi. 1377-2015
http://hdl.handle.net/10803/302110

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Departament de Ciències Ambientals

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DOCTORAL PROGRAMME IN WATER SCIENCE AND TECHNOLOGY

Supervised by:

Dra. Anna M. Romaní Cornet
Institut d’Ecologia Aquàtica
Universitat de Girona

Dr. Andrea Butturini
Departament d’Ecologia
Universitat de Barcelona

This manuscript has been presented to opt for the doctoral degree from the University of Girona

ACKNOWLEDGEMENTS

I would like to send my deepest gratitudes to my advisors Prof. Anna M. Romaní and Prof. Andrea Butturini for giving me the chance to pursue my PhD studies at Universitat de Girona. They are so knowledgeable and professional that, when I am stuck in my doctoral research, they can always help me to find a way out and give me valuable suggestions. I deeply appreciate their instructions and kindness.

The financial support of my PhD studies comes mainly from the European Commission (Erasmus Mundus project TECHNO). I am also thankful to the project GL2011-30151-C02 from the Spanish Ministry of Economy and Competitiveness. All of these financial supports are gratefully acknowledged.

I also have to thank the members of my PhD committee for their helpful career advice and suggestions in general.

Typically, I want to express my great thanks to Anna F. for her kind both in my laboratory experiments and in teaching me the statistical analysis. I will never forget the special day when I arrived at the Girona Station; she picked me up to the University residence "RESA" and helped me to find a nice house.

My sincere thanks go to professors Debouche and Degré as well as to Olivier at Gembloux Agro-Bio Tech·ULg (Belgium) for helping me to correct my text in French.

Besides, I also want to say thanks to professors (Sabater, Garcia, Guasch, Boix, Gascón, Sala, Quintana, Vila and Zamora) in the ecology group and in the department of environmental sciences. It will remain as a precious memory for me to have worked together with them; especially, I really appreciate their team meetings for the Christmas lunch every year. Many thanks are also given to my friends (Anna F., Laura B., Amaraa, Chang, Maria, Juanita, Rob, Núria, Gemma, Meri, Mercè, Núria P., Irene, Jordi C., Jordi, Laura D., Joan, Miquel, Ninew and Pao) I made during my study at UdG. Without them, my life would not be so fruitful and colorful.

In the end, many thanks go to Raquel for her kind help during my visa processes by issuing all necessary documents I needed, and especially to Kariné for her assistance to apply for "La targeta sanitària" for my wife. I want to thank Esther and Gemma for helping me dealing with matters at the laboratory of limnology.

I dedicate this thesis to my wife, Ratha, my son, Justin, and my families for their constant support and unconditional love.

“*Tout est possible à qui rêve, ose, travaille, et n’abandonne jamais.*”

– Xavier Dolan

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SUMMARY

River sediments are composed by a variety of sizes ranging from clay to gravel. When river flowing water and groundwater become overlapped below the streambed, an interface called "hyporheic zone" is created. The sediment physical characteristics play not only a key role in determining permeability of the bulk hyporheic sediments; but also, altogether, they determine microbial biomass and biogeochemical processes in space and time. In fact, these biological processes can occur at different scales (i.e. at the catchment scale, reach scale and sediment-habitat scale) along a stream, and are normally subjected to seasonally-variable changes such as hydrological events. In the context of Mediterranean rivers (such as our study river, La Tordera river, located at the North East of the Iberian Peninsula), floods and droughts are common events which can regulate biological and biogeochemical processes. Generally, hydrological exchange and mixing processes in the hyporheic zone are highly variable and may impact physical and biological variables at daily and seasonal time scales.

The aim of this thesis was to test whether differences in sediment physical characteristics (grain sizes and texture) generate differences in biofilm biomass and metabolic activity, and in nutrient content. It was hypothesized that change of the sediment physical characteristics would affect biological, physical and chemical processes across the surface and subsurface layers; and that this change will be possibly modified by the dry-wet conditions which may happen through sediment depths in the studied Mediterranean river.

To this aim, first a review of the best formulae for the measurement of hydraulic conductivity at our study system was performed. Sediment hydraulic conductivity (K) is an essential parameter to understand sub-superficial flow in fluvial hyporheic systems, and to study solute transport in those systems. In unconsolidated sediments, sediment grain-size distribution is an essential variable, from which several formulae have been proposed to predict K. In this study, we explored the limits and applications of 11 defined formulae to empirically compute K. For this purpose, 36 sand sediment corers of up to 60 cm depth were collected at the downstream reach of the Tordera river during three sampling campaigns (December 2012, June & July 2013).

In order to assess the sediment hydraulic parameters which mostly affect the microbial accumulation at the surface sediment along the Tordera river, a study at the

catchment scale was performed. Twelve sites, from the river source to the mouth, were selected and analyzed for three sampling dates (base discharge in July 2011, high discharge in April & low discharge in July 2012). For all sites and dates, we measured the grain-size distribution and the biofilm bacterial density, chlorophyll-a, and ash free dry-weight (AFDW) content of river surface sediments (uniformly-graded sand).

At the reach scale, we studied the tempo-spatial variation of microbial metabolism in the surface and hyporheic sediments influenced by hydrological conditions at a downstream reach of the Tordera river. We selected four sites including a gradient of hydrological conditions and we sampled interstitial water and sediment (up to 60 cm sediment depth, and up to 1 m for the interstitial water) in two occasions (June & July 2013). Sediments at different depths were used to determine sediment grain-size distribution and texture, organic matter (OM) content, and C, N and P contents. Moreover, extracellular enzyme activities (β-glucosidase, leucine-aminopeptidase and phosphatase) involved in the decomposition of C, N and P organic compounds were measured in the sediment and in the interstitial water.

The obtained results showed that for our study sediments (uniformly graded, moderately and poorly sorted), the USBR, Slichter and Harleman formulas underestimated the K values; while the Beyer and Terzaghi methods overestimated them. On the other hand, our sample characteristics did not fit the domains of applicability for the Krumbein and Monk, and Alyamani and Şen methods. However, two empirical methods (Kozeny-Carman and Sauerbrei) yielded the K values closer to our observed results (*in situ*), and within the K limits given by previous authors. Furthermore, K obtained from the Kozeny-Carman approach showed a better correlation to sediment sorting than K obtained by the Sauerbrei approach. Therefore, our results obtained from a set of sand sediment samples suggest selecting the method proposed by Kozeny-Carman to determine K from grain-size distribution. Thus, for the following studies of the thesis, hydraulic conductivity was estimated by Kozeny-Carman method.

The study at the catchment scale aimed to determine how sediment hydraulic variables affect microbial biomass along the Tordera river. Our results from multiple regression analyses showed: 1) that heterogeneity and sediment interstices significantly predicted the variations in ash free dry weight (AFDW) content in July

2011 and July 2012; 2) that high sand sediment heterogeneity affected positively Chlorophyll-a accumulation, but only at high-flow conditions in April 2012; and 3) that pore spaces and sediment surface area (which increased with % of mud) were relevant for bacterial attachment, and this was found for the three different hydrological periods studied. Bacteria were also related to AFDW content. The study suggests that sediment physical properties differentially affect the microbial biomass – bacteria being the most affected –, and that this effect may change depending on the hydrological conditions.

At the reach scale and considering surface and hyporheic sediment, no significant variations in C, N, P content (or molar ratios) were found in depth. The results from physical sediment properties (i.e. hydraulic conductivity and texture) showed that hydraulic conductivity (K) was significantly different among some sites. There were no significant differences for the percentage of sand and mud (silt and clay) between sites and dates. OM was low and only slightly accumulated in some sites and depths. Further, microbial activities were likely to be regulated by the permeability of streambeds through organic compounds could (spatially) contribute to some microbial activities in sediment depths (e.g. leucine-aminopeptidase and phosphatase activities in June as well as β-glucosidase and phosphatase in July). Furthermore, sediment particles were likely to control nutrients and microbial metabolism. In sediment, it was shown that the percentage of clay favored the β-glucosidase activity and P content by showing positive correlation. At the same time, the % of clay was in the same direction as the peptidase activity in sediment, while the bacterial abundance was strongly associated to K. However, bacteria in interstitial water were shown in a direction of mud (especially with % of clay) whose interconnection was strongly mediated by the porous layers. However, the amount of clay could impact negatively to permeability of the streambeds although its amount was noted very little. Overall, we conclude that the hydraulic conductivity of hyporheic zone is among the most important factors of biogeochemical dynamics involved in microbial processes at the Tordera hyporheic sand downstream reaches.

RÉSUMÉ

Les sédiments fluviaux sont composés de particules de différentes tailles, de l'argile jusqu'au gravier. Lorsque l'eau de la rivière et les eaux souterraines se rejoignent sous le lit de la rivière, se crée une interface appelée "zone hyporhéique". Les caractéristiques physiques des sédiments jouent non seulement un rôle clé dans la détermination de la perméabilité des couches hyporhéiques; mais elles déterminent également une biomasse microbienne et des processus biogéochimiques dans l'espace et le temps. En effet, ces processus biologiques peuvent se produire à différentes échelles (à l'échelle du bassin versant, à l'échelle du tronçon, et à l'échelle du sédiment-habitat) le long de la rivière. Ils sont soumis aux changements saisonniers affectant les événements hydrologiques. Dans le contexte des rivières méditerranéennes (telles que ''La Tordera'', située au nord-est de la péninsule ibérique), les inondations et les sécheresses sont des événements courants qui peuvent contrôler les processus biologiques et biogéochimiques. En général, les échanges et les processus hydrologiques dans la zone hyporhéique sont très variables et peuvent avoir un impact quotidien et saisonnier sur les paramètres physiques et biologiques.

Le but de cette thèse était de tester si des différences dans les caractéristiques physiques des sédiments (la taille des particules et la texture) génèrent des différences au niveau de la biomasse, de l'activité métabolique d'un biofilm microbien et de la teneur en éléments nutritifs. L'hypothèse était que le changement des caractéristiques physiques des sédiments affecte les processus biologiques, physiques et chimiques à travers les couches superficielles et hyporheiques du lit de la rivière; et que ce changement serait éventuellement modifié par les conditions sèches ou humides qui peuvent se produire dans la profondeur des couches de la rivière.

Dans ce but, un examen des formules a tout d'abord été effectué pour calculer la conductivité hydraulique (K) dans notre système d'étude. La conductivité hydraulique de sédiments est un paramètre essentiel pour comprendre l'écoulement souterrain dans les systèmes hyporhéiques fluviaux, et pour étudier le transport de solutés dans ces systèmes. Dans les sédiments non consolidés, la distribution granulométrique des particules est une variable essentielle, à partir de laquelle plusieurs formules ont été proposées pour prédire K. Dans cette étude, nous avons exploré les limites et l'applicabilité de 11 formules définies empiriquement pour estimer K. A cet effet, 36

carottes de sédiments (remaniés) ont été prélevées jusqu'à 60 cm de profondeur en aval de la rivière Tordera pendant trois campagnes d'échantillonnage (décembre 2012, juin et juillet 2013).

Afin d'évaluer les paramètres hydrauliques de sédiments (sableux) qui affectent essentiellement une accumulation microbienne dans les couches de surface le long de la rivière Tordera, une étude à l'échelle du bassin versant a été menée. Pour cette étude, 12 points d'échantillonnage ont été sélectionnés, de la source à l'embouchure de la rivière, et analysés pour trois périodes : débit de base (juillet 2011), débit élevé (avril 2012) et faible débit (juillet 2012). Pour tous les points et toutes les campagnes d'échantillonnage, nous avons mesuré la granulométrie des particules et la densité bactérienne du biofilm, ainsi que la teneur en chlorophylle et en matière organique (AFDW) de sédiments de surface (d'un sable bien classé) de la rivière.

À l'échelle du tronçon de cours d'eau, nous avons étudié la variation spatio-temporelle du métabolisme microbien dans les sédiments de surface et hyporhéiques influencés par des conditions hydrologiques en aval de la rivière Tordera. Dans cette section, quatre points d'échantillonnage ont été sélectionnés suivant un gradient de l'humidité des sols, à partir desquels les sédiments et l'eau interstitielle ont été échantillonnés (à une profondeur de 60 cm pour les sédiments et jusqu'à 1 m pour l'eau) en deux occasions (juin et juillet 2013). Les sédiments prélevés à différentes profondeurs ont été utilisés pour déterminer la granulométrie et la texture, les teneurs en matière organique (AFDW) ainsi qu'en carbone (C), azote (N) et phosphore (P). En outre, les activités enzymatiques extracellulaires (β-glucosidase, leucine-aminopeptidase et phosphatase) participant à la décomposition des composés organiques (de C, N et P) ont été mesurées dans les sédiments et dans l'eau interstitielle.

Pour nos sédiments sableux (granulométrie uniforme, modérément et mal classée), les résultats obtenus ont montré que les formules de USBR, Slichter et Harleman ont sous-estimé les valeurs K; tandis que les méthodes Beyer et Terzaghi les ont surestimées. D'autre part, les caractéristiques de nos échantillons n'étaient pas en adéquation avec le domaine d'applicabilité pour les méthodes de Krumbein et Monk, et Alyamani et Şen. Cependant, deux méthodes empiriques (Kozeny-Carman et Sauerbrei) ont produit les valeurs K proches de nos résultats observés (*in situ*), et dans les limites de K données par d'autres auteurs. En outre, K obtenu par l'approche

Kozeny-Carman a montré une bonne corrélation avec la distribution granulométrique des grains. En cela, les résultats de Kozeny-Carman étaient plus performants que ceux obtenus par l'approche Sauerbrei. Par conséquent, nos résultats obtenus à partir d'un ensemble d'échantillons sableux suggèrent de choisir la méthode proposée par Kozeny-Carman pour déterminer K de la distribution de la taille des particules. Ainsi dans la suite de la thèse, la conductivité hydraulique a été estimée en utilisant la méthode Kozeny-Carman.

Quant à l'échelle du bassin versant, l'étude a visé à déterminer comment les variables hydrauliques de sédiments affectent la biomasse microbienne le long de la rivière Tordera. Nos résultats d'analyses de régression multiple ont montré : 1) que l'hétérogénéité et les interstices de sédiments prédisent significativement la variation de la teneur en matière organique (ou AFDW) en juillet 2011 et 2012; 2) que les sédiments sableux très hétérogènes affectent positivement l'accumulation de chlorophylle, mais seulement pendant les conditions de haut débit en avril 2012; et 3) que les pores et l'aire superficielle disponible de sédiments (augmentée suivant le pourcentage de boue (limon et argile)) sont liées à l'accumulation de bactéries. Cela a été observé pour les trois périodes hydrologiques étudiées. Les bactéries ont été également liées à la teneur en matière organique (AFWD). L'étude suggère que les propriétés physiques de sédiments affectent différemment la biomasse microbienne - les bactéries étant les plus touchées - et que cet effet peut varier selon les conditions hydrologiques.

À l'échelle du sédiment-habitat considérant les sédiments de surface et hyporhéiques, aucune variation significative de teneurs en C, N et P (ou leurs ratios molaires) n'a été trouvée en profondeur. Les résultats des caractéristiques physiques de sédiments (telles que la conductivité hydraulique et la texture) ont montré que la conductivité hydraulique (K) était significativement différente entre certains points d'échantillonnage. Il n'y avait pas de différences significatives pour le pourcentage de sable et de boue (limon et argile) entre les points et les périodes d'échantillonnage. La teneur en matière organique (AFDW) était faible et s'est accumulée légèrement dans certaines sites et à certaines profondeurs. En outre, les activités microbiennes étaient susceptibles d'être réglementées par la perméabilité des couches de sédiments, à l'endroit où les composés organiques pourraient s'accumuler, par exemple les activités de leucine-aminopeptidase et de phosphatase en juin, et celles-ci de β-glucosidase et de phosphatase en juillet. En outre, les particules de sédiments pouvaient contrôler les nutriments et le métabolisme microbien. Dans les sédiments, il a été montré que le pourcentage d'argile favorisait l'activité β-glucosidase et le contenu de P en montrant corrélation positive. En même temps, le % d'argile était également en corrélation avec l'activité de peptidase dans les sédiments, alors que la densité des bactéries était fortement associée au facteur K. Cependant, les bactéries dans l'eau interstitielle étaient particulièrement abondantes dans la boue de sédiments riche en argile; et leur interconnexion était essentiellement due à l’existence des couches poreuses. Par contre, la teneur en argile pourrait avoir un impact négatif sur la perméabilité du lit de la rivière, bien que son contenu fût très faible dans notre étude de cas. Dans l'ensemble, Il a été conclu que la conductivité hydraulique dans la zone hyporhéique est l'un des facteurs importants dans la dynamique biogéochimique et des processus microbiens dans les sédiments sableux en aval de la rivière Tordera.

RESUMEN

Los sedimentos fluviales se componen de partículas de tamaños variables desde arcillas a gravas. Cuando el agua fluvial y subterránea se conectan bajo el lecho fluvial, se crea una interfase llamada “zona hiporreica”. Las características físicas del sedimento juegan un papel importante en determinar la permeabilidad de los sedimentos hiporreicos y, al mismo tiempo, pueden determinar en gran medida la biomasa microbiana y los procesos biogeoquímicos que tienen lugar en gradientes de espacio y tiempo. Estos procesos biológicos pueden tener lugar a distintas escalas (a escala de cuenca, escala de tramo, y escala de hábitat) a lo largo del río, y están normalmente sujetos a cambios que varían de forma estacional como la hidrología. En el contexto de los ríos Mediterráneos (como es el caso de nuestro río de estudio, el río Tordera, situado al nordeste de la Península Ibérica), las avenidas y sequías son hechos comunes los cuales pueden regular procesos biológicos y biogeoquímicos. En general, el intercambio y los procesos de mezcla hidrológicos en la zona hiporreica son muy variables y pueden impactar en las variables físicas y biológicas a escalas estacionales y diarias.

El objetivo de esta tesis era testar si las diferencias en las características físicas del sedimento (tamaño de grano y textura) genera diferencias en el contenido de nutrientes, biomasa y actividad metabólica del biofilm microbiano que se desarrolla en el sedimento. La hipótesis de partida era que los cambios en las características físicas del sedimento afectarían los procesos biológicos, físicos y químicos a lo largo de las capas del sedimento superficial y subsuperficial y que estos cambios se verían probablemente modificados por gradientes de humedad del sedimento (sedimento saturado de agua-sedimento seco) los cuales pueden tener lugar a lo largo de la profundidad del sedimento en el río Mediterráneo estudiado.

Para este objetivo, primero se realizó una revisión de las fórmulas para el cálculo de la conductividad hidráulica en nuestro sistema de estudio. La conductividad hidráulica del sedimento (K) es un parámetro esencial para entender el flujo subsuperficial en sistemas hiporreicos fluviales, y para estudiar el transporte de solutos en estos sistemas. En sedimentos no consolidados, la distribución de tamaños de grano es una variable esencial, a partir de la cual se han propuesto distintas fórmulas para predecir la K. En este estudio, hemos explorado los límites y la aplicabilidad de 11

fórmulas definidas para calcular de forma empírica la K. Con este propósito, se recogieron 36 cores de sedimento de hasta 60 cm de profundidad en el tramo bajo del río Tordera durante tres campañas de muestreo (diciembre 2012, junio y julio 2013).

Para descifrar los parámetros hidráulicos del sedimento que más podían afectar la acumulación de biomasa microbiana en el sedimento superficial a lo largo del río Tordera, se realizó un estudio a escala de cuenca. Para este estudio, se seleccionaron 12 puntos de muestreo, cubriendo des de la cabecera hasta la desembocadura del río y se muestrearon en tres momentos: caudal basal (julio 2011), caudal alto (abril 2012) y caudal bajo (julio 2012). Para todos los puntos y fechas de muestreo se midió la distribución de los tamaños de grano de sedimento y la densidad bacteriana, contenido de clorofila y contenido de peso seco libre de cenizas (AFDW) de los sedimentos superficiales.

A escala de tramo, se estudió la variación espacio-temporal del metabolismo microbiano en los sedimentos superficiales e hiporreicos influenciados por las condiciones hidrológicas en un tramo del río Tordera situado en la parte baja del río. En este tramo, se seleccionaron 4 puntos de muestro incluyendo un gradiente de condiciones hidrológicas y se muestrearon el sedimento y el agua intersticial (hasta una profundidad de 60 cm de sedimento y hasta 1 m para el agua) en dos ocasiones (junio y julio 2013). Los sedimentos recogidos a las distintas profundidades se utilizaron para determinar la distribución de los tamaños de grano y la textura, el contenido de materia orgánica (OM) a partir del peso seco libre de cenizas (AFDW), y el contenido de C, N, y P. Además, se midieron en el sedimento y en el agua intersticial, las actividades enzimáticas extracelulares (β-glucosidasa, leucina-aminopeptidasa y fosfatasa) involucradas en la descomposición de compuestos orgánicos de C, N, y P.

Los resultados obtenidos mostraron que para nuestros sedimentos de estudio (con granulometría uniforme, definidos como “moderately” y “poorly sorted”, es decir, con tamaños de grano distintos), las fórmulas de USBR, Slichter y Harleman subestimaban los valores de K, mientras que los métodos de Beyer y Terzaghi los sobreestimaban. Por otro lado, las características de nuestras muestras no encajaban en el dominio de aplicabilidad para los métodos de Krumbein y Monk, y Alyamani y Şen. A partir de dos métodos empíricos (Kozeny-Carman y Sauerbrei) conseguimos obtener valores de K cercanos a nuestros resultados observados (*in situ*), y dentro de los límites de K dados por otros autores. Además, la K obtenida partir de la aproximación de Kozeny-Carman mostró una correlación mejor con la clasificación por tamaños del sedimento que la K obtenida a partir de la aproximación de Sauerbrei. Así pues, nuestros resultados obtenidos a partir de un conjunto de sedimentos arenosos sugirieron la selección del método propuesto por Kozeny-Carman para determinar la K a partir de la distribución de los tamaños de grano. A partir de esta revisión, para los siguientes estudios de la tesis, la conductividad hidráulica se estimó utilizando el método de Kozeny-Carman.

El estudio a nivel de cuenca tenía como objetivo determinar cómo las variables hidráulicas del sedimento afectaban la biomasa microbiana a lo largo del río Tordera. Nuestros resultados a partir de las regresiones múltiples mostraron que i) la heterogeneidad y los espacios intersticiales del sedimento predecían de forma significativa la variación en el contenido de materia orgánica (AFDW) en julio 2011 y julio 2012; ii) que una elevada heterogeneidad del sedimento afectaba positivamente la acumulación de clorofila pero solamente en condiciones de caudal elevado (abril 2012); y que iii) la porosidad y el área disponible de superficie de sedimento (la cual aumentaba con el % de lodo, es decir las partículas de < 75 µm las cuales incluyen el el limo y las arcillas) eran relevantes para la acumulación de bacterias, y esto se observaba para los tres periodo hidrológicos estudiados. Las bacterias también se vieron relacionadas con el contenido de materia orgánica (AFDW). El estudio sugiere que las propiedades físicas del sedimento afectan de forma diferencial la biomasa microbiana – siendo las bacterias las más afectadas –, y que este efecto puede variar en función de las condiciones hidrológicas.

A escala de tramo y considerando el sedimento superficial y hiporreico, no se encontraron diferencias significativas en profundidad para el contenido de C, N y P (o sus relaciones molares). Los resultados de las propiedades físicas del sedimento (conductividad hidráulica y textura) mostraron que la conductividad hidráulica (K) era significativamente diferente entre algunos puntos de muestreo. No se observaron diferencias significativas en el porcentaje de lodo (limo + arcilla) entre puntos y periodos de muestreo. El contenido de materia orgánica era bajo y solamente se acumulaba algunos puntos y profundidades. Las actividades microbianas eren probablemente reguladas por la permeabilidad del sedimento y la acumulación de compuestos orgánicos en algunos puntos, contribuyendo a las actividades de descomposición microbiana en las distintas profundidades del sedimento (por ejemplo como se observó para las actividades leucina-aminopeptidasa y fosfatasa en junio y para la β-glucosidasa y fosfatasa en julio). Además, las partículas de sedimento podrían controlar los nutrientes y el metabolismo microbiano. En el sedimento se observó que el porcentaje de arcilla favorecía la actividad β-glucosidasa y el contenido de P (correlación positiva). Al mismo tiempo, el % de arcilla también se correlacionó con la actividad peptidasa, mientras que la densidad de bacterias se asoció con la K. Por otro lado, la densidad de bacterias en el agua intersticial eran mayores en condiciones de mayor % de lodo (especialmente de arcilla) y su interconexión al sedimento estaba mediada por las capas porosas. Por otro lado, el contenido de arcilla podría impactar negativamente en la permeabilidad del lecho del río pero en nuestro caso de estudio el contenido era muy bajo. Se concluyó que la conductividad hidráulica en la zona hiporreica es uno de los factores relevantes de la dinámica biogeoquímica y los procesos microbianos en el sedimento hiporreico de los tramos arenosos de la baja Tordera.

RESUM

Els sediments fluvials es composen per partícules de mides variables des d'argiles a graves. Quan l'aigua fluvial i subterrània es connecten sota el llit fluvial, es crea una interfase anomenada "zona hiporreica". Les característiques físiques del sediment juguen un paper important en determinar la permeabilitat dels sediments hiporreics i, al mateix temps, poden determinar en gran mesura la biomassa microbiana i els processos biogeoquímics en l'espai i el temps que hi tenen lloc. De fet, aquests processos biològics poden tenir lloc a diferents escales (a escala de conca, escala de tram i escala d'hàbitat) al llarg del riu, i estan normalment subjectes a canvis que varien de forma estacional com la hidrologia. En el context dels rius Mediterranis (com és el cas del nostre riu d'estudi, la Tordera, situat al nord est de la Península Ibèrica), les avingudes i sequeres són esdeveniments comuns els quals poden regular processos biològics i biogeoquímics. En general, l'intercanvi i els processos de barreja hidrològics a la zona hiporreica són molt variables i poden impactar les variables físiques i biològiques a escales estacionals i diàries.

L'objectiu d'aquesta tesi era testar si les diferències en les característiques físiques del sediment (mides de gra i textura) genera diferències en el contingut de nutrients, biomassa i activitat metabòlica del biofilm microbià que s'hi desenvolupa. La hipòtesi de partida era que els canvis en les característiques físiques del sediment afectarien els processos biològics, físics i químics al llarg de les capes del sediment superficial i subsuperficial i que aquests canvis es veurien probablement modificats per gradients d'humitat al sediment (sediment saturat d'aigua-sediment sec) les quals poden tenir lloc al llarg de la profunditat del sediment en el riu mediterrani d'estudi.

Per a aquest objectiu, primer es va realitzar una revisió de les fórmules per al càlcul de la conductivitat hidràulica en el nostre sistema d'estudi. La conductivitat hidràulica del sediment (K) és un paràmetre essencial per a entendre el flux sub-superficial en sistemes hiporreics fluvials, i per a estudiar el transport de soluts en aquests sistemes. En sediment no consolidats, la distribució de les mides de gra és una variable essencial, a partir de la qual s'han proposats diverses fórmules per tal de predir la K. En aquest estudi, hem explorat els límits i l'aplicabilitat d'11 fórmules definides per tal de calcular de forma empírica la K. Amb aquest propòsit, es van recollir 36 cores de sediment de fins a uns 60 cm de profunditat al tram baix del riu

Tordera durant tres campanyes de mostreig (desembre 2012, juny i juliol 2013).

