UNIVERSIDAD DE MURCIA

ESCUELA INTERNACIONAL DE DOCTORADO

Linking Terrestrial and Aquatic Organic Matter Processing in Fluvial Ecosystems

Uniendo el Procesado Terrestre y Acuático de la Materia Orgánica en Ecosistemas Fluviales

D. Rubén del Campo González

2018

UNIVERSIDAD DE MURCIA

FACULTAD DE BIOLOGIA

Departamento de Ecología e Hidrología

Linking terrestrial and aquatic organic matter processing in fluvial ecosystems

Uniendo el procesado terrestre y acuático de la materia orgánica en ecosistemas fluviales

TESIS DOCTORAL

D. Rubén del Campo González

2018

Directora;

Dr. Rosa Gómez Cerezo

DEPARTAMENTO DE ECOLOGÍA E HIDROLOGÍA
UNIVERSIDAD DE MURCIA

PH.D. THESIS

LINKING TERRESTRIAL AND AQUATIC ORGANIC MATTER PROCESSING IN FLUVIAL ECOSYSTEMS

RUBÉN
DEL CAMPO
GONZÁLEZ

2018

FINANCIACIÓN

El presente proyecto de tesis ha sido financiado mediante fondos de las siguientes instituciones:

- Contrato predoctoral FPU (FPU R-269/2014) del Plan Propio de la Universidad de Murcia.
- Fondos FEDER
- Proyectos de Investigación y Desarrollo del Ministerio de Economía y Competitividad de España (CGL2010-21458).
- Proyectos de la Fundación Séneca. Agencia de Ciencia y Tecnología de la Región de Murcia (19525/PI/14).

AGRADECIMIENTOS

En las páginas anteriores he comenzado con el agradecimiento a las distintas instituciones que han cofinanciado la realización de esta tesis. La financiación económica de una tesis es sin duda imprescindible. Todo el trabajo realizado requiere de un gran esfuerzo económico. Sin embargo, en la investigación como en la vida, el dinero no lo es todo. Para llevar a cabo con éxito una tesis doctoral, además de la inversión económica es absolutamente necesario la existencia de una gran "financiación emocional". Esta tesis es un claro ejemplo de ello, así que en las siguientes líneas me gustaría agradecer a todas esas personas que me han financiado emocionalmente durante los últimos cuatro años. Como seguro que me dejaría a mucha gente importante sin nombrar, voy a intentar decir los menos nombres posibles. Aun así, espero que todos aquellos que leáis estos agradecimientos sepáis encontrar la (gran) parte que os corresponde. Seguro que sí. Aquí va mi más sincero agradecimiento a todas esas personas. Sin vosotros, ni vosotras, esta tesis no habría sido posible, ni yo sería lo que soy.

En primer lugar, es de justicia agradecer al Departamento de Ecología e Hidrología de la Universidad de Murcia y a todas las personas que lo componen el haberme acogido y respaldado durante los más de siete años que llevo trabajando en el mismo desde que empecé como alumno interno. Este agradecimiento va muy especialmente dedicado al grupo de investigación de Ecología de Aguas Continentales. Entré con vosotras al mundo de la investigación siendo prácticamente un crío sin conocimiento alguno, ni de la propia investigación, ni mucho menos de porqué narices iba a ser interesante estudiar las ramblas y secarrales varios de nuestra región. Termino esta andadura predoctoral en este grupo mucho más maduro en ambos sentidos gracias a todas vosotras. Durante todos estos años, no sólo me habéis enseñado a valorar y apreciar nuestros ríos, además me habéis hecho partícipe de vuestra investigación y me habéis formado como investigador, y mucho más importante, como persona. Chary, María Luisa, Marisa, María, muchas gracias por haber estado a mi lado durante todos estos años. Con vosotras he disfrutado y he

aprendido muchísimo. Nunca voy a olvidar mi estancia en este grupo de investigación, porque el investigador que soy hoy es en gran parte gracias a vosotras.

Quiero agradecer también a todas esas personas que han participado y me han echado una mano en esta tesis. Sin vuestra ayuda en el campo o en el laboratorio llegar hasta aquí hubiese sido infinitamente más difícil, posiblemente imposible. Chema, Toñi, Amparo, Almudena, María Botella, Gabriel, Javi, Mercedes, Zeus, Cristina, Pepa, Obdulia… y sin duda me dejo a muchos más… muchas gracias a todas y a todos por haber puesto vuestro granazo de arena en esta tesis.

También agradecer a toda esa gente buena del Departamento de los que he aprendido tantísimo. Desde el primer día que empecé la tesis, tuve muy claro que, si quería tener éxito en mi tesis, debía prestar muchísima atención a todos aquellos que me rodeaban. Habéis sido ejemplos para mí, y os lo agradeceré siempre. Viéndoos progresar en vuestras carreras investigadoras he aprendido de vuestros fallos para no repetirlos, y sobre todo de vuestros aciertos para intentar imitarlos. No sé si esta tesis acabará teniendo más o menos éxito, pero el resultado final es gracias también a todos vosotros.

Dentro de este maravilloso grupo de investigadoras, el agradecimiento más grande de todos es para mí directora, Rosa, o como yo siempre la he considerado, mi madre investigadora. Rosa, esta tesis se ha hecho gracias a ti. Gracias por acogerme y guiarme en lo personal y en lo académico durante todos estos años. Gracias por enseñarme a emocionarme con nuestras ramblas. A no poder esperar ni un segundo para analizar los resultados recién salidos de los análisis de laboratorio para ver qué pinta tenían los resultados y empezar a interpretarlos. Precisamente porque te conozco muy bien, sé que eres una de las mejores investigadoras de todos los que he conocido. Muy pocas personas tienen la capacidad que tú tienes de pensar en nuevas ideas y preguntas a resolver en nuestro campo y llevarlas a cabo. De ti he aprendido que no hace falta grandes recursos logísticos, ni económicos para hacer una gran investigación. Sólo curiosidad, entusiasmo y mucho trabajo. Todo esto me lo llevo de aquí gracias a ti. Muchas gracias, Rosa, de verdad.

Obviamente estos cuatro años no han significado sólo un trabajo descomunal, también han sido unos de los años más divertidos y satisfactorios de mi vida. De esto tiene gran parte de culpa toda esa gente buena de Ecología, la Facultad de Biología o

de AJIUM. No empiezo a decir nombres porque podría llenar páginas sólo con ello, pero os estoy inmensamente agradecidos a todas y a todos por estos maravillosos años de charlas de i+d+c que empezaban con un: "vente! Si nos tomamos un par de cañas y nos vamos. No ves que yo mañana tengo que trabajar también...", pero terminaban en Music a las tantas… Por esos ratos en Letur, o los diversos DoctorFests… Me lo he pasado en grande y os voy a echar mucho de menos a todas y todos. Empezasteis siendo compañeros de trabajo, pero os habéis convertido en algo mucho más grande que eso. Muchas gracias por estos ratos maravillosos e irrepetibles.

Un agradecimiento obligatorio es para toda esa gente que ha liderado y lideran el asociacionismo de jóvenes investigadores en este país. A toda esa gente de AJIUM y la FJI. Con estas líneas quiero expresar mi reconocimiento a todos vosotros y vosotras. Sin duda las condiciones laborales de las que he disfrutado yo y otros tantos investigadores e investigadoras se deben en gran parte a vosotras y vosotros. La lucha sigue. Ánimo.

Siguiendo con los buenos amigos, obviamente no me puedo olvidar de toda la gente que lleva a mi lado desde hace mucho tiempo, siempre apoyándome. Toda esa gente que conocí en la Universidad en la carrera, y que hicieron de esos cinco años unos de los mejores de mi vida. También a mi panda (o más bien banda…) que han estado conmigo prácticamente toda mi vida… Que habría sido de mi sin vosotros y vosotras… Una de las cosas más importantes que una persona puede llegar a tener en su vida es una buena amistad, y yo he tenido la suerte y el privilegio de disfrutar de muchas. Más de lo normal. Para mí ha sido increíble ver la admiración que siempre habéis mostrado por ver como he ido progresando en mi carrera, por valorar lo que hago y hecho, mucho más de lo que yo mismo he podido hacer nunca… por estar ahí siempre que lo he necesitado. Por todo. Millones de gracias.

Va siendo hora de acabar esto, pero obviamente tengo que agradecer, más que a nadie, a los que más tiempo me han tenido que soportar y a los que han hecho de mí la persona que soy a día de hoy. Mi familia. Soy muy consciente que os he agradecido muy, muy poco todo lo que habéis hecho por mí, desde darme la vida, educarme y hacerme una buena persona. A vosotros sí que os lo debo todo. Muchas gracias por estar ahí. Muchas gracias por vuestro orgullo. Lo sois todo para mí.

Nunca os podré devolver todo lo que habéis hecho por mí. Y a los que ya no están, mi abuela Belén y mi abuelo Mariano… especialmente tú, abuelo… nunca olvidaré tu mirada de orgullo cuando te contaba cómo me iba con la carrera o con la tesis. Te fuiste mucho antes de que esto acabase, pero hoy especialmente me acuerdo de ti y me imagino lo satisfecho y orgulloso que estarías de la persona que se ha convertido tu nieto. Allá donde estés, muchas gracias. Nunca te olvidaré.

Quería dejar el último turno a la persona que más ha tenido que soportar las consecuencias no tan positivas de estos cuatro años de duro trabajo… A Patricia, mi pareja, mi vida. Nunca podré devolverte todo lo que me has dado durante estos maravillosos doce años. Gracias por estar siempre, siempre, siempre ahí. Por aguantarme en las malas y disfrutar conmigo en las buenas. Estos últimos meses han sido especialmente duros. Pero si he conseguido aguantar ha sido única y exclusivamente gracias a ti. Muchas gracias mi vida. Te amo más que a nada en este mundo.

Y antes de acabar. El último agradecimiento es para la investigación. Tan minusvalorada por la sociedad, pero tan necesaria y enriquecedora. Gracias por permitirme embarcarme en esta carrera fascinante. Has convertido cada uno de los días de trabajo en una aventura (aunque no siempre buena…). Durante estos cuatro años he tenido la suerte de trabajar en lo que me apasiona. Espero que esta tesis sirva para devolverte un poco de todo lo que me has dado. Muchas gracias. Empezamos.

CONTENTS

Resumen en español

La alternancia de fases terrestres y acuáticas altera la descomposición de la materia orgánica en ríos intermitentes

La descomposición de la materia orgánica (MO) es uno de los principales procesos que controlan los flujos de materia y energía en los ecosistemas de ríos y arroyos, por lo que su estudio es absolutamente necesario para comprender el funcionamiento de estos sistemas. De forma tradicional, todos los estudios a cerca de los aspectos funcionales de los medios acuáticos continentales se han centrado en regiones templadas, por lo que a día de hoy el conocimiento sobre el funcionamiento de los ríos de regiones áridas y semiáridas es muy escaso. Este desconocimiento general, junto a las predicciones de cambio global, en el que se prevé un aumento en el número de los ríos temporales, especialmente en la región mediterránea, pone de manifiesto la necesidad de realizar estudios que permitan mejorar el entendimiento de estos sistemas.

Las regiones áridas y semiáridas se caracterizan por unas condiciones climáticas de altas temperaturas y precipitaciones escasas e irregulares. Estas condiciones climáticas ocasionan que los medios acuáticos de estas regiones estén caracterizados por un gran dinamismo espacio-temporal, marcado por la existencia de eventos hidrológicos extremos, como sequías en verano y crecidas o riadas en las épocas de lluvia. En estos ecosistemas, la dinámica de la MO (transporte y descomposición) está principalmente controlada por los cambios hidrológicos. Principalmente durante los periodos de caudal bajo o sequía veraniega, la MO queda acumulada en las llanuras aluviales de los ríos o en los propios cauces secos, hasta que las lluvias o inundaciones en otoño movilizan la MO acumulada hasta los cauces activos con agua. Dicho de otra forma, en los ríos de regiones áridas el procesado de la MO es llevado a cabo mediante la interacción entre fases terrestres y acuáticas. Durante la fase seca, la MO que queda acumulada en el suelo de las llanuras o en el lecho de los cauces secos (fase terrestre), queda expuesta a diversas condiciones ambientales que modifican su composición química y biodegradabilidad. Sin embargo, todavía se desconoce cómo estos cambios afectan a su posterior descomposición en los ríos, tras la recuperación del flujo hídrico (fase acuática). Por todo ello, la temporalidad hidrológica a la que están sometidos los medios acuáticos en estas regiones las diferencia del resto de regiones bioclimáticas. Así, resulta imprescindible que conozcamos como este factor, y la consecuente interacción entre

los medios terrestre y acuático afectan a la descomposición de la MO, y ampliar así nuestro conocimiento sobre el funcionamiento de los sistemas fluviales en general.

El objetivo principal de esta tesis doctoral es el de analizar los efectos de la temporalidad y la interacción entre los sistemas terrestre y acuático sobre la descomposición de la MO en ríos de regiones áridas. Para ello, se desarrollaron cuatro trabajos científicos independientes, dirigidos a cumplir con cada uno de los objetivos específicos de esta tesis y que se detallan a continuación.

El **primer capítulo** de la tesis tiene como objetivo analizar el efecto de la exposición o "precondicionamiento" de la madera en llanuras aluviales de ríos áridos sobre su composición química, colonización por microorganismos y posterior descomposición en medio acuático. Con este fin, se expusieron en primer lugar depresores linguales de madera de abedul (sustrato entandar) en una llanura aluvial de una rambla árida (Rambla de la Parra) de la Región de Murcia (SE, España) durante cuatro meses. Tras la finalización de esta primera fase de precondicionamiento terrestre, se trasladaron los depresores linguales a distintos ríos de la Región (Corneros, Turrilla y Chícamo) donde se sumergieron y se dejaron descomponer durante tres meses. Para ambas fases terrestre y acuática, se recogieron muestras al final de los periodos de exposición en llanura e inmersión respectivamente, para analizar en el laboratorio la pérdida de peso y MO, los cambios producidos en la composición química de la madera y la actividad microbiana desarrollada sobre ella. Los resultados de este primer experimento demostraron la importancia de la fase de precondicionamiento terrestre en la composición química de la madera. Concretamente, la exposición de la madera bajo las condiciones de la llanura aluvial provocó su empobrecimiento en nutrientes como P o K por lixiviación asociada a lluvias, así como una reducción de su contenido en lignina asociado a una intensa fotodegradación. Esta degradación de la lignina, junto con la colonización de la madera por comunidades de hongos terrestres, provocó que la madera procedente de la llanura o "precondicionada" sufriera un patrón de descomposición, tras su inmersión en los ríos, distinto al observado para madera no precondicionada o control. Así, durante la primera semana de inmersión en el agua la madera precondicionada sufrió un pulso corto e intenso de descomposición microbiana proseguido de un descenso acusado hasta el final de la fase acuática. Estos

resultados implican que el precondicionamiento de la madera en las llanuras de inundación podría cambiar el papel de la madera como recurso de larga duración para las redes tróficas acuáticas, a un recurso con una vida útil muy corta debido a su rápido agotamiento en fuentes de C lábil y nutrientes.

En el **segundo trabajo** ampliamos nuestro punto de mira para analizar cómo distintas condiciones climáticas o del hábitat de las llanuras aluviales, pueden afectar al precondicionamiento de la MO y a su posterior descomposición acuática. Para cumplir con este objetivo expusimos hojarasca (hojas de carrizo, *Phragmites australis*) en dos hábitats de suelo desnudo y cubiertos por vegetación durante 105 días, en tres llanuras con climas distintos: una llanura con clima árido-mediterráneo (Fortuna, Murcia), otra con clima húmedo-mediterráneo (Girona, NE España) y otra con clima frío continental (Brandeburgo, N Alemania). Como en el anterior trabajo, una vez finalizada la exposición terrestre, las hojas se sumergieron esta vez en un único río (Alharabe) en la Región de Murcia durante 90 días. Durante ambas fases, terrestre y acuática, se recogieron muestras de hojas de forma periódica a lo largo de todo el periodo de estudio para analizar la pérdida de MO de la hojarasca, así como los cambios producidos en su composición química y en la actividad microbiana sobre ella. Además del seguimiento de la descomposición de la hojarasca, en este segundo trabajo estudiamos el efecto del precondicionamiento terrestre en la composición química y biodegradabilidad de sus lixiviados (materia orgánica disuelta, MOD), ya que éstos son otro recurso energético esencial para las comunidades microbianas acuáticas. Los resultados de este trabajo demostraron que las distintas condiciones ambientales de las llanuras aluviales (principalmente el clima y la disponibilidad de nutrientes en el suelo) son capaces de modular la alteración química de la hojarasca durante su periodo de exposición en el medio terrestre. Sin embargo, su influencia sobre los lixiviados de la hojarasca fue similar en todos los casos, independientemente de las condiciones ambientales. Todos los lixiviados sufrieron un gran empobrecimiento general de carbono (C) orgánico soluble y nutrientes en todas las llanuras y hábitats de estudio. Por tanto, el precondicionamiento de la hojarasca en las llanuras aluviales provocó una gran depreciación de los lixiviados como recurso energético y de nutrientes para las comunidades heterotróficas fluviales, pero tuvo un efecto variable en la

biodegradabilidad de la hojarasca, dependiendo de los procesos ocurridos sobre ésta durante el precondicionamiento en las llanuras. Concretamente, nuestros resultados indicaron que el balance entre la pérdida de nutrientes por lixiviación, o su retención y conservación en la hojarasca por procesos de inmovilización microbiana durante la fase de llanura, es el factor más importante que determina las tasas de descomposición de la hojarasca en el río.

Una vez analizado el efecto del precondicionamiento de la MO en llanura, el **tercer trabajo** tiene como objetivo analizar el efecto de la temporalidad (intermitencia del flujo hídrico superficial) en el procesado de la MO en ríos áridos. Durante la época de estiaje, los ríos temporales acumulan sobre los lechos secos distintos tipos de detritos vegetales (algas o macrófitos secos, hojarasca semi descompuesta, etc.) y sedimentos. Estos diversos tipos de MO son fuentes potenciales de materia orgánica disuelta (MOD) y nutrientes que se lixivian rápidamente tras el restablecimiento del caudal durante la época de lluvias. Tras ello, la MOD y los nutrientes pasan a la columna de agua dónde representan un recurso importante para las comunidades microbianas aguas abajo. En este trabajo se analizó como distintas condiciones ambientales durante la fase seca (exposición o no, a la radiación solar) pueden afectar a la lixiviación de la MO acumulada en los cauces, analizando tanto su efecto sobre la calidad química de sus lixiviados, como sobre su posterior metabolismo por comunidades microbianas acuáticas. Con este fin, realizamos un experimento en el que simulamos la exposición de distintos sustratos orgánicos (macrófitos, hojarasca y sedimentos) bajo diferentes condiciones de radiación solar mediante un diseño de microcosmos. Para ello, utilizamos tanques de plástico en los que se depositó, de forma independiente, sedimento fresco procedente de distintos ríos y los distintos tipos de sustratos vegetales (hojarasca y macrófitos). Los tanques de plástico con los sustratos orgánicos se colocaron al aire libre en condiciones naturales de luz o sombra en las instalaciones del Servicio de Experimentación Agroforestal de la Universidad de Murcia. El objetivo fue simular la exposición de dichos sustratos a las condiciones ambientales atmosféricas de cauces abiertos (expuestos totalmente a la luz del sol) o forestales (sombreados por la vegetación de ribera). La exposición se realizó durante 60 días. Durante todo el periodo de estudio se recogieron muestras periódicas de los diferentes sustratos

orgánicos, para analizar los cambios secuenciales de su composición química. Una vez finalizado el experimento se extrajeron los lixiviados de todas las muestras recogidas (sumergiendo los sustratos en agua ultra pura durante 24 horas a 4ºC en condiciones de oscuridad y en agitación). Posteriormente se llevaron a cabo los análisis de composición química mediante medidas de absorbancia, fluorescencia y espectrometría de masas de resonancia ion-ciclotrón transformada de Fourier (FT-ICR-MS). Paralelamente se realizó un segundo ensayo para analizar el metabolismo acuático de los lixiviados de los sustratos de estudio. Para ello, se hicieron ensayos de biodegradación con los lixiviados obtenidos de los detritos vegetales tras completar los 60 días de exposición a sequía en condiciones de luz y sombra. Los análisis consistieron en la incubación de los lixiviados junto con un inoculo microbiano durante 8 días y tuvieron como objetivo analizar la pérdida del carbono orgánico disuelto como estima de biodegradación. Los resultados de este experimento mostraron que la exposición de la MO a radiación solar intensa y altas temperaturas durante los periodos de sequía en cauces abiertos provocó una reducción de la calidad química y biodegradabilidad de sus lixiviados asociado a la acumulación de compuestos recalcitrantes. Por el contrario, los ríos temporales forestales no se vieron afectados por esta reducción de la calidad química de la MO al no estar tan expuestos a la radiación solar y elevadas temperaturas, por lo que sus lixiviados mantuvieron una gran calidad y biodegradabilidad. Estos resultados sugieren que el precondicionamiento de la MO en ríos temporales puede derivar en grandes diferencias en su procesado (tanto en las rutas, como en las tasas de biodegradación) dependiendo de que éstos se ubiquen en regiones áridas o húmedas.

Dada la importancia de las condiciones ambientales durante la fase terrestre de acumulación de MO, ya sea en cauces secos o llanuras aluviales, demostrada en los capítulos anteriores, en el **último trabajo** de la tesis analizamos el efecto de la heterogeneidad ambiental del lecho seco de los ríos temporales sobre la diversidad química de la MO y su posterior descomposición acuática. Durante la fase de desecación de los ríos temporales, la fragmentación del caudal origina la aparición de diversos hábitats terrestres y acuáticos a lo largo del cauce, por ejemplo; pequeñas pozas aisladas, sedimentos húmedos y sombreados, zonas secas y expuestas a la radiación solar, etc. Esta diversidad de hábitats provoca que la MO del cauce pueda

quedar retenida bajo una gran heterogeneidad de condiciones ambientales que pueden provocar distintos cambios en su composición química como ya hemos demostrado. Este último trabajo consistió en analizar el efecto de dicha heterogeneidad ambiental sobre la calidad de la MO acumulada en los diversos tipos de hábitats, así como las implicaciones en su posterior descomposición una vez el caudal se restablece. Para ello, realizamos un experimento en dos fases. En la primera simulamos la acumulación y exposición de hojarasca (Aliso, *Alnus glutinosa*) en siete tipos de hábitats terrestres y acuáticos, mediante ensayos de microcosmos en el laboratorio. Posteriormente, utilizamos las hojas procedentes de estos siete tratamientos para hacer diversas mezclas de hojas, esto es, bolsas de malla conteniendo hojas procedentes de varios tratamientos, y en combinaciones crecientes. Por último, las mezclas de hojas fueron incubadas en un único río (Löcknitz, Brandeburgo, Alemania) para estudiar su descomposición acuática siguiendo la misma metodología que para los experimentos de los dos primeros objetivos de la tesis. Los resultados de este último capítulo mostraron que el precondicionamiento de la MO bajo condiciones ambientales heterogéneas provoca una diversificación de su composición química. Este hecho es muy importante ya que, tras el restablecimiento del caudal, toda esta hojarasca químicamente diversificada, es transportada y mezclada aguas abajo hasta quedar retenida en algún punto donde empieza su descomposición acuática. Nuestros resultados mostraron que el incremento en la diversidad química de las hojas, provocó una aceleración de su descomposición en el medio acuático debido a la producción de un estímulo positivo de la actividad microbiana y de los organismos detritívoros.

Por tanto, la compilación de resultados de esta tesis demuestra que la interacción terrestre-acuática, ya sea en base a la interacción espacial entre los ríos y su llanura de inundación, o la interacción temporal entre la fase seca y la fase acuática de los ríos temporales, determina la descomposición y uso final de la MO por los organismos acuáticos y, en consecuencia, los flujos de materia y energía en estos sistemas fluviales. Esta tesis destaca especialmente las diferencias en el funcionamiento ecológico y en los flujos de MO de los ríos de regiones áridas, respecto a ríos de regiones de carácter templado, tradicionalmente mucho más estudiados. Los descubrimientos destacados a lo largo de esta tesis contribuyen a

incrementar el conocimiento sobre los ríos de regiones áridas, lo que posibilita una mejora de su gestión ambiental, lo cual resulta completamente necesario en vista a la actual expansión de las zonas áridas en el planeta.

1. General introduction and objectives

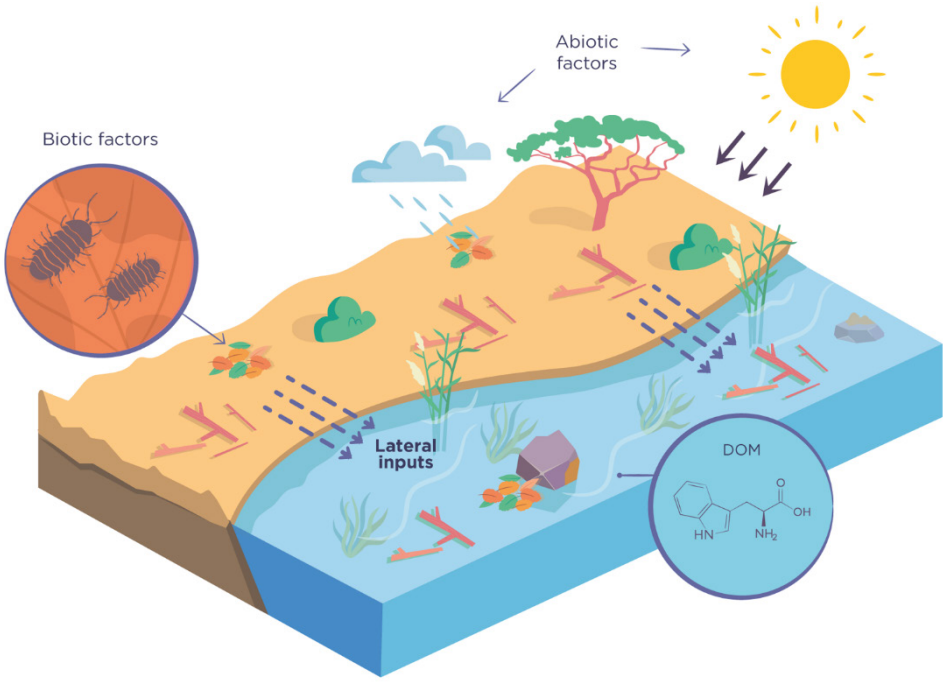

Diagram representing terrestrial-aquatic interactions in a typical arid stream

1.1.Linking terrestrial and freshwater ecosystems: dynamic of coarse organic matter

One of the ecological processes that connect terrestrial and freshwater ecosystems is the organic matter (OM) cycling. The decomposition of OM from riparian vegetation is an essential ecosystem process in fluvial ecosystems as it can support heterotrophic food webs and allows the cycling of carbon (C) and nutrients (Cummins 1974, Webster & Benfield 1986). Leaf and woody litter, stems and fruits may enter rivers directly from the riparian canopy (vertical inputs) and/or laterally from riparian soils, where OM can remain accumulated for several months before accessing the river channel (Benfield 1997, Bell 1975 & 1978). During its accumulation period in floodplains, OM can be exposed to different biotic (soil microbial and invertebrates activity) and abiotic processes (rain leaching, fragmentation, photodegradation) (Fig. 1.1) which may alter its chemical composition, and therefore influence its later processing in fluvial ecosystems (Fellman et al. 2013, del Campo & Gómez 2016). Even so, the effect of this accumulation phase of OM on river functioning has been traditionally overlooked by limnologists, likely because vertical inputs of OM are usually considered more important than lateral contributions in OM budgets in rivers (Wallace et al. 1995, Pozo et al. 1997). However, under certain circumstances, lateral inputs can represent the most important source of OM to the river (Benfield 1997, Jacobson et al. 1999). The relevance of vertical and lateral inputs on OM budgets in rivers depends upon different factors such as climate, riparian species, river geomorphology and flow fluctuations (Benfield 1997, Pozo et al. 1997, Langhans et al. 2013, Sanpera-Calbet et al. 2016). For instance, in more temperate and mesic zones, the main input of allochthonous OM peaks typically with the leaf fall from deciduous riparian forest in autumn (McDowell & Fisher 1976). In these systems, leaf litter can enter the river both by vertical inputs from riparian canopy, but also by lateral inputs after autumnal rains by ensuing floods or surface runoff (Fig. 1.1 and Fig. 1.2a) (Benfield 1997, Bell 1975 & 1978). On the contrary, in arid streams, where riparian vegetation is dominated by perennial woody shrubs (Bruno et al. 2014, Salinas & Casas 2007), the principal source of OM is woody litter entering the stream channel transported

by rains (Fig. 1.2b) (Jacobson et al. 1999). Even more, after rainstorms woody litter can be relocated across the floodplain forming woody debris, or be deposited in dry channels, increasing their exposure time to abiotic and biotic factors (Schade & Fisher 1997, Jacobson et al. 1999, Sponseller & Fisher 2006).

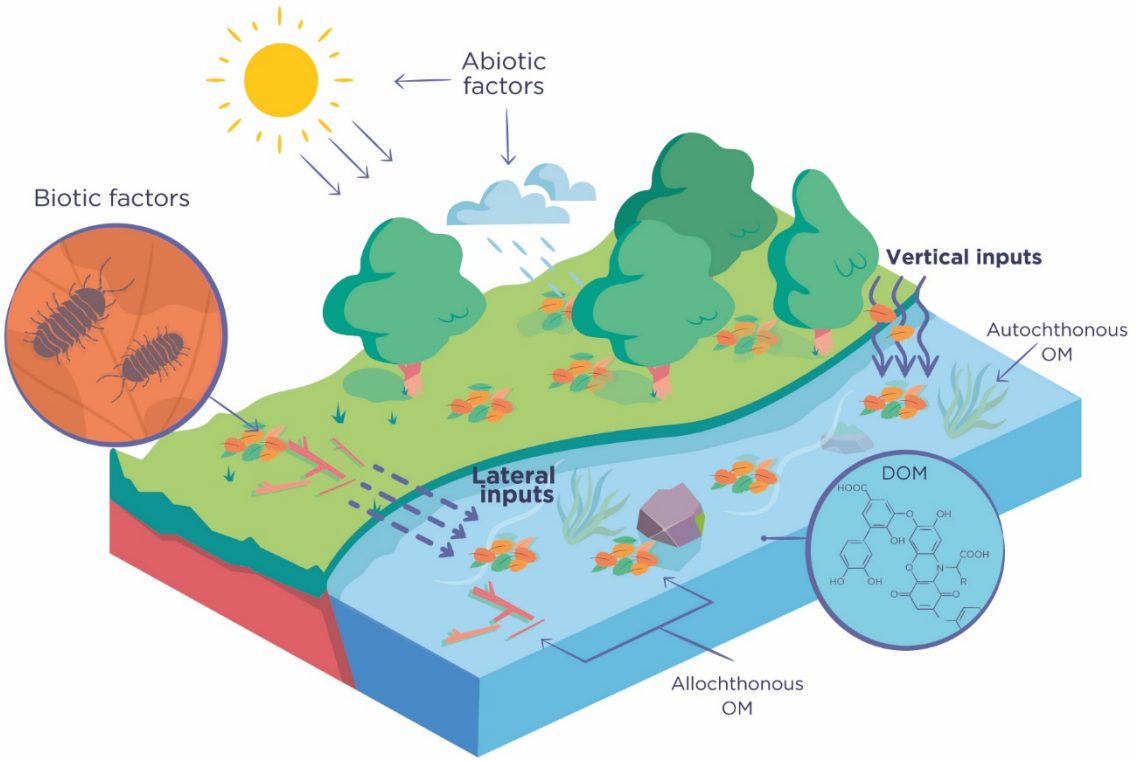

Figure 1.1 Diagram representing terrestrial-aquatic interactions in fluvial ecosystems between the river channel and its floodplain. Horizontal dashed lines represent lateral inputs of OM accumulated in floodplain soils, whereas vertical solid lines indicate direct inputs from riparian canopy.

Therefore, to understand OM dynamic (transport and processing) in fluvial ecosystems it is necessary to consider the terrestrial-aquatic interaction between river channel and its adjacent terrestrial ecosystems. This approach has defined the framework of the present thesis.

Figure 1.2 Pictures of a forested river with leaf litter accumulated in the floodplain under shaded conditions (a) and an arid floodplain with woody debris piles exposed to intense solar radiation (b).

1.2.OM processing in terrestrial and freshwater ecosystems

OM is an essential resource sustaining food webs in a great variety of terrestrial and aquatic ecosystems (Hagen et al. 2012). Its great relevance can be easily recognized based on a numeric perspective. Last estimations suggest that up to 80 or 90% of the global terrestrial plant production (around 122 billion tons of organic C per year according to Beer et al. 2010) can enter the dead OM pool (Cebrian 1999, Zimmer 2008) and to sustain terrestrial brown food web (Gessner et al. 2010). Although this began in terrestrial ecosystems, the highest degradation of organic carbon (OC) occurs in freshwater ecosystems (Battin et al. 2008, Hotchkiss et al. 2015, Catalán et al. 2016), which are able to store, transform and outgas more than a half of the total OC received from terrestrial inputs (Cole et al. 2007, Aufdenkampe et al. 2011, Raymond, et al. 2013). Fluvial ecosystems are considered one of the main hotspots of CO_2 emissions because of its highly efficient processing of terrestrial OC (Raymond et al. 2013, Hotchkiss et al. 2015). Considering the significant role of stream and rivers in global C fluxes, improving our understanding of factors and drivers controlling in-stream C processing is absolutely necessary to know their real contribution in the global C cycle.

The dead OM pool in terrestrial or aquatic ecosystems is composed by any kind of non-living organic residue from animal, fungal, microbial but mainly plant

origin (Zimmer 2008). OM has been traditionally classified by its size as: coarse particulate OM (CPOM; > 1 mm), fine particulate OM (FPOM; 0.45 µm–1 mm) and dissolved OM (DOM; < 0.45 µm). CPOM includes leaf or woody litter, while FPOM is composed by little pieces of fragmented plant litter or faeces of invertebrates. On the other hand, DOM is a diverse mixture of thousands of organic molecules with different origin (Tank et al. 2010). The decomposition process transforms CPOM into FPOM and DOM in both terrestrial and freshwater ecosystems by both biotic (microbial and detritivore activity) and abiotic mechanisms (leaching, physical abrasion) (Fig. 1.3). Because CPOM (hereafter referred always as POM) and DOM represent the main OM sources for food webs in both terrestrial and aquatic ecosystems (Kalbitz 2000, Zimmer 2008, Tank et al. 2010), this thesis focuses on these two fractions.

1.2.1.POM

Contrary to terrestrial ecosystems, the principal source of POM in freshwater ecosystems is allochthonous, coming from the surrounding terrestrial ecosystems (Cummins 1974). Allochthonous POM in rivers is mainly composed by leaves and wood (Wallace et al. 1995, Webster et al. 1999). Leaf litter is usually considered the largest and the most energetic C source for the aquatic food webs due to its higher nutritional quality and faster processing rates regarding wood (Wallace et al. 1995, Gulis et al. 2008). Even so, woody litter can represent an important resource in fluvial ecosystems, especially in arid streams (Jacobson et al. 1999), because it is a long-lasting resource that increases the pool of nutrients (Romero et al. 2005) and the stored carbon (Elosegi et al. 2007).

POM breakdown is carried out by the decomposition process, which involves a great variety of biological and physical mechanisms that transform the OM into their different products (Fig. 1.3). The main three mechanisms driving this process in terrestrial and aquatic ecosystems are leaching, microbial degradation and fragmentation by detritovores and/or abiotic agents (Berg & McClaugherty 2003, Graça et al. 2015). Although these mechanisms are common for both ecosystems, there are some differences in the decomposition performance in aquatic or terrestrial

ecosystems (Wagener et al. 1998, Treplin & Zimmer 2012). Leaching consists of the abiotic release of soluble DOM compounds (mainly simple carbohydrates, amino acids and polyphenols) and nutrients (N, but specially P, K and Mg) (Fig. 1.3) (Suberkropp 1976, Webster & Benfield 1986, Hongve et al. 2000, Wang & D'Odorico 2008). The influence of leaching on POM is clearly much higher in rivers than in soils due to the abrasive power of continuous water flow, which can cause up to 25% of POM mass loss during the first weeks of immersion (Webster & Benfield 1986). On the contrary, leaching in terrestrial ecosystems is limited to occur during storm episodes, causing severe changes of the chemical composition of POM (Lambert et al. 1980, Hongve et al. 2000), but a slighter influence on mass loss than in rivers because of the discontinuous action of rains (Treplin & Zimmer 2012). Even so, in terrestrial ecosystems other factors such as soil movement, wind or even the impact of rain drops can also promote POM abrasion and fragmentation (Throop & Archer 2009).

Microbial degradation is likely the most important process during POM decomposition (Fig. 1.3) (Wang & D'Odorico 2008, Graça et al. 2015). Fungi and bacteria achieve the POM degradation through extracellular enzyme activities (Moorhead & Sinsabaugh 2000), such as cellulases, hemicellulases or phenol oxidases to obtain energy (Sinsabaugh et al. 1992, Sinsabaugh et al. 2002), and aminopeptidases or phosphatases to uptake nutrients (Sinsabaugh & Moorhead 1994, Sinsabaugh et al. 2008). Although fungi are considered the main responsible for the microbial decomposition through the degradation of complex molecules such as lignin or cellulose (Duarte et al. 2010), bacteria also have an important role in the decomposition of more simple compounds (Romaní et al. 2006a), acting at advanced states of POM decomposition (Wagener et al. 1998). There are great differences in the composition of microbial communities between aquatic and terrestrial ecosystems (Table 1.1) (Bärlocher & Boddy 2016). Aquatic fungal communities are mainly dominated by hyphomycetes, a group extremely adapted to running waters thanks to the shape of their conidia, which facilitates their dispersal and the adherence to plant litter substrata (Bärlocher & Kendrick 1974). On the contrary, terrestrial fungal communities are much more diverse (Bärlocher & Boddy 2016), which also derives into their higher diversity of extracellular enzymes capabilities

(Bärlocher & Boddy 2016). Although terrestrial fungal species are not supposed to be able to sporulate in aquatic ecosystems (Bärlocher & Kendric 1974), some studies have shown that they can survive in aquatic ecosystems and even actively participate in POM decomposition, at least during the first days of immersion (Nikolcheva & Barlocher 2004, Nikolcheva et al. 2005, Voronin 2014).