Per tal de desxifrar els paràmetres hidràulics del sediment que més podien afectar l'acumulació de biomassa microbiana al sediment superficial al llarg del riu Tordera, es va realitzar un estudi a escala de conca. Per a aquest estudi, es van seleccionar 12 punts de mostreig, cobrint des de la capçalera del riu fins a la desembocadura, i es van mostrejar en tres moments: cabal basal (Juliol 2011), cabal elevat (Abril 2012) i cabal baix (Juliol2012). Per a tots els punts i dates de mostreig es va mesurar la distribució de les mides de gra de sediment i la densitat bacteriana, contingut de clorofil·la i contingut de pes sec sense cendres (AFDW) dels sediments superficials.

A escala de tram, es va estudiar la variació espacio-temporal del metabolisme microbià als sediments superficials i hiporreics influenciats per les condicions hidrològiques en un tram del riu Tordera situat al tram baix del riu. En aquest tram, es van seleccionar 4 punts de mostreig incloent un gradient de condicions hidrològiques i es van mostrejar el sediment i l'aigua intersticial (fins a una profunditat de 60 cm de sediment i fins a 1 m per l'aigua) en dues ocasions (juny i juliol 2013). Els sediments recollits a les diferents profunditats es van utilitzar per a determinar la distribució de les mides de gra i la textura, el contingut de matèria orgànica (OM) a partir del pes sec sense cendres (AFDW), i el contingut de C, N i P. A més a més, es van mesurar al sediment i a l'aigua intersticial, activitats enzimàtiques extracel·lulars (β-glucosidasa, leucina-aminopeptidasa i fosfatasa) involucrades en la descomposició de compostos orgànics de C, N, i P.

Els resultats obtinguts mostraren que per als nostres sediments d'estudi (amb granulometria uniforme, del tipus "moderately" i "poorly sorted", és a dir, amb mides de gra força diferents), les fórmules de USBR, Slichter i Harleman subestimaven las valors de K, mentre que els mètodes de Beyer i Terzaghi las sobreestimaven. Per altra banda, les característiques de les nostres mostres no encaixaven en el domini d'aplicabilitat per als mètodes de Krumbein i Monk, i Alyamani i Şen. A partir de dos mètodes empírics (Kozeny-Carman i Sauerbrei) aconseguírem obtenir valors de K propers als nostres resultats observats (*in situ*), i dins el límits de K donats per altres autors. A més a més, la K obtinguda a partir de l'aproximació de Kozeny-Carman mostrà una correlació més bona amb la classificació per mides del sediment que la K obtinguda a partir de l'aproximació de Sauerbrei. Així doncs, els nostres resultats obtinguts a partir d'un conjunt de sediments sorrencs suggeriren la selecció del mètode proposat per Kozeny-Carman per a determinar la K a partir de la distribució de mida de gra. A partir d'aquesta revisió, per a la resta d'estudis de la tesi, la conductivitat hidràulica es va estimar utilitzant el mètode de Kozeny-Carman.

L'estudi a nivell de conca tenia com a objectiu determinar com les variables hidrològiques del sediment afectaven la biomassa microbiana al llarg del riu Tordera. Els nostres resultats a partir de les regressions múltiples mostraren que i) l'heterogeneïtat i els espais intersticials del sediment predeien de forma significativa la variació en el contingut de matèria orgànica (AFDW) al Juliol 2011 i Juliol 2012; ii) que una elevada heterogeneïtat del sediment afectava positivament l'acumulació de clorofil·la però només en condicions de cabal elevat (abril 2012); i que iii) la porositat i l'àrea disponible de superfície de sediment (la qual augmentava amb el % de fang, és a dir de partícules < 75 μm les quals inclouen el llim i l'argila) eren rellevants per a l'acumulació de bacteris, i això es va observar per als tres períodes hidrològics estudiats. Els bacteris també es van veure relacionats amb el contingut de matèria orgànica (AFDW). L'estudi suggereix que les propietats físiques del sediment afecten de forma diferencial la biomassa microbiana – sent els bacteris els més afectats –, i que aquest efecte pot variar en funció de les condicions hidrològiques.

A escala de tram i considerant el sediment superficial i hiporreic, no es van trobar diferències significatives en profunditat en el contingut de C, N, i P (o les seves relacions molars). Els resultats de les propietats físiques del sediment (conductivitat hidràulica i textura) mostraren que la conductivitat hidràulica (K) era significativament diferent entre alguns punts de mostreig. No es van observar diferències significatives en el percentatge de fang (llim + argila) entre punts i períodes de mostreig. El contingut de matèria orgànica era baix i només s'acumulava en alguns punts i profunditats. Les activitats microbianes eren possiblement regulades per la permeabilitat del sediment i l'acumulació de compostos orgànics en alguns punts, contribuint a les activitats de descomposició microbiana en les diferents profunditats del sediment (per exemple com s'observà per a les activitats leucina-aminopeptidasa i fosfatasa al juny i per la β-glucosidasa i fosfatasa al juliol). A més a més, les partícules de sediment podrien controlar els nutrients i el metabolisme microbià. En el sediment es va observar que el percentatge d'argila afavoria l'activitat β-glucosidasa i el contingut de P (correlació positiva). Al mateix temps, el % d'argila també es correlacionà amb l'activitat peptidasa, mentre que la densitat de bacteris s'associà amb la K. Per altra banda, els bacteris en l'aigua intersticial eren majors en condicions de major % de fang (especialment d'argila) i la seva interconnexió al sediment estava mitjançada per les capes poroses. Per altra banda, el contingut d'argila podria impactar negativament a la permeabilitat del llit del riu però en el nostre cas d'estudi el contingut era molt baix. Es va concloure que la conductivitat hidràulica a la zona hiporreica és un dels factors rellevants de la dinàmica biogeoquímica i els processos microbians al sediment hiporreic dels trams sorrencs de la baixa Tordera.

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1

General introduction

Relevance of riverbed and hyporheic sediments

Riverbed sediments are usually composed by different grain sizes (ranging from clay to gravel) are a relevant site for microbial colonization and biogeochemical processes. The hyporheic zone is the interface between flowing water and groundwater located below the streambed. In hyporheic zone, the key physical elements are stones, gravel, sand, silt and organic matter, together with complex, seasonally-variable patterns of interstitial flow (Fraser & Williams, 1997). It becomes necessary to provide practical procedures (i.e. sieving and hydrometer test) to measure distribution of particle sizes for a given sediment, and to estimate their physical properties. With those physical properties, we are able to link them to subsurface flow, as well as to the biogeochemical processes within any submerged sediments (made practically of liquid and solid phases; Avnimelech *et al.,* 2001).

The surface and hyporheic sediment can be studied at different spatial scales, the catchment scale, the reach scale and the sediment habitat scale (Boulton *et al.,* (1998); see Figure 1.1). At the catchment scale, upstream-downstream differences are expected due to changes in slope, flow velocity and transport of particles. At the reach scale, patchiness between upwelling and downwelling zones are crucial to determine flow direction in the subsurface and hyporheic sediment, determining the biogeochemical processes taking place. At the sediment habitat scale, the distribution of grain sizes and organic matter affect the development of a microbial biofilm which is highly responsible for the biogeochemical processes. Further, knowing the physical sediment characteristics plays a role specially in determining sediment permeability (i.e. hydraulic conductivity and porosity), sediment bulk density and hydraulic parameters within these three scales. The sediment texture is also important to water-holding capacity and the amount and nutrient-holding abilities in a given sediment. However, the hydraulic conductivity is one of the most important characteristics of water-bearing formations (Alyamani & Şen, 1993), and enhancing understanding exchange processes between river water and hyporheic layers.

Link of hydrology and sediment properties to biogeochemical processes

Fluvial systems transport sediments in suspension and in bed-load together with bacteria adhering to the transported particles (Fisher *et al.,* 2003). Bacterial activity in sediments deposited in river channels can control the ecosystem metabolism of rivers (Allan, 1995; Fischer & Pusch, 2001). In riverbed structures, bacteria colonizing sediment grains are apparently influenced by dynamics of bedforms due to overlying river flow; whereas water exchange between sediments and the overlying river flow is also accomplished by turnover of the sediment structures themselves. At high rates of turnover, the propagation of sediment structures is fast (Nikora *et al.,* 1997). Consequently, this evolution of bedforms causes changes in their hydraulic characteristics affecting the microbial accumulation within surface and subsurface layers. In contrary to that microbial concentration, increasing loads of fine sediments in the hyporheic substrates are considered as one of the major threats to stream ecosystems (Wood & Armitage, 1997; Bo *et al.,* 2007; Geist, 2011; Larsen *et al.,* 2011; Mueller *et al.,* 2013). For example, deposition of fine sediments often affects hyporheic zone functioning by reducing hydrological exchanges at the water-sediment interface and by increasing the organic matter content of surface sediments – these two factors usually occur concurrently to control biogeochemical processes in sediments (Navel *et al.,* 2012).

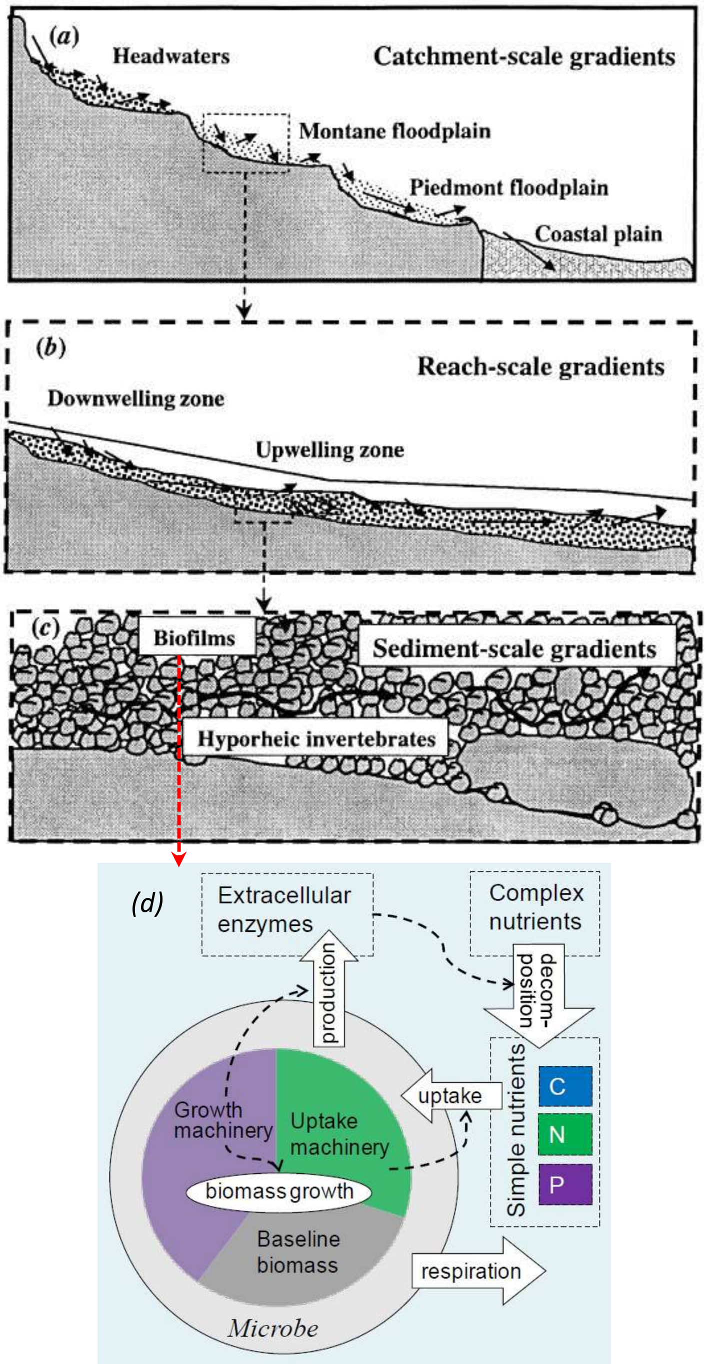


Figure 1.1*: Lateral diagrammatic view of the hyporheic zone (HZ) at three spatial scales (after Boulton et al., 1998). At the catchment scale (a), the hyporheic corridor concept predicts gradients in relative size of the HZ, hydrologic retention, and sediment size (Stanford & Ward, 1993). At the reach scale (b), upwelling and downwelling zones alternate, generating gradients in nutrients, dissolved gases, and subsurface fauna. At the sediment habitat scale (c), microbial and chemical processes occur on particle surfaces, creating microscale gradients. Arrows indicate water flow paths. (d) Microbial*

biomass consists of baseline biomass, uptake machinery and growth machinery (e.g. RNA). The biomass fraction of uptake and growth machinery determines the maximum capacity for uptake and production (biomass plus enzymes), respectively. The biomass fractions, each with a fixed C:N:P ratio, also determine the total biomass stoichiometry. Three types of extracellular enzymes are considered, defined by the element they target: C-, N-, and P-acquiring enzymes. The effect of each enzyme depends on its production rate, its efficiency, and the availability of complex resource (Franklin et al., 2011).

Interstitial water flow is supposed to influence the activity of microorganisms of the sedimentary biofilms (Fisher *et al.,* 2003). When interstitial flow is stagnant, these micro-organisms mainly depend on molecular diffusion of nutrients to their attachment sites. Under the conditions of significant interstitial flow, however, bacteria can make use of nutrients and substrates transported into the sediment interstices. Together with the development extracellular polymeric substances (EPS), which were found to be higher with early colonizing bacteria (Artigas *et al.,* 2012), the biofilms may envelop sediment grains changing their frictional characteristics and may even cause individual particles to conglomerate into larger structural elements (Or *et al.,* 2007).

River sediments are known as key habitats for organic matter cycling mainly due to microbial heterotrophs forming biofilms (Push *et al.,* 1998). Following Lock (1993), stream biofilms are complex communities of auto and heterotrophic microorganisms embedded in a polysaccharide matrix (bacteria, protozoans, fungi and algae), and have been long recognized as major sites of energy transduction with a vital role in nutrient trapping (Freeman & Lock, 1995; Pusch *et al.,* 1998). Further, biofilms can store organic compounds, retaining and transforming them in the presence of extracellular enzymes produced by the biofilm bacteria (Lock, 1993). In this thesis, we focused on bacteria which are relevant for sediment metabolism. It has been shown that the hydrolytic activity of hyporheic bacterial biofilms was relevant and considered the main mechanism linked to hydrological exchanges (Battin, 2000; Tonina & Buffington, 2009).

The relative activities of the functional classes of extracellular enzymes provide both a measure of nutrient availability and ecosystem metabolism (Hill *et al.,* 2012). Although it is recognized that microbial metabolism in sedimentary deposits depends on a continuous supply of nutrients and oxygen from the overlying river flow, the relationships between characteristics of microbial activity and characteristics of flow and the movable riverbed have rarely been examined (Fisher *et al.,* 2003). Nutrients and oxygen are normally transported from overlying flow into interstitial waters via turbulent flow (pumping) (Elliot & Brooks, 1997) and moveable sediments (turnover) (Elliot & Brooks, 1997; Packman & Bencala, 2000). Both pumping and turnover mechanisms favor the substrate available for bacterial activity. Stream benthic microbial communities are characterized not only by their microbial composition but also by their metabolism and role in the OM cycling within the stream ecosystem (Marxsen & Witzel, 1990; Romaní & Sabater, 2001; Fellows *et al.,* 2006). They may change depending on the availability of nutrients in their surrounding media. For example, microbial heterotrophs (bacteria and fungi) have the capacity to break down high molecular weight molecules into low molecular weight compounds using extracellular enzymes, and thereafter assimilate those small molecules through the cell membrane (Figure 1.1, d). Some extracellular enzymes are expressed constitutively by the microbes, but many more are induced only under specific circumstances (Arnosti, 2003). Enzymatic activities related to organic matter decomposition processes are in general stimulated by increasing the available organic substratum. Thus, peptidase activity will be stimulated by increasingly available proteins or peptides while polysaccharide degrading enzyme activities (among other, e.g. β-glucosidase) will be stimulated by increasingly available hemicellulose, cellulose and other polysaccharides. However, enzyme activities and the use of specific materials might be also modulated by nutrient imbalances of the microbial community (C:N:P molar ratios; Sterner & Elser, 2002). At the same time, the organic matter use and thus the expression or inhibition of a specific enzyme is affected by the availability and nutrient ratios of dissolved inorganic nutrients in stream water (Alvarez & Guerrero, 2000; Sala *et al.,* 2001; Romaní *et al.,* 2004).

Link of sediment physical properties to biogeochemical processes in Mediterranean stream hydrology

Streams are dynamic systems, especially in regions with variable hydrology such as the Mediterranean region (Gasith & Resh, 1999). Combined to these active processes, climate change represents an emerging problem for those ecosystems by increasing the frequency and duration of drought periods, with potentially important effects on fluvial biogeochemical processes (Zoppini & Marxsen, 2011). For example, the structure of benthic substrata changes with episodes of high and low water, so the respective contribution of the microbial community to organic matter cycling also varies (Artigas *et al.,* 2009). Flooding events can rapidly impact on stream biota through scouring and abrupt changes in streambed morphology (Holmes *et al.,* 1998). In the studies of Gibert *et al.* (1990) and Vervier *et al.* (1992), geomorphology and hydrology largely determine interactions between surface and interstitial habitats, and the permeability of the hyporheic zone in space and time. In general, it must be emphasized that hydrological exchange and mixing processes in the hyporheic zone are highly variable and may change on anything between daily and seasonal time scales (Brunke & Gonser, 1997).

For some instances, Mediterranean headwater streams are subjected to high variability of hydrological and physicochemical characteristics, which can strongly affect biofilm composition and functioning, and may determine changes in the biogeochemical cycles and energy fluxes (Acuña *et al.,* 2005; Romaní *et al.,* 2006; Artigas *et al.,* 2009). Such biogeochemical processes are strongly modified during droughts (Ylla *et al.,* 2010, 2011; von Schiller *et al.*, 2011; Vázquez *et al.,* 2011), which in the Mediterranean region mostly occur in summer, and usually extend for several weeks or months (Gasith & Resh, 1999; Butturini *et al.,* 2008). During droughts groundwater inflow is reduced, superficial water progressively disappears, and the stream becomes intermittent, separated first in isolated pools, and finally completely dries up (Sabater & Tockner, 2010). However, as a result of small-scale exchange processes, heterogeneous interstitial flow patterns establish gradients even without groundwater influence (Triska *et al.,* 1989; Meyer, 1990; Vervier *et al.,* 1992). Therefore, several literatures suggest the Mediterranean streams naturally cease to flow could serve as a template to understand better the biogeochemical and ecological implications of drought in more temperate regions (Timoner *et al.,* 2012; Arce, 2013).

Objectives of this thesis

The main objective of this thesis was to test whether differences in sediment physical characteristics (i.e. grain sizes and texture) generate differences in biofilm biomass and metabolic activity and nutrient content in the surface and subsurface layers. It was hypothesized that change of the sediment physical characteristics would affect biological, physical and chemical processes across the surface and subsurface layers; and that this change will be possibly modified by the dry-wet conditions which may happen through sediment depths in the studied Mediterranean river.

To this aim, the thesis is divided in three sections. In the first section (§ 3.1), we reviewed 11 formulae to calculate hydraulic conductivity by using sediment samples collected at the downstream of the Tordera river. In the second section (§ 3.2), we computed the surface sediment physical parameters from grain-size distribution data of 12 sites sampled at the catchment scale to study microbial accumulation within bed-stream layers. In the third section (§ 3.3), we studied the tempo-spatial changes in microbial metabolism and activity in the surface and subsurface of riverbed (at the reach-scale), which were subjected to the variation in hydrological conditions at downstream of the Tordera river. The specific objectives linked to each section are the following:

a) To evaluate several distinct empirical formulae to predict the permeability of river sediments from granulometry measurements (especially in sandy sediments such as those from the Tordera river as an example),
b) To assess the sediment physical characteristics (from particle size distribution), which could influence the microbial biomass of surface sediment (such as ash free dry-weigh (AFDW), Chlorophyll-*a* and bacteria accumulation) along the main course of the Tordera river, and
c) To assess the microbial processes in sediment depths and in a gradient of hydrological conditions at a reach-scale (located at downstream of the Tordera river).

2

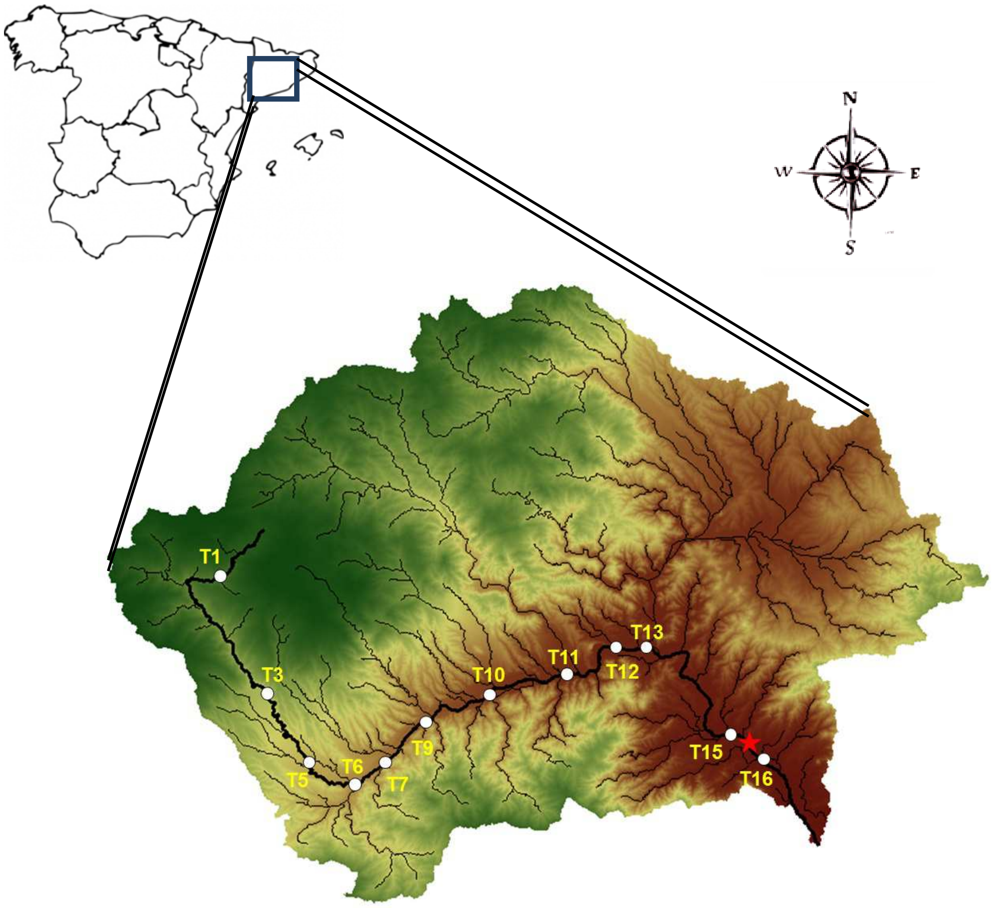
General methods

Study site

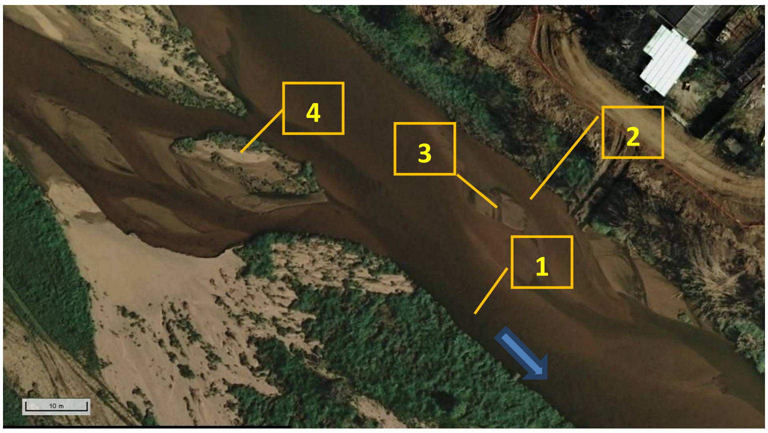
La Tordera river (865 km^2 watershed area) is a 3rd order stream located in the northeast of Catalonia, Spain. The river in its main stream is about 60 km length (Figure 2.1). Climate is Mediterranean sub-humid with severe droughts in summer. Mean annual rainfall ranges from 1000 mm on the mountains to 600 mm at the coast (Rovira & Batalla, 2006). With the exception of a small dam in headwaters, there are no reservoirs along its course. The 77% of the catchment is forested and the remaining 16% and 7% are crops (in the alluvial deposits) and urban-industrial areas respectively (Caille, 2009). Population density ranged from less that 100 (headwaters) to 20000 inhab/km^2 (urban zones).

Granulometry analysis

In this study, grain-size distribution tests were performed to study, especially, sediment physical characteristics. Granulometry was analyzed by the Standard Test Method for particle size analysis of soils following the Designation: ASTM D422 –63 (reapproved 1998). This test method covers the quantitative determination of the distribution of particle sizes in soils. Particle size analysis is the measurement of the proportion of the various sizes of primary soil particles as determined usually either by their capacities to pass through required sieves of various mesh size or by their rates of settling in water (Figure 2.2). The procedures for testing are as follows.



(a)



(b)

Figure 2.1*: The Tordera river and catchment area (a). T1 to T16 represent the sampling sites to examine the microbial colonization along its main course (see § 3.2). The (red)*

star represents the selected reach (b) (near to the Mediterranean Sea mouth) to analyze the sediment granulometry and the microbial processes across the dry-wet hyporheic boundary (§ 3.1 and 3.3). Arrow represents the flow direction.

APPARATUS

Balances – Sensitive to 0.01 g for weighing the material passing a No. 10 (2 mm) sieve, and a balance sensitive to 0.1% of the mass of the sample to be weighed for weighing the material retained on a No. 10 sieve.

Stirring apparatus – A mechanical stirring device consisting of an electric motor mounted to turn a shaft at a speed not less than 10,000 revolutions per minute without a load, with a replaceable stirring paddle made of metal, plastic or hard rubber and a dispersion cup.

Hydrometer – an ASTM 152-H hydrometer, conforming to the requirements of Specification E100, graduated to read in either specific gravity of the suspension or g per liter of suspension.

Thermometer – Accurate to 0.5°C.

Sedimentation cylinder – A glass cylinder essentially 457 mm in height and 63.5 mm in diameter, and marked for a volume of 1000 mL. The inside diameter shall be such that the 1000 mL mark is 36±2 cm from the bottom on the inside.

Sieves – A full set of sieves of square-mesh woven-wire cloth conforming to the requirements of Specification E11.

Water bath or constant-temperature room – A water bath or constant-temperature room for maintaining the soil suspension at a constant temperature during the hydrometer analysis. A satisfactory water tank is an insulated tank that maintains the temperature of the suspension at a convenient constant temperature at or near 20°C. In cases where the work is performed in a room at an automatically controlled constant-temperature, the water bath is not necessary. Other methods that can provide the required temperature control are acceptable.

Oven – A thermostatically controlled an oven capable of maintaining a temperature of 110±5^{o}C for drying the sieve analysis samples.

Beaker – A beaker of 250 mL capacity or a pint mason jar.

Timing device – A watch or clock with a second hand.