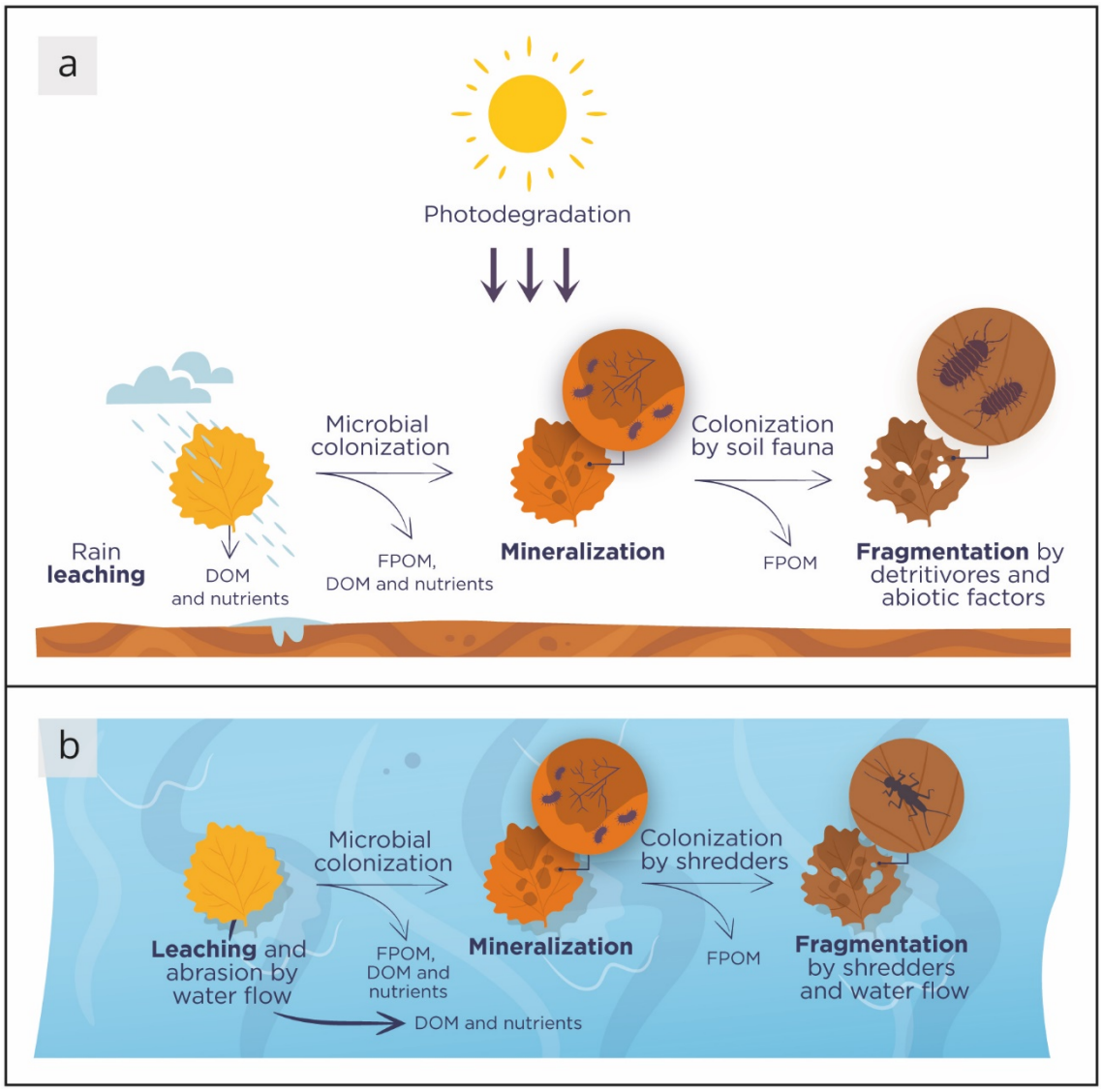

Figure 1.3 Diagram showing the biological and physical mechanisms involved in the decomposition process in terrestrial (a) and aquatic (b) ecosystems.

Microbial degradation causes severe changes in the chemical composition of the POM (Whickings et al. 2012). In addition to the loss of OC compounds by mineralization, microbial activity alters the nutrient content of OM by the

immobilization of N and P from the environment (Suberkroop & Chauvet 1995, Gulis & Suberkroop 2003). Nutrient immobilization is usually more efficient and quicker in aquatic ecosystems than in terrestrial ones due to the higher nutrient availability in the water column than in soils (Wagener et al. 1998), but it is strongly dependent on the initial chemical composition of POM (Webster & Benfield 1986, Woodward et al. 2012). Associated with N immobilization, microbial activity can derive an increment of lignin composition due to the polymerization of phenolic compounds (Melillo et al. 1984). In the end, the immobilization of nutrients in POM together with its softening by the partial degradation of structural C compounds results in an increase of palatability and the nutritional quality of POM in a process called "conditioning" which is critical for the subsequent processing of POM by the detritivores (Cornut et al. 2015, Tant et al. 2015). Detritivore activity and physical abrasion (water flow in aquatic ecosystems and soil burial or wind in terrestrial ones) contribute to the fragmentation of the softened POM and the consequent mass loss (Fig. 1.3) (Graça 2001, Wall et al. 2008, Throop & Archer 2009). Similar to microbial communities, the diversity of animal species participating in decomposition is much higher in terrestrial ecosystems than in aquatic ones mainly due to the higher residence time of litter resources in the former one (Table 1.1) (Gessner et al. 2010).

Both aquatic and terrestrial decomposition are subjected to the very same abiotic and biotic drivers: environmental conditions (Gavazov et al. 2014, Delgado-Baquerizo et al. 2015), POM chemical composition (García-Palacios et al. 2015, Boyero et al. 2017), and the structure and composition of the decomposer communities (Fukami et al. 2010, Santschi et al. 2017) (Table 1.1). However, the degree of influence of any of these drivers is completely different in aquatic and terrestrial ecosystems (Wagener et al. 1998, Treplin & Zimmer 2012, Graça et al. 2015). Probably, the most evident difference between both ecosystems is the constant supply of water and nutrients in the aquatic ones that triggers higher microbial activity and consequently higher decomposition rates than in terrestrial ecosystems (Wagener et al. 1998, Treplin & Zimmer 2012). Other environmental conditions, but mainly temperature, are also important regulators of decomposer activity (Aerts 2006, Boyero et al. 2016).

Table 1.1 Summary of the main differences between mechanisms and drivers involved in the decomposition process in terrestrial and aquatic ecosystems.

		Aquatic	Terrestrial
Mechanisms			
Abiotic		Higher leaching and abrasion by water flow or sediment transport.	Lower leaching just during storm events. Abrasion by soil burial, wind or rain impact. Photodegradation.
Biotic	Microbial decomposers	Lower diversity. Usually without ligninolytic. capabilities. Not clear successional stages.	Higher diversity. With ligninolytic capabilities. Complex and well-structured successional stages.
	Detritivores	Lower diversity. r-strategist.	Higher diversity. K-strategist.
Drivers			
Environmental conditions	Moisture	Not limiting, except during the dry phase of intermittent rivers.	Limiting, especially in arid regions.
	Temperature	Moderate fluctuations.	Strong diel and/or seasonal fluctuations.
	Nutrients	Normally not limiting due to the continuous supply by water flow.	Frequently limiting, but it depends on the type of soil.
	Oxygen	Limiting in stagnant or lentic water bodies.	Not limiting in well aerated soils.
POM chemistry	Secondary metabolites	Rarely inhibiting due to leaching or dilution.	Inhibit microbial and/or detritivore activity.

Water also plays an important role in this regard, as its buffer capacity provides a more favourable and stable environment for decomposer communities than terrestrial systems, which are much more prone to diel or seasonal changes in temperature and also moisture that can limit decomposer activity (Wagener et al. 1998).

The chemical composition of POM and the relative proportion among their elements, is a crucial driver of its decomposition pathways and rates in both, aquatic and terrestrial environments (Gessner et al. 2010, García-Palacios et al. 2015). The composition in C, N, P and lignin are traditionally considered the most important chemical regulators of microbially-driven leaf litter decomposition and therefore to define the POM quality (Melillo et al. 1984, Zhang et al. 2008, García-Palacios et al. 2015). Both microbial decomposers and detritivores tend to prefer POM rich in labile C compounds and nutrients to maximize the energy intake (Graça 2001, Talbot & Treseder 2012) and to avoid imbalances between the C:N:P ratio of POM and their own body tissues (Güssewell & Gessner 2009, Frainer et al. 2015a). However other micronutrients, such as P or Ca, K or Mg, can also play a very important role in decomposition, limiting the growth or activity of decomposers, especially in nutrient-poor ecosystems (García-Palacios et al. 2015). Besides the relevance of particular chemical quality on decomposition, many studies have proved that the increase of leaf litter species diversity in mixtures can produce positive (synergistic) or negative (antagonistic) non-additive effects on decomposition (that is, changes that cannot be predicted from decomposition rates of individual fractions of the mixture) (Gessner et al. 2010, Handa et al. 2014). In general, positive effects of leaf litter mixing dominate on negative ones (Gartner & Cardon 2004, Lecerf et al. 2011). They are mainly associated with complementarity interactions among leaf litter species composing the mixture (Handa et al. 2014, Tonin et al. 2017). Examples are the emergence of facilitation reactions among species with contrasting chemical quality, or the increase in the diversity and availability of the essential resources necessary for the metabolism and growth of decomposers (Gessner et al. 2010, Lecerf et al. 2011).

In addition to the bottom-up control exerted by leaf litter chemistry on decomposition, the composition, structure and diversity of the decomposer

communities can control decomposition rates by top-down regulation reactions (Srivastava et al. 2009, Cheever & Webster 2014). Thus, the increase of biodiversity of microbial communities has been proved to increase microbial decomposition due to facilitative interactions among species, such as the partitioning of resources in communities with species that can produce complementary enzymes for the degradation of different C compounds (Gessner et al. 2010, Fukami et al. 2010).

1.2.2.DOM

DOM represents a pivotal energy source for river network as it is the largest pool of OM circulating in running waters (Fisher & Likens 1973, Karlsson et al. 2005, Battin et al. 2008). From an aquatic ecosystem perspective DOM is conventionally classified as autochthonous or allochthonous. Autochthonous DOM results from in-stream production, derived from the exudates of both autotrophic and heterotrophic organisms, and it is regarded as highly labile and biodegradable due to high content in compounds with low molecular weights such as sugars, amino acids or simple organic acids (Bertilsson & Jones 2003, Wetzel 2003). On the contrary, allochthonous DOM originates from terrestrial vegetation and may enter aquatic ecosystems in diverse ways; from soil runoff and plant litter leaching (Fig. 1.1 and 1.3) to groundwater discharge (Allan & Castillo 2007, Hernes et al. 2017). Allochthonous DOM is believed to be mostly recalcitrant due to its humic character and high aromaticity and molecular weight (Sinsabaugh & Foreman 2003, Allan & Castillo 2007). However, this simplified picture of considering DOM as more or less bioavailable just depending on its origin is greatly questioned nowadays. Recent studies have showed that allochthonous DOM can be more bioavailable and a pivotal energy source for aquatic microbial communities than previously thought (Marín-Spiotta et al. 2014, Wiegner et al. 2015). Indeed, leaf litter leachates are considered extremely bioavailable due to their high concentration of fresh DOM, barely diagenetically altered (Sun et al. 2003, Cleveland 2004 & 2014). On the other hand, many factors influence the chemical composition and biodegradability of terrestrial DOM; from the type of soils, vegetation or land uses found in the catchment (Lambert et al. 2017, Seifert et al. 2016), to the landscape position of soils

or the DOM hydrological path (Marín-Spiotta et al. 2014, Yates et al. 2016, Laudon & Sponseller 2017).

Besides its origin, in-stream processes occurring once DOM enters the river network are major modulators of DOM chemistry and quality. During its transport along the river continuum, DOM undergoes complex transformations derived from diverse processes such as flocculation, sedimentation (Aitkenhead-Peterson et al. 2003, von Wachenfeldt & Tranvik 2008), photodegradation (Moran & Covert 2003), or partial microbial biodegradation (del Giorgio 2003, Findlay 2003), which alter its chemical composition and bioavailability, modulating its potential metabolism in downstream ecosystems (Moran & Zepp 1997, Sinsabaugh & Foreman 2003). As for POM, environmental conditions in fluvial systems, such as nutrient availability (Wickland et al. 2012) or temperature (Raymond & Bauer 2000) can also modulate the DOM biodegradation. Moreover, the main external factor dominating DOM biodegradation rates is the water residence time (Battin et al. 2008, Catalán et al. 2016, Casas-Ruíz et al. 2017). In fluvial ecosystems, water residence time depends mainly on flow velocity and discharge. Therefore, strong hydrological fluctuations can lead to the drastic alteration of the streams and rivers ability to retain and degrade DOM (Casas-Ruíz et al. 2017).

Contrary to POM, DOM biodegradation is mainly assumed by bacterial communities in biofilms (Fischer 2003), riverbed sediments (Kaplan et al. 2008) or the hyporheos (Battin et al. 2003). Bacteria can uptake very simple DOM molecules such as sugar monomers directly from the water, but they usually produce extracellular enzyme activities to degrade complex DOM compounds into smaller and simpler ones before being assimilated (Fischer 2003, Findlay 2003). DOM is considered an important source of energy in soils too (Kalbitz et al. 2000, Hongve et al. 2000), where about 10 to 40% of DOM may be easily decomposed by microbial communities. However, both leaching and complex adsorption reactions to mineral fractions of soils can complicate the uptake of DOM by terrestrial microorganisms (Kalbitz et al. 2000).

1.2.3.POM and DOM dynamics in river networks

OM particle size determines their dynamics and biodegradation pathways in fluvial ecosystems (Suberkropp 1998, Allan & Castillo 2007). One of the main reasons is their varying amenability to be transported through the river networks (Wallace et al. 1995, Battin et al. 2008). Owing to its small size, DOM is the most exported OM fraction in river networks (Wallace et al. 1995, Webster et al. 1999). Due to the short time residence of running waters, DOM is not immediately degraded once it enters the stream but exported downstream, at the same time that it is chemically transformed, and partially biodegraded. Therefore, DOM can be considered as a long lifetime resource that increases its relevance in the aquatic metabolism as we move downstream in the fluvial network (Hotchkiss et al. 2015). The largest size of POM fosters a much higher "retentiveness" than DOM. Consequently, it is transported along the river network by pulses (Battin et al. 2008). Once POM enters the stream, mainly in the forested upper part of the catchment, it is mainly retained and eventually transported downstream during high flow events (Wallace et al. 1995, Battin et al. 2008). In comparison with DOM, the higher retention of POM, together with its bigger size and fresher state, usually allow for faster decomposition rates (except wood or needles) that transform POM in a short lifetime resource, which is likely more "locally" consumed.

Although the current understanding about POM and DOM dynamics and processing is quite extensive, most of this knowledge is based on temperate, mesic regions and perennial rivers, that results in an over-representation of this kind of ecosystem and a biased comprehension of global C cycles. Since climate, flow regimes and the riparian vegetation structure are major modulators of both the dynamics and processing of OM in running waters (Larned 2000, Schade & Fisher 1997, Acuña & Tockner 2010, Larned et al. 2010), expanding our understanding of C fluxes to less known geographical regions such as arid areas, and to rivers subjected to high hydrological fluctuations such as intermittent rivers, is a crucial step to improve the realism of global C cycles.

1.3.OM dynamics in intermittent streams: the role of hydrological fluctuations

In perennial fluvial ecosystems, OM dynamics between the floodplain and the river channel is mainly regulated by flow fluctuations. Thus, OM remains accumulated in floodplain soils during periods of low or base flow, till eventual floods or storm events transport it into the river channel (Bell & Sipp 1975, Baldwin 1999). However, the highest control of OM dynamics by flow fluctuations occurs in intermittent rivers. Intermittent rivers are characterized by the succession of extreme hydrological fluctuations, ranging from severe drought to floods events (Vidal-Abarca et al. 1992, Datry et al. 2014). Surface and groundwater flow fluctuations alter the hydrological connectivity of intermittent rivers through dry-wet cycles that contract and expand the river network and consequently alter the redistribution of nutrients and OM within the catchment (Stanley et al. 1997, Bernal et al. 2013). The decrease of water flow during contraction reduces the lateral connection of intermittent river with the catchment and fragments the longitudinal connectivity through the river network, which can finish with the formation of isolated pools or the complete desiccation of the riverbed (Fig. 1.4).

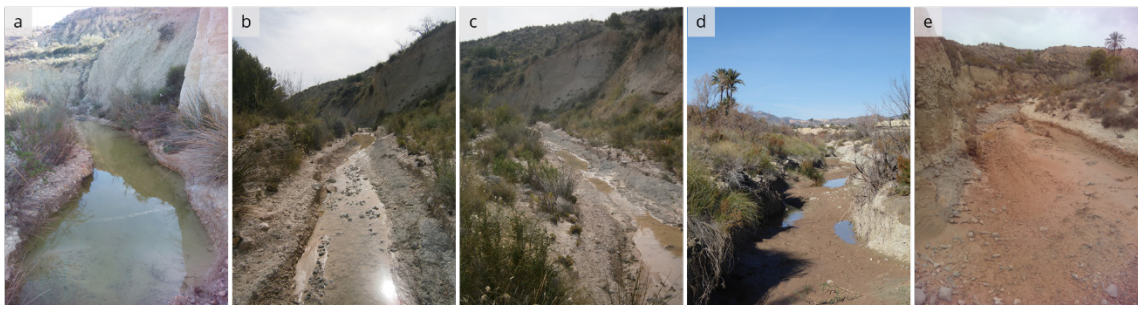

Figure 1.4 Flow variations over the hydrological cycle in the intermittent stream Parra (Murcia. SE, Spain): High flow after flow resumption (a), base flow conditions (b), low flow during stream contraction (c), stream fragmentation with isolated pools (d) and dry phase after complete drying.

The lateral disconnection between catchment and stream during the contraction decreases and even stops the supply of nutrients and OM from

surrounding terrestrial ecosystems (von Schiller et al. 2011, Bernal et al. 2013, von Schiller et al. 2015). Stream fragmentation increases the spatial heterogeneity of both the concentration of nutrients and DOC (Gómez et al. 2009, Siebers et al. 2015) and the chemical diversity of DOM (Vázquez et al. 2011, Casas-Ruíz et al. 2016). In addition to these changes, different POM materials coming from various sources (e.g. dead macrophytes or biofilms, terrestrial leaf or woody litter, fish carcasses or DOM flocculated in or on riverbed sediments) can remain on dry riverbeds for an extended time during the drying phase length (Sanpera-Calbet et al. 2016, Datry et al. 2018) and undergo notable changes in chemistry (Dieter et al. 2011 & 2013). The wet period starts with the resumption of surface water flow that usually occurs after autumn rainstorms. At this moment, the river network extend recuperates and even increases the connection of the river with its catchment (Stanley et al. 1997, Sponseller et al. 2013). Flow resumption results in the export of huge loads of nutrients (mainly N) and humic DOM downstream, coming from the dry riverbed but also from the surrounding terrestrial soils (Vidal-Abarca et al. 2001, Heffernan & Sponseller 2004, Hladyz et al. 2011, Arce et al. 2014, von Schiller et al. 2015).

Hydrological fluctuations derived from flow intermittency not only control nutrients and OM dynamics, but also the structure and functioning of biological communities from biofilm (Romaní et al 2013, Sabater et al. 2016) to invertebrate assemblages (Leberfinger et al. 2010, Sánchez-Montoya et al. 2018) and important processes in ecosystem functioning as OM decomposition (Datry et al. 2011, Abril et al. 2016, Duarte et al. 2017). So, considering that intermittent rivers occupy more than 50% of global river networks (Datry et al. 2014) and their presumable expansion in the near future according to expectations of climate change models (IPCC 2013), it is no wonder how fast the attention on intermittent rivers has increased during the last years (see Larned et al. 2010, Acuña et al. 2014, Datry et al. 2014, Leigh et al. 2016, Datry et al. 2018).

Of particular interest is the functioning of OM processing in intermittent rivers as pulsed bioreactors where OM is subjected to repeated cycles of transport, decomposition and retention when water has vanished (Larned et al. 2010). Under this assumption, OM mainly decomposes while water is present. In contrast, the dry phase is considered a static period when OM is merely retained on the dry riverbeds

until the flow resumption. This traditional assumption can be justified due to the well demonstrated, slowing effect of non-flow periods on OM processing as a result of microbial decomposer activity limitation by desiccation and the disappearance of shredders (Corti et al. 2011, Bruder et al. 2011, Foulquier et al. 2015, Abril et al. 2016). However, recent studies have proved that dry phase is not a static phase but an active period of chemical transformation of the OM retained before its aquatic decomposition during the wet phase in a process called "preconditioning" (Dieter et al 2011 & 2013).