SAMPLE

- The field sampling soils, coarse and fine aggregate mixtures, shall be the same as for coarse aggregate in 7.4 as described in ASTM Practice C136. For example, the nominal maximum size of particles 9.5 mm, the minimum test sample size is being considered for 1000 g.
- The soil samples as received from the field shall be dried thoroughly in air at room temperature or in an oven at the temperature not exceeding 60^{o}C. Spread the field samples on trays or on plastic sheeting. Thoroughly mix and roll the samples to break up clods. A representative sample is obtained after splitting the sample on the No. 10 (2 mm) sieve.
- The mass of air-dried (or a drying oven) soil selected for the purpose of tests, as prescribed in ASTM Practice D421, shall be sufficient to yield quantities for mechanical analysis. The size of portion passing the No. 10 sieve shall be approximately 115 g for sandy soil and approximately 65 g for silt and clay soils. The size of the portion retained on the No. 10 sieve shall depend on the maximum size of particles according to the following schedule:

Nominal diameter of largest particles, mm	Approximate minimum mass of portion, g
9.5	500*
19	1000

** According to ASTM Method C136, analysis of results of testing of 300 g and 500 g test samples from Aggregate Proficiency Test Samples 99 and 100 (Sample 99 and 100 were essentially identical) indicates only minor differences due to test sample size.*

PROCEDURE

- *Hygroscopic moisture*

A hygroscopic moisture sample is taken when preparing the soil for testing. Approximately a 10-15 g sample portion of the fraction passing the No. 10 (2 mm) sieve is used for the determination of the hygroscopic moisture. Weigh this sample to the nearest 0.01 g, dry to a constant weight in an oven at a temperature of 110±5°C. Weigh to nearest 0.01 g and record weight.

- *Dispersion of soil sample*

a) When the soil is mostly of the clay and silt sizes, weigh out a sample of air-dry soil of approximately 50 g. When the soil is mostly sand the sample should be approximately 100 g.

b) Place the sample in the beaker of 250 mL capacity and cover with 125 mL of sodium hexametaphosphate solution prepared using distilled or demineralized water at the rate of 40 g/L. Solutions should be prepared frequently (at least once a month) or adjusted to a pH of 8 or 9 by means of adding sodium carbonate (approximately 5 g after AASHTO Method T88). Bottles containing solutions should have the date of preparation marked on them.

c) After being placed in the beaker, stir until the sample is thoroughly wetted. Allow to soak for at least 16 hours.

d) After the soaking period, the contents of the beaker shall be washed into a dispersion cup. During this process some soil may stick to the side of beaker. Using the plastic squeeze bottle filled with distilled water, wash all of the remaining soil in the beaker into the dispersion cup (or mixer cup). Distilled or demineralized water shall be added until the cup is little more than half full and the contents dispersed for a period of 1 minute with the mechanical stirring apparatus.

- *Hydrometer test*

e) Before at the end of soil soaking period. Take a 1000 mL graduated cylinder and add 875 mL of distilled water *plus 125 mL* of deflocculating agent (the solution of that one prepared at 40 g/L in step b) to it. Mix the solution well. Put the cylinder in a constant-temperature bath set at $20^{o}C$[1]. Small variations of temperature do not introduce differences that are of practical significance and do not prevent the use of corrections derived as prescribed. When temperature of the bath or room attains the approved temperature, then put the 152-H hydrometer in the cylinder. Record the reading (**at the top of the meniscus should be read**). This is the zero correction F_z, which can be positive or negative. Also, observe the meniscus correction F_m. For the 151-H hydrometer the composite correction is the difference between this reading and one; for the 152-H hydrometer it is the difference

[1] The basic temperature for hydrometer test is $20^{o}C$ and variations in temperature from this standard temperature produce inaccuracies in the actual hydrometer readings. In the case of actual temperature of the test may not be $20^{o}C$, the temperature correction (F_T) may be approximated as $F_T = -4.85+0.25 \cdot T$, where F_T is the temperature correction to observed reading (can be either positive or negative); T is temperature of the test between 15 and $28^{o}C$.

between the reading and zero.

f) Immediately after dispersion (from Step d), transfer the soil-water slurry to the second glass sedimentation cylinder, and add distilled or demineralized water until the total volume is 1000 mL. Make sure that all of the soil solids are washed out of the dispersion cup (or mixer cup). Use the palm of the hand over the open end of the cylinder or secure the rubber stopper on the top of the cylinder, mix the soil-water well by turning the cylinder upside down approximately 60 turns for a period of 1 minute to complete the agitation of the slurry. Count the turn upside down and back as two turns.

g) Put the second cylinder into the constant-temperature compartment in a convenient location next to the cylinder described in Step e. Carefully insert the hydrometer, about 20 to 25 seconds before reading is due to approximately the depth it will have when the reading is taken, into the cylinder containing the soil-water suspension. The hydrometer should float freely and not touch the wall of the sedimentation cylinder. Take hydrometer readings at the following intervals of time (measure form the beginning of sedimentation), or as many as may be needed, depending on the sample or the specification for the material under test at 2, 5, 15, 30, 60, 250 and 1440 minutes.

h) After each reading[2], take the temperature of the suspension by inserting the thermometer into the suspension.

- *Sieve analysis*

i) After taking the final hydrometer reading, transfer the suspension to a No. 200 (75 μm) sieve and wash with tap water until the wash water is clear. Transfer the material on the No. 200 sieve to a suitable container, dry in an oven at 110±5°C and make a sieve analysis of the proportion retained. The set of sieves is No. 10 (2 mm), No.16 (1.18 mm), No. 30 (600 μm), No. 50 (300 μm), No. 100 (150 μm) and No. 200 (75 μm) or as many as may be needed.

[2] It is important to remove the hydrometer immediately after each reading and place it into the cylinder next to it (that one described in Step e). Reading shall be taken at the top of the meniscus formed by the suspension around the stem, since it is not possible to secure the readings at the bottom of the meniscus.

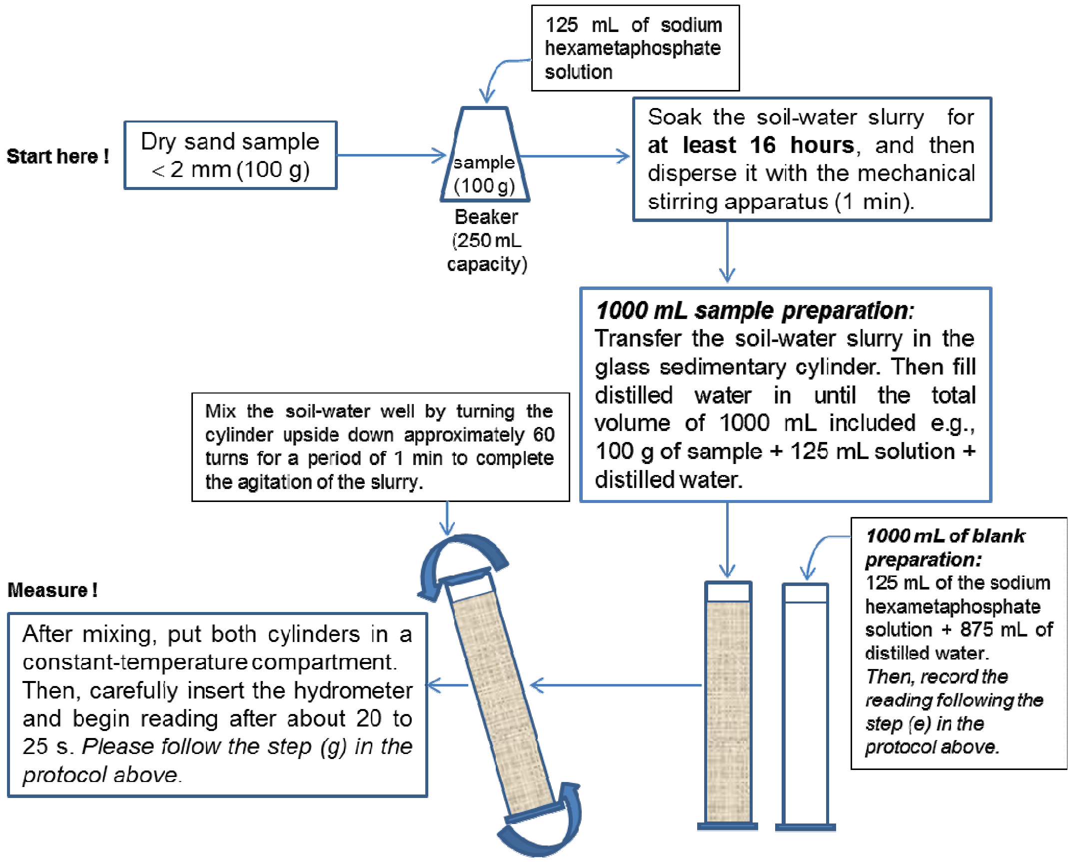


Figure 2.2*: Summary of main steps of the hydrometer test procedure (steps a to h). See an ASTM 152-H hydrometer plotting in Figure 2.3.*

j) For sieve analysis of portion retained on No. 10 (2 mm) sieve, the sieving is conducted as following:

- Place the material retained on the No. 10 (2 mm) sieve in a pan, cover with water, and allow to soak until the particle aggregations become soft. After soaking, wash the material on a No. 10 sieve in the following manner: place an empty No. 10 sieve on a bottom of clean pan and pour the water from the soaked sample into the sieve. Add sufficient water to bring the level approximately 12.7 mm above the mesh of the sieve. Transfer the soaked material to the sieve in increments not exceeding 0.45 kg, stirring each increment with fingers while agitating the sieve up and down. Crumble or mash any lumps that have not slaked, using the thumb fingers. Raise the sieve above the water in the pan and complete the washing operation using a small amount of clean water. Transfer the washed material on the sieve to a clean pan before placing another increment of soaked material on the sieve. Dry the material retained on the No. 10 sieve at a temperature of 110±5°C for use in the particle-size analysis.

- Weigh the portion of test sample approximately 300 g (or 500 g in the case of having sufficient material). Separate this portion retained on the No. 10 (2 mm) sieve into a series of fraction using the 75 mm, 50 mm, 37.5 mm, 25 mm, 19 mm, 9.5 mm, No. 4 (4.75 mm), and No. 10 sieves, or as many as desired. The nest is shaken for 10 minutes[3].

CALCULATIONS

- *Hygroscopic moisture correction factor*

The hydroscopic moisture correction factor is the ratio between the mass of the oven-dried sample and the air-dry mass before drying. It is a number less than one, except when there is no hygroscopic moisture.

- *Percentages of soil in suspension*

1) Calculate the oven-dry mass of soil, passing the No. 10 (2 mm), used in the hydrometer analysis by multiplying the air-dry mass by the hygroscopic moisture correction factor.
2) The percentage of soil remaining in suspension at the level at which the 152-H hydrometer is measuring the density of the suspension may be calculated as follows:

$$P = (R_{cp}.\alpha /W) \times 100 \qquad \text{(eq. 1)}$$

Where: P, percentage of soil remaining in suspension at the level at which the hydrometer measures the density of the suspension;

R_{cp}, hydrometer reading with composite correction applied, for the calculation of

[3] Conduct the sieving operation by means of a lateral and vertical motion of the sieve, accompanied by a jarring action in order to keep the sample moving continuously over the surface of the sieve. In no case turn or manipulate fragments in the sample through the sieve by hand. Continue sieving until not more than 1% by mass of the residue on a sieve passes that sieve during 1 minute of sieving. When mechanical sieving is used, test the thoroughness of sieving by using the hand method of sieving as described above or limit the quantity of material on a given sieve so that all particles have opportunity to reach sieve openings a number of times during the sieving operation. For sieves with openings smaller than No. 4 (4.75 mm), the quantity retained on any sieve at the completion of the sieving operation shall not exceed 7 kg/m^2 of sieving surface area. For sieves with openings No. 4 or larger, the quantity remained in kg shall not exceed the product of [2.5 $\times$ (sieve opening, mm) $\times$ (effective sieving area, m^2)].

percent finer, as R_{cp}= R + F_T – F_z where R is the observed hydrometer readings corresponding to times of measurement;

α, correction factors, given in Table 2.1, are to be applied to the reading of 152-H hydrometer. The values shown on the scale are computed using a specific gravity of 2.65;

W, oven-dry mass of soil in a total test sample represented by mass of soil dispersed.

- *Diameter of soil particles*

The diameter of a particle (finer than 0.075 mm) corresponding to the percentage indicated by a given hydrometer reading shall be calculated according to Stokes' law, on the basis that a particle of this diameter was at the surface of the suspension at the beginning of sedimentation and had settled to the level at which the hydrometer is measuring the density of the suspension.

$$v = [(\gamma_s - \gamma_w)/18\eta]\cdot D^2 \qquad \text{(eq. 2)}$$

Where v, is terminal velocity (cm/s) of fall of a sphere through a liquid equal to L/*t* – L is effective depth (cm) and *t* is time (s); γ_s, is specific weight of soil solids (g/cm^3); γ_w, is unit weight of water (g/cm^3); η is viscosity of water (g.s/cm^2); D is diameter of soil particle (cm).

In the test procedure described here, the ASTM 152-H type hydrometer will be used. If a hydrometer is suspended in water in which soil is dispersed (Figure 2.3), it will measure the specific gravity of the soil-water suspension at the depth L.

$$\frac{L\,(cm)}{t\,(min)\times 60} = \frac{\gamma_s\,(g/cm^3)\text{-}\gamma_w\,(g/cm^3)}{18\eta\,(g.s/cm^2)}\left[\frac{D\,(mm)}{10}\right]^2 \qquad \text{(eq. 3)}$$

$$D\,(mm) = \sqrt{\frac{1800\eta}{60\,(\gamma_s - \gamma_w)}}\sqrt{\frac{L}{t}} \qquad \text{(eq. 4)}$$

$$\text{or } D\,(mm) = A\sqrt{\frac{L}{t}} \qquad \text{(eq. 5)}$$

In which, A = $\sqrt{\frac{30\eta}{\gamma_s - \gamma_w}}$; L is expressed as (cm) and t is (min).

For actual calculation purposes, we also need to know the values of A. An example of this calculation is γ_s = G_s γ_w, in which G_s is the specific gravity of soil solids (if not known, assume 2.65 for the calculation) and γ_w is assuming equal to 1 g/cm^3. The factor A is determined as follows.

$$A = \sqrt{\frac{30\eta}{(G_s - 1)\gamma_w}} \quad \text{(eq. 6)}$$

Where: *A*, constant depending on the temperature of the suspension and the specific gravity of the soil particles. Values of *A* for a range of temperatures and specific gravities are given in Table 2.3. The value of *A* does not change for a series of readings constituting a test, while values of L and *t* do vary.

Table 2.1*: Values of correction factor α for the different specific gravities of soil particles.*

Specific gravity	Correction factor[4]
2.95	0.94
2.90	0.95
2.85	0.96
2.80	0.97
2.75	0.98
2.70	0.99
2.65	1.00
2.60	1.01
2.55	1.02
2.50	1.04
2.45	1.05

[4]*For use in equation for percentage of soil remaining in suspension when using ASTM 152-H Hydrometer.*

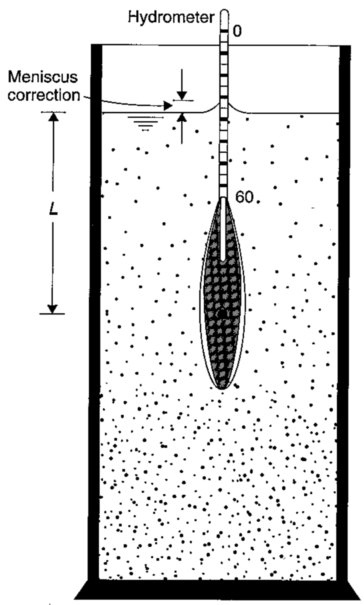


Figure 2.3*: ASTM 152-H hydrometer suspended in water in which soil is dispersed.*

***Table 2.2**: Variations of L with the hydrometer* readings.*

Actual Hydrometer Reading	Effective Depth L, (cm)	Actual Hydrometer Reading	Effective Depth L, (cm)
0	16.3	31	11.2
1	16.1	32	11.0
2	16.0	33	10.9
3	15.8	34	10.7
4	15.6	35	10.6
5	15.5	36	10.4
6	15.3	37	10.2
7	15.1	38	10.1
8	15.0	39	9.9
9	14.8	40	9.7
10	14.7	41	9.6
11	14.5	42	9.4
12	14.3	43	9.2
13	14.2	44	9.1
14	14.0	45	8.9
15	13.8	46	8.8
16	13.7	47	8.6
17	13.5	48	8.4
18	13.3	49	8.3
19	13.2	50	8.1
20	13.0	51	7.9
21	12.9	52	7.8
22	12.7	53	7.6
23	12.5	54	7.4
24	12.4	55	7.3
25	12.2	56	7.1
26	12.0	57	6.9
27	11.9	58	6.8
28	11.7	59	6.6
29	11.5	60	6.5
30	11.4		

* ASTM 152-H Hydrometer

- *Plotting graphs*

a) When the hydrometer analysis is performed, a graph of the test results shall be made, plotting the diameters of the particles on a logarithmic scale as the abscissa and percentages smaller than the corresponding diameters to an arithmetic scale as the ordinate. To complete the curve, plot the sieve size versus the percent passing from the sieve analysis.

b) A soil classification shall be made by determining the percentages of clay (below 2 µm), silt (75 µm to 2 µm) and sand (2 mm to 75 µm) present in the sample. These percentages are grouped into soil texture "classes", which have been organized into a "soil textural triangle". Use the soil texture triangle to estimate the soil texture classification (Figure 2.4).

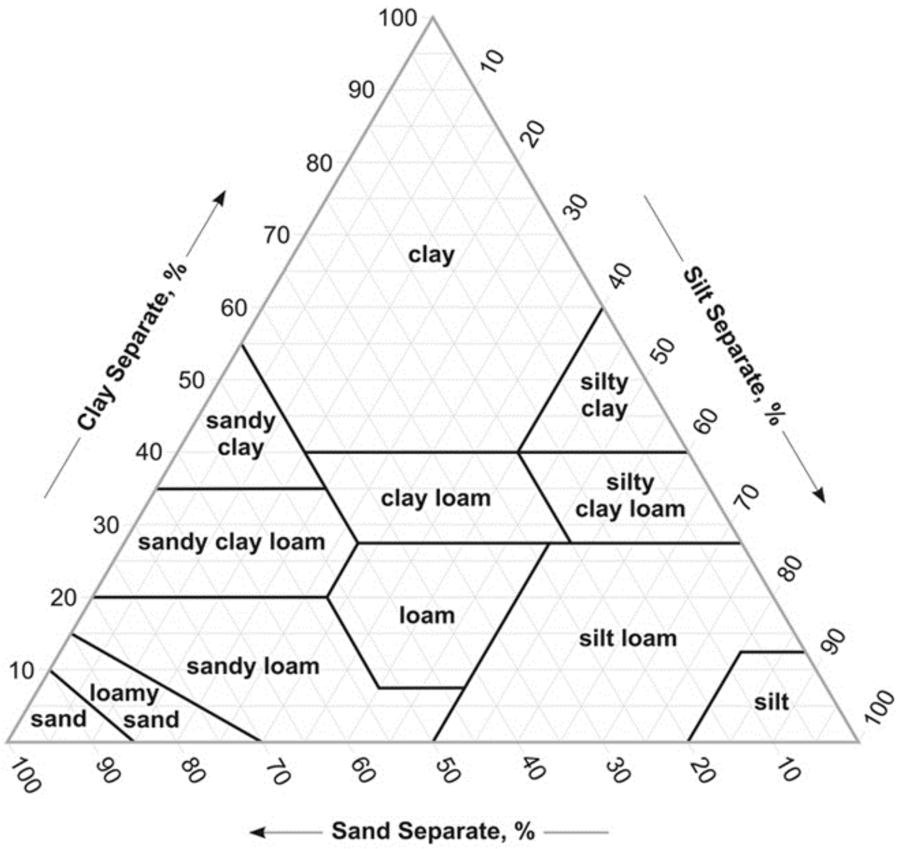


***Figure 2.4**: USDA Soil Texture Triangle. Diagram showing 12 of the main soil classes (Source: United States Department of Agriculture).*

PHYSICAL PARAMETERS OF SEDIMENT

Grain size distribution allows us to obtain several relevant physical characteristics of the sediment as presented in Table 2.4. The specific calculations to measure granulometry and the related physical properties are described in each chapter.

Sediment microbial biomass

Organic matter (or ash free dry-weight, AFDW) – Dry weight (DW) was estimated after drying the fresh material (1 cm^3 sediment samples) at 60^{o}C until constant weight (at least 4-6 days). The dried material was burned in a muffle furnace at 450^{o}C for 4 hours. Samples were then kept in a desiccator while cooling down to room temperature, and then re-weighed in order to obtain the ash free dry weight (AFDW) as a measure of organic matter (OM) content. OM content was expressed as (mg AFDW / g DW).

Table 2.3*: Values of A for use in equation for computing diameter of particle in hydrometer analysis.*

Temp. (^{o}C)	Specific gravity of soil particles						
	2.50	2.55	2.60	**2.65**	2.70	2.75	2.80
16	0.01505	0.01481	0.01457	**0.01435**	0.01414	0.01394	0.01374
17	0.01486	0.01462	0.01439	**0.01417**	0.01396	0.01376	0.01356
18	0.01467	0.01443	0.01421	**0.01399**	0.01378	0.01359	0.01339
19	0.01449	0.01425	0.01403	**0.01382**	0.01361	0.01342	0.01323
20	0.01431	0.01408	0.01386	**0.01365**	0.01344	0.01325	0.01307
21	0.01414	0.01391	0.01369	**0.01348**	0.01328	0.01309	0.01291
22	0.01397	0.01374	0.01353	**0.01332**	0.01312	0.01294	0.01276
23	0.01381	0.01358	0.01337	**0.01317**	0.01297	0.01279	0.01261
24	0.01365	0.01342	0.01321	**0.01301**	0.01282	0.01264	0.01246
25	0.01349	0.01327	0.01306	**0.01286**	0.01267	0.01249	0.01232
26	0.01334	0.01312	0.01291	**0.01272**	0.01253	0.01235	0.01218
27	0.01319	0.01297	0.01277	**0.01258**	0.01239	0.01221	0.01204
28	0.01304	0.01283	0.01264	**0.01244**	0.01255	0.01208	0.01191
29	0.01290	0.01269	0.01249	**0.01230**	0.01212	0.01195	0.01178
30	0.01276	0.01256	0.01236	**0.01217**	0.01199	0.01182	0.01165

The ASTM 152-H hydrometer is calibrated up to a reading of 60 g/L at a temperature of 20^{o}C for soil particles having the specific gravity of 2.65.

Chlorophyll-a – Chl *a* was extracted from the sediments with 90% acetone for 12 hours at 4^{o}C in the dark. Then, to ensure complete extraction, sediment samples were sonicated for 1 min two times in a sonication bath (Selecta, 40 W and 40 kHz). 10 mL of extract were passed through 1.4 µm filters (GF/C Whatman) and Chl *a* concentration was further determined using a spectrophotometer (Shimadzu V-1800, Kyoto, Japan) following the method described in Jeffrey and Humphrey (1975). Results were expressed as µg Chl *a* per g DW of sediment.

Bacterial density – Each sample (1 cm^3 of sediment and 10 mL of water) was sonicated for 60s (Selecta, 40W and 40 kHz) and homogenized. Then, 1 mL was collected and diluted with 9 mL of solution “B” (7.54 g NaCl; 0.99 g Na_2HPO_4; 0.36 g NaH_2PO_4; 50 mL of formaldehyde 37% (pH 7); 1 g $Na_4P_2O_7 \cdot 10H_2O$ 99% at 0.1% final concentration; 5 mL of Tween 20 detergent at 0.5% final concentration). This solution was previously filtered through 0.2 µm filter before mixing with the sample. Solution "B" helps the sediment bacteria to disperse and avoid aggregation between bacterial cells. We agitated the diluted samples with a vortex, and then placed them in a shaker during 30 min at 150 rpm in dark and at room temperature. After shaking, we kept the samples in the fridge at 4^{o}C during 10 min. Next, samples were sonicated 60s and mixed; and then 1 mL was placed in a clean autoclaved 2 mL Eppendorf. One mL of Nycodenz (optiprep, density gradient medium) was added later to each extracted sample by a syringe at the bottom of the 2 mL Eppendorf, following Fazi *et al.* (2008). Then we placed the samples in the centrifuge carefully (with any further mixing) (MicroCL 17R). The samples were centrifuged at 4^{o}C during 90 min at 14000 rpm, and then we collected very carefully ~ 2 mL of supernatant by separating a pellet at the bottom. Finally, we kept the samples ready for measuring in new autoclaved Eppendorf 2 mL. Next step, we diluted the samples 1:2 before adding 5 µL of stain at the final 500 µM concentration (Syto 13 with size 250 µL, catalog number S-7575) into each sample, and then kept all samples in a dark condition during 15 min. At the final step, we added 5 µL of bead solution (10 µL of 10^{6} beads/mL, FISHER 1.0 µM). We measured the samples with the flow cytometer (FACS calibur). Results from the cytometer were obtained as cells per mL and transformed to cells per sediment dry weight (DW) used for each subsample and after considering all dilution steps.

Extracellular enzyme activities and water chemistry

Extracellular enzyme activities and water chemistry were only included in § 3.3, thus the analysis is described in that section.

__Table 2.4__: Summary of some relevant sediment physical parameters.

Parameter	Description
Effective diameter (d_x)	d_x represents a grain diameter for which x% of the sample will be finer than it. In other words, x% of the sample by weight is smaller than diameter d_x.
Coefficient of uniformity (C_u)	A coefficient related to the size distribution of a granular material, such as sand; obtained by dividing one size of grain (60% of the grains are smaller than this size, by weight) by a second size (10% of the grains are smaller than this size, by weight).
Porosity (n)	Porosity or void fraction is a measure of the void (i.e. empty) spaces in a material, and is a fraction of the volume of voids over the total volume, between 0 and 1, or as a percentage between 0 and 100%.
Hydraulic conductivity (K)	Hydraulic conductivity describes the ease with which a fluid (usually water) can move through pore spaces or fractures. It depends on the intrinsic permeability of the material, the degree of saturation, and on the density and viscosity of the fluid.
(Dry) bulk density (BD)	It is defined as the mass of many particles of the material divided by the total volume they occupy.
Void ratio (e)	The ratio of the volume of the void space to the volume of the solid particles.
Sediment heterogeneity	It is defined by Li and Reynolds (1995) as ‘‘the complexity and/or variability of a system property in space and/or time’’
Coefficient of sorting (S_o)	Grain-size homogeneity of the sample (heterogeneity > 1) (Rempel *et al.,* 2000)
mud (silt and clay)	A mixture of water and some combination of silt and clay. They usually form after rainfall or near water sources.

3

Results

3.1

Limits and potentiality of eleven empirical hydraulic conductivity methods: a technical description and a critical review

3.1.1 BACKGROUND AND AIMS

In general, the hydraulic conductivity represents the ability of a porous medium to transmit water through its interconnected voids (Alyamani & Şen, 1993). Hydrologists have been concerned with the determination of relationships between hydraulic conductivity and grain-size distribution parameters since the work by Hazen (1893). The grain-size distribution (sorting) analysis has been in turn of interest to geologists since its introduction by Krumbein (1934), because the grain-size is the most fundamental property of sediment particles, affecting their entrainment, transport and deposition (Blott & Pye, 2001). Relating hydraulic conductivity to grain-size distribution of soil porous media relies on the statistical parameters such as effective diameter, geometric mean, median, and standard deviation. Based on these variables, permeability (i.e. hydraulic conductivity and porosity) of a sediment can be estimated from grain-size distribution curves using some empirical approaches. Alternatively, the permeability can be determined in the laboratory using permeameters, as well as by classical analytical methods involving pump tests *in situ*. However, accurate estimation of the permeability in the field environment is limited by the lack of precise knowledge of aquifer geometry and hydraulic boundaries (Uma *et al.,* 1989). Laboratory tests, on the other hand, can be problematic in the sense of obtaining representative samples and they need, very often, long testing times (Odong, 2007). Most importantly, since information about the textural properties of soils is more easily obtained, a potential alternative for estimating hydraulic conductivity of soils is from grain-size distribution. In hydromechanics, it would be more useful to characterize the diameters of pores rather than those of the grains, but the pore size distribution is very difficult to determine and thus the approximation of hydraulic properties are mostly based on the easy-to-measure grain size distribution as a substitute (Cirpka, 2003). Also, in other fields of research such as chemistry and engineering, grain-size distribution, particle shape (Tickell *et al.,* 1938), and particle packing (Furnas, 1931; Graton *et al.*, 1935) have been indicated to explain variations in porosity.