1.4.Environmental conditions during preconditioning modulate OM quality and biodegradability

When exposed in floodplains or dry riverbeds, OM may be affected by abiotic (photodegradation, rain leaching, soil burial) and biotic processes (microbial colonization and grazing by terrestrial invertebrates). The prevalence of one or another factor depends on the combination of temperature, moisture, incidence of solar radiation and soil/sediments nutrient availability (Parton et al. 2007, Wall et al. 2008, Austin 2011). These factors are modulated both at global scale by climate, but also at local scale by microenvironmental conditions (Aerts 1997, Dent et al. 2006, Gavazov et al. 2014, Delgado-Baquerizo et al. 2015). In arid environments, where water scarcity limits microbial activity, photodegradation is considered the most important decomposition force due to the intense incidence of solar radiation on OM (Austin 2011). Photodegradation has a global relevance because it can cause a direct OM mass loss by photolysis reactions (Fig. 1.1) (Austin et al. 2006, Brandt et al. 2009, Rutledge et al. 2010), which attack principally aromatic, recalcitrant compounds (mainly lignin and phenols) due to their capacity to absorb solar radiation (Austin & Ballaré 2010, Austin et al. 2016). In turn, the photodegradation of lignin can facilitate subsequent microbial decomposition as it facilitates the access of extracellular hydrolytic enzymes to cellulosic polysaccharides (Wang et al. 2015, Austin et al. 2016, Lin et al. 2018). On the contrary, in more humid areas, microbial decomposition is usually the most important process degrading OM exposed in floodplains or dry riverbeds (Gavazov et al. 2014, García-Palacios et al. 2016), but

also altering OM chemistry by the consumption of labile compounds and the increase of refractory ones by polyphenols polymerization reactions (Melillo et al. 1984, Baldwin 1999). Another important transformation agent during the OM preconditioning period can be rain leaching, as it may reduce the availability of nutrients and labile C compounds (Fig. 1.1 and 1.3a) (Hongve et al. 2000, Bechtold et al. 2003, Schrumpf et al. 2006). Although leaching could only be considered important under specific climates, sporadic rains in arid areas can also cause more severe changes in OM chemistry than expected, due to both photodegradation and high temperatures which increase OM solubility (Gallo et al. 2009, Dieter et al. 2013, Fellman et al. 2013).

Both floodplains and intermittent riverbeds are considered highly heterogeneous ecosystems (Langhans et al. 2008, Datry et al. 2014). Floodplain and river channel morphology, in combination with the structure and composition of riparian vegetation, are major modulators of the spatial configuration of different habitats along fluvial networks (Fig. 1.2). Specially, the distribution of the riparian canopy organises a spatial mosaic of highly irradiated, open canopy areas and shaded, closed-canopy patches, which results in a great variety of micro-environmental conditions. Furthermore, intermittent rivers can exhibit a great spatial and temporal diversity in habitat conditions during their drying phase (Datry et al. 2014), such as areas exposed or unexposed to intense solar radiation) (Fig. 1.4e), anoxic and dark conditions in stagnant pools (Fig. 1.4e), or areas subjected to wet and dry cycles associated with rainy summer events. This variety of terrestrial and aquatic habitats can appear, coexist and disappear during the course of the drying phase, creating a shifting mosaic of habitats where OM can accumulate and be exposed to very different preconditioning situations, which ultimately result in different OM resources with contrasting chemical quality and biodegradability (Wickings et al. 2012). Finally, once they reach the water, the diverse preconditioned OM can be subjected to different metabolism pathways (Berggren & del Giorgio 2015). Therefore, analysing how heterogeneous environmental conditions affect OM during its preconditioning is essential to understand its final fate in fluvial ecosystems and the consequences for the functioning of aquatic ecosystems.

1.5.OM processing after flow resumption: hot biogeochemical moments for fluvial ecosystems

Once water flow is re-established in intermittent rivers, all the preconditioned POM is mobilized and transported downstream (Datry et al. 2018), accompanied or not, by POM from floodplain soils depending on the magnitude of rewetting fronts. At the same time, the wetting of riverbed sediments and POM promotes the release of large amounts of DOM and nutrients (Romaní et al. 2006a, Arce et al. 2014, Merbt et al. 2016, Bianchi et al. 2017). As a result, the flow resumption triggers the increase of concentrations of both OM and nutrients in advancing wetted fronts, exceeding baseflow concentrations by several orders of magnitude (Corti & Datry 2012), which can result in hot moments (sensu McClain et al. 2003) of microbial activity that are pivotal for river ecosystem functioning (Acuña et al. 2007, Gallo et al. 2014, Bianchi et al. 2017, Datry et al. 2018). However, this assumption needs to be treated cautiously. Considering that the preceding history of preconditioning can alter drastically the OM chemical quality and biodegradability, it can ultimately trigger very different metabolic responses by the aquatic communities (Berggren & del Giorgio 2015).

Finally, we have to consider that during the transport of POM during the rewetting phase, diverse materials that have been preconditioned under very different environmental conditions can be indifferently mixed and deposited together at some point downstream in the river. Likely, the mixing of POM chemically diverse can cause non-additive effects on its decomposition (analogous to the leaf species biodiversity response explained above) (Lecerf et al. 2011, Stoler et al. 2016), leading to enhanced or decreased OM metabolism by microbial and metazoan consumers.

So far, previous studies analysing the effect of terrestrial-aquatic interactions on OM processing in freshwater ecosystems, show contrasting results and have not yet elucidated the potential implications of such interaction in either global C fluxes or ecosystem functioning in rivers. Meanwhile some studies show a negative effect of the OM preconditioning, decreasing its quality, biodegradability and processing

rates in the rivers due to the accumulation of recalcitrant compounds (Baldwin 1999, Fellman et al. 2013, Jian et al. 2016); others show no effects on OM processing rates (Dieter et al. 2011 & 2013), or even positive effects associated with the degradation of lignin during the preconditioning phase (Pu et al. 2014). These seemingly contradictory previous results, can be due to two main reasons. Firstly, the interpretation of the global effect of terrestrial preconditioning on OM dynamics, is extremely complicated within studies analysing singularly POM and DOM processing. Secondly, different methodological approaches have been used (laboratory vs field experiments) and under very different environmental conditions. The combination of these two factors makes previous works hardly comparable. Therefore, the performance of more holistic experiments analysing in a combined way the influence of terrestrial preconditioning under different environmental conditions on both POM and DOM aquatic processing is absolutely necessary to achieve a more comprehensive knowledge of C fluxes between terrestrial and aquatic ecosystems.

1.6. Objectives

This thesis dissertation aims to evaluate how the interaction between terrestrial and aquatic ecosystems modulate POM and DOM processing in freshwater ecosystems. In order to do that, we studied two clear examples of terrestrial-aquatic interactions in fluvial ecosystems that likely occur at different periods of time: the interaction between the stream and its floodplain and the interaction between wet and dry phases of intermittent rivers. As it has previously been introduced, environmental conditions during the exposure of OM to terrestrial habitats are major drivers transforming its chemical quality and biodegradability (phase of preconditioning). Thus, across this thesis we simulated the exposure of various OM types to different climatic and micro-environmental conditions using different approaches: field, mesocosms and laboratory incubations, to address how differences in the environmental conditions during the preconditioning phase can ultimately influence the final OM aquatic processing.

As far as we know, this thesis represents the first compilations of works that combines the study of the terrestrial preconditioning on POM and DOM fractions under homogeneous conditions for their comparison, using a great variety of habitats to analyse the influence of the environmental conditions. Therefore, this thesis aims to increase the current understanding of how terrestrial-aquatic interactions can modulate OM processing and thus, to contribute to an improvement of the current global models of C fluxes.

The results of this thesis are structured in four chapters, which have been written as independent publications and follow a chronological order. The specific objectives of each chapter are:

- **Chapter III. Exposure of wood in floodplains affects its chemical quality and its subsequent breakdown in streams:** In this first work, we aimed to analyse the effect of floodplain preconditioning on the chemical quality of wood and its subsequent aquatic decomposition. In this chapter we focused on wood as the major fraction of allochthonous OM in arid streams.
- **Chapter IV. Linking terrestrial and aquatic carbon processing: Environmental conditions of floodplains control the fate of leaf litter inputs in rivers:** In this study we analysed how contrasting climatic and micro-environmental conditions during floodplain preconditioning affect both the chemical quality and the aquatic processing of leaf litter inputs in streams.
- **Chapter V. Dry phase conditions prime wet-phase dissolved organic matter dynamics in intermittent rivers:** In this work, we aimed to analyse how contrasting environmental conditions during the dry phase of intermittent rivers, mainly related to differences in their riparian vegetation canopy (i.e. forested vs open streams) alter the quantity, quality and biodegradability of DOM leached from plant litter and sediments accumulated in their dry riverbeds, during a later rewetting phase.
- **Chapter VI. Flow intermittence alters carbon processing in rivers through the chemical diversification of leaf litter**: Based on all previous results, in this last chapter we aimed to address how the mixing of leaf litter preconditioned under

diverse situations, as naturally occur in intermittent rivers during their dry phase, affect their later aquatic decomposition.

2. General Methods

This thesis is mainly based on experiments studying the decomposition and transformation of OM in terrestrial ecosystems and its effect on fluvial ecosystems. The methodological approach used for this includes: field experiments developed "in situ" (Chapter III and IV) in different European floodplains and streams from the Segura River Basin (SE of Spain), experiments developed in outdoor microcosms (Chapter V), or combining laboratory and field experiments (Chapter VI). Here, we present a compilation of the methods employed and parameters analysed along this thesis to study the terrestrial and aquatic processing of POM and DOM (Table 2.1).

Table 2.1 Summary of the analysed parameters and methods employed in the study of POM and DOM in the present thesis with indication to the Chapters where these techniques were used.

Chapter	Study substrate	POM			DOM (POM leachates)	
		Chemical composition	*Microbial activity*	*Fungal biomass*	*Chemical composition*	*Biodegradation*
III	Wooden sticks	Lignin, C, nutrients	FDA	Ergosterol		
IV	Reed leaf litter	Cellulose, lignin, C, nutrients	CBH	Ergosterol	DOC, nutrients, spectroscopic characterization	R_{az}-R_{ru}
V	Plant litter and sediments				DOC, nutrients, spectroscopic characterization PARAFAC, FT-ICR-MS	Bioassay (BDOC)
VI	Leaf litter mixtures	FTIR, C, nutrients	Respiration rate	Ergosterol		

FTIR = Fourier-transform infrared spectroscopy; FDA = fluorescein diacetate hydrolysis; CBH = cellobiohydrolase activity; PARAFAC = parallel factor analyses; FT-ICR-MS: Fourier-transform ion cyclotron mass spectrometry. R_{az}-R_{ru} = Resazurin-Resorufin.

2.1.Environmental characterization of study sites

Environmental conditions including climatic and habitat features are fundamental regulators of the OM processing in both terrestrial and aquatic ecosystems. Therefore, to measure water and air temperature, soil humidity, solar radiation, nutrient availability in the environment, as well as flow velocity and discharge in streams alongside experiments, is a basic requirement to understand the changes that occurred in the OM through its decomposition. Specific characteristics such as geographical location, altitude, lithology and land uses in study sites are described in detail in each Chapter.

2.1.1. Climatic conditions

When necessary (all Chapters, except VI) we obtained UV and global solar radiation, air relative humidity, air temperature and total precipitation from the closest meteorological station to the sampling site. In addition, and depending on the case, floodplain soil and water temperature were measured over the experiment in hourly intervals using iButtons temperature loggers (iBCod 22L, Alpha Mach Inc., Mont St. Hilaire, Canada).

2.1.2. Floodplain soil and riverbed sediment properties

To characterize floodplain soils and riverbed sediments we measured their moisture, texture, OM and nutrient content (C, N, P, K, Ca, Mg, Na, S) in Chapter IV and V, respectively. To measure soil moisture, we estimate the gravimetric water content (GWC) (Robertson et al. 1999) from fresh soil samples in monthly intervals. Soil texture was estimated using the flied determination of FAO (2006) and OM by the loss during ignition of dried soil samples (24 h at 105 °C, followed by 550 °C for 4 h). C and N content in soils were analysed in sieved samples (through 2 mm) by a LECO Tru-Spec CN analyser (LECO Corp., MI, USA) and the content of P, Na, K, Ca, Mg and S by an ICP-OES analyser ICAP 6500Duo, Thermo Scientific (Thermo Fisher Scientific Inc., MA, USA).

2.1.3. Stream characterization

In Chapters III, IV and VI we characterized the studied stream reaches by collecting stream water samples and measuring "in situ" environmental variables at reach scale. Water conductivity, dissolved oxygen (DO), pH and temperature were measured using handheld sondes (Intellical HQD, Hach Lange, Loveland, USA). Additionally, water column depth, water sheet width and flow velocity were measured at habitat scale. Flow velocity was measured with a current meter (MiniAir2; Schiltknecht Co., Zurich, Switzerland). Surface discharge at reach scale was estimated as the product of the average water velocity and the average water depth of the cross-sectional area of the reach at two different points.

Stream water samples were obtained from the stream thalweg and filtered in the field through pre-ashed glass fibre filters (GF/F) (Whatman, Maidstone, UK) and transported to the laboratory on ice to be analysed within 24 h after being collected. Water samples were analysed for nitrogen (NO_3^- and NH_4^+) and soluble reactive phosphorus (SRP) by standard colorimetric methods (APHA 2005) in a Systea EasyChem autoanalyser (Frosinone, Italy). Dissolved nitrogen concentration (DIN) was calculated as the sum of NO_3^- and NH_4^+. Dissolved organic carbon (DOC) was analysed in pre-acidified samples with a Shimadzu TOC-5000A Total Organic C (TOC) analyser (MD, USA).

2.2.POM processing in terrestrial and aquatic ecosystems

We carried out three POM decomposition experiments (Table 2.1) in this thesis, one using wood (Chapter III) and two using leaf litter (Chapters IV and VI). Methods to study wood and leaf litter decomposition were quite similar but had some slight differences.

2.2.1. Wooden sticks decomposition

In Chapter III we used standard wooden sticks (untreated tongue depressors made of *Betula* sp.), hereafter sticks. Standard wooden sticks have proved to perform

similarly to natural wood or leaf litter (Arroita et al. 2012, Gulis et al. 2008, among others). Hence this substrate is very often employed in freshwater studies (Abril et al. 2015, Tank & Winterbourn 1995). In our experiment 5 sticks (15 × 2 × 0.2 cm) were set up on plastic meshes (a set) attaching them using fishing line (Fig. 2.1a). Prior to constructing the stick sets, each wooden stick was air-dried, weighed and numbered with an aluminium tag. Once in the field, stick sets were nailed down to the floodplain ground or to the riverbed depending on the case. After finishing the field incubation, we retrieved individual sticks, which were kept in cold and dark conditions in plastic bags or placed into borosilicate tubes filled with stream water, depending on sticks came from the floodplain or from the stream, respectively (Fig. 2.1b). Once back in the laboratory we washed each stick individually with tap water and then we obtained three subsamples (1 cm long) for subsequent analyses (Fig. 2.1c). The day 0 of each decomposition experiment, we carried an extra set of wooden sticks to the field to consider handling losses and to determine the initial ash-free dry mass (AFDM) of sticks. To do this, the sets were carried to the field, and deployed with the rest of the samples. Then handling controls were returned to the laboratory the same day. There, handling controls were air-dried and weighed to obtain their air-dry mass. Finally, we oven dried (60 °C, 24 h) and weighed again to measure their oven dry mass.

Figure 2.1 Wooden sticks deployed in floodplains, directly exposed to atmospheric and soil conditions (a). Stick samples removed from the stream and kept in borosilicate tubes filled with stream water, for their processing in the laboratory (b); Processing of stick samples in the laboratory to obtain analytical subsamples (wood stick pieces of 1 cm width were cut with scissors) (c).

2.2.2. Leaf litter decomposition

In Chapter IV and VI we performed decomposition experiments using the litter bag technique (Bärlocher 2005). *Phragmites australis* (reed, Chapter IV) and *Alnus glutinosa* (alder, Chapter VI) were selected as leaf litter species. Senescent reed leaves were directly picked from standing plants in a wetland close to Murcia (SE of Spain). Alder leaves were collected in fresh state directly from trees in the floodplain of Löcknitz river in Brandenburg (NE of Germany). Collected leaf litter were air-dried for two weeks and then kept under dark conditions in cardboard boxes until experiment was performed. Leaves with evidences of fungal colonization were discarded.

For the elaboration of litter bags, we enclosed 3-6 g of air-dried leaf litter in mesh bags with different mesh sizes according to the objectives of each study. In Chapter IV, we used only coarse mesh bags (4 mm) as we were interested in the total breakdown process, meanwhile we used both coarse (8 mm) and fine mesh bags (0.2 mm) in Chapter VI, since we wanted to isolate the microbial decomposition process (fine bags) from the combined action of shredders and microbes (coarse bags) (Fig. 2.2a).

To estimate leaf litter decomposition in stream, mesh bags were tied to iron rods and then fixed on the riverbed (Fig. 2.2c). Similar to wooden sticks, an extra set of litter bags were prepared to use them as handling control. At each sampling period, litterbags were retrieved from the stream and kept in cold and dark conditions in plastic bags till laboratory processing, where each bag washed individually above a 250 µm sieve to collect invertebrates. Finally, from each litterbag we selected five random leaves to cut various sets of five leaf disks for subsequent analyses using a cork borer (11 mm diameter) and avoiding the central vein (Fig. 2.2b). Collected invertebrates from litterbags were preserved with alcohol (70%) till taxonomic identification. Number of sampling campaigns, parameters analysed in the leaf litter, and other details of sampling strategy varied among studies and are detailed in each specific Chapter.

Figure 2.2 Coarse mesh bags (red, 8 mm) and fine mesh bags (black, 0.2mm) (a); Processing of leaf litter samples in the laboratory to obtain analytical subsamples (sets of 5 leaf litter disks, 11 mm) (b); Litterbags were immersed in a stream tied to iron rods, which were fixed on the streambed (c).

2.2.3. Decomposition rates calculation

Decomposition rates were calculated by the mass loss estimation between the initial and final ash-free dry mass (AFDM) of sticks or plant litter (Bärlocher 2005), depending on the study. We used handling controls to estimate both the initial AFDM and handling loss (i.e. the difference in the dry mass of handling controls before and after be deployed in the field). We estimated the humidity correction factor as the relationship between the oven dry mass and the air-dry mass of handling controls (Bärlocher 2005). After each sampling, one subsample from sticks (stick piece of 1cm) or litterbags (set of 5 leaf disks), together with the remaining material of each sample, were placed individually in aluminium pans, oven-dried (60 °C, 24 h) and weighed to obtain the oven dry mass (DM). Then, the subsample was ignited (500 °C, 4 h) to determine the final percentage of AFDM (%AFDM). We obtained the litterbag AFDM as the product of %AFDM and the litterbag DM. Finally, we computed the percentage of AFDM remaining (%AFDMr) by dividing the

%AFDM at each sampling date by initial %AFDM. Initial %AFDM was corrected first by the humidity correction factor and taking into consideration handling losses.

We calculated the decomposition rates using an exponential decay model:

$$Mt = M_0\, e^{-kt}$$

where k is the decomposition rate, M_0 the initial AFDM and M_t the remaining AFDM after a period t, measured in days or degree-days to consider the influence of temperature (Petersen & Cummins 1974, Bärlocher 2005). Degree-days units were estimated by summing average daily temperatures recorded during the field incubation.

2.2.4. Changes in POM chemical composition

Besides environmental conditions, the chemical composition of POM is one of the most important drivers controlling POM decomposition. Thus, in every Chapter we analysed changes in POM chemical composition through its decomposition in terrestrial or aquatic ecosystems. In Chapters III, IV and VI, we separated and grounded up to 1.5 g of oven-dried (60 °C, 24 h) stick or leaf litter material to analyse their composition in structural compounds (cellulose and lignin) and main elements (C, N, P and micronutrients) (Table 2.1). Cellulose and lignin concentrations were analysed following the method described by Goering and Van Soest (1970). C and N were analysed with a LECO Tru-Spec CN analyser, whereas P, Na, K, Ca, Mg and S were analysed by an ICP-OES analyser. The elemental chemical composition, cellulose and lignin results were expressed in relative terms as concentration (% of DM). In Chapter III, we also calculated the content of lignin, C and nutrients in mass terms (g) and then we calculated the percentage of loss or increase of each specific element or compound in relation to the initial mass as follows (Romero et al. 2005):

$$[1 - (DMf\ Xf / DMi\ Xi)] \times 100$$

where DM_f is the final dry mass of the stick on each sampling date, X_f is the final element or compound concentration (%) in the stick, DM_i is the initial dry mass of the stick, X_i is the initial element or compound concentration (%).