It has become increasingly important to being able to accurately estimate the hydraulic conductivity of unlithified sediments for different engineer applications, such as natural filtration projects (Rosas *et al.,* 2014) or the use of soil columns to assess the removal of pathogens, algae, and trace organic contaminants (Lewis & Sjöstrom, 2010). At the same time, Alyamani and Şen (1993) define the hydraulic conductivity as one of the most important characteristics of water-bearing formation since its magnitude, pattern and variability significantly influence the ground-water flow patterns and contaminants dissolved in the water through the soils (Salarashayeri & Siosemarde, 2012). Furthermore, streambed hydraulic conductivity is a primary factor controlling the efficiency of collector wells (Zhang *et al.,* 2011), and is an important parameter in modelling the surface and ground water mixing in the hyporheic zones (Lautz & Siegel, 2006).

The objectives of this study were to (1) apply 11 empirical relationships to calculate hydraulic conductivity of unconsolidated river sediments from grain size distribution, and (2) compare and select a suitable empirical formula among them for further estimating the hydraulic conductivity of hyporheic sediments, especially in our case study of the Tordera river. For this purpose we measured sediment grain-size distribution from 36 sand sediment samples and calculated sediment physical characteristics such as texture, the effective diameter, the coefficient of uniformity, and the defined porosity. Based on these sediment physical characteristics, we calculated hydraulic conductivity by applying 11 different mathematical approaches. In addition, we reviewed the limits of the applicability for each formula, and matched the hydraulic conductivity (K) with the sorting for all the sediment samples using the inclusive standard deviation proposed by Krumbein and Monk (1943).

3.1.2 METHODS

Sediment collection

The fieldwork of this study was conducted at the downstream reach of the Tordera river (northeast of Spain, see Figure 2.1, General methods). Three sampling campaigns were performed (December 2012, June and July 2013). On each sampling campaign, samples from four different sites were collected by using a Multisampler (E-365-04.02.SA) to obtain sediment core samples from surface to 60 cm depth. For each site, the sediment was divided in three fractions (0-5, 20-30 and 50-60 cm depths). The sediment was dried (105^{o}C, for 24 hours) and particle size distribution was analyzed.

Sediment particle size analysis

The particle size analysis of the collected sediment samples was performed following the results of sieving and hydrometer tests through the ASTM method D422-63 (reapproved 1998, see the details in General methods). This method covers the quantitative determination of the distribution of particle sizes in soils. In our sediment samples, there were very few particle sizes $>$ 9.5 mm found at each depth of all sites. A representative sample of the sediments (about 300 g) was run through a set of sieves

(9.5 mm, 4.75 mm, 3 mm, 2 mm, 1.18 mm, 600 μm, 300 μm, 150 μm and 75 μm) to break the sample subset into size classes by sieve testing (for the coarser fractions). The finer size fractions (less than 75 μm diameter) were determined by sedimentation process using a hydrometer. In this case, about 100 g of sample < 2 mm was used.

For each sediment sample, several parameters were calculated from grain-size distribution results by mediating the effective diameters and phi sizes read at different percentages; those parameters were geometric mean grain diameter (GM_{ξ}), inclusive phi standard deviation (or sorting, $\sigma_{ø}$), coefficient of uniformity (C_u), coefficient of curvature (C_c), defined porosity (*n*) and hydraulic conductivity following the empirical formulae given below.

Empirical determination of hydraulic conductivity

The hydraulic conductivity (K) of unconsolidated materials has been related empirically to grain-size distribution by a number of investigators (Hazen, 1893; Krumbein & Monk, 1943; Harleman *et al.,* 1963; Masch & Denny, 1966; Wiebenga *et al.,* 1970). In this type of estimation, the obtained K values are non-directional (not linked to flow path directions) due to the procedure of complete mixing during the process of grain-size analysis (Lu *et al.,* 2012). In our study, a series of 11 empirical methods were used to estimate the K values from the grain-size distribution; nine methods are defined in Table 3.1 while two further methods are described as follows.

Krumbein and Monk (1943) proposed an empirical equation whose formula is nearly similar to the set of formulas given in Table 3.1. They used a statistical approach for the determination of the hydraulic conductivity (K) using a transformation of the grain-size distribution to a logarithmic frequency distribution and by incorporating various moments into an empirical equation (Rosas *et al.,* 2014). Their equation was developed empirically using very well sorted sediment samples ranging from -0.75 to 1.25 phi in mean grain-size, and with standard deviations ranging from 0.04 to 0.80 phi. In this case, the calculation of the intrinsic permeability (k) from the grain-size analysis is as follows:

$$k = 760\ (GM_{\xi})^2 \cdot \exp^{-1.31 \cdot \sigma_{ø}} \qquad \text{(eq. 1)}$$

Where *k*, intrinsic permeability in darcy – named after Henry Darcy (1856); GM_{ξ}, geometric mean grain diameter (mm); σ_{ϕ}, the standard deviation of diameter in phi scale. The geometric (graphic) mean is based on three points at specific % coarser particles (by weight) and is equal to $(\phi_{16}+\phi_{50}+\phi_{84})/3$. The inclusive (graphic) standard deviation, whose formula includes 90% of the distribution that is calculated as $(\phi_{84}-\phi_{16})/4 + (\phi_{95}-\phi_{5})/6.6$, and is the best overall measure of sediment sorting. In order to facilitate graphical presentation and statistical manipulation of grain-size frequency data, Krumbein (1934) further proposed that grade scale boundaries should be logarithmically transformed into phi (ø) values, using the expression $\phi=-\log_2(d)$, where d is the grain diameter (mm). Distributions using these scales are termed “log-normal”, and are conventionally used by sedimentologists (Visher, 1969; Middleton, 1976).

Table 3.1*: Formulas and applicable domains of empirical grain-size analysis methods*[a] *to determinate the hydraulic conductivity (K) in cm/s.*

Method	Function of porosity f(*n*)	Effective diameter (d_x)	Coefficient (β)	Domain of applicability
Beyer	1	d_{10}	$6\times10^{-4}\times\log(500/C_u)$	0.06 mm < d_x < 0.6 mm, 1 < C_u < 20
Hazen-original	$1+10\cdot(n-0.26)$	d_{10}	6×10^{-4}	0.1 mm < d_x < 3 mm, C_u < 5
Harleman	1	d_{10}	6.54×10^{-4}	N/A
Kozeny	$n^3/(1-n)^2$	d_{10}	1/180	Silts, sands, and gravelly sand; d_x < 3 mm
Kozeny-Carman	$n^3/(1-n)^2$	d_{10}	8.3×10^{-3}	Large-grain sands
Sauerbrei (from Vukovic & Soro, 1992)	$n^3/(1-n)^2$	d_{17}	3.75×10^{-3}	Sand and sandy clay
Slichter	$n^{3.287}$	d_{10}	1×10^{-2}	0.01 mm < d_x < 5 mm
Terzaghi	$\left(\frac{n-0.13}{\sqrt[3]{1-n}}\right)^2$	d_{10}	(between 6.1×10^{-3} & 10.7×10^{-3})	Large-grain sands
USBR (from Vukovic & Soro, 1992)	1	d_{20}	$4.8\times10^{-4}\times(d_{20})^{0.3}$	Medium-grain sands, C_u < 5

[a] *The general empirical formula takes the form of* $K=(g/\nu)\cdot\beta\cdot f(n)\cdot d_x^2$ *in which* ν *is kinematic viscosity set to* 1.002×10^{-2} cm^2/s *at* 20^oC *when g is the acceleration due to gravity equal to 980* cm/s^2. d_x *(cm), the value (as originally read in mm) is defined as the effective grain diameter with x% cumulative weight of the sample determined by*

the grain-size distribution curves (Lu et al., 2012). Additionally, n is porosity derived from the empirical relationship with the coefficient of uniformity (Vukovic & Soro, 1992): $n=0.255\cdot(1+0.83^{C_u})$ where C_u is the coefficient of uniformity and is defined as $C_u=d_{60}/d_{10}$ (Holtz & Kovacs, 1981). d_{60} and d_{10} represent the grain diameter (mm) for which, 60% and 10% of the sample respectively, are finer than, which are readily obtained from the particle size distribution curve (Abdullahi, 2013). USBR stands for U.S Bureau of Reclamation.

The Krumbein and Monk's permeability (*k*), in darcy, can be converted into the hydraulic conductivity (K), in cm/s, using the relation:

$$K = k\cdot(g\cdot\rho/\mu) \qquad \text{(eq. 2)}$$

Where *k*, intrinsic permeability, in units of cm^2, converted from equation (1) with the conversion factor which is equal to 9.87×10^{-9} cm^2/ darcy; ρ, density of water set to 0.9982 g/cm^3 at 20°C; g, acceleration of gravity is equal to 980 cm/s^2; μ, dynamic viscosity of water set to 0.01 g/cm.s at 20°C.

Another alternative procedure to estimate the hydraulic conductivity (K) from the grain-size analysis was that given by Alyamani and Şen (1993). In this case, the proposed formula is clearly distinct to those listed in Table 3.1 and Eq. 2, and includes a variant from Hazen formulation (Hazen, 1893). The method relates the hydraulic conductivity, in cm/s, to the initial slope and intercept of the grain-size distribution curve, and is defined as:

$$K = 1.505\cdot[I_o + 0.025\cdot(d_{50}-d_{10})]^2 \qquad \text{(eq. 3)}$$

Where I_o is the x-intercept (mm) of the straight line formed by joining d_{50} and d_{10} of the grain-size distribution curve; d_{50} is the mean grain diameter (mm) for which 50% of the particles are finer by weight, and d_{10} is the effective grain diameter (mm).

Data treatment

The results from the hydraulic conductivity for the three sampling periods were compared by ANOVA followed by a multiple comparison test (Tukey *t*-test). The hydraulic conductivity calculated for each method was related to sediment sorting by regression analysis. Furthermore, correlation between K obtained by different approaches was performed. The SPSS v.19 program was used for statistical analysis.

3.1.3 RESULTS

The physical characteristics of the sediment samples collected in the Tordera river are summarized in Tables 3.2 and 3.3. Results showed that, after the hydrometer test, a small amount of mud (silt and clay) was recorded in our samples. Approximately 90% of samples (by weight) were fallen in the coarser fraction (coarse sand and very fine & fine gravel) for all sampling periods. In addition, nearly all samples were uniformly graded due to their coefficient of uniformity (C_u) less than 4, except a sample of site 4 in July ($C_u \sim 4.13$). The sediment sample patterns in June and July coincided, so that their diameters at any percentages were approximately identical (see Figure 3.1). Comparatively, samples from December showed larger particle sizes than those from June and July for the above 75 µm fraction. Thus, sand sediments from December were of coarser grain sizes, and therefore they produced significantly higher K values than those from June and July (Tukey *t*-test, $p < 0.05$).

Table 3.2: *Statistical parameters[b] from the cumulative percent (passing) frequency distribution curves.*

ID	d_{10} (mm)	d_{17} (mm)	d_{20} (mm)	d_{30} (mm)	d_{50} (mm)	d_{60} (mm)	C_u	C_c	*n*	Sand (%)	Silt (%)	Clay (%)
121	0.80	1.18	1.43	2.16	2.77	3.08	3.88	1.92	0.38	94.22	5.04	0.74
122	0.83	1.18	1.42	2.14	2.75	2.98	3.58	1.87	0.39	94.09	5.28	0.63
123	0.84	1.20	1.50	2.13	2.62	2.95	3.57	1.87	0.39	94.57	4.57	0.87
124	0.80	1.31	1.54	2.12	2.56	2.82	3.59	2.05	0.39	96.10	3.34	0.57
061	0.65	0.79	0.87	1.10	1.61	1.97	3.03	0.94	0.40	94.22	5.04	0.74
062	0.60	0.72	0.78	0.95	1.43	1.72	2.90	0.88	0.40	94.09	5.28	0.63
063	0.48	0.61	0.66	0.86	1.36	1.72	3.56	0.88	0.39	94.57	4.57	0.87
064	0.59	0.76	0.83	1.10	1.68	1.99	3.41	1.03	0.39	96.10	3.34	0.57
071	0.59	0.72	0.77	0.96	1.46	1.82	3.11	0.86	0.40	94.22	5.04	0.74
072	0.66	0.80	0.87	1.10	1.71	1.97	2.98	0.92	0.40	94.09	5.28	0.63
073	0.58	0.74	0.81	1.07	1.72	2.09	3.57	0.93	0.39	94.57	4.57	0.87
074	0.54	0.70	0.77	1.02	1.74	2.19	4.13	0.88	0.37	96.10	3.34	0.57

[b] two prefix numbers in ID represent the sampling periods, e.g. 12 was December 2012, 06 was June 2013 and 07 was July 2013 since its suffixes represent the sampling sites. Values for each ID were calculated as an average from triplicate samples at surface (0-5 cm), 20 cm and 50 cm depths. d_x (mm) value corresponds to which x% (e.g., read at 10, 17, 20, 30, 50 and 60%) of the sample is finer, and was calculated from the phi size formula given by Krumbein (1934) above. The coefficient of uniformity (C_u) for

uniformly graded soil containing particles of the same size is less than 4, and $C_c = d^2_{30}/(d_{10} \cdot d_{60})$ called the coefficient of curvature lies between 1 and 3 for gravels and sands (Murthy, 2002). n is porosity of sediment expressed dimensionless in our calculation. These parameters were used to compute hydraulic conductivity (K) described in Table 1 and equation 3.

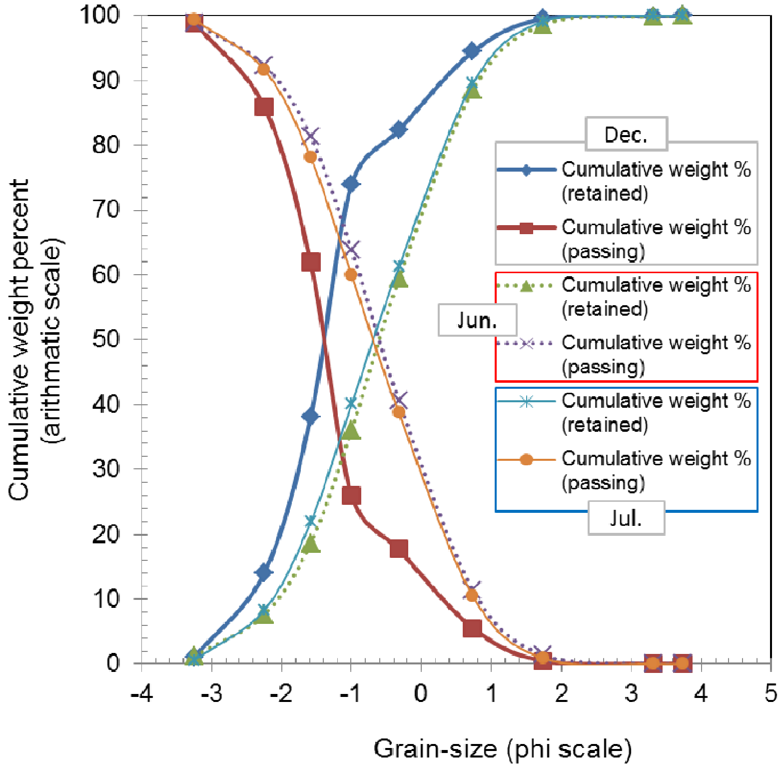


***Figure 3.1**: Grain-size distribution curves. These curves were drawn from sieving results (averaged 12 samples for each period).*

On the other hand, the sand sediments had skewness values (S_k) in the range of 0.06 to 0.38, -0.12 to 0.07, and -0.14 to 0.11 for December, June and July, respectively; while their kurtosis values (S_G) ranged respectively from 1.32 to 1.94, 0.94 to 1.05 and 0.87 to 1.11 for December, June and July (all values not shown, their average given in Table 3.3). In addition, the sorting of the sizes around the average was lying between the boundary of moderately and poorly sorted (Blott & Pye, 2001) for December (σ_ϕ = 0.75 –1.19), while for June and July sorting was approximately σ_ϕ = 0.96 –1.31.

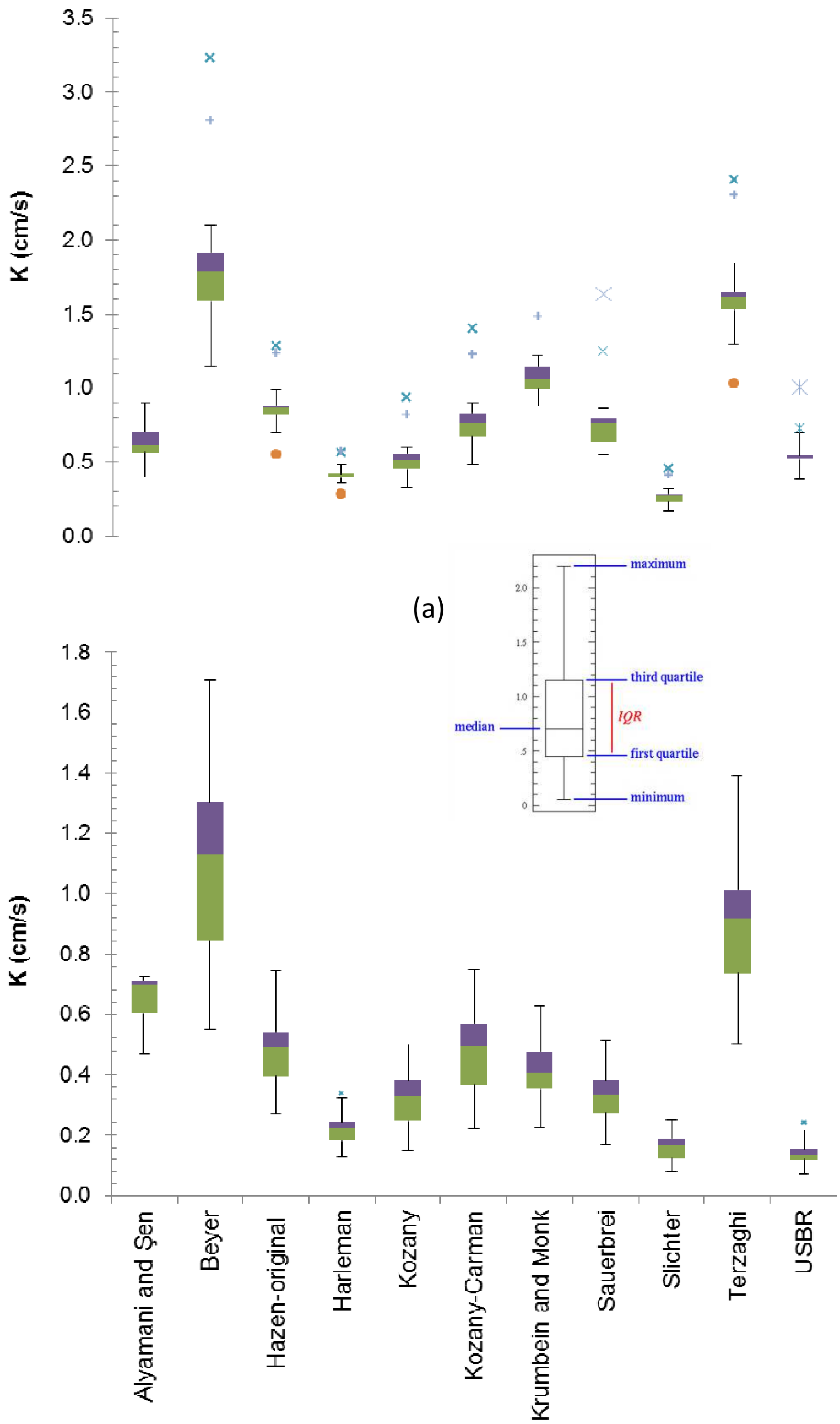


Figure 3.2: *Boxplots showing the hydraulic conductivity (K) values, in cm/s, estimated from 11 empirical methods. Figure 3.2 presents 12 samples in December 2012 (a), and 24 samples in June and July 2013 (b) (plotted together as their grain-size distribution curves were appeared similarly). (*) and (+, × and •) showed respectively the extreme values and outliers.*

Table 3.3*: Statistical parameters[c] from the cumulative percent (retained) frequency distribution curves.*

ID	ϕ_5 (mm)	ϕ_{16} (mm)	ϕ_{25} (mm)	ϕ_{75} (mm)	ϕ_{80} (mm)	ϕ_{84} (mm)	ϕ_{95} (mm)	GM_ξ (mm)	σ_ϕ	S_k	S_G
121	-2.80	-2.17	-1.92	-0.94	-0.57	-0.17	0.81	-1.27	1.05	0.28	1.56
122	-3.06	-2.39	-1.99	-0.98	-0.59	-0.20	0.66	-1.35	1.11	0.15	1.54
123	-2.65	-2.13	-1.86	-0.98	-0.58	-0.17	0.78	-1.23	1.01	0.27	1.67
124	-2.71	-2.05	-1.79	-0.97	-0.66	-0.28	0.90	-1.23	0.99	0.23	1.80
061	-2.49	-1.68	-1.37	0.04	0.21	0.38	0.93	-0.66	1.03	-0.02	0.99
062	-2.58	-1.65	-1.26	0.22	0.37	0.51	1.08	-0.55	1.09	-0.09	1.01
063	-2.80	-1.74	-1.33	0.41	0.59	0.76	1.40	-0.48	1.26	-0.08	0.99
064	-2.55	-1.76	-1.42	0.07	0.27	0.46	1.19	-0.68	1.12	0.06	1.03
071	-2.37	-1.67	-1.35	0.21	0.37	0.49	1.05	-0.57	1.06	-0.05	0.90
072	-2.34	-1.72	-1.30	0.02	0.22	0.37	0.92	-0.71	1.01	0.07	1.02
073	-2.78	-1.90	-1.54	0.11	0.31	0.51	1.14	-0.72	1.20	0.02	0.98
074	-2.83	-2.00	-1.63	0.15	0.38	0.54	1.25	-0.75	1.25	0.02	0.94

[c] See ID details' description in Table 2. These parameters were used to compute K by the Krumbein and Monk's method (see Eq. 2). The subscript in the phi terms (ϕ_x) refers the grain-size at which x% (e.g., read at 5, 16, 25, 75, 80, 84 and 95%) of the sample is coarser than that size. As for the phi size corresponding to the 50% mark on the cumulative, either percent passing or retained, frequency distribution curves; the ϕ_{50} values were estimated with the expression given by Krumbein (1934) (see the formula mentioned above and use the d_{50} values in Table 3.2 for the calculation). Inclusive skewness (S_k) was used to describe the degree of asymmetry for a given distribution and is equal to $(\phi_{16}+\phi_{84}-2\cdot\phi_{50})/2\cdot(\phi_{84}-\phi_{16}) + (\phi_5+\phi_{95}-2\cdot\phi_{50})/2\cdot(\phi_{95}-\phi_5)$. Graphic kurtosis ($S_G$), used to describe the degree of peakedness for a given distribution, is defined as $(\phi_{95}-\phi_5)/2.44\cdot(\phi_{75}-\phi_{25})$; (Masch & Denny, 1966).

Figure 3.2 shows the hydraulic conductivity (K) estimated by the 11 empirical formulas. In the calculation of K values using these formulas, the water temperature was assumed to be at 20^{o}C for each campaign. Nearly all of the empirical formulas produced some outliers for the samples in December (mostly samples from 50 cm depth at site 4, and surface depth at site 3), except for that of Alyamani and Şen (Figure 3.2a). In contrary, all formulas seemed not to give much variation in K values for the sand samples in June and July, except two outliers (surface samples at site 1 in June) that were detected for the Herlaman and USBR approaches (Figure 3.2b). From K results estimated by the Kozeny-Carman approach, e.g. when we excluded all outliers K varied between 0.48 and 0.90 cm/s in December (Figure 3.2a), and between 0.22 and 0.75 cm/s in June and July (Figure 3.2b). The middle 50% scored from 0.67 to 0.83 cm/s in December, and from 0.37 to 0.57 cm/s in June and July; thus, the interquartile K ranges were 0.16 cm/s (0.83–0.67) and 0.21 cm/s (0.57–0.37); respectively. The median K values for the entire grade distributions of the Kozeny-Carman approach were 0.77 cm/s in December, and 0.49 cm/s in June and July. We can say that the lowest scoring 25% ranged between 0.48 and 0.67 cm/s in December, and between 0.22 and 0.37 cm/s in June and July; while their highest scoring 25% varied between 0.83 and 0.90 cm/s, and between 0.57 and 0.75 cm/s; respectively.

Just by looking at the graphs, the Terzaghi method is the second highest estimator of K after the Beyer method in all sampling dates; while the lowest is for the Harleman formula, and the next lowest is for the Slichter for December data (Figure 3.2a). For June and July data (Figure 3.2b), the lowest estimator is for the USBR relation, and the next lowest is for the Slichter formula followed by that of Harleman.

The different K estimates were significantly correlated among them (r from 0.82–0.99, $p < 0.001$) except for that of Alyamani and Şen (r between 0.10–0.27, p from 0.115–0.551). Significant regressions were found between sorting and K estimates (R^2 from 0.20–0.55, $p < 0.001$) excepting the Alyamani and Şen formula (R^2=0.03, p=0.326). The highest coefficient of determination was that found for the regressions between sorting and K estimated by Kozeny-Carman, shown in Figure 3.3. The regression obtained from Sauerbrei formula, which gave similar K to our field estimates as discussed below, is also shown in Figure 3.3.

3.1.4 DISCUSSION

The particle size distribution data have been commonly used to estimate the hydraulic conductivity (K) of sediments using a variety of different empirical equations (Rosas *et al.,* 2014). From the present analysis, it is clear that each of the empirical equations yields different K estimates. This was not surprising since other authors also find different formulas gave a range of K values for the same sediment by a factor of 10 or even up to ±20 times (Vukovic & Soro, 1992), and then it becomes relevant to select the best appropriate formula to apply for a specific sediment sample.

In Tables 3.1 and 3.2, according to the applicability domains of the empirical methods, 6 samples were not applicable to the Beyer approach because their effective diameter (d_{10}) was greater than 0.6 mm. The Beyer method is most useful for analyzing heterogeneous samples with well-graded grains (Pinder & Celia, 2006), so it is not applicable for our uniformly graded samples. As our samples were not very well sorted, the Krumbein and Monk approach might not be also the most convenient for our study site samples, although it produced K values similar to our field direct measurements (0.20 to 0.75 cm/s estimated by *in situ* infiltration measurements, unpublished data from authors). On the other side, the USBR, Slichter and Harleman formulas underestimated the K values for sand sediments of the Tordera river; while the Beyer and Terzaghi methods overestimated them (Figure 3.2 a & b). Similarly, Vukovic and Soro (1992), and Cheng and Chen (2007) also reported K underestimation when applying USBR and Slichter methods. On the other hand, in the case of the Terzaghi formula, Odong (2007) assured that it gives low K values in contrast to our findings, even though this author used the same average sorting coefficient (β) as us. For the Harleman formula, our K results confirmed underestimated as similarly found by Lu *et al.* (2012).

Rosas *et al.* (2014) comparing 20 different empirical equations highlighted Hazen and Kozeny-Carman ones as the most commonly used to estimate K values from grain-size distribution. Carrier (2003) recommended that the Hazen method should be retired and the Kozeny-Carman be adopted because the Hazen method for predicting the permeability of sand is based only on the d_{10} particle size, whereas the Kozeny-Carman method (proposed by Kozeny and later modified by Carman) is based on the entire particle size distribution, the particle shape, and the void ratio (Kozeny, 1927, 1953; Carman, 1937, 1956). As a consequence, the Hazen method might be less accurate than that of Kozeny-Carman. This agrees with the K results of Odong (2007) confirming that the Kozeny-Carman formula proved to be the best estimator of most samples analyzed, and may be the case, even for a wide range of other soil types. In parallel, the results presented in the study of Chapuis and Aubertin (2003) show that, as a general rule, the Kozeny-Carman method predicts fairly well the saturated hydraulic conductivity of most soils.

As highlighted in the "Results" (see § 3.1.3) for the Kozeny-Carman method, it also seems that this method provided reliable K estimates (see the average values for December as well for June and July) closer to the range of our K values estimated *in situ*. At the same time, for the case study of sand sediments our results are situated within the K limits given by Freeze and Cherry (1979) which oscillated from 0.0001 to 1 cm/s. K values from Kozeny-Carman method, however, estimated some high values for some samples from December in our study (Figure 3.2a); and also underestimated a sample in the study of Odong (2007), this was because of that the formula is not appropriate if the particle distribution has a long, flat tail in the fine fraction (Carrier, 2003). On the other side, there is a large consensus in the geotechnical literature that the hydraulic conductivity of compacted clays (clay liners and covers) cannot be well predicted by the Kozeny-Carman equation (Chapuis & Aubertin, 2003). Despite the adoption of the Kozeny-Carman equation, the classical soil mechanics textbooks maintain that it is approximately valid for sands (Lambe & Whitman, 1969; Taylor, 1948), but it is not valid for clays. However, recently Steiakakis *et al.* (2012) showed that the Kozeny-Carman equation provides good predictions of the K estimates of homogenized clayey soils compacted under given compactive effort, despite the consensus set out in the literature.

Altogether, our study results suggest that the Kozeny-Carman formula can be successfully applied to our coarse loose sand sediments because they contained very little content of clay about 0.7% (Table 3.2). Also, the K values estimated by the Kozeny-Carman formula showed remarkably the highest correlation with the particle size distribution (Figure 3.3) compared to other empirical approaches in this study. Alternatively, respecting to the obtained results, the approach which is likely to be also chosen together with the Kozeny-Carman formula would be the Sauerbrei approach. Both K values obtained from Kozeny-Carman and Sauerbrei approaches were significantly correlated ($r=0.926$, $p < 0.001$). In the case of Sauerbrei approach, K values ranged from 0.17 to 0.87 cm/s and it can be also applicable to the sand samples (Table 3.1, Figure 3.2 a & b). But, the K obtained by the Sauerbrei approach was less correlated to sorting (Figure 3.3).

Looking at the results from Alyamani and Şen, K values (0.4 to 0.9 cm/s) were in the higher limits of K given above and values were poorly correlated to the K estimated by Kozeny-Carman formula (r=0.217, p=0.202). At the same time, the Alyamani and Şen method is particularly very sensitive to the shape of the grading curve and is more accurate for well-graded sample (Odong, 2007) and thus not useful for our uniformly graded samples. Moreover, this approach gave no significant link between K and sorting.

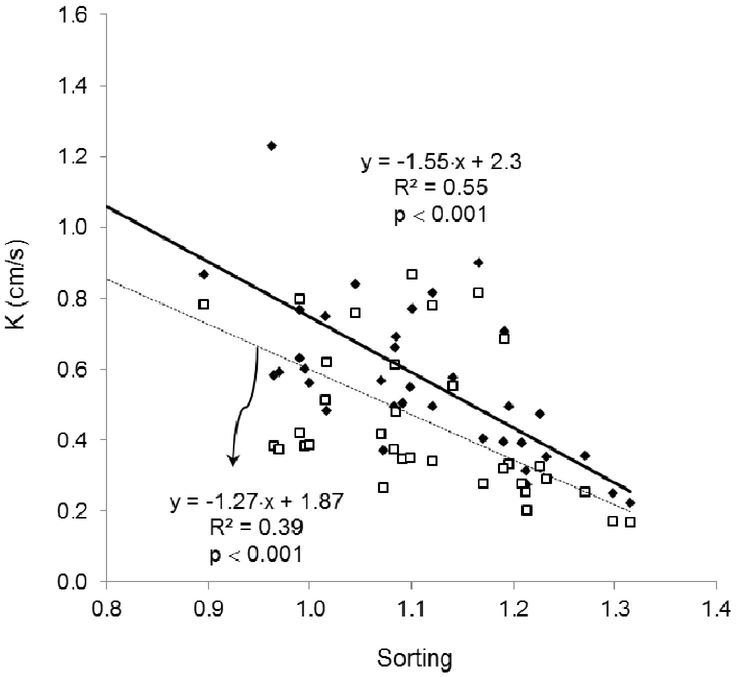


***Figure 3.3**: K values predicted by the Kozeny-Carman (♦) and Sauerbrei (□) formulas versus the sorting of streambed samples. The sorting was calculated using inclusive (graphic) standard deviation (Krumbein & Monk, 1943).*

In summary, hydraulic conductivity (K) is correlated to soil properties like pore size and particle size distribution, and soil texture, and this link can be, in part, defined by specific empirical formulas. Most of the empirical relations use the effective diameter (d_{10}) value, as a specific aspect of the grain-size distribution that can impact significantly on the permeability of the porous layers. We need to be prudent about the domains of applicability of each empirical formula. In the end, although from our data, we suggest using Kozany-Carman approach to calculate K from grain-size distribution; next step would be measuring K with experimental approaches to check the choice. Interestingly, Song *et al.* (2009), Lu *et al.* (2012) and Rosas *et al.* (2014) showed some correlations between the outcomes from experimental approaches (such as a standard constant head described in Tanner and Balsillie (1995), falling-head standpipe permeameter tests, a permeameter test) with those predicted by the empirical relations but still no new empirical equations have been developed that accurately predict K from these relationships yet. After doing so, the more reliable K estimates we obtain, the more we could understand about the changes in hydrogeochemical processes in the hyporheic zone, since it plays a key relevant role in fluvial ecosystems functioning (Triska *et al.,* 1993; Valett *et al.,* 1994).

3.2

How do sediment physical characteristics affect microbial biomass in a Mediterranean river?

3.2.1 BACKGROUND AND AIMS

River sediments usually comprise a heterogeneous mixture of particles of different sizes, origins and surface features (Santmire & Leff, 2007). The physical characteristics of the sediment defined by its granulometry (size distribution of sediment particles) determine not only the surface area available for microbial colonization, but also characteristics linked to water flow at the habitat scales (i.e. permeability including hydraulic conductivity and porosity, see § 3.1).

Environmental factors such as nutrient content, light and temperature have been shown to significantly define the microbial biomass found in riverbeds (Stock & Ward, 1989). However, much less attention has been paid on the potential effect of sediment physical properties on benthic microbial biomass. The sediment physical properties may be relevant for the attachment and development of organisms (Biggs & Hickey, 1994; Ditsche-Kuru *et al.,* 2010; Ditsche *et al.,* 2014; Tonetto *et al.,* 2014), as it has been shown for bacterial density (Eisenmann *et al.,* 1999). The sediment grain-size composition determines the heterogeneity and surface roughness of the substratum which, in turn, creates fine-scale patterns of near-bed flow that influence organic matter retention and algal accumulation (Cardoso-Leite *et al.,* 2015) as well as the distribution of benthic organisms (Culp *et al.,* 1983; Hart *et al.,* 1996). Previous studies (Williams, 1980; Poff & Ward, 1990) underlined that physical spatial heterogeneity at the microhabitat scale in streambeds is especially important for biological colonization given intimating association between organisms and bed characteristics. Thus, hydraulic and substratum conditions have been identified as two factors that affect community composition, and the abundance and distribution of the constituent populations (i.e. Statzner *et al.,* 1988; Cobb *et al.,* 1992; Quinn & Hickey, 1994). At a larger scale, the study results of Rempel *et al.* (2000) indicated that hydraulic conditions, as well as substratum texture and the distribution of organic matter, represent major gradients in large gravel-bed rivers. According to Fisher *et al.* (2003), the evolution of bedforms and the exchange processes with the river flow influenced bacteria colonizing sediment grains in riverbed structures. However, Santmire and Leff (2007) concluded seasonal variations in abundance of microbial were predominant while differences among sediment sizes were of secondary importance.

Sediment physical characteristics may be further affected by temporal dynamics (as important factors in lotic ecosystems) usually linked to hydrology (Ward, 1989). However, the impact of such seasonal changes may differ among varying substrates. For example, in some cases, the organic matter content of the sediments varied among size classes (i.e. coarser and finer fractions) and seasons (Santmire & Leff, 2007), whilst algae (mainly diatoms) and bacteria prevail in the hard and relatively inert substrata such as cobbles and gravels (Stevenson, 1996; Battin *et al.,* 2003). Following Kaplan and Bott (1989), the high Chl *a* levels measured in sediments (kept in the dark) indicate that fine particles are continually deposited on the sediment surface under base-flow conditions. In that study, it is also shown that the aggrading sediment habitat constantly changes, quite possibly before strong interactions between autotrophs and heterotrophs can become established (Kaplan & Bott, 1989).

As the sediment physical parameters are of particular interest because they can lead to the identification of mechanisms controlling the streambed colonization (Kaplan & Bott, 1989) – from here, this study focuses on the influence of sediment physical parameters on algae and bacteria colonization and OM deposition of surface sediments along a river continuum from headwaters to downriver alluvial reaches. In an attempt to reveal some of the findings in the large scale, the study includes twelve sampling sites along the Tordera river, and includes three sampling periods (July 2011, April and July 2012) defined by base, high and low discharges; respectively. For all sites and periods the sediment physical properties and microbial biomass were measured. It was hypothesized that the microbial biomass of the surface riverbed at different sites would depend on the surrounding sediment physical characteristics, and that those physical factors would be dependent on hydrological conditions. More specifically we expect greater effect of sediment physical characteristics on colonizing microbial biomass during basal and low flows when more OM accumulation might be trapped thus affecting microbial biomass development. However, the sediment physical characteristics during high flow period may also affect algal development due to determining nutrient availability from the flowing water.

3.2.2 METHODS

Study site and sampling

Twelve sampling sites were selected along the main stream of the Tordera river (starting 3 km from its source to 54 km near to its mouth) for three sampling periods (Figure 2.1, General methods). The mean discharge for the three study periods were 185.97 L/s (ranged between 11.30–473.34) in July 2011, 744.74 L/s (203.76–1541.00) in April 2012 and 34.43 L/s (1.98–143.47) in July 2012.

Each sampling date, three surface sediment samples (5 cm depth and 4.5 cm in diameter) were collected at random with a methacrylate corer at each sampling site. Samples for all sites were collected on the same sampling day to avoid any effect of atmospheric phenomena (i.e. sudden rain).

Subsamples of 1 cm^3 (~ 1 g) of sediment for each sampling site were used to measure organic matter (OM) content, chlorophyll-*a* content and bacterial density. Subsamples for bacterial density were preserved with formalin (2%) and 10 mL of filter-sterilized river water (0.2 µm nylon filter) at $4^{o}C$ until analysis. Subsamples for algal biomass were preserved at $-20^{o}C$ until analysis. Once in the laboratory, fresh subsamples for organic matter content were directly placed in the oven at the same day. The three replicate sediment subsamples were measured for each microbial variable and no replicate for the granulometry measurement.

Sediment physical properties

We used the existing data previously performed (by a sieve analysis) with a representative sample of the surface sediments (about 100 g). That specimen was run through a set of sieves (3.0 mm, 2.0 mm, 1.25 mm, 800 µm, 300 µm, 100 µm and 63 µm). Based on the grain-size distribution curves obtained, we estimated the sediment physical characteristics whose formulae are listed in Table 3.4 as following.

***Table 3.4**: Summary of sediment physical parameters calculated in the study after granulometry measurements.*

Parameter	Symbol	Formulae	Units
Porosity	n	$0.255\cdot(1+0.83^{Cu})$	None
(Dry) bulk density	BD	$2.65\cdot(1-n)$	g/cm^3
Void ratio	e	$n/(1-n)$	None
Hydraulic conductivity (from Kozeny-Carman's eq., see Table 3.1)	K	$(g/\nu)\cdot 8.3\times10^{-3}\cdot n^3/(1-n)^2\cdot d_{10}^2$	cm/s
Sediment heterogeneity[a]	-	d_{84}/d_{50}	None
Coefficient of sorting	S_o	$\sqrt{d_{75}/d_{25}}$	None
mud (silt and clay)	-	$\frac{\text{Weight}^b < 63\ \mu m}{\text{Total weight}}\cdot 100$	[%]

Note: d_x (cm) represents the effective particle diameter corresponding to x% cumulative (from 0 to 100%, e.g. it was originally read (in mm) at 10, 25, 50, 60, 75 or 84% for our calculation) underside particle size distribution curves. C_u is coefficient of uniformity equal to d_{60}/d_{10}. ν is kinematic viscosity set to 1.002×10^{-2} cm^2/s at 20^oC, when g is the acceleration due to gravity equal to 980 cm/s^2. [a] High heterogeneity is greater than 2, when less than 2 is low heterogeneity (Cardinale et al., 2002). [b] Proportion by weight of the total samples less than 63 μm.

Sediment microbial biomass

Organic matter content, chlorophyll-*a* and bacterial density were measured with the methods described in details in General methods.

Data treatment

The effect of sediment physical properties on sediment microbial biomass was analyzed by multiple regression analysis by using the parameters describing sediment properties included in Table 3.4 as independent variables. Multiple regressions were run with organic matter (or AFDW) content, Chl *a* content and bacterial density, as dependent variables. Multiple regressions were performed for all data together, and then for each sampling period independently to select for possible changing interactions depending on the season. Selection of independent variables included in the regression was performed by "backward" method and considering collinearity less than 9 VIF (variance inflation factor) (Neter *et al.,* 1996). Multiple regressions were performed with SPSS v. 19. Selected regressions were then used for the calculation of expected values and those were related to measured values by linear regression.

3.2.3 RESULTS

Sediment physical characteristics

During the three samplings, the sand sediment sampled along the main course of the Tordera river had coefficient of sorting (S_o) between 1 and 2, except a sample at 37 km sampling site in July 2012 which was of 2.42. As shown in Figures 3.4 a, b and c, the sediment physical patterns did not appear the same. The sand sediments comprised 28.56% (dry weight) of very fine gravel (VFG, 2-4 mm), 71.33% of sand (2 mm - 63 µm) and 0.10% of mud (less than 63 µm) in July 2011; 21.46% of VFG, 78.45% of sand and 0.10% of mud in April 2012; and 42.45% of VFG, 57.41% of sand and 0.14% of mud in July 2012. At each sampling site, most of samples showed low heterogeneity ($d_{84}/d_{50} < 2$). Mean (dry) bulk density for each period was about 1.59 g/cm^3 (July 2011), 1.61 g/cm^3 (April 2012) and 1.67 g/cm^3 (July 2012). Mean hydraulic conductivity (K) was differently changed following the scour by running water at 0.51 cm/s in July 2011, at 0.30 cm/s in April 2012, and at 0.48 cm/s in July 2012. The average void ratio (e) was about 0.67 in July 2011, 0.65 in April 2012 and 0.60 in July 2012.

Sediment microbial biomass

The patterns of ash free dry weight (or AFDW), chlorophyll (Chl) *a* content and bacterial density were not the same seasonal fluctuations at each sampling site (Figure 3.5). AFDW, Chl *a* content and the bacteria in the Tordera river sediments were the highest in July 2011 and specially increased in mid to lower sites; whereas the lowest was reported in April 2012. The average of AFDW, Chl *a* and the bacteria were respectively 53.19 (±8.82) mg/ g DW, 12.37 (±5.10) µg/ g DW and 1.98 (±1.30) cell×10^9/

g DW in July 2011; 5.61 (±0.99) mg/ g DW, 3.91(±0.82) μg/ g DW and 0.14(±0.03) cell×10^9/ g DW in April 2012; and 10.66 (±3.13) mg/ g DW, 4.92(±1.62) μg/ g DW and 1.25(±0.28) cell×10^9/ g DW in July 2012.

Effect of sediment physical properties to sediment microbial biomass

Multiple linear regression analysis was used to determine models for predicting ash free dry weight (AFDW), Chl *a* content and bacterial density from the sediment physical characteristics including the effective diameter (d_{10}), porosity (*n*), (dry) bulk density (BD), void radio (e), hydraulic conductivity (K), sediment heterogeneity (d_{84}/d_{50}), coefficient of sorting (S_o), % very fine gravel (VFG), % sand and % mud (silt and clay) for the three study periods (July 2011, April and July 2012).

As it can be seen in Tables 3.5, 3.6, and 3.7, it was found that all the full samples' models to predict AFDW, Chl *a* content and the bacterial density were not significant, but several significant regressions were obtained when considering each sampling period individually.

Table 3.5*: Equations obtained after multiple linear regressions for ash free dry weight (AFDW) as dependent variable. Independent variables and slopes are included for the regressions obtained with all samples together or independently for each period (July 2011, April and July 2012). Adjusted coefficient of determination (R^2) and significance of each regression are also shown. B and β respectively stand for unstandardized and standardized coefficients.* ***.*** *Low case letters indicate significance of correlation of each independent variable to whole regression, ^{a} <0.001, ^{b} <0.01, ^{c} <0.05.*

Variable	Full samples		July 2011		April 2012		July 2012	
	B	β	B	β	B	β	B	β
n							-499.36	-1.50^a
BD			1476.05	0.77^b				
K					-2.28	-0.36		
d_{84}/d_{50}	71.50	0.45^c	184.23	0.89^b	1.70	0.36	40.46	0.94^b
S_o	-42.98	-0.19					-68.91	-1.47^a
mud			-310.78	-0.46^c				
y-intercept	-41.15		-2590.94		3.09		239.18	
adj. R^2	*0.09*		*0.74*		*0.24*		*0.66*	
p	*0.081*		***0.005***		*0.118*		***0.001***	

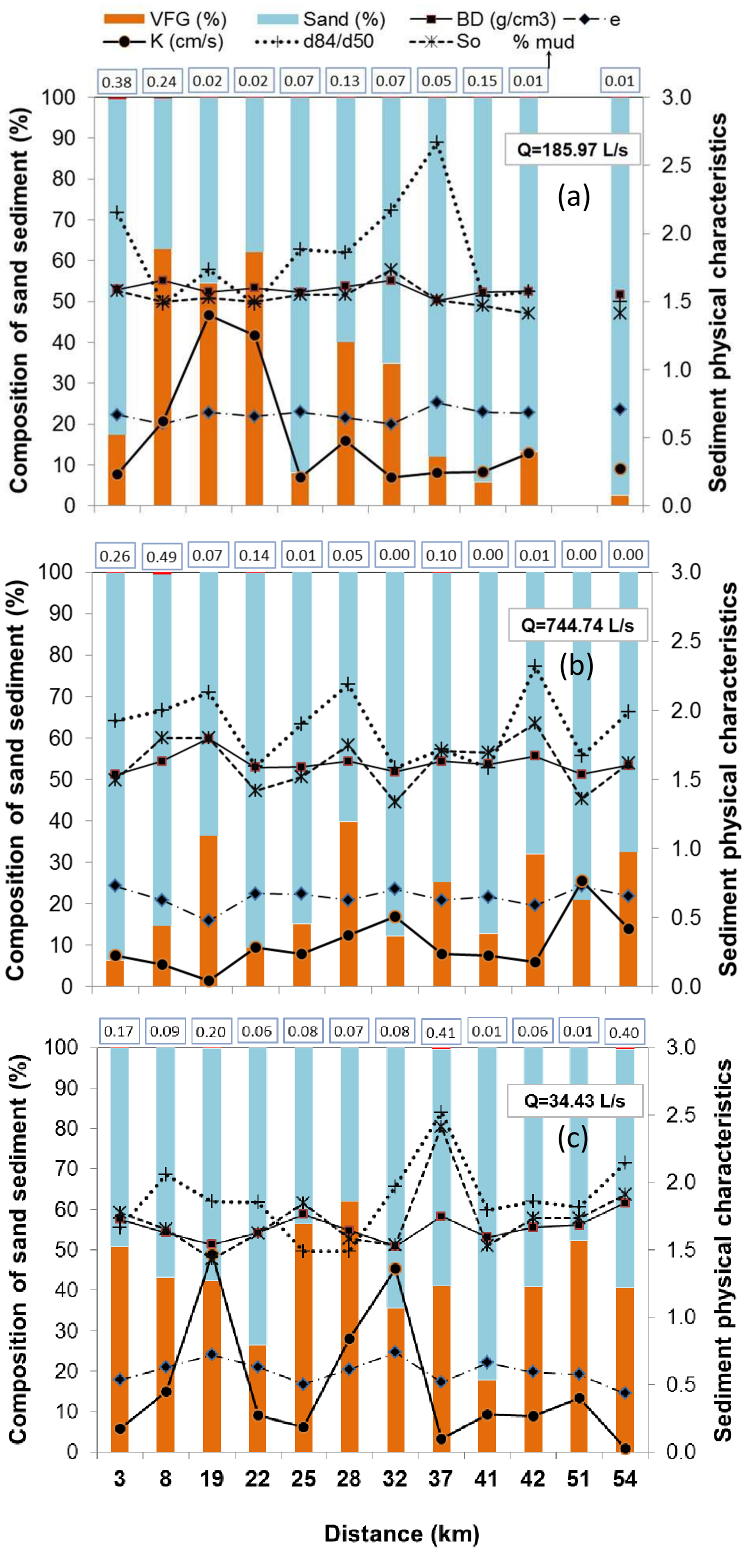


***Figure 3.4**: Sediment physical characteristics at the 12 sampling sites along the Tordera river for the three study periods (a was July 2011; b and c were April and July 2012, respectively). VFG and Q respectively represent very fine gravel and discharge. See other abbreviations in Table 3.4.*

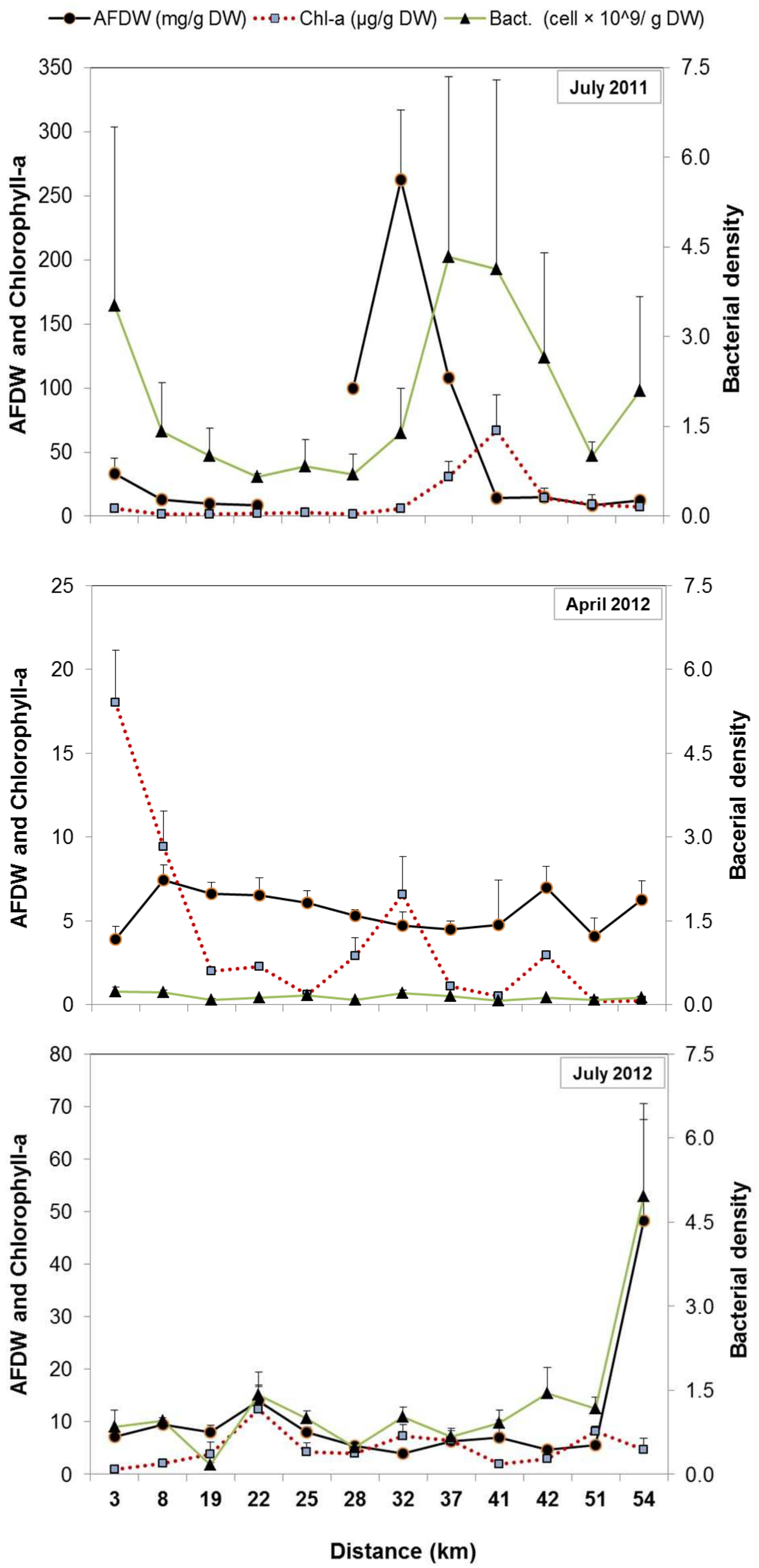


Figure 3.5*: The evolution of microbial biomass (AFDW, Chl a, and bacteria) at the 12 sampling sites along the Tordera river for the three study periods.*

In Table 3.5, in July 2011, the sediment heterogeneity (d_{84}/d_{50}) followed by bulk density (BD) significantly positively predicted value of AFDW, while % mud significantly negatively predicts value of it. In July 2012, sediment heterogeneity (d_{84}/d_{50}) also predicted AFDW content, and the porosity (*n*) and the coefficient of sorting (S_o) variables significantly negatively predicted AFDW content.

***Table 3.6**: Equations obtained after multiple linear regressions for the content of Chlorophyll-a (µg/ g DW) as dependent variable. See details in Table 3.5 legend.*

Variable	Full samples		July 2011		April 2012		July 2012	
	B	β	B	β	B	β	B	β
d_{10}	-20.76	-0.33						
d_{84}/d_{50}					16.86	0.82^c	4.99	0.44
mud							-8.14	-0.35
e	57.27	0.36^c	67.65	0.16				
Sand			0.35	0.41	0.48	1.04^b		
y-intercept	-18.60		-57.74		-65.44		-3.34	
adj. R^2	*0.12*		*0.09*		*0.47*		*-0.09*	
p	*0.052*		*0.284*		***0.023***		*0.608*	

Any significant regression was obtained for the data in the highest discharge period (April 2012) – in the case of Chl *a* (Table 3.6), significant regression was only obtained for that date, and it was reported that % sand and sediment heterogeneity (d_{84}/d_{50}) explained a significant amount of the variance in the value of Chl *a*. However, % sand significantly predicted value of Chl *a* in April 2012 higher than the (d_{84}/d_{50}) predictor did.