2.2.4.1. Fourier-transform infrared spectroscopy

There are alternatives to classical procedures explained above to measure the chemical composition of POM. One option is the Fourier-transform infrared spectroscopy (FTIR) used in Chapter VI (Table 2.1). FTIR spectroscopy allows for the identification of a great variety of organic C compounds, such as carbohydrates, lignin, cellulose or fats, through the vibrational characteristics of their structural chemical bonds (Parikh et al. 2014). There are several reasons which have promoted the use of this method in studies of biogeochemistry of soil OM (Lammers et al. 2009, Tatzber et al. 2011, Sánchez-González et al. 2017) and POM decomposition experiments (Dighton et al. 2001, Duboc et al. 2012, Liu et al. 2016a); such as its quick and easy laboratory procedures, or its precision and completeness of information in terms of OM biochemistry. Nevertheless, it has also some drawbacks, such as, the need for high level of expertise in organic biochemistry to interpret FTIR information, or in that the results are semi-quantitative. This requires the use of statistical multivariate analyses to extract useful information from the raw data.

FTIR spectra (as absorbance in units of cm^{-1}) were measured from 400 to 4000 cm^{-1} at a resolution of 4 cm^{-1} with 200 scans per sample. We measured a blank spectrum (pure KBr pellet) of every 5 samples, as a background, to remove the influence of ambient air. We transformed raw data applying vector normalization and baseline adjustment. Then, we extracted heights of seventeen peaks from 800 to 1800 cm^{-1} corresponding to main functional groups of carbohydrates, cellulose, lignin and phenolic compounds (Dighton et al. 2001, Duboc et al. 2012, Liu et al. 2016a). Details of FTIR results interpretation are described in the method section of Chapter VI.

2.2.5. Microbial activity during POM processing

Microbial activity is likely the main responsible for OM decomposition in most terrestrial and aquatic ecosystem. Microbial decomposition allows the mineralization of OM into their inorganic constituents, but also facilitates the action of detritivores by increasing OM palatability. Thus, to analyse microbial activity

over the decomposition process give us information about the decomposition pathways and its response to environmental changes. A great variety of techniques to analyse microbial activity exist. They mainly differ in their degree of specificity, varying from generalist evidences of microbial activity such as heterotrophic respiration, to very specific activities on decomposition, such as the degradation of particular compounds by extracellular enzymes. In this thesis, we estimated microbial activity by the fluorescein diacetate hydrolysis technique (FDA) in Chapter III, by the cellobiohydrolase activity in Chapter IV and by a respiration assay in Chapter VI.

2.2.5.1. FDA

The FDA method (Claret & Boulton 2003) is based on the release of a fluorescent substrate (fluorescein) as results of hydrolytic esterase activity. This technique has been used frequently in litter breakdown studies to estimate decomposer activity (e.g. Corti et al. 2011; Datry et al. 2011), since the resulting data are strongly correlated with ATP content and cell density (Stubberfield & Shaw 1990, Gillian & Duncan 2001). We used the FDA technique to estimate microbial activity in sticks in Chapter III. For this, each stick piece (1 cm width) was placed into 12 mL falcon vials and incubated with 3 mL of phosphate buffer (pH 7.6) and 0.2 ml of fluorescein diacetate (FDA) in a water bath at 30 °C for 30 min. Then, the reaction was stopped adding 3 ml chlorofolm:methanol and placing the vials into ice. Finally, absorbance was measured at 490 nm with a Shimadzu UV1700 (Shimadzu Corporation, Kyoto, Japan) and converted to µmol of fluorescein using a standard curve. Microbial activity was expressed as µmol FDA g^{-1} AFDM h^{-1}.

2.2.5.2. Extracellular enzymatic activities

Cellulose is the principal structural polysaccharide of vascular plants, consequently it supposes one of the main sources of C and energy for microbial communities during leaf litter decomposition (Sinsabaugh et al. 1992). Cellobiohydrolase activity (EC 3.2.1.91) is the main extracellular enzyme involved in the degradation of

cellulose. In Chapter IV we assessed cellobiohydrolase activity (CBH) as an indicator of the microbial decomposer activity in the leaf litter using the fluorogenic model substrate 4-Methylumbelliferyl β-D-cellobioside (MUF-cellobioside) (German et al. 2011). For this, fresh leaf litter disk sets were placed into 12 mL falcon vials with 4 mL of acetic acid buffer (50 mM, pH = 5) and sonicated for 3 minutes in a water bath at room temperature. Afterwards, the MUF-cellobioside was added to each sample to reach the enzymatic saturation concentration at 0.5 mM. Samples were incubated for 90 minutes on a shaker at room temperature and in darkness. At the end of incubation, the enzyme reaction was stopped adding 2 mL of glycine - NaOH buffer (0.05 mM, pH 10.4) to the samples. CBH activity was quantified by fluorescence measurement at 365 nm excitation and 450 nm emission (Hitachi F4500; Hitachi Instruments Inc., Japan).

Due to the interference of the leaf litter sample and of different reagents on the emission of fluorescence by MUF, we prepared a set of controls according to German et al. (2011) to ensure we had the most accurate estimation of the CBH activity through the application of the MUF standard curve. For this, we prepared: blanks (controls for the abiotic degradation of MUF-cellobioside using tubes with all reagents but no leaf litter disks); controls of the colour of the sample (fluorescence emitted from the sample without MUF-cellobioside); MUF standards and quench controls (MUF standards with leaf litter disks to control the interferences in MUF fluorescence caused by the absorbance emitted by the sample). CBH activity results were expressed as μmol MUF-cellobioside g^{-1} AFDM h^{-1}.

The saturation concentration was estimated in a preliminary assay incubating leaf litter samples in a gradient of increasing concentrations of MUF-cellobioside, till detection of the substrate concentration where CBH was saturated. To assay hydrolytic enzymes under saturation concentrations results extremely important as it increases the power of the analysis to detect differences in the enzymatic activity of different treatments due to microbial communities are expressing their potential enzymatic activity (German et al. 2011).

2.2.5.3. Microbial respiration

Perhaps, the most popular and easiest way to analyse microbial decomposer activity is through microbial respiration assays in closed chambers (Tank et al. 2010). This method is extremely simple, as it only consists of measuring changes in dissolved oxygen concentration in closed chambers filled with water, but at the same time, it is the most holistic, as it integrates the respiration of every microorganism and extracellular activity involved in the decomposition of OM. In Chapter VI, we conducted a respiration assay consisting in the measurement of oxygen consumption rates on different leaf litter mixtures as a proxy for microorganism metabolism. For this, we incubated 12 leaf discs (11 mm) obtained from leaf litter mixture in 250 mL sealed vials filled with mineral water (Volvic) at room temperature in a water bath. As microbial inoculums we used 10 mL of river water filtered by 0.7 µm pre-combusted glass fibre filters (Whatman GF/F). DO concentrations were measured 13 times over 24 days with a needle-based micro-optode (Oxygen Microsensor PM-PSt7; PreSens, Regensburg, Germany) using a stand-alone, portable, fibre-optic O_2 meter (Microx 4 trace; PreSens, Regensburg, Germany). 10 vials were filled with water as a respiration control. Oxygen consumption rates (day^{-1}) were computed as the decomposition rate (see above) but using the decrease of dissolved oxygen concentration over time on a daily basis.

2.2.6. Fungal biomass

Fungi are the most important actors involved in the decomposition process (Gessner & Chauvet 1994, Duarte et al. 2010). So, quantifying fungal biomass is usually as important as microbial activity to describe and understand the evolution of the decomposition process. The most common indicator of living fungal biomass is ergosterol, as it is a major membrane component only found in fungi (Gessner 2005). We used ergosterol to quantify fungal biomass in sticks and leaf tissue (Martin et al. 1990, Gessner 2005) in Chapters III, IV and VI. In Chapter III, we followed the protocol of Martin et al. (1990), consisting in a sequential extraction by methanol and hexane. Methanol was used instead of ethanol as an extraction alcohol because

of its observed greater extraction efficiency. Wooden stick pieces were oven-dried (60 °C, 24h) and weighed at the end of the extraction. In Chapter IV and VI, frozen leaf litter disks from each leaf litter sample were lyophilized and weighed. Then, lipids were extracted using a KOH-methanol solution at 80 °C for 30 min. The extracted lipids were purified using solid-phase extraction cartridges (Waters Sep-Pak®, Vac RC, 500 mg, tC18 cartridges, Waters Corp., Milford, MA, USA). Finally, ergosterol was eluted using isopropanol and quantified by HPLC with absorbance detection at 282 nm (Agilent 6220; Agilent Technologies, Silicon Valley, CA, USA). Ergosterol concentration was always expressed as µg ergosterol g^{-1} AFDM.

2.2.7. Macroinvertebrates density

Macroinvertebrates found in leaf litter coarse mesh bags in Chapters IV and VI were separated and preserved in 70% ethanol until sorting. Individuals were counted and identified to family or genus level. The density of total individuals and shredders were expressed as individual g^{-1} AFDM.

2.3. DOM characterization and processing in aquatic ecosystems

DOM is a diverse mixture of thousands of organic molecules with different origin. One of the principal sources of DOM for aquatic ecosystems is the leaching of terrestrial plant litter. In intermittent rivers, riverbed sediments, autochthonous vegetal detritus, such as dead macrophytes or biofilm, and allochthonous material accumulated in dry channels during drought episodes. All of them may result in a noticeable although pulsed DOM input after flow resumption. In this thesis, we focused on analysing how different terrestrial environmental conditions affect to the production of plant litter or sediment leachates, their chemical composition and how they are metabolized by aquatic microbial communities.

2.3.1. Leachates preparation

In Chapter IV and V, we prepared aqueous leachates from leaf litter and riverbed sediments (Fig. 2.3) to analyse the chemical composition of the leached DOM and DOC and nutrient concentration. Leachates were extracted in pre-combusted glass beakers flasks, with Milli-Q water in the darkness and under gently stirred by shaking at 4 °C for 24 h. We used mass:water volume ratio of 1:800 and 1:5 (g:mL) for leaf litter and sediments, respectively. After 24 h, leachates were immediately filtered through pre-combusted glass fibre filters (Whatman GF/F) into different vials. Sediment leachates were centrifuged (10 min, 4500 rpm) before filtration. Aliquots for the various analyses were stored in acid-washed and pre-combusted glass vials. Leachates samples were stored in the fridge or frozen depending on the analyses. DOM chemical composition through spectroscopic measurements was always analysed in fresh leachates the same day of preparation.

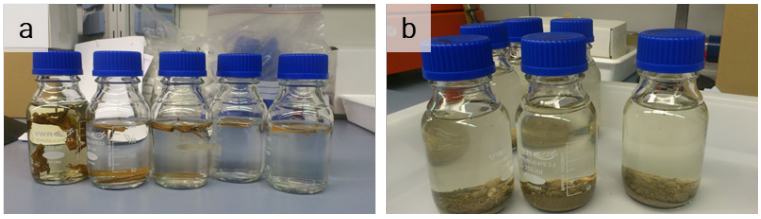

Figure 2.3 Aqueous leachates prepared from plant litter substrates, such as dead macrophytes and leaf litter (a) and from riverbed sediments (b).

2.3.2. DOC and nutrient concentration in leachates

DOC and nutrients (NO_3^-, NH_4^+ and SRP) were analysed using the same techniques employed for stream water characterization (see 2.1.3.).

2.3.3. Spectroscopic DOM characterization

Spectroscopic measurements (absorbance and fluorescence) are the most extended techniques to analyse the chemical composition of DOM as they provide reliable

information through fast, inexpensive and easy to implement methodologies (Jaffé et al. 2008, Fellman et al. 2010). Spectroscopic measurements are based on the capacity of certain DOM molecules to absorb part of the light spectra. Therefore, this method has some recognized drawbacks, as it only provides information about a reduced fraction of the DOM pool (40-60%), and it does not allow the identification of specific biomolecules, or their concentration. However, absorbance but mainly fluorescence metrics have been demonstrated to provide excellent information of chemical and functional properties of DOM as well as biogeochemical and ecological processes which occurred in the past (Jaffé et al. 2008). This converts spectroscopic DOM characterization in a very useful and valuable tool to understand DOM dynamics in aquatic ecosystems. Spectroscopic measurements range from very basic absorbance indexes providing information about DOM aromaticity or molecular size, to fluorescence indexes reflecting the source, redox state or biological reactivity of DOM. In addition, we can recognize individual components of the fluorescence signature of DOM through multivariate analyses of excitation emission matrices (EEMs), which provide a more detailed information about biochemical composition and origin of DOM (Stedmon & Bro 2008, Fellman et al. 2010).

In Chapter V, we recorded absorbance scans and EEMs simultaneously using an Aqualog® (Horiba Scientific, Japan); whereas, in Chapter IV we used a spectrophotometer Shimadzu UV1700 and a fluorescence spectrophotometer Hitachi F450. Fluorescence intensities were measured at excitation wavelengths ranging from 250 to 600 nm (5 nm increments) and emission wavelengths from 212 to 620 nm (2 nm increments). EEMs were corrected for blanks (Milli-Q water) and inner filter effects (Stedmon & Bro 2008). We then characterised composition of DOM by a range of indices. From absorbance data we computed the ratio of absorptions at 250 and 365 nm (E_2:E_3) (De Haan & De Boer 1987), the spectral slope for 275–295 nm ($S_{275–295}$) (Helms et al. 2008), the ratio between slopes in wavelength regions 275-295 to 350-400 nm (Sr) (Helms et al. 2008) and $SUVA_{254}$, calculated as the decadal absorption coefficient at 254 nm relative to DOC (mg L^{-1}). The indices E_2:E_3, $S_{275–295}$ and Sr have been reported to correlate inversely with DOM average molecular weight (De Haan & De Boer 1987, Helms et al. 2008), while $SUVA_{254}$ is

an accepted surrogate for DOM aromaticity (Weishaar et al. 2003). From EEMs we calculated the humification index (HIX) and the fluorescence index (FI). HIX was calculated as the peak area of the emission wavelengths from 435 to 480 nm divided by the peak area of the emission wavelengths from 300 to 445 nm, at an excitation wavelength of 254 nm (Zsolnay et al. 1999). As its own name indicates, HIX is used as an indicator of humic-like DOM content or extent of humification. The FI was calculated as the ratio of emission intensity at 450 nm to that at 500 nm for an excitation wavelength of 370 nm (McKnight et al. 2001), and it is often used as an indicator of DOM source or microbial decomposition (i.e. ~1.3 values suggest terrestrial source, whereas ~1.8 values suggest the dominance of microbially derived DOM).

In addition, in Chapter V, we performed parallel factor analysis (PARAFAC) on EEMs using the DOMFluor Toolbox (version 1.7; Stedmon & Bro 2008) for Matlab (version 7.11.0, MathWorks, Ismaning, Germany). PARAFAC is a multivariate modelling technique that allows the decomposition of the complex mixture of fluorescence signals of DOM into their individual fluorescent components and the estimation of the relative contribution of each component to total DOM fluorescence (Stedmon & Markager 2005). We followed common PARAFAC modelling guidelines including split-half validation and multiple random model initializations (see Stedmon & Bro 2008 for a complete development of the analytical procedures).

2.3.4. Ultrahigh-resolution mass spectrometry

In Chapter V, for an in-depth characterization of DOM, we used ultrahigh-resolution Fourier-Transform Ion Cyclotron Mass Spectrometry (FT-ICR-MS). This novel technique is characterized by a very high-resolution capability that allows the identification of specific biomolecules conforming DOM through the assignment of molecular formulae to peaks measured by mass spectrometry (Kujawinski 2002). Evidently, the main disadvantage of this technique is the high expertise in biochemistry required both to carry out the laboratory and the data analysis procedures. Very briefly, the method consists of a combination of analytical

procedures to decompose a sample into their constituent ions. For this a liquid sample is firstly ionized, so released ions can react to a magnetic field and be identified by their mass to charge ratio (m/z). Then, excited ions signal is fourier-transformed to get the final mass spectrum of the sample (Kujawinski 2002), from which we can proceed to the assignment of molecular formulae.

In our case, leachates samples were adjusted to 5 ppm carbon in 1:1 ultrapure water/methanol prior to broadband mass spectrometry on a 15 Tesla Solarix FT-ICR-MS (Bruker Daltonics, Bremen, Germany) in electrospray ionization (ESI) negative mode (400 accumulated scans, 2 sec ion accumulation time) searching for masses from 153 to 1000 Da. Following internal calibration, peaks with S/N>1 were exported from Bruker-DataAnalysis software for further data analysis using in-house code in R. First, we computed method detection limits similar to Riedel & Dittmar (2014) as upper limits of one-sided 99 and 99.9% confidence limits of intensities of 'noise' peaks (MDL_{99} and $MDL_{99.9}$). Here, noise peaks were sampled randomly from all available spectra and for each nominal mass in mass ranges defined by mass defects not normally occurring in natural OM (mass defect intervals of [-0.5, -0.2] and [+0.4, +0.5] Da around nominal masses). We then pooled peaks>$MDL_{99.9}$ and generated a kernel density profile for each nominal mass using peak-specific m/z (rounded to 10^{-6} Da) and full-width-at-half-maximum as local kernel bandwidth. Local maxima in the density profile were used as a masterlist, to which peaks of individual spectra were matched in order to achieve a matrix of aligned peaks across all spectra. This was done in a step-wise sequence involving ever smaller peaks (quantile thresholds) to generate the kernel density profiles and repeatedly recalibrating spectra to mean m/z computed across spectra at each step. This procedure resulted in a matrix totalling 82 k compounds with an improved alignment of small peaks. After removal of approximately 67% singlets and 72 contaminants with known mass we assigned molecular formulae to mean m/z assuming single-charged deprotonated molecular ions and Cl-adducts for a maximum elemental combination of $C_{100}H_{250}O_{80}N_4P_2S_2$, with a mass tolerance of 0.6 ppm, and using the following restrictions: agreement with the nitrogen rule, positive integer double bond equivalent for uncharged molecule, minimum $C_1H_1O_1$, P<(O+1), S<(O+1), H:C within [0.3, 2.5], O:C and N:C within [0,1], H≤2C+2+N,

at least 1 O for each P or S. We then used unequivocal CHO assignments to partition m/z measurement error into a systematic and a truly random component (Savory et al. 2011). The random component could successfully be modeled as linearly dependent on peak intensity (lower m/z error for intense peaks). A sizeable number of formulae could then be ruled out by applying a refined tolerance for a formula´s mass error computed as:

$$tolerance\ (ppm) = z_{99} \cdot \sqrt{err_{sys}{}^2 + (err_{rnd}/\sqrt{N})^2}$$

where z_{99} is the 99% quantile of the normal distribution, err_{sys} and err_{rnd} are systematic and random error, respectively, and N is the frequency of occurrence across all samples. This m/z error tolerance was modelled as formula- and peak-specific as it depended on N and on peak-intensity, which linearly predicted err_{rnd}. We then checked for isotope confirmation of all potentially valid formulae using generated isotope intensity patterns (up to 6 most prevalent daughter peaks considering isotopes of all elements except P) and based on adequate mass shift(s) and adequate intensity ratio(s) (approx. ±35% as determined from unequivocal CHO assignments) of isotopic daughter peaks to the monoisotopic, parent peak (Koch et al. 2007). A single daughter isotope peak sufficed for confirmation of a suggested sum formula, 2 daughter peaks were minimum for sum formulae with Cl, which has abundant secondary isotopes and produces prominent daughter peaks besides those produced by exchange of ^{12}C by ^{13}C. In case of multiple formula assignments to the same mean m/z, we gave preference to formulae involved in longer homologous series; here, length of a series was based on simultaneous consideration of CH_2, CO_2 and H_2O as chemical building blocks for aliphatic, acid-based and alcohol-based elongation (Koch et al. 2007). In this data-processing, formula assignment, m/z error partitioning, isotope confirmation and homologous series assessment were repeated in two iterative steps, during which the systematic error was lowered from 0.03 to 0.05 ppm by m/z recalibrations done similarly to Savory et al (2011). In contrast to these authors, we used general additive models (Hastie & Tibshirani 1990, Venables & Ripley 2002) of error dependent on m/z rather than segment-wise polynomial fits of m/z on ion cyclotron frequency. Before statistical analysis, we further filtered the FT-ICR-MS dataset based on a 'replicate

filter', i.e., any singlet molecular formula determined for a set of 3 replicates was deleted (Payne et al. 2009). The final dataset consisted of approx. 19 k masses and 1851 sum formulae covering on average 46% of total spectrum intensity.