For bacterial density, significant regressions were obtained for the three study periods, predictors differently contributed to each model to predict the bacterial density for each study period, but no significant regression was obtained when analyzing all data together (Table 3.7). For example, void ratio (e) did significantly predict value of the bacteria in July 2011 while % mud did not. However, % mud added statistically significantly to the prediction of the bacteria in April 2012. However, neither void ratio nor % mud was involved in the model predicting the bacteria in July 2012. During that study campaign, other predictors' model (such as *n*, d_{84}/d_{50} and S_o) were able to account for 89.5% of the variance in the bacterial density.

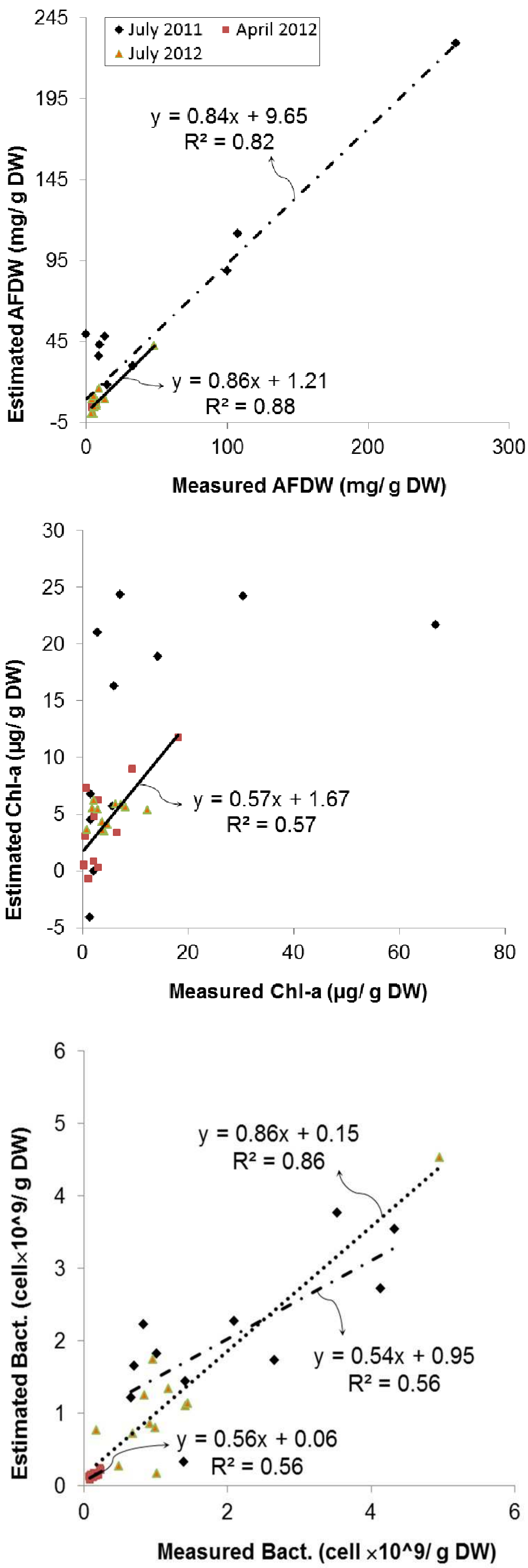


***Figure 3.6**: Relationship between measured and estimated values for AFDW, Chl a and bacteria in the Tordera river sediment (by using multiple regression equations given in Tables 3.5, 3.6, and 3.7).*

Table 3.7*: Equations obtained after multiple linear regressions for bacterial density (cell×10^9/ g DW) as dependent variable. See details in Table 3.5 legend.*

Variable	Full samples		July 2011		April 2012		July 2012	
	B	β	B	β	B	β	B	β
n							-51.03	-1.53[a]
e			21.57	0.73[c]	0.33	0.40		
d_{84}/d_{50}							3.95	0.91[a]
S_o	-1.14	-0.18					-6.78	-1.43[a]
mud	3.39	0.34	6.36	0.53	0.24	0.63[c]		
y-intercept	2.61		-13.09		-0.09		24.50	
adj. R^2	*0.04*		*0.44*		*0.47*		*0.86*	
p	*0.191*		***0.041***		***0.024***		***< 0.001***	

From the multiple regressions expected versus observed measures showed several significant correlations especially for the bacteria underlying the predictive power of sediment characteristics for sediment bacteria (Fig. 3.6). In the case of Chl a, significant correlation was only obtained during high discharge (April 2012) while that of AFDW was not. However, the significant correlation for AFDW was detected during the base and low discharges (July 2011 and 2012).

3.2.4 DISCUSSION

This study shows that physical properties of river surface sediments affect the accumulation of bacteria, algae and organic matter (OM); and that this effect is different depending on hydrological events and microbial group considered. As hypothesized, bacterial density is strongly shaped by sediment physical properties since they rely significantly on OM (or AFDW) accumulation in river sediment (Romaní & Sabater, 2001). AFDW was also well predicted by the physical sediment properties excepting during the high discharge period. The lowest prediction power of sediment physical properties was obtained for Chl *a* suggesting that other key environmental variables such as nutrients and light may greater determine algal biomass variation (Acuña *et al.,* 2007).

The specific sediment characteristics selected as better predictors may highlight on relevant processes determining microbial biomass. In the case of AFDW, different predictive parameters were selected for the different hydrological periods. During the lowest discharge period (July 2012), reduced porosity of sediment affected positively AFDW content (Table 3.5, Figure 3.6); this happened when the surface layers of our system were composed by the highest amount (> 42 % of dry weight) of very fine gravel (VFG). It is suggested fine particles (< 2 mm) transported in the flowing surface water may intrude into stable gravel-beds and progressively reduce pore spaces, thereby causing decreasing seepage rates (Brunke, 1999). In other studies (Beyer & Banscher, 1975; Schalchli, 1993; Lake, 2003), fine sediment that passes the coarse armor layer may accumulate beneath the armor layer; and then, if low discharge continues a compact layer that reduces the porosity and hydraulic conductivity of the streambed may develop and stabilize the streambed against erosion (internal colmation). Beyer and Banscher (1975) underlined a thicker layer of fine particulate organic matter reduces the permeability of this compacted streambed (external colmation). This is consistent with our study results. Similarly, in addition to what occurred in the lowest flowing water period, we noticed that higher sediment heterogeneity and bulk density significantly enhanced the accumulation of AFWD in basal flow period (July 2011, Table 3.5, Figure 3.6). When eroded soil particles (with a certain amount of mud, in our study) fill pore spaces due to running water (Brunke, 1999); consequently, the sediment layers became compacted and bulk density of sediment increased. For the highest discharge period (April 2012), no significant regression was obtained for AFDW probably due to the major effect of discharge and the shear stress power to control organic matter accumulation, which was reduced during this period because of an increase in flowing water depth and flow velocity (Gashith & Resh, 1999).

In contrast to AFDW, algal biomass seems to be determined by sediment physical properties only for the highest discharge period. Our study results showed that higher heterogeneity of the sand sediment did predict higher values of Chl *a* during the highest discharge conditions in April 2012 (Table 3.6, Figure 3.6), which is consistent with the results of Cardinale *et al.* (2002). They highlighted physical habitat heterogeneity had an immediate and significant impact on the primary productivity of stream algae, probably as a result of alterations to near-bed flow velocity and turbulence intensity. At the same time, high flow velocity at the micro-habitat scale can enhance algal development due to greater nutrient availability (Lock & John, 1979;

Romaní & Marxsen, 2002).

Interestingly, the sediment bacterial density might be significantly predicted from sediment physical properties both for higher and lower discharge study periods. In some instances, densities of bacteria on streambed substrata can increase due to colonization and growth, and decrease from death and lysis, predation, burial in depositional zones, and losses to transport by scour (Lawrence & Caldwell, 1987). In the lower discharge period, bacterial density was significantly predicted by lower porosity and higher heterogeneity, as similarly found OM content, suggesting similar processes control both OM and bacteria during this period. Moreover, Cole *et al.* (1988) have shown that levels of bacterial production in sediments are significantly correlated with sediment organic content for a broad range of aquatic habitats – therefore, this case happened to our study during drought conditions in July 2012 (see Tables 3.5 & 3.7). In consequence, previous studies (Taylor & Jaffé, 1990; Cunningham *et al.,* 1991; Vandevivere & Baveye, 1992; Vandevivere *et al.,* 1995; Clement *et al.,* 1996) have revealed the attachment and growth of bacteria on a solid matrix may induce changes in porous medium properties, such as the porosity and permeability. That is why; the porosity of sediment layers in July 2012 became reduced.

In contrast, during basal and high discharge periods, other physical sediment properties seem to control bacterial density, including mud content and void ratio (Table 3.7, Figure 3.6). These parameters may be linked to grain surface area available for bacterial attachment. In this sense, Marxsen (2001) observed higher production rates for the bacteria on the sandy grains than on the coarse particles. Similarly, Romaní and Sabater (2001) showed bacterial biomass was higher in finer river sediment substrata from stream-edge, also containing higher percentage of mud. Typically, in the study of Santmire and Leff (2007), bacterial abundance was higher on the smallest particles (0.125 mm - 0.85 mm) – a situation which may result from differences in the surface area available for colonization. In these two periods, the higher percentage of mud particles coincides with higher void ratio in predicting enhanced bacterial density in surface sediments. Probably, mud in suspension in such porous sediment thanks the bacterial accumulation processes when the sediment layers became loose. In porous media, as in other aqueous environments, Cunningham *et al.* (1991) observed that microbial cells may exist in suspension or adsorb firmly to solid surfaces comprising the effective pore spaces – in which the finer suspended particles also entered and thus increase more surface area that can serve for bacterial attachment. However, the wide distribution of pore velocities introduces considerable variation in the processes of adsorption; and the net flow direction exerts a dominant influence on interstitial colonization (Brunke & Gonser, 1997).

In summary, multiple regressions for predicting the attached microbial biomass by organic matter (or AFDW), algae and bacterial density due to variations in sediment properties were analyzed, and then validated by coupling the expected versus observed measures. Not all sediment physical properties (as predictors) contributed at the same time to the equations obtained in this study. This could be explained that the selection of the physical factors was discharge dependent. The discharge did not, however, significantly predict the microbial accumulation along the Tordera river; although it is considered as one of the major factors in flowing water systems. The amount of mud (silt and clay) might compete the spaces with AFDW, but mud has been seen to be a factor predicting the value of the bacteria (i.e. especially during high discharge). To conclude, our results suggest that heterogeneity and interstices of sediment significantly predicted the variations in value of AFDW; and that high sand sediment heterogeneity played a significant role in the Chlorophyll-*a* accumulation in high-flow conditions; and besides pore spaces and the surface area (increased more with % of mud) for the attachment were significantly detected in this study, bacteria were also related to AFDW content.

3.3

Microbial processes across the dry-wet hyporheic boundary in a Mediterranean river

3.3.1 BACKGROUND AND AIMS

The sediment habitat in rivers plays a critical role in transformation, accumulation and release of organic and inorganic molecules transported by the water. Due to their long residence time and high microbial biomass and activity, surface and hyporheic sediments are centers of high biogeochemical reactivity (Romaní & Sabater, 2001; Hall *et al.,* 2012). Changing the physical and chemical conditions of interstitial water during the exposure time with the sediment, especially in fine substratum, might determine changes in the development of bacterial communities (Mueller *et al.,* 2013). Constantly affected by the deposition of allochthonous and autochthonous organic matter (Avnimelech *et al.,* 2001), river sediments are generally colonized by microbial biofilms, which constitute a biological complex consortium mainly of bacteria and fungi, which are commonly responsible for most metabolic activity in small streams (Findlay *et al.,* 1993). Sediment microbial biofilm communities are capable to uptake, storage and transform fluvial dissolved organic matter and nutrients from the bulk water (Pusch *et al.,* 1998; Battin & Sengschmitt, 1999). Microbial communities in streams play a key role for the turnover of organic matter and pollutants (Fischer *et al.,* 1996, 2002; Findlay, 2010), and specifically, within the hyporheic zone, biofilm structure and metabolic activity are known to control the matter fluxes and hydrochemical conditions (Hancock *et al.,* 2005). On the other hand, sediment physical properties also influence microbial metabolism (Jones, 1995).

In our Mediterranean study system no input (or few) of groundwater was expected, where rivers and streams are usually affected by severe hydric stress, normally in the summer (Gasith & Resh, 1999). During drought periods, sediment permeability and texture can regulate the water availability in the interstitial environment. Examples of previous studies indicated that the drastic changes in discharge are common in Mediterranean streams and determine important changes in the quality of the available organic matter flowing in the streams (Vázquez *et al.,* 2007). In such severe changes in discharge, a patchiness of drought and wet streambed surfaces occur where the differential sediment water content might be affecting microbial activity (Romaní *et al.,* 2006). However, these sediment habitats, as well as decaying leaves, provide refuge for microorganisms and organic matter storage, being the habitats more resistant to drought (Ylla *et al.,* 2010).

Hyporheic zone physical properties widely change in space (and time) creating physicochemical gradients in the sediment interstices (Brunke & Gonser, 1997). Mueller *et al.* (2013) reported that content of dissolved O_2, electric conductivity, pH, redox potential and nitrite strongly differed between coarse and fine substratum. Further, the dissolved O_2, organic carbon, microbial biomass, and hydrolytic activity declined with depth (especially in the first meters), and changed over time, apparently in response to varying in discharge (Chafiq *et al.,* 1999). Within this context, the main objective of this study was to explore how sediment moisture spatial heterogeneity influences the biofilm functioning in a sandy alluvial reach of a Mediterranean river. Sediment samples were collected in four sampling sites at different depths (up to 60 cm) for two periods (June and July 2013) with different hydrological conditions (from moderate flood to drought) in order to cover the wider spectra of sediment moisture conditions. In this study, we are working with sediments with high hydraulic conductivity (K) and porosity. Therefore, we expected a rapid water exchange between surface and interstitial water and thus no large differences in physical and chemical conditions in depth (i.e. no anaerobic layers, no large differences in water temperature). In consequence, under this premise, we expected the variations in microbial activity and density would be related to organic content and moisture availabilities within the sediments.

3.3.2 METHODS

Study site

We conducted this study at a downstream reach of the Tordera river. The selected reach was on its main course marked with (red) star in Figure 2.1a (see General methods). It was characterized by a large coarse sand sediment deposit (moderately and poorly sorted) on the active channel migration (over time). The cross-section consists of low percentage of mud (silt and clay) particles, and appears as a subterranean layer with high permeability within bed-sediments (composed of nearly 90% by weight of mixed coarse sand-gravel).

Sampling strategy

The selected reach was sampled under moderate high flow (Q=2200 L/s, June 2013) and drought (Q=30 L/s, July 2013) conditions. Four sampling sites were selected within the studied reach following a gradient of hydrological and moisture conditions: Site 1 was in a fast flowing water on the river channel, Site 2 was located in slow flowing and shallow water, Site 3 was located where no flowing water but sediment was water saturated, and Site 4 was located at surface drought sediment but wet in depth (Figure 2.1b). Since the bed channel morphology has moved over time, we tried to maintain these conditions for each site accordingly during the two study periods.

Sediment cores were performed at each site by Eijkelkamp Multisampler (E-365-04.02.SA). For each site we collected sand samples from surface layer (0-5 cm depth), 20 cm (15-25 cm depth) and 50 cm (45-60 cm depth). From each core, we collected about 1.0 kg of sediment for each layer and placed them in plastic cylindrical vials in order to minimize sediment disruption. Three replicates were considered for each site and depth. At each sampling site, we also pumped the interstitial water (or the subsurface water in pore spaces) from hyporheic zone using piezometers with a hand pump (Solinst, mod 140) at the different depths of 20, 50 and 100 cm. From each layer we collected 50 mL of water. At the same time, samples from flowing river channel water were collected.

During each sampling campaign, temperature (T), pH, dissolved oxygen (DO) and electrical conductivity (EC) were measured *in situ* with the portable devices (WTW 340i) for all water samples collected (river and interstitial water).

Both sediment and water samples were kept in a cool box preserved at 4^{o}C, and then transported for being analyzed in laboratory. The sediment and water samples were used to determine three extracellular enzyme activities (i.e. β-glucosidase, leucine-aminopeptidase and phosphatase) which were measured on the same sampling day or maximum the following day in order to not over-and/or infra-estimate microbial metabolism measurements. Samples for bacterial cell density in sediment and water were fixed with formalin 2% with 10 mL of filter sterilized river water (0.2 µm, Nylon filter) and kept at room temperature until analysis (see the details in General methods).

In sand sediments, we performed likewise the distribution of particle sizes (sieve and hydrometer analyses) as well as determination of organic matter (OM) (see General Methods), carbon (C), nitrogen (N) and phosphorus (P) contents. The water samples for physicochemical parameters such as nitrate (NO_3), sulfate (SO_4), soluble reactive phosphorus (SRP), ammonium ($N\text{-}NH_4$), total nitrogen (TN) and dissolved organic carbon (DOC). Further, three dissolved organic matter quality optical parameters - namely, the humification Index (HIX), fluorescence Index (FI), and SUVA254. These indices provide information about origin (terrigenous or aquatic), potential bioavailability for microbiota and aromaticity of dissolved organic matter.

Samples for DOC were 3% acidified with HCl 2M and stored refrigerated for posterior analysis. Samples for HIX, FI and SUVA254 were filtrated with nylon filters 0.2 µm (Whatman).

Chemistry of water samples

Soluble reactive phosphorus (SRP) in water was measured using the molibdate method following Murphy and Riley (1962). Nitrate (NO_3) and sulphate (SO_4) were analyzed with a Metrohm ion chromatograph. Total nitrogen (TN) was determined by oxidative combustion and chemoluminescence. Ammonium ($N\text{-}NH_4$) concentration was measured using the salicylate method of Reardon *et al.* (1966). Dissolved organic carbon (DOC) was determined via combustion in a Shimatzu (model TOC-5000) total carbon analyzer provided with an infrared sensor.

The humification index (HIX) was calculated according to Zsolnay *et al.* (1999) as the ratio of the emission spectrum (excited at 255 nm) integral over the spectral range of 434–480 nm, to the integral of emission spectrum over the spectral range of 330–346 nm (excited at the same wavelengths). The Fluorescence Index (FI) was measured (by the method of McKnight *et al.* (2001)) with a spectrofluorophotometer (Shimadzu

RF-5301 PC), and SUVA254 values were determined by dividing the UV absorbance measured at λ = 254 nm by the DOC concentration (Weishaar *et al.,* 2003) and are reported in the units of liter per milligram carbon per meter.

Sediment physical characteristics

The particle size distribution curves (for June and July 2013, see § 3.1) were used to estimate certain sediment physical characteristics such as the effective grain size (d_{10}) corresponded to 10 percent finer particles, the porosity (*n*) and the hydraulic conductivity (K). As for the percentage of sand and mud (silt and clay) in the particles finer than 75 μm, they were estimated to determine the soil texture as well as to follow their interaction with biological processes, especially the percentage of clay in sand sediment.

Sediment C, N and P content

Samples of 1 cm^3 of wet sediment (approx. 1 g wet mass, 3 replicates per each depth) were placed in plastic vials and stored at - 20^oC until the analysis of C, N and P contents following the method of Zimmermann *et al.* (1997). Sediment samples were unfrozen in drying-oven at 105^oC during at least 24 hours. After being completely dried, the sediments were crushed with a mortar to achieve the homogenous grain sizes of ~ 1 mm in diameter for measuring P. After that step, samples were first digested and further analyzed with the method described for the soluble reactive phosphorus (SRP) analysis in water by Murphy and Riley (1962). We prepared ~ 20 mg of dry weigh of homogenized sediment samples, and placed in glass tubes. We added 20 mL of Milli-Q water and 2 mL of oxidation reagent (5 g $K_2S_2O_8$ plus 3 g H_3BO_3 in 100 mL of NaOH 0.375 M) in the sediment samples, and then digested in an autoclave (90 minutes at 110^oC, Grasshoff *et al.,* 1983). After this basic digestion, the samples were filtered with GF/F filters (0.7 μm, glass fiber filter Whatman). A volume of 10 mL of each digested sample was taken for measuring the P content. The standard solutions were prepared from 1000 mg/L PO_4-P mother solution (4.39 g KH_2PO_4 diluted in 1000 mL Milli-Q water). For the next step, we added to each sample and standard solution 1 mL of reagent 1 (0.2 g $C_4H_4O_7SbK$; 111 mL H_2SO_4; 11.2 g $[(NH_4)_6Mo_7O_{24}\cdot4H_2O]$), and then shook properly with vortex mixer. Immediately, we added 0.2 mL of reagent 2 (27 g $C_6H_8O_6$ diluted in 500 mL Milli-Q water) into both sample and standard solutions, and shook properly. The final prepared solutions were kept in dark condition during 45 minutes. Finally, we measured the color intensity of the sample and standard solutions with 880 nm spectrophotometer (UV-1800, Shimadzu).

For determining C and N contents, 5 mL of Milli-Q water were added to the samples, and then sonicated during 5 min and mixed with vortex. We extracted the volume ~ 5 mL from each sample to clean vials. We added again 5 mL of Milli-Q to each rested sample, and followed exactly the first step above to improve the extraction. The collected extracts from samples (10 mL) in each clean vial were dried at 70^{o}C during 4 days. After being completely dried, we weighed a dry mass of ~ 2 mg (from 1 to 3 mg available) of the homogenized sediment extracts into silver capsules (9 mm height, 5 mm diameter) to the nearest 0.001 mg with an ultra-micro balance. Then, we closed the silver capsules (not to lose the mass), so that the samples were ready for the combustion at 975^{o}C using an elemental analyzer (2400 Series II CHNS/O, Perkin Elmer). C, N and P contents were expressed as % in weight (i.e. g C per 100 g of sediment dry weight (DW)).

Extracellular enzyme activities

Enzymatic activities of leucine-aminopeptidase (EC 3.4.11.1), β-glucosidase (EC 3.2.1.21), and phosphatase (EC 3.1.3.1-2) were analyzed in sand sediments and in interstitial and river water samples from the Tordera river. These potential enzyme activities were determined spectrofluorometrically by following the methodology described by Romaní and Sabater (2001). Samples of 1 cm^3 of wet sediment (three replicates per each depth) and 4 mL in case of interstitial and river water samples were used for the analysis. We added 4 mL of the synthetic water (12 mg/L Na_2SO_4, 20 mg/L Na_2SiO_3, 30 mg/L $CaCl_2$, 1 mg/L KCl, 2 mg/L $MgSO_4$ and 20 mg/L $NaHCO_3$ in autoclaved Milli-Q water in order to reproduce the chemical composition of pristine river) into the sediment sample vials, and then added 120 μL of the artificial substrate for each enzyme (MUF-methylumbelliferyl-β-D-glucoside, MUF-phosphate, AMC (aminomethyl-coumarin)-leucine; for β-glucosidase, phosphatase and leucine-aminpeptidase measurements, respectively). All three enzyme assays above were conducted at 0.3 mM final substrate concentration (substrate saturating conditions). After adding the artificial substrates of MUF or AMC, incubation of samples was performed during one hour at stream water temperature in a shaking bath (IKA® KS260 basic) and in the dark. Together with the samples we also incubated a blank (autoclaved Milli-Q water) to control abiotic degradation of the artificial substrate, and several standards of MUF and AMC. After incubation, 4 mL of glycine buffer (pH 10.4) were added to samples in order to stop the microbial metabolism and reach the maximal fluorescence for MUF and AMC. This buffer solution was prepared (1 L) by diluting 196.4 mL of glycine (37.534g/500 mL) with NaOH (8g/L). Standard solutions of MUF and AMC were used (0.1-100 μM for sediment samples, and 0.01-1 μM for water samples).

Before fluorescence measurements, sediment samples were centrifuged at 2000 rpm during 2 minutes to avoid that sediment particles interfere in fluorescence measurements. Then, fluorescence of samples (approximately 300 μL) was measured at 365-455 nm (excitation-emission, wavelengths; respectively) for MUF-substrates and at 364-445 nm for AMC-substrates using a fluorimeter (TECAN, Infinite M200 PRO).

Statistical treatments

Data analyses and comparison of the mean values of observations were performed using the IBM SPSS v. 9. Differences among sampling dates (June and July 2013), sites and depths for C, N, P content, OM content, bacterial density and extracellular enzyme activities were analyzed by a three-way multivariate analysis of variance (MANOVA) using triplicate samples for each factor. Further analyses by considering the two periods and the four sites separately were also performed in order to being able to distinguish differences between sites and depths for each period after applying a Tukey-b multiple comparison test. Multivariate redundancy analysis (RDA) was used to investigate on the relationships between the sand physical variables and the microbial sediment microbial metabolism, and the relationship between sand physical variables and water chemistry and microbial metabolism. RDA analyses were performed by using R. All observations data were log-transformed ($y = \log [x+1]$) to improve normality and homogeneity of variance. The significant variables were selected by forward selection method. All tests were considered significant at $p < 0.05$. Correlations between some variables were examined using Pearson’s correlation coefficient.

3.3.3 RESULTS

Physicochemical variables of water

Water physicochemical properties during the two sampling dates are summarized in Table 3.8. Due to no significant differences in depth, the values are summarized per sites and periods. In June, the temperature of interstitial water, dissolved O_2, SRP and $N\text{-}NH_4$ were not different among four sampling sites, but there were significant differences in the concentrations of EC (df=3, F=25.495, $p < 0.001$), NO_3 (df=3, F=11.423, p=0.004), SO_4 (df=3, F=45.796, $p < 0.001$) and TN (df=3, F=10.770, p=0.005), which showed the lowest values at site 4 (dry-wet condition, Tukey *t*-test, $p < 0.05$). However, there was no significant difference among depths in June. In contrast, in July, there were not physicochemical differences among sites.

In June, SRP concentration was higher in river water than in interstitial water, but not in July when a significant increase in SRP along the depth (at 1 m depth) was detected (df=3, F=6.122, p=0.024).

Our results showed NO_3 was negative correlated with DO (n=24, r=-0.426, p=0.038); while TN was negative correlated with SO_4 (n=24, r=-0.464, p=0.022), and also with EC (n=24, r=-0.513, p=0.01). $N\text{-}NH_4$ remained *quasi*-constant during both dates, its content was found positively correlated with TN and NO_3 (n=24, r=0.424, p=0.039; n=24, r=0.367, p=0.078; respectively). The temperature in river water was lower than in interstitial water, and also negative correlated with TN and NO_3 (n=24, r=-0.509, p=0.011; n=24, r=-0.750, $p < 0.001$; respectively).

Dissolved organic carbon (DOC) was at an average of 1.81 (±0.05) mg/L and no differences between sampling dates, sites or depths were detected. Indices related to DOM quality were also not varying among factors excepting HIX which was higher in interstitial than in river water during both periods.

Sediment physical parameters

Overall, there were significant differences in values of hydraulic conductivity (df=3, F=8.367, p=0.02) and porosity (df=3, F=13.773, $p < 0.001$) among four sites for both sampling dates (June and July 2013). In this study, hydraulic conductivity (K) was significantly higher at site 1 (under fast flowing water) and lower at site 3 (saturated sediment) in June (Table 3.9); while it was significantly higher at site 2 (under slow flowing water) and lower at site 4 (dry-wet depth site) in July. There was no significant difference for the percentage of sand and mud (silt and clay) between sites and dates. In addition, we did not see differences for these parameters among depths. K was found to correlate negatively to SRP in interstitial water (n=14, r=-0.724, p=0.003) and P sediment content (n=24, r=-0.481, p=0.017), but positively to OM content (n=24, r=0.499, p=0.013).