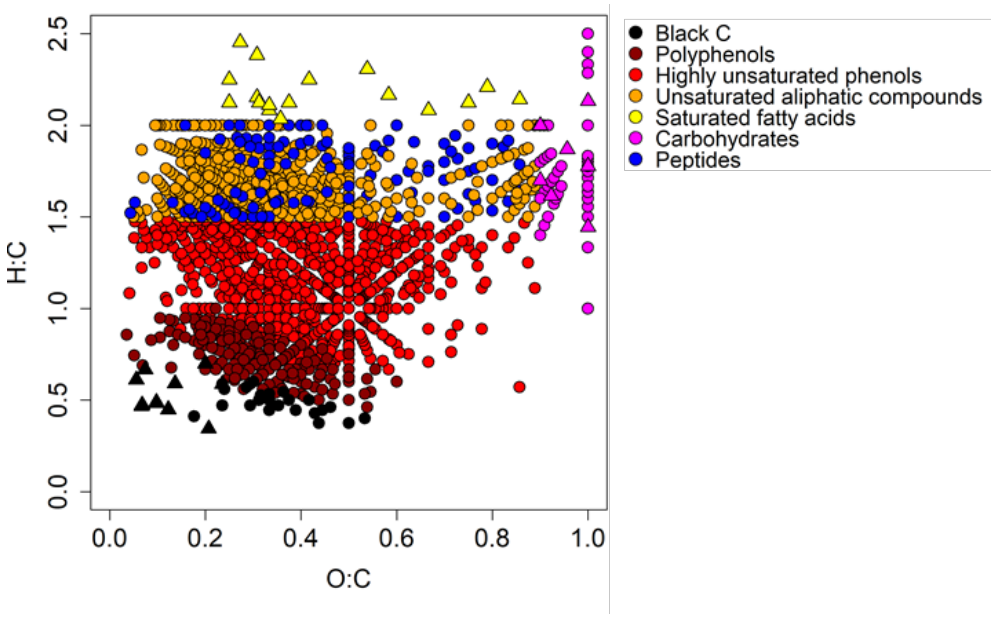

Figure 2.4 Representation of the principal molecular groups found in leaf litter leachates from Chapter V by FT-ICR-MS using van Krevelen plots. These graphs allow the identification of the different molecules (dots) which are present in DOM samples by their distribution in the van Krevelen space. This is defined by atomic O:C and H:C ratios. For instance, a molecule with low O:C and H:C ratios is within the black C or polyphenol region (black and dark red dots), whereas a molecule with high O:C and H:C is within the carbohydrate region (pink dots).

FT-ICR-MS data is graphically presented in van Krevelen plots, which show identified sum formulae in a space defined by O:C (oxygen richness) and H:C (saturation) ratios (Fig. 2.4); plotting order was random to avoid bias created by systematic overplotting of thousands of compounds. To condense the rich mass-spectrometric information, we grouped formulae into 12 non-overlapping molecular groups (Lesaulnier et al. 2017) based on elemental composition and derived structural information such as double bond equivalents (DBE) and a computed aromaticity index (Koch & Dittmar 2006). We then summarised the raw monoisotopic peak intensities of all formulae assigned to a molecular group. Finally,

we obtained the relative intensity of each molecular group (i.e. the intensity of a single molecular group divided into the total intensity of all groups), their total number of differing formulas (counts) and the relative counts (i.e. number of formulas of each group divided by the total number of differing formulas - molecular richness -). As further response variables we computed the average molecular mass.

2.3.5. Biodegradation of DOM from leachates

In this thesis we talk about bioavailability, biodegradability and biodegradation referring to DOM or leachates. Although all these three terms are conceptually close, there are some subtle differences among them. We must consider differences among these three terms for a total understanding of the different experimental approaches carried out in each Chapter. According to Marschner & Kalbitz (2003), DOM bioavailability describes the potential of microorganisms to uptake and use DOM. On the other hand, DOM biodegradation is considered the consumption of organic compounds by microorganisms and is quantified by the decay of DOC or DOM, or by the increase in CO_2. In other words, bioavailability is a requirement for subsequent biodegradation (Marschner & Kalbitz 2003). Lastly, we talk here about leachates biodegradability when using evidences of microbial heterotrophic activity in leachates as a proxy of DOM uptake or biodegradation. Thus, in Chapter IV, we estimated the microbial biodegradability of the leached DOM by measuring the microbial metabolic activity associated with the leachates using the R_{az}-R_{ru} (resazurin-resorufin) chemical system following Haggerty et al. (2008) and González-Pinzón et al. (2012). In Chapter V, we determined both DOC bioavailability (BDOC) and biodegradation as the DOC decay in leaf litter incubations (Servais et al. 1989).

2.3.5.1. R_{az}-R_{ru}

The R_{az}-R_{ru} chemical system is a metabolic tracer, which is based on the transformation of R_{az} into R_{ru} under reduced conditions generated by microbial respiration. For this, leachate samples were previously filtered by 0.2 µm cellulose

acetate membrane filters (ABLUO ® GVS, Sanford. USA) and a R_{az} solution was added to result in a concentration of 200 µg R_{az} L^{-1} in the vials. These solutions were then spiked with a microbial inoculum consisting of an aqueous extract of sediment from a river filtered by a GF/F filter. The incubations lasted for 1 h and we measured the increase of R_{ru} over this time by measuring the fluorescence at 571 nm excitation and 602 nm emission. Results of microbial metabolic activity associated with leachates were expressed using the R_{ru} production rates (mmol R_{ru} g^{-1} AFDM h^{-1}).

2.3.5.2. Biodegradation assays

BDOC in the leachates was experimentally determined by 8-day incubations of 500 mL 0.2 µm sterile-filtered (pre-rinsed nylon membrane filters, Whatman) leachates diluted to 15 mg L^{-1} DOC and adjusted to a C/N/P ratio of 100/10/1 by adding nutrient solutions (NH_4-NO_3 and K_2HPO_4). These solutions were prepared in acid-washed and pre-combusted bottles and inoculated with a common natural microbial inoculum (GF/F-filtered river water) at a volumetric ratio of 1:10. The bioassay bottles were loosely capped with combusted aluminium foil to avoid contamination yet allow continuous oxygenation. Incubations were performed at room temperature and in dark conditions to avoid photosynthetic activity. From each bottle, six samples for DOC analyses and spectroscopic measurements were taken at 0, 1, 2, 4, 6, 8 days. BDOC was calculated as the difference in DOC concentration at days 0 and 8 and expressed as percentage of the initial concentration (%BDOC). DOC decay rates were computed from exponential fits of DOC concentration over incubation time.

Finally, it is worth noting that there are two key differences between the biodegradation assays carried out in Chapter IV and III. Firstly, in Chapter IV, we analysed leachates biodegradability indirectly through the measurement of a microbial metabolic activity; whereas, in Chapter V we measured directly DOM biodegradation in leachates as the disappearance of DOC during the laboratory incubation. Secondly, in Chapter V, we adjusted all the leachates to the same DOC and nutrient concentration previously to the incubation, so only the chemical quality of DOM varies between the studied treatments, being a strict prerequisite when

discussing DOC bioavailability. On the contrary, in Chapter IV we did not adjust either DOC and nutrients concentration, therefore both factors varied among leachates samples and affected the potential biodegradability of each leachate sample.

3.

Exposure of wood in floodplains affects its chemical quality and its subsequent breakdown in streams

Parra floodplain and Corneros, Turrilla and Chícamo streams (top to bottom)

Abstract

In stream ecosystems, coarse organic matter from the riparian vegetation, a key food resource, is often retained in the floodplains before reaching the channel. During floodplain exposure, organic matter can be affected by abiotic and biotic processes ("preconditioning"), which alter its quality and affect its subsequent decomposition in streams. We analysed the effect of floodplain preconditioning on wood quality (lignin, C, N, P, K, among others), and its subsequent aquatic breakdown, paying special attention to microbial activity. We simulated preconditioned standard wooden sticks on one arid stream floodplain for 3 and 4 months, and then monitored their breakdown in three different streams, together with control (non-preconditioned) sticks. Preconditioning reduced lignin mass and C:N and lignin:N ratios, caused the leaching of soluble nutrients such as P and K, as well as N immobilisation by microbes. These changes enhanced the breakdown of wood in the first week of immersion but had no effect on breakdown rates after 4 months of incubation in the streams, although N immobilization was diminished. Our results suggest that terrestrial preconditioning could alter the role of wood as a long-lasting nutrients and energy source for freshwater ecosystem.

3.1. Introduction

In most forested streams, leaf litter is the most important fraction of allochthonous organic matter inputs (Benfield et al. 1997, Wallace et al. 1995) and is thus an essential energy source for ecosystems (Fisher and Likens 1973, Kuehn 2015, Tank et al. 2010). Additionally, wood litter can represent an important energy source for aquatic heterotrophs and also a relevant structural support for microorganisms that grow on its surface (i.e. Golladay & Sinsabaugh 1991, Rabeni & Hoel 2000). Whereas leaves are rapidly flushed away, wood litter is a long-lasting resource that increases the pool of nutrients (Romero et al. 2005) and stored carbon and significantly contributes to the energy flux in streams (i.e. Elosegi et al. 2007). In arid and semiarid regions, where deciduous vegetation is scarce, or even absent, and riparian vegetation is dominated by perennial woody shrubs (Bruno et al. 2014, Salinas et al. 2007), wood litter may represent an essential energy resource for freshwater ecosystems functioning (Jacobson et al. 1999).

The dynamics and breakdown of organic matter in streams are affected by climate, flow regimes and the riparian vegetation structure (Larned et al. 2000, Schade & Fisher 1997). In dryland streams, before entering the stream channels, most coarse organic matter from terrestrial and riparian vegetation is usually transported by floods and retained in floodplains (Jacobson et al. 1999), where its preconditioning may have a substantial effect on its subsequent use by aquatic organisms (Fellman et al. 2013, Pu et al. 2014). These dynamics differ from those occurring in more humid regions, where the bulk of leaf litter inputs from riparian trees tend to enter the stream channel directly (Pozo et al. 1997, Winterbourn 1976). Today arid regions occupy almost 40% of the land surface (Safriel et al. 2005), but are likely to increase as a result of ongoing climate change (Döll & Schmied 2012, Reynolds et al. 2007). Therefore, it is important to improve the knowledge on the effects of preconditioning on breakdown.

When exposed in floodplains, organic matter may be affected by abiotic (solar radiation, wind, rain and soil burial) and biotic factors (microbial colonisation and grazing by invertebrates). However, in dry areas, abiotic factors usually have a stronger influence on organic matter breakdown than biotic ones due to water

scarcity (Austin 2011, King et al. 2012, Whitford & Wade 2002). In these environments, photodegradation can be an important process in organic matter breakdown as it can affect mass loss and chemical composition (Austin & Ballaré 2010, Brandt et al. 2010, Day et al. 2007, Gallo et al. 2009). For instance, photodegradation can degrade recalcitrant compounds, such as lignin or phenolic compounds, which absorb solar radiation (Austin & Ballaré 2010, Gallo et al. 2009). This fact has been proved to increase the leaching of soluble phenolic compounds (Fellman et al. 2013, Gallo et al. 2009) and to facilitate access of microorganisms to labile C resources (Foereid et al. 2010, Henry et al. 2008, Pu et al. 2014). Nevertheless, photodegradation may also reduce organic matter quality by promoting the leaching of nutrients and labile C compounds (Dieter et al. 2013). Hence, it is well known the positive linear relationship between organic matter decomposition rates and N and P concentration (e.g. Enriquez et al. 1993). As the chemical composition of organic matter is a key factor in determining its decomposition and use by microorganisms (Webster & Benfield 1986, Zhang et al. 2008), any change in organic matter quality that occurs during its exposure in floodplains is expected to have implications on its subsequent aquatic decomposition. Previous studies found contrasting effects of terrestrial preconditioning of leaf litter on its aquatic decomposition. Some showed increased decomposition rates (Fellman et al. 2013, Pu et al. 2014), whereas others reported the opposite or no effects (Dieter et al. 2011, 2013, Mora 2014). Given the potential significance of organic matter preconditioning in floodplains, which could also extend to more humid streams in the near future, this study aimed to analyse the effect of a long exposure period of wood in a stream floodplain on both its chemical quality and its subsequent aquatic decomposition. Special attention was paid to leaching and microbial activity in streams. For this purpose, we compared changes in chemical quality, aquatic decomposition and microbial activity between preconditioned and non-preconditioned wood. We hypothesised preconditioning to affect wood chemical composition and to increase aquatic breakdown rate due to greater microbial activity.

3.2. Material and methods

3.2.1. Experimental approach

To carry out this study, we designed an experiment divided into two phases, firstly we simulated wood exposure in a stream floodplain during a summer drought (terrestrial phase); then we simulated its arrival at streams (aquatic phase). In both phases, we analysed changes in wood quality, breakdown rates and microbial activity. For this experiment we used standard wooden sticks (untreated tongue depressors made of *Betula* sp.), hereafter *sticks.* Standard wooden sticks have proved to perform similarly to natural wood or leaf litter (Arroita et al. 2012, Gulis et al. 2008). Hence this substrate is very often employed in freshwater studies (Abril et al. 2014, Tank & Winterbourn 1995).

3.2.2. Study site

The experiment was carried out in four streams located in the Segura River Catchment in Murcia (SE Spain). The terrestrial phase was undertaken in the floodplain of a temporary stream, called Rambla de la Parra (1º05'20'', 38º13'51''), whereas the aquatic phase took place in the perennial stream reaches of the Chícamo (1º01'09'', 34º14'25''), Turrilla (1º53'13'', 37º46'28'') and Corneros (1º51'39'', 37º43'37") streams. All three streams are located in an arid region (Peel et al. 2007) and have a Mediterranean climate (< 300 mm average annual rainfall and an average annual temperature of 18 °C) (SHC 2007). Marls and limestones are the dominant lithology of these streams, characterised by their low flow discharges, broad channels, and wide floodplains with sparse riparian vegetation.

3.2.3. Field procedures

For the terrestrial phase, we made stick sets as described in Chapter II, section 2.2.1. The field procedures began in July 2012 by installing 10 stick sets in an open fully

exposed area of the Rambla de la Parra floodplain. One month later in August, 10 more stick sets were placed in the same area to test the effect of 4 and 3 months of floodplain exposure; that is, the typical length of a summer drought in this area.
10 sticks from the 3-months exposure and 8 sticks from the 4-months exposure (as we lost two sticks), were used to analyse the effect of floodplain exposure on the chemical composition and microbial activity. All the other sticks were preserved for the experimental aquatic phase.

To carry out the aquatic phase, we constructed new stick sets. Each set was made up of the 3- and 4-month exposed sticks, plus the control sticks (with no prior floodplain exposure). At each sampling date, 4 replicates of 3- and 4-month exposed sticks and control sticks were removed from each stream. Stick replicates were distributed between four different stick sets. Sticks sets were staked firmly to the streambed along a 50-metre long reach on similar substratum, and pools were avoided. Long axes of sticks were oriented in parallel to the direction of flow. Stick sets were placed in streams in early December 2012 and were retrieved on two sampling dates: after 7 days to analyse leaching (December 2012); after 4 months to estimate breakdown rates (April 2013). Because of vandalism, we lost all stick sets after the first sampling date (7 days) in Chícamo stream. See section 2.2.1. for further explanation about stick collection and processing. During the aquatic phase we characterized stream reaches as indicated in section 2.1.3.

3.2.4. Laboratory procedures

Once in the lab, we cut three subsamples (1 cm long) from each stick to analyse wood mass loss, chemical composition, microbial activity (FDA) and fungal biomass (ergosterol). See sections 2.2.3., 2.2.4., 2.2.5.1. and 2.2.6., respectively for necessary explanations of the different methods.

3.2.5. Data analysis

For the terrestrial phase, we analysed any differences in OM loss, ergosterol concentration and FDA activity, between the sticks exposed for 3 and 4 months in

the floodplain, using a T-test analysis. Chemical composition of the sticks in terms of concentration (% of DM) was analyzed using one-way ANOVA with the floodplain exposure period as fixed factor (with 3 levels of floodplain exposure, 0-, 3- and 4-month). Changes in sticks chemical composition in terms of mass, before and after floodplain exposure, were analysed using a repeated-measures ANOVA (RM-ANOVA), with the floodplain exposure period (3- and 4-month) as a fixed factor and time (before and after floodplain exposure), as the repeated-measure factor. For the aquatic phase, we analysed differences in OM loss, chemical composition in terms of concentration, ergosterol concentration and FDA activity between the 3- and 4-month preconditioned sticks and the control sticks (non-preconditioned) using a generalised randomised block design (GRBD). In this design, the study streams were used as the block factor and the preconditioning as the fixed factor (with 3 levels, the control, the 3- and the 4-month floodplain preconditioning). Replication within each block allowed us to analyse any interactions between preconditioning factor and blocks. Significant interactions were checked using simple effects analyses. Tukey's *post hoc* test was used when the fixed factor showed significant differences. Changes in the chemical composition of sticks in terms of mass, before and after the aquatic phase, were analysed using a mixed model design, employing the preconditioning as the fixed factor, streams as the block factor and time (before and after the aquatic phase) as the repeated-measure factor. We examined the relationships between studied variables (i.e. %OM loss, wood quality, FDA activity and ergosterol concentration), and the relationship between these variables and environmental variables, (i.e. water nutrient concentration, flow velocity and so on), by simple linear regression models and Pearson correlations. Prior to the analyses, we checked the assumptions of normality and homoscedasticity with Shapiro-Wilk and Levene tests. The logarithm and square transformations of the data were used to meet these assumptions whenever necessary. In those cases when data did not perfectly meet the assumptions of homoscedasticity and normality, GRBD was deemed an acceptable method given its robustness to moderate violations of test assumptions (Box 1954). All the analyses were performed by SPSS (version 19.0/SPSS Inc., Illinois, USA).

Table 3.1 Physicochemical characteristics (mean ± SE; n = 15) of the study streams reaches. In parentheses, we provide the minimum and maximum values for each measured variable.

	Corneros	Turrilla	Chícamo
Discharge (L s^{-1})	103.3 ± 23.6	14.8 ± 0.9	25.3 ± 1.4
	(36. 8 - 262.3)	(10.4 - 18.5)	(17.6 - 31.8)
Flow velocity (m s^{-1})	0.46 ± 0.04	0.23 ± 0.03	0.36 ± 0.06
	(0.36 - 0.55)	(0.13 - 0.36)	(0.33 - 0.51)
Water column depth (cm)	12.17 ± 1.17	7.94 ± 0.52	5.50 ± 0.27
	(8.50 - 16)	(6.40 - 10.25)	(4.75 - 6.25)
Water conductivity (µS cm^{-1})	1305 ± 1	8783 ± 19	2592 ± 11
	(1926 - 1315)	(8.726 - 8836)	(2584 - 2600)
DOsat (%)	112.8 ± 1.9	113.4 ± 2.1	103.9 ± 0.8
	(111.4 - 114.1)	(112.5 - 114.2)	(103.2 - 104.2)
Temperature (ºC)	14.56 ± 0.75	11.79 ± 0.84	14.55 ± 0.98
	(14.40 - 14.68)	(11.34 - 12.24)	(14.38 - 14.76)
pH	8.37 ± 0.01	8.07 ± 0.01	8.40 ± 0.01
	(8.37 - 8.40)	(8.04 - 8.11)	(8.39 - 8.41)
NH_4^+ (mg l^{-1})	0.068 ± 0.019	0.113 ± 0.014	0.027 ± 0.005
	(0.024 – 0.137)	(0.083 – 0.164)	(0.010 – 0.044)
NO_3^- (mg l^{-1})	1.74 ± 0.20	2.38 ± 0.39	3.15 ± 0.30
	(1. 26 - 2.26)	(1.15 - 3.47)	(3.09 - 4.71)
DIN (mg l^{-1})	1.81 ± 0.19	2.50 ± 0.39	2.65 ± 5.45
	(1.38 - 2.29)	(1.23 - 3.56)	(0.05 - 32.41)
SRP (µg l^{-1})	0.65 ± 0.56	0.55 ± 0.60	0.08 ± 0.07
	(0.00 - 3.08)	(0.00 - 3.28)	(0.0 - 0.41)

DOsat: Dissolved oxygen saturation; DIN: dissolved inorganic nitrogen; SRP: soluble reactive phosphorous.