Table 3.8*: Physicochemical characteristics of water (mean (±SE)) collected at four sites (downstream) of the Tordera river during June and July 2013.*

Site	Dissolved oxygen (mg/L)	Electrical conductivity (µs/cm)	T. (°C)	pH	NO_3 (mg/L)	SO_4 (mg/L)	SRP (mg/L)	N-NH_4 (mg/L)
				June				
River	8.05	511.67	16.90	8.11	1.14	34.75	0.48	0.014
1	5.64 *(±0.24)*	501.33 *(±9.21)*	19.47 *(±0.88)*	7.65 *(±0.05)*	1.57 *(±0.02)*	34.63 *(±0.21)*	0.48 *(±0.02)*	0.022 *(±0.01)*
2	6.93 *(±0.49)*	527.67 *(±2.91)*	20.30 *(±0.21)*	8.04 *(±0.01)*	1.31 *(±0.01)*	34.35 *(±0.24)*	0.40 *(±0.03)*	0.011 *(±0.00)*
3	7.28 *(±0.12)*	521.00 *(±1.53)*	19.70 *(±0.21)*	7.97 *(±0.01)*	1.20 *(±0.03)*	34.65 *(±0.11)*	0.51 *(±0.02)*	0.011 *(±0.00)*
4	5.88 *(±0.76)*	461.50 *(±2.04)*	21.75 *(±0.29)*	7.95 *(±0.03)*	1.13 *(±0.12)*	30.29 *(±0.52)*	0.46 *(±0.04)*	0.013 *(±0.00)*
				July				
River	10.03	606.67	23.60	8.32	0.35	48.66	0.22	0.014
1	7.82 *(±0.46)*	594.33 *(±2.33)*	27.83 *(±0.59)*	7.92 *(±0.05)*	0.59 *(±0.10)*	45.99 *(±2.15)*	0.42 *(±0.06)*	0.011 *(±0.00)*
2	7.44 *(±0.85)*	591.00 *(±4.36)*	26.07 *(±0.13)*	8.09 *(±0.13)*	0.48 *(±0.09)*	41.26 *(±7.07)*	0.40 *(±0.06)*	0.009 *(±0.00)*
3	5.94 *(±0.47)*	603.78 *(±3.73)*	28.27 *(±0.35)*	7.89 *(±0.04)*	0.70 *(±0.03)*	46.15 *(±2.12)*	0.45 *(±0.02)*	0.011 *(±0.00)*
4	6.37 *(±0.08)*	597.67 *(±1.09)*	28.15 *(±0.37)*	7.89 *(±0.04)*	0.70 *(±0.04)*	47.13 *(±0.49)*	0.51 *(±0.02)*	0.011 *(±0.00)*

Organic matter content

The organic matter (OM) content was not reported significantly difference between June and July (see Table 3.10). The average value of OM was about 0.5 (±0.31) % of g DW. In June, there was significantly higher value at surface (0-5 cm) of site 2 (Tukey *t*-test, $p < 0.05$), while no significant differences were found among depths for the other sites (1, 3 and 4). Overall, in July, there was significantly higher OM content at 50 cm depth, while lower OM content was recorded at surface layer (Tukey *t*-test, $p < 0.05$).

***Table 3.8**: continued*

Site	TN (mg/L)	DOC (mg/L)	HIX	FI	SUVA254
			June		
River	0.72	1.85	4.77	1.56	1.72
1	0.97 *(±0.03)*	1.69 *(±0.32)*	4.97 *(±0.47)*	1.58 *(±0.02)*	0.70 *(±0.39)*
2	0.81 *(±0.02)*	1.95 *(±0.28)*	5.52 *(±0.31)*	1.57 *(±0.01)*	2.09 *(±0.90)*
3	0.75 *(±0.01)*	1.88 *(±0.13)*	5.06 *(±0.03)*	1.59 *(±0.02)*	1.99 *(±0.77)*
4	0.70 *(±0.07)*	1.93 *(±0.11)*	6.18 *(±0.29)*	1.57 *(±0.00)*	2.12 *(±0.55)*
			July		
River	0.40	1.82	2.51	1.63	1.92
1	0.61 *(±0.07)*	1.74 *(±0.09)*	2.99 *(±0.16)*	1.67 *(±0.02)*	1.70 *(±0.03)*
2	0.58 *(±0.06)*	1.71 *(±0.02)*	3.14 *(±0.14)*	1.68 *(±0.01)*	1.64 *(±0.34)*
3	0.73 *(±0.02)*	1.80 *(±0.09)*	2.98 *(±0.11)*	1.65 *(±0.01)*	2.04 *(±0.16)*
4	0.70 *(±0.04)*	1.73 *(±0.00)*	3.01 *(±0.14)*	1.71 *(±0.06)*	1.99 *(±0.03)*

Microbial activity in sand sediment

The enzymatic activities of β-glucosidase, peptidase and phosphatase within sand sediment showed significantly differences between June and July (Table 3.10). All three extracellular enzymes showed higher values during the summer low discharge (in July) compared to those at the same year in June: slightly higher for the β-glucosidase activity, but much higher for the peptidase and phosphatase activities (Figure 3.7). Changes in extracellular enzyme activities between sites varied depending on the sampling date and sampling depth (Date × Site and Site × Depth effects).

Table 3.9: *Summary of hydraulic conductivity (K, cm/s) values, estimated by the Kozeny-Carman formula (see* § 3.1*), among sites and depths in June and July 2013 (Tukey t-test p < 0.05). Lower cases letters represent the significant differences among sites. The bold values are the highest and lowest significant levels.*

Site	June				July			
	Surface	20 cm	50 cm	Mean	Surface	20 cm	50 cm	Mean
1	0.75	0.50	0.58	**0.61**^{a}	0.37	0.56	0.55	0.49^{ab}
2	0.50	0.60	0.50	0.53^{a}	0.63	0.59	0.69	**0.64**^{a}
3	0.25	0.27	0.35	**0.29**^{b}	0.40	0.40	0.47	0.42^{ab}
4	0.31	0.49	0.57	0.46^{ab}	0.35	0.22	0.39	**0.32**^{b}

Table 3.10: *Summary of multivariable analysis in sediment during June and July 2013. The values indicate probabilities after the three factors ANOVA for date (D) effect, S (site) effect, d (depth) effect and their interactions. n.s stands for non-significance.*

Source of variation	β-Glu	Pep	Pho	OM	C	N	P	Bacterial density
D	**<0.001**	**<0.001**	**<0.001**	*n.s*	0.003	**<0.001**	*n.s*	0.005
S	**<0.001**	0.010	0.010	0.028	*n.s*	*n.s*	*n.s*	**<0.001**
d	**<0.001**	*n.s*	0.010	*n.s*	*n.s*	*n.s*	*n.s*	0.001
D × S	**<0.001**	**<0.001**	**<0.001**	*n.s*	*n.s*	*n.s*	0.037	*n.s*
D × d	*n.s*	**<0.001**	**<0.001**	0.044	*n.s*	*n.s*	*n.s*	0.023
S × d	**<0.001**	**<0.001**	0.001	*n.s*	0.003	0.001	*n.s*	0.038
D × S × d	*n.s*	**<0.001**	**<0.001**	*n.s*	*n.s*	*n.s*	*n.s*	0.003

The β-glucosidase activity in June showed the lowest values at sites 2 and 4, while in July the lowest activity was measured at site 3. In both sampling periods, β-glucosidase activity significantly decreased in depth being the lowest at 50 cm depth (Tukey *t*-test, $p < 0.05$). However, this depth gradient varied among sites, being more evident at sites 3 and 4 (Site × depth effect, Table 3.10).

In June, leucine-aminopeptidase activity was the lowest at sites 1 and 2; while it was the highest at these two sites in July (Figure 3.7). Peptidase activity at 50 cm depth was higher than at surface sediment in June (Tukey *t*-test, $p < 0.05$), especially for sites 3 and 4. However, in July, peptidase decreased at 20 and 50 cm depth (Figure 3.7).

In June, phosphatase activity at site 1 was the lowest, but in contrast activities were higher at sites 1 and 2 in July (Figure 3.7). Phosphatase activity in June significantly decreased at 20 cm depth (Tukey *t*-test, $p < 0.05$, Figure 3.7), but it showed large variability among sites. In July, phosphatase activity showed an increase in depth at sites 1 and 2, but a decrease at site 3.

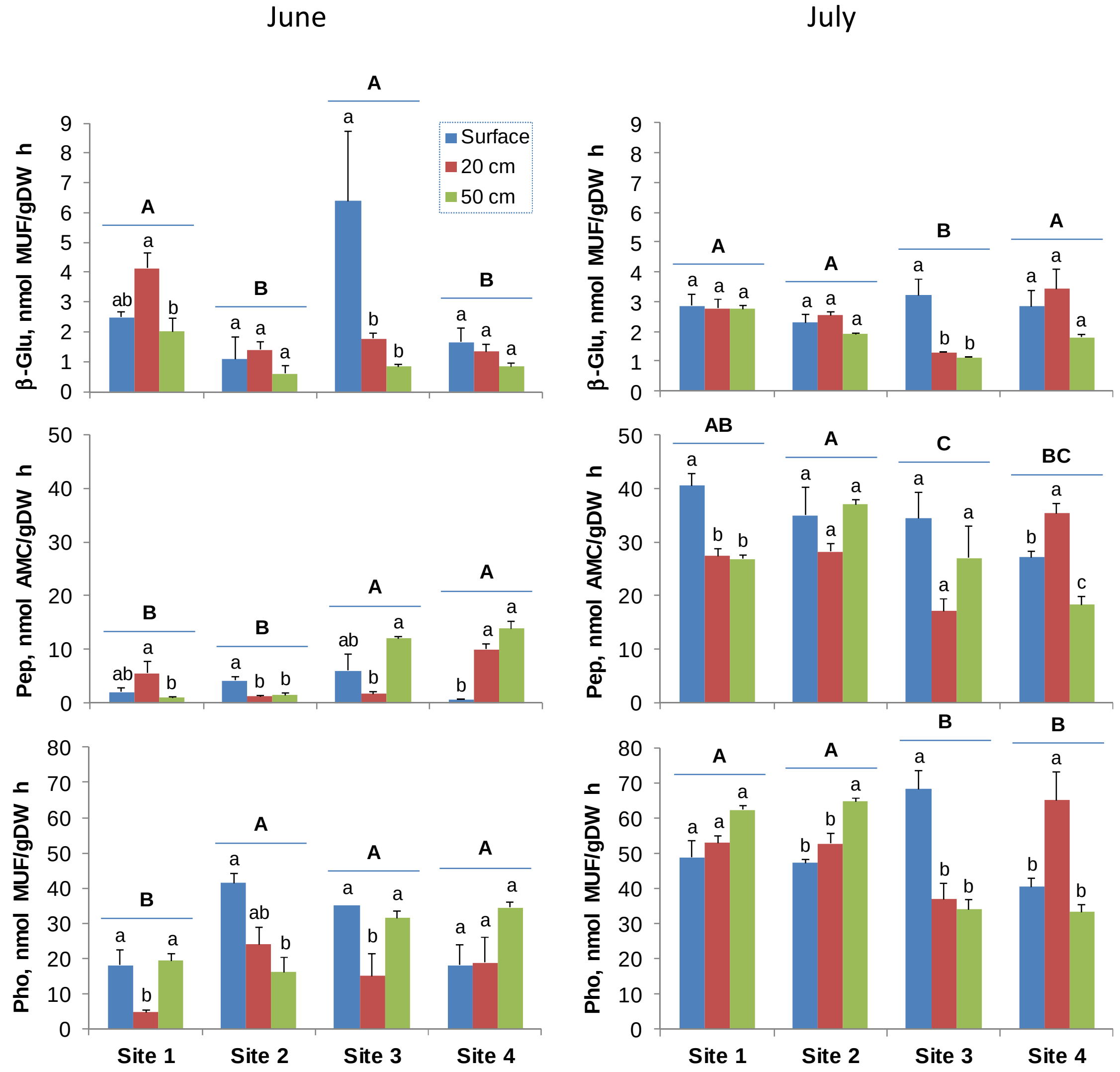


Figure 3.7*: Microbial activities (i.e. β-glucosidase, peptidase and phosphatase) in the sand sediment at the study reach during June and July 2013. The capital letters (A is the greatest compared to B then C) represent the significant differences among sites, while differences among depths are shown with small letters, after Tukey's multiple comparison tests (p <0.05).*

Microbial activity in interstitial water

The enzymatic activities of β-glucosidase, peptidase and phosphatase in interstitial water were significantly different between sampling periods (Table 3.11). As shown in

Figure 3.8, β-glucosidase was higher in June than in July. However, β-glucosidase activity did not show significant differences between sites and depths. Peptidase activity was higher in river water than in interstitial water both in June and in July, and lower at site 4. Phosphatase activity also showed higher values in river water than in interstitial water in July, while in June activity in interstitial water decreased only at sites 3 and 4.

For both June and July, there was lower value at site 4 for peptidase activity, and at sites 3 and 4 for phosphatase. During both dates, the activities of peptidase and phosphatase decreased significantly in depth showing simultaneously higher value at 20 cm layer (Tukey *t*-test, $p < 0.05$).

Table 3.11*: Summary of multivariable analysis in water during June and July 2013. The letters D, S and d represent date, site and depth; respectively. n.s stands for non-significance.*

Source of Variation	β-Glu	Pep	Pho	Bacterial Density
D	0.020	**<0.001**	**<0.001**	**<0.001**
S	0.027	*n.s*	**<0.001**	0.007
d	*n.s*	**<0.001**	**<0.001**	0.011
D × S	*n.s*	*n.s*	0.011	**<0.001**
D × d	*n.s*	**<0.001**	**<0.001**	0.035
S × d	*n.s*	**<0.001**	**<0.001**	*n.s*
D × S × d	*n.s*	**<0.001**	**<0.001**	**<0.001**

Sediment C, N and P content

Mean C, N and P contents of the studied sediments were of 0.0089(±0.0068), 0.0011(±0.0008) and 0.0029(±0.0012) % in weight, for C, N and P, respectively. The C and N contents were significantly different during the two sampling dates (June and July), but not for the P content (see Table 3.10). All nutrients elements (C, N and P) were noted more higher in July than in June. The difference in the C and N contents in depth depended on the site (Site x depth effect, Table 3.10).

In June, there were generally significant differences in C and N contents among sites (df=3, F=5.462, p=0.005; df=3, F=5.923, p=0.004, for C and N, respectively) – e.g. the highest values were measured at site 4 (followed by sites 3, 2 and 1) for C, and sites 3 and 4 (followed by sites 2 and 1) for N; while it remained unchanged for the P content. Overall, no C, N or P content was significantly different among depths during this period (see Figure 3.9).

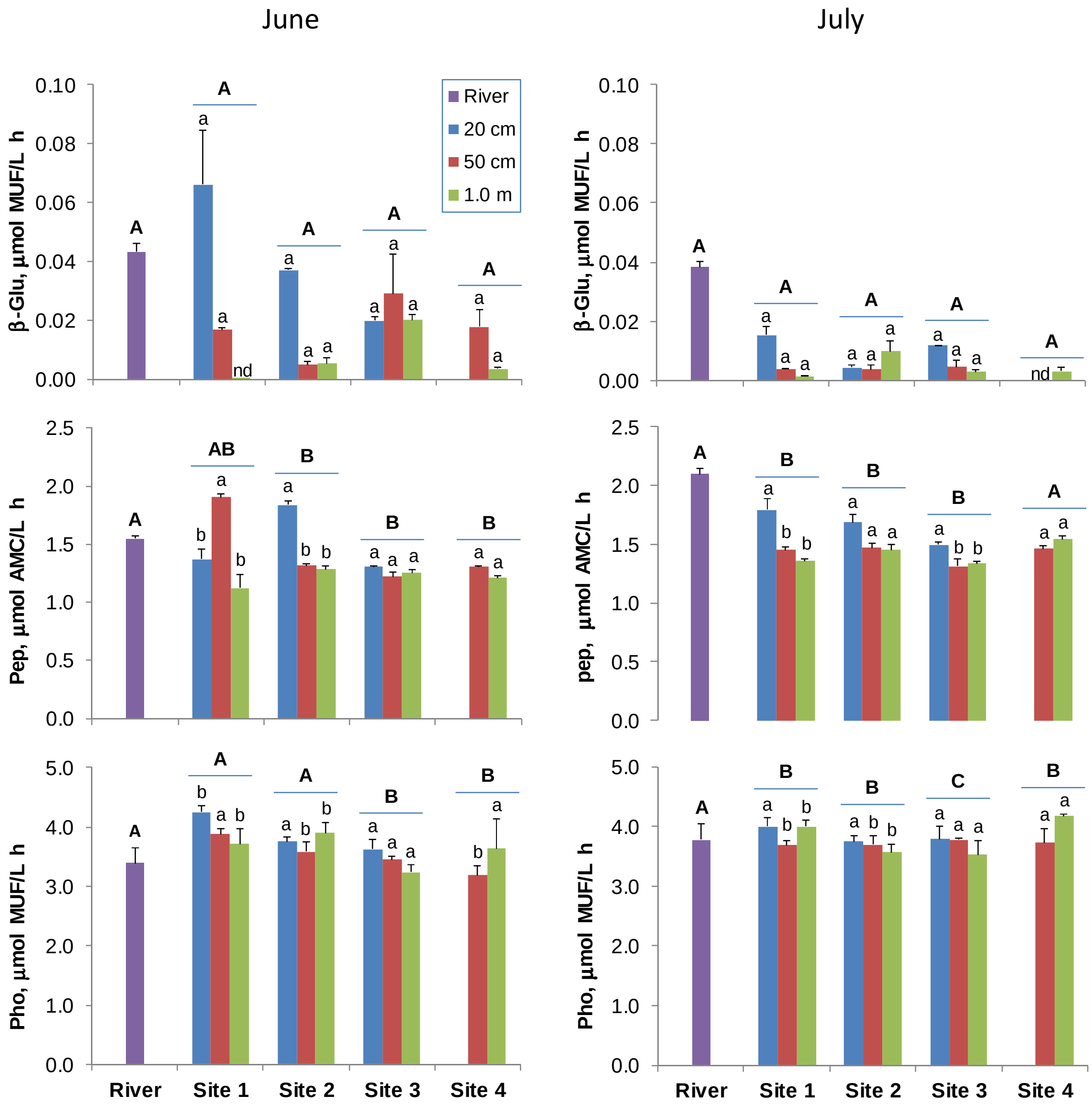


Figure 3.8: *Microbial activities (i.e. β-glucosidase, peptidase and phosphatase) in water during June and July 2013. The capital letters (A is the greatest compared to B then C) represent the significant differences among sites, while among depths were shown with small letter. n.d stands for non-detection.*

In July, the P content was reported significantly (df=3, F=3.730, p=0.025) – higher at site 4 and lower at site 2; whereas the C and N contents remained unchanged among sites. Similar to that observed in June, there were not significant differences of C, N or P content in depth in July. As shown in Figure 3.9, there was no significant difference in P content in depth, but in general higher values were measured at the deeper depths.

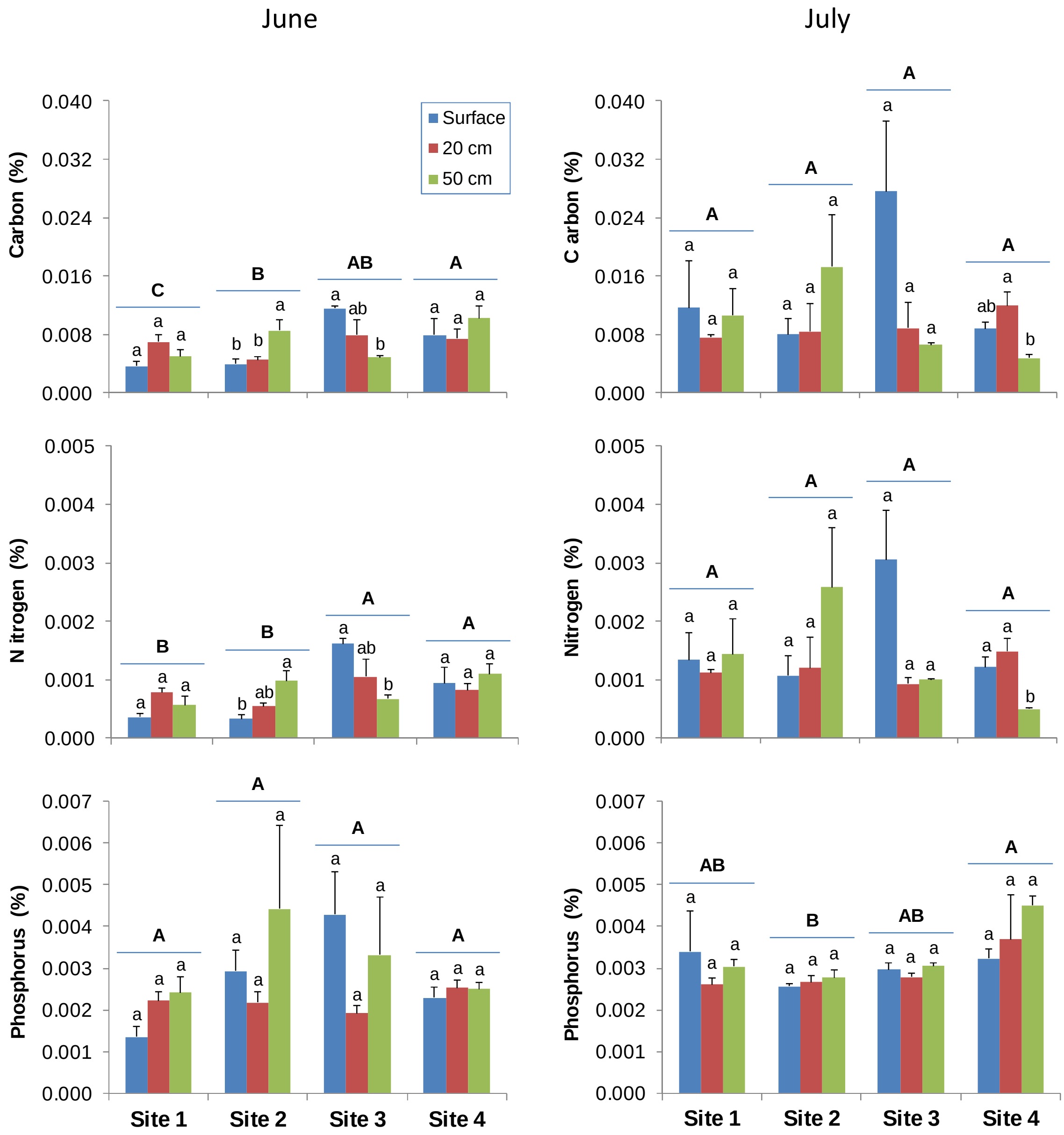


Figure 3.9*: Percentage of C, N, P contents in sediment during June and July 2013. The capital letters (A is the greatest compared to B then C) represent the significant differences among sites, while among depths were shown with small letters.*

Bacterial density in sediment and water

Bacterial density in sediment samples was significantly higher in July than in June (Table 3.10, Figure 3.10). In both periods there were significantly differences among sites and among depths. In June, bacterial abundance was higher at site 1, and lower at site 2. Bacterial cells were less abundant at 50 cm depth and higher at surface (0-5 cm) depth for overall sites during June (Tukey *t*-test, $p < 0.05$). In July, the bacterial density also showed higher values at site 1, but lower at site 4 (dry-wet condition). In this period, differences in bacterial density in depth were depending on the site (site $\times$ depth; F=5.027, p=0.02).

The bacterial density in water was significantly different during both sampling dates, higher values being measured in June (Table 3.11, Figure 3.10). In June, the highest bacterial density was measured in river water, while in July bacterial density was the highest at the hyporheic site 1. During both periods, the bacteria were in general less abundant at 100 cm depth, but showed higher values in river flowing water (Tukey *t*-test, $p < 0.05$).

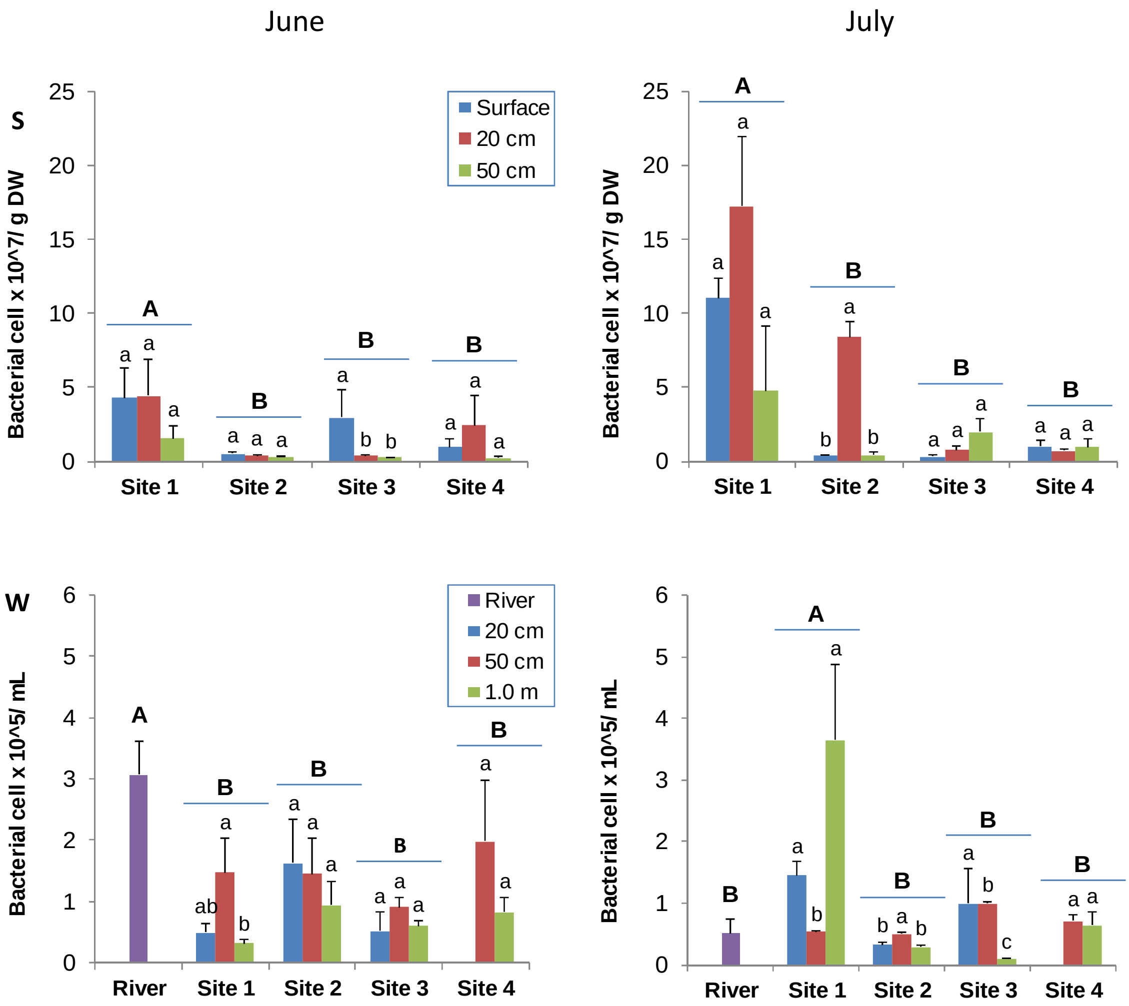


***Figure 3.10**: Bacterial density in sediment (S) and in water (W) during June and July 2013. The capital letters (A is the greatest compared to B then C) represent the significant differences among sites, while among depths were shown with small letters.*

Relationships between physicochemical and microbiological parameters

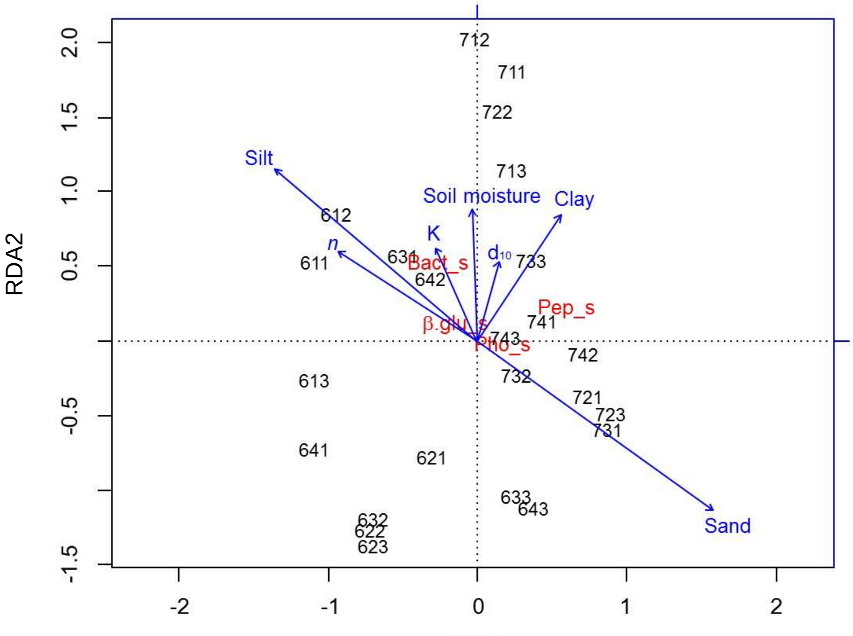
The microbial activities (β-glucosidase, leucine-aminopeptidase and phosphatase) in sediment and water samples were likely associated with the environmental factors (see Table 3.12). In water, apart from that correlation reported, TN was negatively correlated to C and N contents (n=14, r=-0.585, p=0.028; n=14, r=-0.637, p=0.014; respectively).

Table 3.12*: Summary of the Pearson correlation (r) and sample size in sediment and water during June and July 2013. The suffixes s and w represent sediment and water samples, respectively. Significant levels (2-tailed): ** p < 0.01, * p < 0.05.*

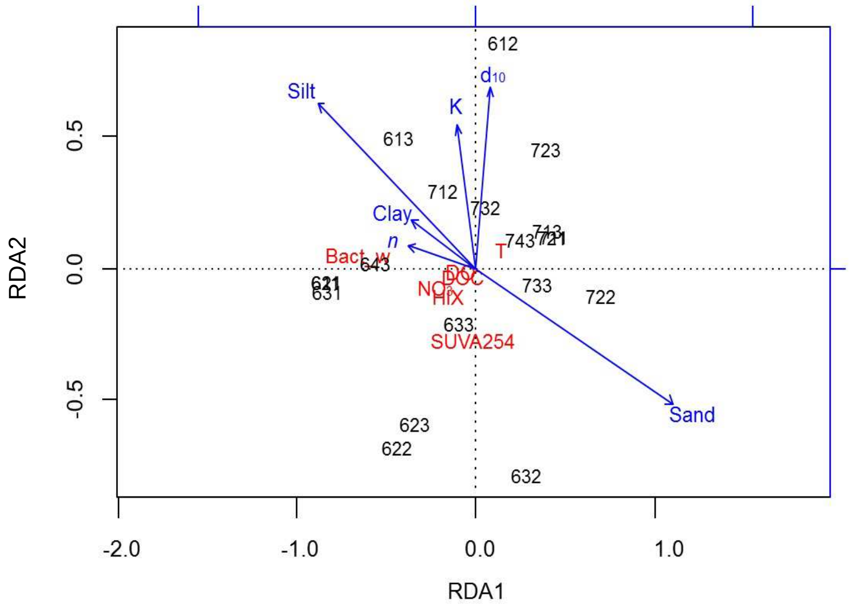
Variable	C	N	DO	T	EC	NO_3	SO_4	NH_4	TN	HIX	K	% clay
β-glu_s								0.66* 14				0.57** 24
Pep_s	0.56** 24	0.62** 24		0.80** 14	0.72** 14	-0.90** 14	0.84** 14		-0.81** 14			
Pho_s	0.63** 24	0.68** 24		0.66** 14	0.60* 14	-0.88** 14	0.68** 14		-0.91** 14			
β-glu_w				-0.41* 24				0.72** 24		0.41* 24		
Pep_w			0.50* 24								0.54* 14	
Pho_w							0.43* 24					

In sediment, the RDA which explained 27.2% of total variance showed a spatial distribution of the samples due to microbial parameters mainly separated by date, samples from June being separated to those from July (Figure 3.11a). These results showed the peptidase activity was relevant in summer (July), during which the amount of clay was also higher. As for the bacterial abundance, it coupled highly associated with the hydraulic conductivity (K) value. β-glucosidase activity followed the porosity and silt vectors, whereas phosphatase activity variability between sites and depths was not very significant.

In interstitial water, 33.2% of the total variance is explained by the RDA analysis. The results showed the association between bacterial density and porosity. The amount of clay was more likely to contribute to the bacteria abundance than that of silt as clay and porosity have smaller angle between their vectors (n=24, r=-0.497, p=0.014). In this study, the variations of NO_3 and HIX, SUVA 254 and bacteria in water were shown a direction opposite to temperature. Variation of SUVA254 was also opposite to hydraulic conductivity (Figure 3.11b).



(a)



(b)

Figure 3.11*: Multivariate analyses in sediment (a), and in water (b). Three number code for the samples indicate the date (6-June,7-July), the site (1, 2, 3, 4), and the depth (1-surface, 2-20 cm, 3-50 cm for sediment, but 1-20 cm, 2-50 cm, 3-100 cm for water).*

3.3.4 **DISCUSSION**

This study revealed high hyporheic hydraulic conductivity (K) (between 0.22 and 0.75 cm/s, Table 3.9) in comparison to the limits (from 0.0001 to 1 cm/s for sand) given by Freeze and Cherry (1979). The porosity ranged between 0.37 and 0.41, and was similar to the results from other authors (e.g. from 0.29 to 0.49; Morris & Johnson, 1967). In the streambed deposits and alluvial sediments, the variability and distribution of K within hyporheic zone act as key factors determining the volume of large-scale and small-scale exchange processes (Brunke & Gonser, 1997), as well as determining the residence time of water within the riverine aquifer. In addition to those processes, other authors indicated the direction of the exchange processes varies with the hydraulic gradient when flow (volume/ unit time) depends on sediment permeability; whereas the local conditions are substantially influenced by the permeability and roughness of the sediments (Triska *et al.,* 1989; Meyer, 1990; Vervier *et al.,* 1992; Bencala, 1993). Within the streambed interstices, organic matter and nutrients can be retained, transformed and stored (Gibert *et al.,* 1990). Conjugated with these processes, those constituents can vary with the permeability (high or low ranges) of the layers in which they passed.

The specific physical conditions at our study reach, showing high hydraulic conductivity (K) and hence high permeability, determined high connectivity in depth. Hoelhn *et al.* (1983) and Gölz *et al.* (1991) reported that the quality of the downwelling surface water is normally altered during its passage through the first meters of the infiltrated sediments. However, in our study site, several physical and chemical parameters remained *quasi*-constant at each depth of all sites during both moderate high and low discharges (Table 3.8). For example, in the case of temperature not statistically significant differences between depths were detected. It has been described that, generally, no sudden decrease in temperature occurs in the hyporheic zone and the fluctuations become lagged and attenuated with the increase of depth and distance from infiltration site (Brunke & Gonser, 1997). Similarly, no difference in water temperature was detectable in depth even between fine and coarse substratum (Mueller *et al.,* 2013). Additionally, temperature was also considered as a factor regulating exchanges of water between river and hyporheic zone (as well as among the subsurface layers), since the K value varies with temperature due to the viscosity and density of water which are temperature dependent. For example, in small streams with significant diel variations in stream temperature it was shown that reduced afternoon stream flows were caused mainly by increased infiltration rates due to increased K (Constantz *et al.,* 1994). Brunke and Gonser (1997) also noted that temperature regime in the interstices is important for the groundwater and fluvial system because the microbial activity is temperature dependent. Although the effect of temperature depth gradient in our study site was negligible, it seems that some small temperature changes may be enough to affect microbial activity since temperature was significantly and positively correlated to the activities of leucine-aminopeptidase and phosphatase in sediment. In a larger scale, increasing mean temperatures during drought period in July might be also responsible for the higher extracellular enzyme activities measured in this period (Figures 3.7 & 3.8).

A part from temperature, other physicochemical parameters from our study sites underlined high connectivity in depth. In the case of oxygen, there was no decrease in depth in contrast to what was reported from other studies. In general, the oxygen content declines with increasing depth and lateral distance from the channel until it reaches the more constant conditions of groundwater (Schwoerbel, 1961, 1964; Poole & Stewart, 1976; Pennak & Ward, 1986; Valett *et al.,* 1990; Triska *et al.,* 1993). Hyporheic metabolic activity in depth can cause reductions in the oxygen saturation from 100% in the surface water to 0% in the interstices, this depending on the residence time of the water (Brunke & Gonser, 1997). On the contrary, the main reason for having still high oxygen availability in depth in our study may be linked to the high K values (given above). This concurs with the study of Mueller *et al.* (2013) suggesting high K value easily allowed a possible exchange between the free flowing water and hyporheic zone. This condition generally causes a high supply of dissolved oxygen and high export rates of metabolic products (nitrite or ammonium) resulting in no or small differences in pH and electric conductivity between interstitial zone and free-flowing water (Table 3.8). Moreover, the exchanges (between free-running water and hyporheic zone) are strongly influenced by hydraulic gradients at the surface and in depth; and by the sediment characteristics such as K and streambed porosity (Harvey & Bencala, 1993; White, 1993). Anyway, as published by Geist and Auerswald (2007) only in coarse and well-sorted substratum, the intense exchanges between the free flowing water and the hyporheic zone are possible.

Under aerobic conditions, nitrifying bacteria oxidize ammonium ($N\text{-}NH_4$) to nitrate (NO_3). As published by Triska *et al.* (1993), sites with short travel times (i.e. thanks to high permeability) tended to be high in DO and nitrate (ranging from 0.5 to 1.3 mg NO_3/L is said to be relatively high in the study of Kasahara and Hill (2007)), but low in ammonium (see Table 3.8). However, in our study these processes may occur in low rate since not clear changes in N-NH_4 or NO_3 were measured in depth. In general, oxygen seems to be the dominant regulator of the denitrification process, with a threshold of at least 10 µmol/L (~ 0.32 mg/L) (Tiedje, 1988), although there is evidence for aerobic denitrification (Robertson & Kuenen, 1984). Due to this high oxygen in our sediment, low denitrification would be expected. However, in our study results, we noted that only dissolved O_2 was negatively correlated to NO_3, not to N-NH_4. Contrariwise, Claret *et al.* (1997) reported some hyporheic processes (i.e. nitrification and denitrification) are influenced by the distribution of fine sediments and organic matter (OM). Similarly that early mentioned study, the stimulation of biological processes by high very fine sediment content, as well as by nutrient inputs, led to the decrease in oxygen content (Chafiq *et al.,* 1999).

Heterotrophic microbial processes are fuelled by both dissolved and particulate organic matter (Wetzel, 1983). In streams, most biological processes in the hyporheic zone are linked to the use of buried material such as the storage of OM (Pusch, 1996; Naegeli & Uehlinger, 1997; Webster *et al.,* 1999; Minshall *et al.,* 2000; Sobczak & Findlay, 2002; Lamberti & Gregory, 2006). In this sense, higher extracellular enzyme activities were related to sites of OM accumulation (de Haan *et al.,* 1993). On the other hand, Findlay *et al.* (1986) reported that bacterial production was directly related to the organic content of the sediment, but our results did not generally show this clear evidence; and none of enzymatic activities, neither bacterial density was correlated to the OM content. In our study river reach, OM was low and only slightly accumulated in some sites and depths, which might determine specific increases in enzyme activities for some periods and depths. This occurred during moderate high discharge (June), when higher peptidase and phosphatase activities at the surface of site 2 occurred together with higher OM content (Figure 3.7). This higher OM content in surface sediment in June might be also responsible for the highest bacterial density in sediment together with the β-glucosidase and phosphatase activities at the surface (0-5 cm) of site 3 in June (Figures 3.7 & 3.10), coinciding with higher C and N content (Figure 3.9). Similarly, during the low flow (July), phosphatase and leu-aminopeptidase activities in sediment were higher at deeper (50 cm) depth where OM was also higher.

However, in our study reach other factors seem to be further affecting changes in extracellular enzyme activity but OM content that could be OM quality. Bacterial production might be controlled more by the composition of OM than by its absolute amount (Bärlocher & Murdoch, 1989; Münster & Chróst, 1990). In this sense, there is a gradient for the β-glucosidase activity to decrease in depth indicating a reduced use of polysaccharides in depth and thus probably a reduced availability. In contrast, the use of peptides was increasing in depth suggesting greater availability of nitrogen organic compounds in depth. However, these two enzyme activity gradients were most pronounced at sites 3 and 4, the ones being either with no flowing water or dried at the surface, which indicates an accumulation of polysaccharides at the drying sediment surface but the deep sediment (at 50 cm depth) being a store of organic nitrogen compounds. However, any significant changes in C, N and P content (or molar ratios) were observed in depth. Only slight changes in DOM quality were observed as the increase in the HIX index at the interstitial water, especially at site 4, indicating a higher degree of humification. DOM composition (i.e. HIX) could indicate the substrate value of DOM for respiration (Kalbitz *et al.,* 2003).

Interstitial biofilms are undoubtedly affected by the physical characteristics of the streambed (such as particle sizes and porosity) that have an effect on resource availability such as carbon supply and inorganic nutrients (Findlay & Sobczak, 2000; Findlay *et al.,* 2002). From our results, sediment particles were likely to control nutrients and microbial metabolism. In sediment, it was shown that the percentage of clay favored the β-glucosidase activity and P content by showing positive correlation. In the study of Bretschko (1994), particle sizes and their distribution are the most influential on sediment functioning while the significance to fluvial metabolism of transformations in interstitial water increases with sediment permeability (such as K) and with the stream water velocity, both of which increase water velocity in the deeper interstitial layers. As shown in Figure 3.8 and Table 3.9, K was significantly higher at site 1 and regulated the enzymatic activities in interstitial water (phosphatase and leucine-aminopeptidase) during moderate high discharge (June). During low discharge (July), these both enzyme activities might be also controlled by K value at site 2 where K was shown significantly the highest.

The hyporheic habitat is characterized by complex physicochemical gradients. For aquatic ecologists, the knowledge of the interstitial space created by the arrangement of particles in bed-sediments (Bretschko, 1992) is of major importance to better understand the physical, chemical and biological processes that take place in the streambeds (Gibert *et al.,* 1990; Vervier *et al.,* 1992). In our study, the hydraulic conductivity (K) was high allowing easily the hydrological connection within zones, as well as the interaction of substances among depths. From the multivariate analysis (RDA), the % clay was pointed in a direction of the peptidase activity but opposite to that of β-glucosidase in sediment, while the bacterial abundance was strongly associated to K (see Figure 3.11a). In Figure 3.11b, bacterial density in interstitial water was shown in a direction of mud (especially with % clay), due to more available surface area for bacterial attachment (see § 3.2.4), whose interconnection was strongly mediated by the porous layers. However, the amount of clay could impact negatively ($n=24$, $r=-0.553$, $p=0.005$) to permeability of the streambeds. Overall, we conclude that the hydraulic conductivity of hyporheic zone is among the most important factors of biogeochemical dynamics involved in microbial processes at the Tordera hyporheic sand downstream reaches. At our study reach, DO and temperature may not well, however, control the patterns of microbial activities within our sediments, which are contrary to the findings of other authors (Findlay *et al.,* 1993; Hendricks, 1993). The dissolved O_2 was not prevented from reaching the deeper levels by the low physical barrier such as low % mud (silt & clay). In this study, microbial activities were likely to be affected by the permeability of streambeds through which organic compounds could (spatially) contribute to some microbial activities. This could explain that the microbial activities may be regulated by other factors such as sediment matrices, very fine particles, organic matter and nutrients. As for the OM content, it involved in controlling microbial activities at some sites during moderate high and low discharges, while the P content was found to be favored due to % clay in sediment.

4

General discussion

Relevance of hydraulic conductivity in river sediments

Hyporehic zone plays an important role in the ecological processes of lotic ecosystems. Thus, studying the interstitial areas in bed sediments (sensu Bretschko, 1992) – as well as biological relationships between the hyporheos and its environment – is of major importance for aquatic ecologists to better understand the physical, chemical and biological processes that take place in the streambeds (Triska *et al.,* 1989; Vervier *et al.,* 1992; Ward & Palmer, 1994).

Simplification of natural habitats is a growing global concern demanding that ecologists better understand how habitat heterogeneity influences the structure and functioning of ecosystems (Cardinale *et al.,* 2002). As hypothesized in this thesis, the sediment physical characteristics (i.e. permeability and texture) affect the attached microbial biomass and biogeochemical processes across the dry-wet boundaries of streambeds in a Mediterranean system (for our case, La Tordera river). Therefore, selection of a suitable approach to well describe the bed permeability (especially the hydraulic conductivity, K) is crucial. In this study, the Kozeny-Carman equation that fits with several criteria for our sand sediment (see the details in § 3.1.4) was thought to be the best model for computing K. It is known that the sediment grain-sizes and sediment depth affect the available interstitial spaces (Maridet & Philippe, 1995), where a certain amount of microbial accumulation would vary with those physical variables that normally are flooding dependent. At the same time, since the hydraulic conductivity is the measure of the ease with which fluid flows through the porous material (Alyamani & Şen, 1993), certain relationships are expected to exist between hydraulic conductivity and biogeochemical activities. For example, previous studies suggest the water flowing in the pore system carries dissolved chemical substances (Triska *et al.,* 1993; Valett *et al.,* 1994), particulate and dissolved organic matter (Lenting *et al.,* 1997).

Coupling and uncoupling between organic matter, bacteria and chlorophyll in sandy river sediments

After initial transient and abiotic storage, hyporheic organic matter is mobilized and transformed by the biota (Brunke & Gonser, 1997). The interstitial storage of particulate organic matter is influenced mainly by particle size distribution that seems relevant for bacterial retention (Dong *et al.,* 2002). At the same time, interstitial flow and flood episodes (i.e. spates) usually involve bedload movement which either import or release matter depending on the season. Several authors reported that the stored OM in the hyporheic zone may account for up to 82% of the total OM retained in streams (Smock, 1990; Jones, 1997). Similarly, a previous study reported that the hyporheic zone stored about three times more OM than the benthic surface (Valett *et al.,* 1990). At our study reach, the average OM content of 5.0 mg/g DW was similar to that measured in the study of another Mediterranean river (~ 5.5 mg/g DW after a flood in the Riera Major (NE, Spain); see Romaní *et al.*, 1998). Though this obtained OM content was low, its magnitudes led to see clear control on some enzymatic activities at some depths of our sand sediment (see § 3.3.4).

Interestingly, in general bacterial density was coupled to OM content in sediment. The sediment biofilm has been described as a site for OM degradation where bacteria play a significant role on C cycling, in comparison to the biofilm grown on rocks and cobbles (epilithic biofilm) (Romaní & Sabater, 2001). Both OM and bacteria in our study river system were at the same time favored by hydraulic conductivity (K) and % mud (Figure 4.1). Apparently, these two factors (K and % mud) can drive opposite processes since it has been described that sediments with high % of mud (especially of clay) usually show a reduction of their hydraulic conductivity due to reduction of porosity. At the same time, for our case it seemed that % of mud (silt and clay) might act as a key role in regulation water and nutrients in deeper layers. Alternatively, the clogging of sediments caused by the deposition of particles in streambeds can decrease sediment permeability, and hence greatly affect hyporheic microbial processes (Nagaro *et al.,* 2010). In such a fine sediment profile, water transport and organic matter supplies might be also low (Mermillod-Blondin *et al.,* 2014). Although the model results of Kim (2006) showed that the permeability and porosity of porous media could be altered due to bacterial deposition and growth on the solid matrix. In our study site, bacterial growth was probably not enough to any significant reduction in porosity, still higher bacterial density being related to high hydraulic conductivity. However, in comparison to other findings, cell density in our study site was low, between two-three orders of magnitude per g DW lower than those in the study of Romaní *et al.* (1998).

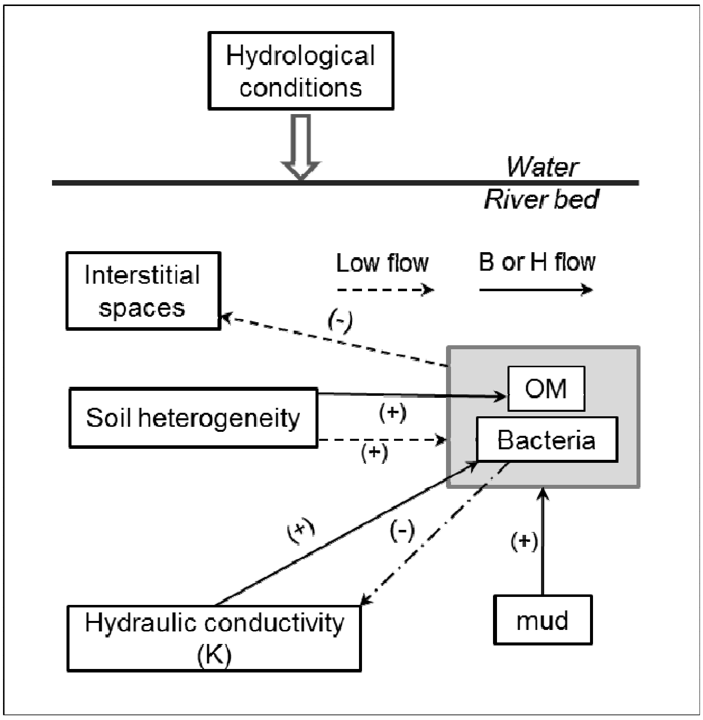


***Figure 4.1**: Summary of the relationships between the sediment physical characteristics and biofilms biomass from the studied Tordera river characterized by its sand sediment. OM, B and H represent organic matter, base and high discharges, respectively. Arrows show the interactions between variables. The sign on each arrow indicates either a*

positive or negative effect. The line-doted arrow indicates the theoretical relationship between bacteria and K described by several authors (e.g. Kim, 2006).

Chlorophyll-*a* also accumulates in river sediments, although in our study we find that sediment physical characteristics were much less relevant than for OM and bacteria. Algal development might be probably more dependent on nutrient content in flowing water. However, previous results of Scott *et al.* (2008) suggest that algal and bacterial production are decoupled by nutrient enrichment; and that algae might rely more heavily on bacterial-regenerated nutrients than on streamwater nutrients to support production in nutrient-poor streams. In parallel, Lock *et al.* (1984) found the highest densities of bacteria in the Muskeg River were associated with the highest densities of Chl-*a*. However, apparently no link bacteria-algae were recorded for our case; only bacteria linked to OM content in sand sediments (see § 3.2.4). The use of organic matter by biofilms differs according to the nature of the benthic substrata (organic or inorganic; rock or sand) (Peterson, 1996; Romaní & Sabater, 2001; Romaní *et al.,* 2004) and thus, being enhanced in the sediment habitat with probably lower relevance of algae.

Processes in the sediment of intermittent rivers

Mediterranean climate regions are characterized by long summer droughts that usually involve flow intermittency in low- to mid-order streams. Flow intermittency implies flow cessation, drying and subsequent rewetting of the streambed, and affects both autotrophic and heterotrophic processes (Timoner *et al.,* 2012). During drought episodes, the flowing water channel connection is lost but still hyporheic water flow might be relevant, but in few amount. If ecosystem metabolism was profoundly affected by stream intermittency (Acuña *et al.,* 2005), benthic microbial density and biomass are also affected by physical, chemical and biological conditions in the stream ecosystem (Hill *et al.,* 1995; Dodds *et al.,* 1996). For instance, streambed desiccation had clear effects on the functioning of stream biofilms. Autotrophic biomass decreased by 80% with streambed desiccation, but recovered rapidly after flow resumption (Holmes *et al.,* 1998; Timoner *et al.,* 2012). After a drought episode, the microbial community at the river sediment might not only actively use the available dissolved organic matter (Romaní *et al.,* 2006), but also bacteria would use the particulate OM accumulated as noted in our study during low discharge (July 2012). Interestingly, at our study site 4 (dry-wet conditions), which was dry at the surface but wet in depth, the maintenance of humidity at the deep sediment was very relevant for the maintenance of microbial activity (i.e., for peptidase). This indicates that although drought periods typically occur, metabolic activity is kept in humid deeper sediments.

In our study site, higher OM and bacterial content in sediment is related to the amount of mud as well as high hydraulic conductivity (K). High K and flow through interstitial spaces may provide greater nutrient and fresh OM input to the hyporheic zone thus favoring bacterial colonization. These obtained results might be linked to sediment granulometry of our studied river sediment, which is mainly sandy with a very low % mud. Although a few amounts (nearly negligible if compared to that of sand and very fine gravel (VFG)) were recorded, the mud particles may favor microbial colonization in the porous layers; because they contain adsorbed compounds on their surfaces available for the bacterial attachment (Brunke, 1999). At that time, it seems that in our sandy river sediment slight increase in mud content may increase microbial colonization and activity as well as OM accumulation.

5

Conclusions

Limits and potentiality of eleven empirical hydraulic conductivity methods: a technical description and a critical review

- Hydraulic conductivity (K) has relationship with sediment properties included pore-sizes (which are difficult to be determined) and grain-size distribution (easy-to-measured) by a standard method.
- To select an empirical formula, we need to pay attention to its domains of applicability; and to couple the empirical results to laboratory measurement. In the end, although from our data, we suggest using Kozany-Carman approach to calculate K from grain-size distribution.

How do sediment physical characteristics affect microbial biomass in a Mediterranean river?

- Heterogeneity and interstices of sediment significantly predicted the variations in ash free dry weight (AFDW) content.
- High sand sediment heterogeneity affected positively Chl *a* accumulation, but only at high-flow conditions.
- Pore spaces and sediment surface area (which increased with % of mud) were

relevant for the bacterial attachment, and this was found for the different hydrological periods studied. Bacteria were also related to AFDW content.

- The study shows that sediment physical properties differentially affect the microbial biomass– bacteria being the most affected–, and that this effect may change depending on the hydrological conditions.

Microbial processes across the dry-wet hyporheic boundary in a Mediterranean river

- High K value prevents the water chemistry (among other, the dissolved O_2 and temperature) not to decrease along the depth profiles.
- High hydraulic conductivity (K) of the sediment layers favors, for our case, downwelling flow into the bed-sediment depths – in which the ongoing activity of microorganisms and activity was measured especially at the accumulation of OM within some depths.
- The microbial processes at the surface and hyporheic sediment where different in the two study periods, in general, microbial activities were higher in July than in June.
- In some sites, depth gradients in microbial activity occur, and a general decrease in β-glucosidase and increase in peptidase was observed, indicating a major use of carbon compounds in surface sediments but a greater use of nitrogen compounds in depth.
- Although the amount of mud (especially clay) is little compared to the larger fractions (sand and gravel), but it managed to control some microbial activities (i.e. β-glucosidase, peptidase) and nutrients such as P, as well as impact on permeability of layers.

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