3.3. Results

3.3.1. Climatic and environmental variables

Rambla de la Parra floodplain received during the terrestrial phase an average UV radiation of 71160 J m^{-2} and an average global solar radiation of 520425 KJ m^{-2}. Daily average air and soil temperatures were 21.13 °C and 24.58 °C, respectively, whereas average air relative humidity was 58.92%. Total precipitation was 174 mm and was concentrated on a few days during the last two months (AEMET). With regard to the aquatic phase, the main differences between streams were in flow discharge and water conductivity (Table 3.1), being both variables negatively correlated. On the contrary chemical parameters as DO saturation, pH and nitrogen concentration were similar in all streams (Table 3.1). In all streams, DIN concentration was higher than 1 mg l^{-1} and was dominated by NO_3^- (Table 3.1). On the contrary, SRP concentration was very low and N:P molar ratios exceeded 6000, indicating potential P limitation.

3.3.2. Changes in wood chemical composition

Compared with other organic substrates (e.g. Enriquez et al. 1993), sticks were characterised by low N and P concentrations (0.13% and 0.009%, respectively) and high lignin concentration (14.6%), showing consequently high C:N and lignin:N, and a low N:P ratios (Table 3.2). Floodplain exposure caused a significant variation both in terms of concentration and mass in all elements in sticks, except of lignin, C and N, which showed only a significant variation in mass (Table 3.2). Mass of lignin (RM-ANOVA: $p = 0.047$), C (RM-ANOVA: $p < 0.0001$), P (RM-ANOVA: $p < 0.0001$) and K (RM-ANOVA: $p = 0.001$) in sticks, decreased significantly during the 3 and 4 months of floodplain exposure (Table 3.2). P and K fell around 60%, whereas lignin and C lost only 3 to 7% regarding their initial mass. On the contrary, the N mass in sticks increased significantly (24%) (RM-ANOVA: $p = 0.003$), as it did Ca (RM-ANOVA: $p < 0.0001$)

and Na (RM-ANOVA: $p < 0.001$). Elemental ratios showed a significant decrease in C:N (one-way ANOVA: $p = 0.039$), lignin:N (one-way ANOVA: $p = 0.007$) and a significant increase in N:P (one-way ANOVA: $p < 0.001$) (Table 3.2).

Stream immersion for 7 days caused significant differences in the chemical composition between the floodplain preconditioned sticks and the controls sticks. The preconditioned sticks showed higher C mass loss than the control sticks (Mixed Model: $p < 0.0001$) (Table 3.3). On the contrary, whereas the control sticks were subjected to a P and K mass loss (around 55% and 75%, respectively), their mass increased in the preconditioned sticks (Mixed model: $p < 0.0001$). Both the preconditioned and the control sticks showed a significant increase of N, Ca, Mg and Na mass (Mixed model: $p < 0.0001$). Ca, Mg and Na increments were above the 100% regarding their initial mass, and they were higher in the preconditioned than in the control sticks (Table 3.3). Respecting elemental ratios, both the preconditioned and the control sticks underwent a significant drop of C:N (GRBD: $p < 0.0001$) and a significant increase of N:P (GRBD: $p < 0.0001$).

Stream immersion for 4 months did not cause any differential change between the floodplain preconditioned sticks and the control sticks in their chemical composition in terms of concentration (Table 3.4). Both stick groups underwent a sharp drop of C and lignin mass (both around 20% and 30%) (Mixed model: $p < 0.0001$). The preconditioned sticks experienced a higher increase in P and K mass than the control sticks, nevertheless both sticks groups were characterized by wide variability of P and K mass. Finally, Ca, Mg and Na mass increased in sticks until the aquatic phase ended (Mixed model: $p < 0.0001$) with no differences between sticks groups (Table 3.5). In both cases, a significant decrease in the C:N and lignin:N ratios was observed (GRBD: $p < 0.0001$). On the contrary, the control sticks showed only a significant increase of N:P (GRBD: $p < 0.0001$; simple effects: $p < 0.0001$) (Table 3.4). However, the principal difference in the chemical composition between the preconditioned and the control sticks after this period was found in N mass net change (Fig. 3.1).

Table 3.2 Chemical composition in terms of concentration (% of DM), elemental ratios and net changes (expressed in %) of lignin and the main chemical elements, regarding their initial mass, after sticks floodplain exposure. Negative values indicate chemical component loss. Values are the mean ± SE (n = 8-10).

	Chemical composition						Net changes			
	INITIAL		3M		4M		3M		4M	
C:N	A	402 ± 40	B	302 ± 22	B	297 ± 27				
N:P	A	16.1 ± 1.6	B	53.9 ± 3.6	B	56.4 ± 5.6				
Lignin:N	A	135 ± 15	B	90 ± 6.5	B	89 ± 8.2				
Lignin		14.6 ± 0.6		14.5 ± 0.6		14.4 ± 0.6	*	-4.7 ± 4.1	*	-7.4 ± 3.6
C		48.0 ± 0.2		48.4 ± 0.2		47.9 ± 0.3	*A	-3.3 ± 0.5	*B	-5.7 ± 0.5
N		0.13 ± 0.01		0.17 ± 0.01		0.17 ± 0.02	*	24.1 ± 9.1	*	23.9 ± 11.3
P	A	0.009 ± 0.001	B	0.003 ± 0.000	B	0.003 ± 0.000	*	-66.6 ± 1.4	*	-67.7 ± 2.0
K	A	0.027 ± 0.003	B	0.012 ± 0.001	B	0.010 ± 0.000	*	-58.8 ± 2.4	*	-64.7 ± 1.0
Ca	A	0.051 ± 0.003	B	0.074 ± 0.003	B	0.087 ± 0.003	*A	38.4 ± 4.8	*B	61.4 ± 5.2
Mg	A	0.011 ± 0.001	B	0.007 ± 0.000	AB	0.010 ± 0.000	*A	-36.3 ± 3.6	*B	-18.2 ± 2.1
Na	A	0.001 ± 0.000	B	0.006 ± 0.000	C	0.008 ± 0.001	*A	463 ± 27	*B	641 ± 54

Sticks groups are identified as follows: Initial, sticks in which initial composition was measured; 3M, 3-months floodplain exposed sticks; 4M, 4-months floodplain exposed sticks. Ratios represent the relation between elements or compounds concentrations. The means followed by different letters in a row indicate significant differences between sticks groups (ANOVA: $p < 0.05$). * Express significant differences (RM-ANOVA: $p < 0.05$) in the chemical composition in terms of mass before and after floodplain exposure.

Table 3.3 Net changes (expressed in %) of the main chemical elements, regarding their initial mass, after the first 7 days of sticks stream immersion. Negative values indicate chemical component loss. Values are the mean ± SE (n = 4).

		CT				3M				4M		
		CHI	COR	TU		CHI	COR	TU		CHI	COR	TU
C	*A	-1.34 ± 1.24	-0.39 ± 0.55	-0.30 ± 0.20	*B	-4.15 ± 0.93	-4.27 ± 0.68	-3.91 ± 0.60		-2.59 ± 0.27	-3.16 ± 0.44	-2.84 ± 0.62
N	*A	50.7 ± 6.4	56.9 ± 7.6	95.8 ± 9.3	*B	45.7 ± 10.5	66.1 ± 4.5	65.3 ± 10.6	*B	28.5 ± 4.1	58.8 ± 16.0	74.3 ± 11.6
P	*A	-62.1 ± 1.3	-51.1 ± 10.0	-49.5 ± 7.8	*B	78.8 ± 37.8	47.4 ± 17.0	80.7 ± 31.6	*B	36.6 ± 6.0	84.7 ± 18.9	107.3 ± 31.7
K	*A	-85.4 ± 2.8	-83.5 ± 5.8	-72.8 ± 3.5	*B	52.3 ± 24.5	43.7 ± 10.0	60.5 ± 11.8	*C	123 ± 26	139 ± 30	136 ± 17
Ca	*A	204 ± 30	228 ± 40	161 ± 29	*B	172 ± 14	112 ± 12	137 ± 12	*C	231 ± 49	192 ± 15	170 ± 29
Mg	*A	177 ± 36	350 ± 58	374 ± 42	*B	587 ± 76	755 ± 61	892 ± 55	*C	540 ± 79	735 ± 66	905 ± 127
Na	*	69540 ± 1581	7525 ± 3011	10091 ± 2392	*	1383 ± 319	1025 ± 602	2319 ± 649	*	1383 ± 86	1035 ± 452	2080 ± 641

Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks, non-preconditioned sticks. The means followed by different letters in a row indicate significant differences between sticks groups (GBRD: $p < 0.05$). ***** Express significant differences (Mixed model: $p < 0.05$) in the chemical composition in terms of mass before and after 7 days of stream immersion.

Table 3.4 Concentration (% of DM) of the main chemical elements and elemental ratios in sticks after 4 months of stream immersion. Values are the mean ± SE (n = 3-4).

		CT			3M			4M	
		COR	TU		COR	TU		COR	TU
C:N	*	111 ± 15	144 ± 8	*	106 ± 12	180 ± 13	*	107 ± 11	181 ± 8
N:P	*	50.7 ± 16.2	72.7 ± 7.2		47.4 ± 14.0	68.3 ± 7.5		57.5 ± 19.1	55.6 ± 6.7
Lignin:N	*	30.1 ± 2.0	41.7 ± 1.2	*	30 ± 1.7	47.6 ± 3.6	*	31 ± 4.1	43.1 ± 2.5
Lignin		12.8 ± 1.1	13.4 ± 0.6		13.5 ± 1.1	12.3 ± 0.5		13.3 ± 0.5	11.1 ± 0.7
C		46.1 ± 0.2	45.9 ± 0.3		46.7 ± 0.3	46.4 ± 0.2		46.3 ± 0.2	46.5 ± 0.4
N		0.44 ± 0.07	0.32 ± 0.02		0.45 ± 0.06	0.26 ± 0.02		0.44 ± 0.04	0.26 ± 0.01
P		0.014 ± 0.009	0.005 ± 0.001		0.013 ± 0.006	0.004 ± 0.000		0.012 ± 0.006	0.005 ± 0.001
K		0.031 ± 0.018	0.015 ± 0.001		0.032 ± 0.017	0.014 ± 0.001		0.029 ± 0.014	0.018 ± 0.002
Ca		0.22 ± 0.01	0.36 ± 0.07		0.25 ± 0.04	0.33 ± 0.06		0.23 ± 0.02	0.26 ± 0.01
Mg		0.061 ± 0.005	0.070 ± 0.003		0.063 ± 0.001	0.073 ± 0.003		0.061 ± 0.004	0.077 ± 0.003
Na		0.043 ± 0.008	0.16 ± 0.01		0.044 ± 0.007	0.17 ± 0.01		0.048 ± 0.011	0.17 ± 0.02

Data from Chícamo stream are absent because sticks were lost before 4-months sampling period. Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks, non-preconditioned sticks. Ratios represent the relation between elements or compounds concentrations. * Express significant differences (Mixed model $p < 0.05$) in the chemical composition in terms of mass before and after 4 months of stream immersion.

Table 3.5 Net changes (expressed in %) of lignin and main chemical elements regarding their initial mass, after 4 months of sticks stream immersion. Negative values indicate chemical component loss. Values are the mean ± SE (n = 3-4).

	CT			3M			4M	
	COR		TU	COR		TU	COR	TU
Lignin	*	-34.4 ± 2.9	-19.3 ± 3.8	*	-38.8 ± 1.9	-28.5 ± 3.6	*	-37.4 ± 4.9	-37 ± 4.5
C	*	-27.5 ± 2.6	-15.6 ± 2.5	*	-35.8 ± 4.7	-19 ± 1.0	*	-34.9 ± 2.9	-21.1 ± 1.3
N	*A	149 ± 28	117 ± 10	*B	76.7 ± 7.5	31.5 ± 10.5	*B	72.7 ± 9.6	23.2 ± 6.9
P	A	14.8 ± 66.0	-53.9 ± 6.6	B	157 ± 102	5.3 ± 7.7	B	145 ± 123	26.8 ± 13.7
K	*A	-17.2 ± 45.6	-51.8 ± 2.8	B	70.6 ± 77.4	2.8 ± 8.0	B	85.2 ± 82.7	46.8 ± 19.0
Ca	*	219 ± 19	526 ± 137	*	130 ± 48	275 ± 71	*	74.3 ± 19.3	137 ± 5
Mg	*	324 ± 20	470 ± 45	*	473 ± 37	743 ± 51	*	333 ± 34	557 ± 25
Na	*	2689 ± 370	11978 ± 791	*	389 ± 41	2302 ± 127	*	313 ± 92	1684 ± 159

Data from Chícamo stream are absent because sticks were lost before 4-months sampling period. Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks, non-preconditioned sticks. The means followed by different letters in a row indicate significant differences (GBRD: $p < 0.05$) between sticks groups. * Express significant differences (Mixed model: $p < 0.05$) in the chemical composition in terms of mass before and after 4 months of stream immersion.

Whereas the control sticks underwent a continuous increment in N mass along the stream immersion period (from 0 to 120 days), the preconditioned sticks registered only a N mass increase during the first 7 days of immersion, afterward their N mass stopped to increase, or even began to decrease in Turrilla (Fig. 3.1). Therefore, at the end of the aquatic phase, the control sticks had significantly higher N mass than the preconditioned sticks (Table 3.5).

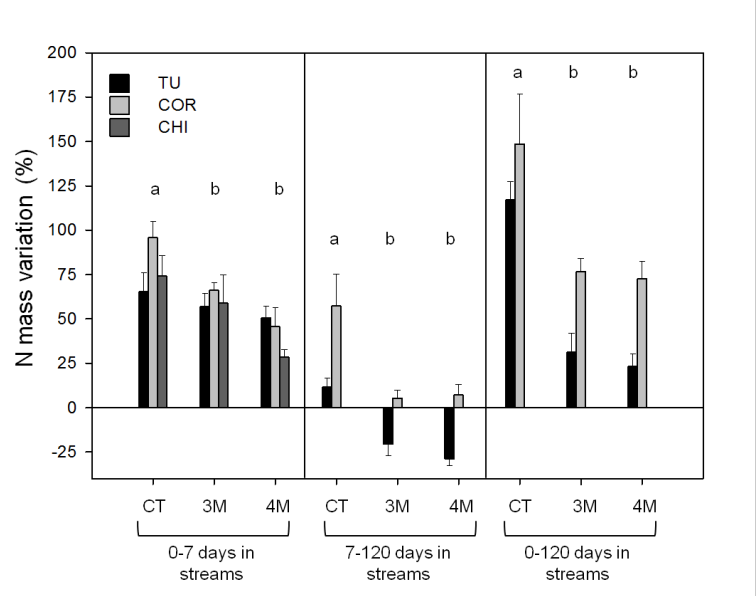

Figure 3.1 Net changes in sticks N mass (expressed in %) for the considered period: among 0-7 days of stream immersion (left); among 7-120 days of stream immersion (centre); among 0-120 days of stream immersion (right). Positive values indicate N mass increment and negative values N mass loss. Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks, non-preconditioned sticks. Error bars show SE. Different letters indicate significant differences (GBRD: $p < 0.05$) between sticks groups within each period. Data from Chícamo after 7 days of stream immersion are absent because sticks were lost.

3.3.3. OM loss

Floodplain exposure caused significant differences in OM loss between the 3-month (5.15%) and 4-month (7.15%) periods (T-test: $p < 0.0001$) (Fig. 3.2), which

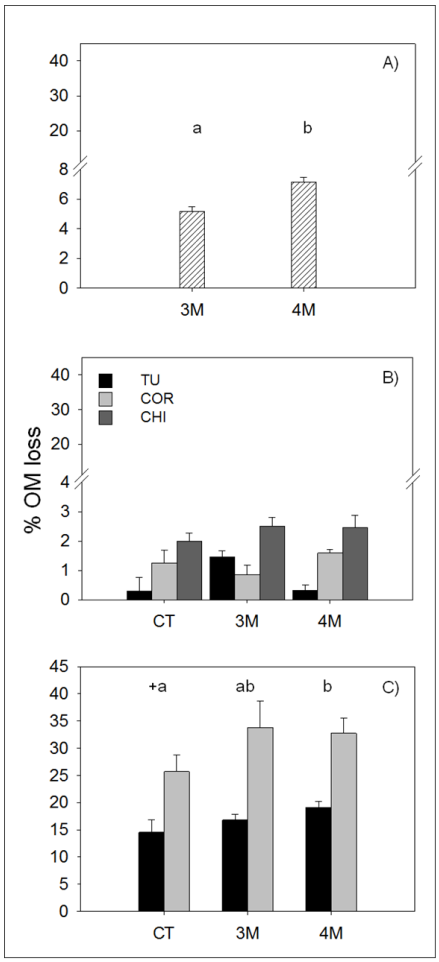

Figure 3.2 OM loss (mean ± SE) estimated after A) wood floodplain exposure (n = 8-10); B) 7 days of stream immersion (n = 4) and C) 4 months of stream immersion (n = 3-4). Data from Chícamo stream are absent because sticks were lost before 4-months sampling period. Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks: non-preconditioned sticks. Different letters indicate significant differences between sticks groups (GBRD: $P < 0.05$). The letters accompanied by symbol "+" indicate marginally significant differences (GBRD: $P = 0.062$) between stick groups.

corresponded to a breakdown rate of 0.0006 ± 0.0003 d^{-1}. After the first 7 days of stream immersion, both the preconditioned sticks and the control sticks showed a similar low OM loss (GRBD: $p = 0.852$) (Fig. 3.2), which ranged from a minimum value of 0.30% registered in Turrilla to a maximum value of 3.60% in Chícamo. However, after the 4 months of stream immersion, only the 4-month preconditioned

sticks showed marginally significant, higher OM loss (18-33%) than the control sticks (15-26%), (GBRD: $p = 0.062$; Tukey test: $p = 0.047$) (Fig. 3.2). Aquatic breakdown rates ranged from 0.0015 to 0.0035 d^{-1} in Turrilla and Corneros respectively, for the preconditioned sticks, and from 0.0013 to 0.0029 in Turrilla and Corneros respectively, for the control sticks. Independently of the stick group, mass loss in Corneros stream was significantly higher than in Turrilla (GRBD: $p = 0.005$). Sticks OM loss was related highly and positively with the C ($R^2 = 0.97$, $p < 0.0001$) and lignin loss ($R^2 = 0.61$, $p < 0.0001$), whereas it was correlated with N concentration in sticks ($r = 0.68$, $p < 0.0001$), in both the

terrestrial and aquatic phases. OM loss also was related highly and positively with the ergosterol concentration in sticks ($R^2 = 0.77$, $p < 0.001$) in the aquatic phase.

3.3.4. Microbial activity

After floodplain exposure, noticeable FDA activity and ergosterol concentration were detected in sticks, with no differences between the 3- and the 4-month exposed sticks (T-test: $p > 0.05$) (Fig. 3.3 and 3.4). After 7 days of stream immersion, the preconditioned sticks showed significantly higher FDA activity (GRBD: $p = 0.001$; Tukey test: $p < 0.0001$) and ergosterol concentrations (GRBD: $p = 0.002$; Tukey test: $p < 0.0001$) than the control sticks, showing both variables a considerably increase regarding the floodplain exposure period (see Fig. 3.3 and 3.4). The preconditioned sticks showed FDA values ranged from 0.74 to 2.81 $\mu molgMO^{-1}h^{-1}$, whereas ergosterol ranged from 0.60 to 3.73 $\mu g\ AFDM^{-1}$. On the contrary, the control sticks showed negligible values for both parameters (Fig. 3.3 and 3.4). Such as differences disappeared after the 4 months of stream immersion, when FDA activity and ergosterol concentration were similar for both stick groups (GRBD: $p = 0.138$; $p = 0.222$, for FDA and ergosterol respectively) (Fig. 3.3 and 3.4). From 7 days to 4 months of stream immersion, FDA activity increased only in the control sticks (Fig. 3.3), whereas ergosterol concentration showed an increased in both the floodplain preconditioned and the control sticks, although its increase in the control sticks was much higher (Fig. 3.4). At 4 months of stream immersion, ergosterol concentration in sticks was significantly higher in Corneros than in Turrilla (GRBD: $p = 0.009$), whereas FDA activity showed similar values in both streams. FDA activity and ergosterol concentration correlated only highly and positively at the first 7 days of stick immersion ($r = 0.91$, $p < 0.0001$). After 4 months in streams, ergosterol concentration in sticks correlated highly and positively with the N concentration in sticks ($r = 0.85$, $p < 0.0001$).

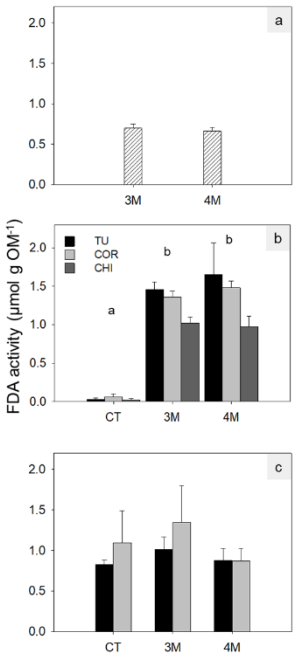

Figure 3.3 FDA activity (mean ± SE) found after A) wood floodplain exposure (n = 8-10); B) 7 days of stream immersion (n = 4) and C) 4 months of stream immersion (n = 3-4). Data from Chícamo stream are absent because sticks were lost before 4-months sampling period. Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks: non-preconditioned sticks. Different letters indicate significant differences (GBRD: $P < 0.05$) between sticks groups.

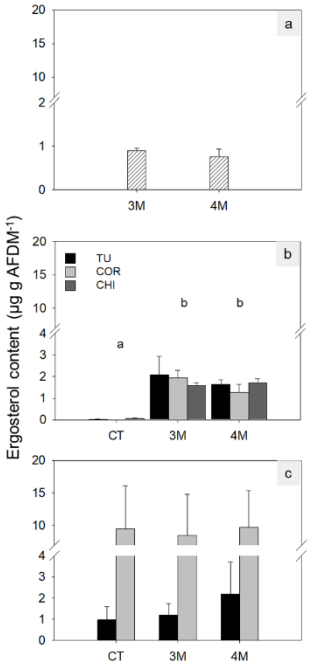

Figure 3.4 Ergosterol concentration (mean ± SE) found after A) wood floodplain exposure (n = 5); B) 7 days of stream immersion (n = 3) and C) 4 months of stream immersion (n = 3). Data from Chícamo stream are absent because sticks were lost before 4-months sampling period. Sticks groups are identified as follows: 3M, 3-months floodplain preconditioned sticks; 4M, 4-months floodplain preconditioned sticks; CT, control sticks, non-preconditioned sticks. Different letters indicate significant differences (GBRD: $P < 0.05$) between sticks groups.

3.4. Discussion

As it was expected, floodplain exposure greatly affected the chemical composition of wood. Some soluble nutrients were lost and, according to the C:N and lignin:N ratios, the wood chemical quality improved. Yet, contrary to what we initially hypothesized, preconditioning did not increase stick breakdown rate in streams, although it accelerated microbial activity on the first days of immersion.

3.4.1. Effects of floodplain exposure on OM loss and chemical quality

Floodplain exposure caused OM, nutrients (P and K) and lignin loss in wood. Our results support that these losses could be the results of a combined effect of microbial activity and abiotic processes, such as photodegradation and leaching by rains. Despite the fact that we do not have direct evidences of the solar radiation effect on wood, the high lignin loss rate registered in such short time period could be an indicator of photodegradation (i.e. Austin & Ballaré 2010). In arid and semiarid terrestrial systems, photodegradation is usually the main responsible for OM breakdown and lignin degradation (Austin & Ballaré 2010, Day et al. 2007, Gallo et al. 2009, Henry et al. 2008), due to water scarcity limits microbial degradation (Austin 2011, King et al. 2012). However, after sporadic rainfall events, as summer rainstorms, OM breakdown can be enhanced by microbial degradation and leaching (Brand et al. 2010, Henry et al. 2008, Mora 2014). In addition, some works have shown how microbial activity can contribute significantly to OM decomposition and lignin degradation despite water scarcity in arid systems and lignin recalcitrance (Araujo et al. 2012, Dirks et al. 2010, Wang et al. 2015). One reason could be that alkaline pH of arid land soils can support high oxidative enzyme potentials, which favours the degradation of recalcitrance compounds as lignin (Stursova & Sinsabaugh 2008).

Wood exposure in floodplain during 4 months resulted in a high leaching of the most soluble compounds, such as P and K. Similar results have been described in wood decomposition studies carried out in forest soils of much more humid zones (Lambert et al. 1980, Romero et al. 2005). Despite the huge differences in precipitation regimes between the study zones, the existence of similar nutrient loss rates highlights the high potential of rainstorm events for soluble compounds leaching from OM in arid systems. In addition, we must also consider that photodegradation and OM drying at high temperatures could enhance OM solubility (Bärlocher 1992, Feng et al 2011, Gallo et al. 2009). Contrarily to the observed loss of P, a noticeable rise in N mass was observed, suggesting net microbial immobilisation (Homyak et al. 2008, Parton et al. 2007). Although P and K are also required by microorganisms, their leaching would have exceeded their

immobilisation (Lambert et al. 1980, Romero et al. 2005). Because both N and P are often limiting microbial activity (i.e. Güssewell & Gessner 2009) the relative accumulation rates of both elements in floodplains are of interest to aquatic decomposition. Just as other works have reported in forests (Lambert et al. 1980, Romero et al. 2005), floodplain exposure caused the increment of the N:P ratio in wood, suggesting a potential P limitation. Enhanced leaching can cause two contrary effects on wood quality with negative and positive effects respectively on breakdown: on the one hand, impoverishment of nutrients (Dieter et al. 2013); on the other hand, leaching of recalcitrant phenolic compounds (Fellman et al. 2013, Gallo et al. 2009, Wang et al. 2015). Thus, the increase in N together with the loss of C and lignin, resulted in a wood quality improvement, as showed by the lower C:N and lignin:N ratios (e.g. Melillo et al. 1983). Floodplain exposure caused also changes in other nutrients such as Ca, Mg or Na. Thus, whereas wood was enriched in some elements present at high concentration in our soils (e.g. Ca, Na), other elements were released by leaching or decomposition (e.g. Mg) (Blair 1988, Gosz et al. 1973). All these elements have an important function for microorganisms physiology (e.g. as enzyme cofactors, being part of cell structures, and so on) (see Jellison et al. 1997), but they are needed in very low concentrations, so it is difficult that their impoverishment can cause a microbial limitation. On the other hand, some elements, as Mn or Fe, can be toxic for fungi at very high concentration, but it is difficult that occur at natural conditions (Jellison et al. 1997).

3.4.2. Effects of wood preconditioning on its aquatic breakdown

Floodplain preconditioning enhanced microbial activity and C mass loss in streams during the first days of wood immersion. However, after longer time periods (4 months), preconditioning did not affect wood aquatic breakdown rates (see Fig. 3.5). Despite the evidences provided by previous studies (Dieter et al. 2013, Fellman et al. 2013), preconditioned wood did not produce any net input of nutrients into the aquatic ecosystem by leaching, as most of them were lost in the floodplain. Conversely non-preconditioned wood showed a significant leaching of soluble nutrients as P and K, evidencing similar ranges of P mass loss than previous works

(e.g. Díez et al. 2002). Wood preconditioning did lead a greater C mass loss in streams. Although we cannot discard that some of this C mass loss could be due to leaching, we hypothesize that the enhanced activity of the terrestrial fungal communities in sticks into the streams was the main responsible factor. Our results of FDA activity and ergosterol concentration in sticks during the first days of their stream immersion support this idea. Several works have shown how terrestrial fungal species present in leaf or wood litter can survive and be active at least on the first days they are immersed in streams (Barlöcher & Kendrick 1974, Nikolcheva et al. 2005, Shearer et al. 2007, Voronin 2014). We hypothesize that the sum of higher water and nutrient availability in streams, together with the lower lignin content in preconditioned wood (i.e. easier access to labile C sources) could have had a synergistic effect on terrestrial fungi communities increasing their decomposer activity. This hypothesis agrees with other studies (Foereid et al. 2010, Henry et al. 2008, Pu et al. 2014), who have shown how the leaf litter quality improvement by the effect of photodegradation can enhance afterward microbial activity when the water availability in the surrounding environment increases. Also, in line with our results, Fukamani et al. (2010) show how wood breakdown rates can change depending on which fungal species colonise it first, due to important changes at community level. In this sense, in addition to the described changes in chemical quality, the effect of OM preconditioning on fungal communities composition seems to be a key point determining its breakdown rates in aquatic systems.

After the first days of increased microbial activity and C loss in preconditioned wood, we observed a decline in both processes after 4 months of wood immersion. As consequence breakdown rates in preconditioned and non-preconditioned wood were similar at that time (Fig. 3.5). The decline of microbial activity in preconditioning sticks at the end of the aquatic phase was supported by the net changes of N mass in sticks. During decomposition, microorganisms tend to immobilise N from the surrounding environment, enriching OM in N (Danger et al. 2015, Melillo et al. 1983, Pastor et al. 2014). However, if abiotic breakdown dominates on microbial activity, the N mass of OM falls (Parton et al. 2007). Thus, net changes of N mass can be used as an indicator of microbial activity. In our case, this suggests that distinctive wood breakdown patterns had occurred depending on

wood preconditioning (Fig. 3.5). In the case of wood exposed in floodplain, the initial phase of high microbial activity, evidenced by FDA and ergosterol results, could have caused a rapid depletion of labile C forms and consequently a gradual reduction of the microbial activity as the drop in the N mass in sticks suggests. On the contrary, in non-preconditioned wood, fungal colonization and activity could have slowly but constantly risen along the wood immersion period, until to reach similar values of OM loss than those found in preconditioned wood, just as our results show. This hypothesis is in the line of the results obtained by Mora (2014), who found different breakdown patterns in terrestrial preconditioned and non-preconditioned leaves. This study describes how aquatic microbial communities degraded the cellulose more efficiently in preconditioned leaves on the first days of stream immersion, whereas microbial communities present in non-preconditioned leaves had to apply more energy in lignin degradation to access more labile C resources.

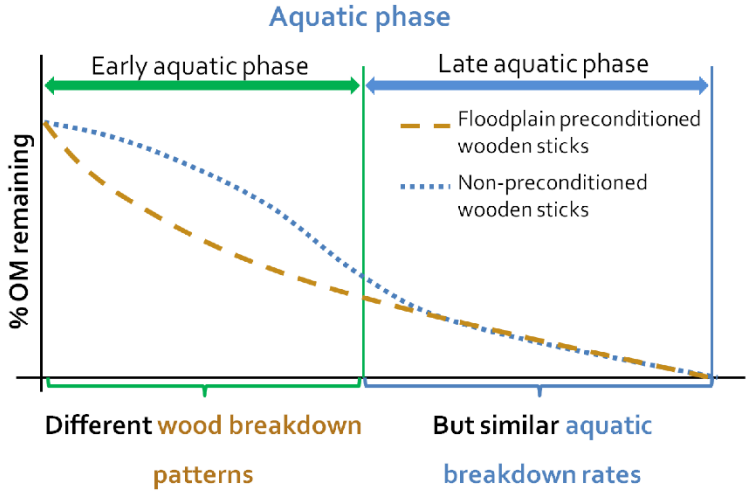

Figure 3.5 Conceptual scheme of the different aquatic breakdown patterns for preconditioned and non-preconditioned wood during early and late aquatic phases.

At the end of aquatic phase, the environmental differences among streams had a higher effect on wood breakdown rates than floodplain preconditioning, which highlights the importance of the stream environmental conditions in OM breakdown. Corneros and Turrilla streams showed great differences in discharge,

flow velocity and water conductivity. In this sense, our results reflect a negative effect of water conductivity on breakdown rates and fungal biomass, and a positive effect of stream discharge on both variables, just as a recent study that include these streams shows (Gómez et al. 2015). Thus, the stronger effect of streams environmental conditions could have masked partially the effects of wood preconditioning on its aquatic breakdown at long term. Finally, our result of no-effect of preconditioning in aquatic decomposition contradict those reported in previous studies (Dieter et al. 2011, 2013), where a negative effect of preconditioning by UV-B radiation was observed on leaf litter aquatic decomposition. There are two principal reasons that could explain this divergence. The use of distinct substrates (i.e. wooden sticks instead of leaf litter); and the different preconditioning methods employed (i.e. 3-4 months of natural conditions in the floodplain, instead of 21 days of UV-B radiation in the laboratory). Due to wooden sticks have been proved to perform similarly to leaf litter against driving factors (Arroita et al. 2012, Gulis et al. 2008), differences could be more related to the different experimental approaches. In particular, the absence of microbial activity during leaves preconditioning in the laboratory could be an important element determining such as different results. It is well-known that conditions in mesocosms or laboratory approaches are quite different from the natural ones (Morin 1998), so one of the most important challenges for ecology researchers is to develop experimental designs which are able to mimic natural conditions as real as possible.

3.4.3. Ecological implications

Our results suggest that wood preconditioning in floodplains could change its role from a long-lasting resource for freshwater food webs (Díez et al. 2002, Tank et al. 2010) to a short-term resource, due to the reduction of nutrients and C availability for heterotrophic aquatic communities. However, our study also stresses the need to analyse changes in C quality through preconditioning in order to definitely know its effect on aquatic decomposition. This work highlights how important the interactions between terrestrial and aquatic systems in C processing can be, just as previous works with leaf litter have also shown (Dieter et al. 2011, 2013, Langhans

et al. 2008, Mora 2014, Pu et al. 2014). Storage of OM in floodplains and/or dry riverbeds susceptible of preconditioning, is an extended situation which is even expected to increase affecting to more humid areas as a result of ongoing climate change (Döll & Schmied 2012, Hirabayashi et al. 2013, Reynolds et al. 2007). Therefore, given the potential significance of OM preconditioning on aquatic systems functioning, future works along these lines are clearly required.

3.5. Conclusions

Floodplains preconditioning could turn wood from a long-lasting resource for freshwater microorganisms into a short-term resource. Soluble elements were mostly leached in floodplains as results of summer rainfalls, among other factors, whereas microbial activity appeared to contribute, probably together with photodegradation and leaching, to the C and lignin loss. Such as changes, as well as the presence of an active terrestrial fungal community, indeed controlled aquatic breakdown during the first seven days of wood immersion in streams. This period was characterised by short, rapid and intense microbial degradation which was followed by a final decline at 4-month of aquatic immersion, we suspect, as easily available compounds were diminished. At this moment environmental stream conditions could be more important controlling wood breakdown rates. Although not addressed in this study, the analysis of the effect of preconditioning on fungal communities composition is a key aspect to be analysed.

3.6. Acknowledgements

We thank Gonzalo González for his advice on data analysis and Hellen Warbuton for revising English. We also thank the anonymous reviewers for providing helpful comments on earlier versions of this manuscript. This research was funded by the European Regional Development Fund (FEDER) and by the Spanish Ministry of Economy and Competitiveness through Project CGL2010-21458. R. del Campo was funded by a PhD contract (FPU R-269/2014) from the University of Murcia.

4.

Linking terrestrial and aquatic carbon processing: Environmental conditions of floodplains control the fate of leaf litter inputs in rivers



Parra, Tordera and Demnitzer streams

Abstract

In fluvial ecosystems, leaf litter can be retained in floodplains for several months before entering the river as lateral inputs. During this period, abiotic and biotic factors modulated by environmental conditions of floodplain can alter the chemical quality of leaf litter and consequently alter its later processing in the river. Thus, we analysed the effect of contrasting environmental conditions of floodplains on the chemical composition of particulate and dissolved organic matter (POM and DOM) fractions of leaf litter and their subsequent processing in the river. To do that, we firstly exposed reed leaf litter under open- and closed-canopy habitats of three floodplain sites with contrasting climate (arid-Mediterranean, humid-Mediterranean and continental), to finally monitor its decomposition in a river using litterbags and the biodegradation of their leachates in a parallel laboratory assay. Floodplain exposure dropped leachates biodegradability due to the reduction of nutrients and labile DOC independently of the floodplain site and habitat. Conversely, contrasting environmental conditions among floodplain sites caused the chemical differentiation of leaf litter exposed in each site, which in turn translated into different decomposition rates in the river. Our results point out that the nutrient balance in leaf litter during its floodplain exposure (impoverishment by leaching vs enrichment by immobilization) was the main driver of decomposition rates in the river. Therefore, our results demonstrate that the exposure of leaf litter in floodplains had important implications on the role of leaf litter as nutrient and energy subsidy for aquatic decomposer communities.

4.1. Introduction

Leaf litter is an essential energy resource for food webs in a great variety of terrestrial and aquatic ecosystems (Zimmer 2008, Hagen et al. 2012). In fluvial ecosystems, leaf litter from the riparian vegetation can represent the most important source of particulate organic matter (POM) for aquatic microbial decomposers and invertebrate consumers (Wallace et al. 1995, Tank et al. 2010), but also of dissolved organic matter (DOM) and nutrients by the leaching short after its input into rivers (Bernhardt & McDowell 2008, Wymore et al. 2015). Although the fraction size of DOM and POM is a fundamental modulator of their processing by aquatic communities (Suberkropp 1998, Allan & Castillo 2007), the chemical quality is likely the main factor influencing its use by different food web compartments (Frainer et al. 2015a, Boyero et al. 2017). Leaf litter chemical quality mainly relies on leaf traits of plant species (Wieder et al. 2008, Schindler & Gessner 2009); however, in some cases the pathway of leaf litter inputs can affect it also.

A great part of riparian leaf litter can fall directly into the river, but also in the floodplain soils, where it can accumulate and remain for several months before they enter the river by ensuing floods or surface runoff (Bell & Sipp 1975, Baldwin 1999, Sponseller & Fisher 2006, Fellman et al. 2013). During the accumulation of leaf litter in floodplains, the decomposition process begins, and leaf litter can undergo important changes in its chemistry and biodegradability as a consequence of the "ageing" or "preconditioning" caused by abiotic (mainly photodegradation and rain leaching) and biotic processes (biological activity of terrestrial microbes and invertebrates) (Baldwin 1999, Fellman et al. 2013, Pu et al. 2014, del Campo & Gómez 2016). Considering that in some rivers, the lateral input of leaf litter from the floodplain can be the most important source of organic matter (OM) (Benfield 1997, Jacobson et al. 1999), any chemical alteration of leaf litter occurring during its floodplain preconditioning may have important implications for its subsequent processing in the river.

The relative contribution of biotic and abiotic processes to leaf litter preconditioning in floodplains is modulated by environmental conditions such as temperature, moisture, nutrients availability in soils or the solar radiation exposition,

which in turn, are regulated at both regional and habitat scales (Austin 2011, Wang et al. 2014, Delgado-Baquerizo et al. 2015). At regional scale, these conditions can vary among floodplains due to climatic or land use differences (Aerts 1997, Graça & Poquet 2014). For instance, in open floodplains of warm arid regions, the intense solar radiation can favour the photodegradation of POM, which can break up recalcitrant compounds such as lignin (Austin & Ballaré 2010), and thus enhance the chemical quality of POM (Pu et al. 2014, del Campo & Gómez 2016). On the other hand, in forested floodplains of temperate and mesic regions, the higher nutrient and water availability in soils can facilitate the microbial decomposition of leaf litter (Gavazov et al. 2014, Delgado-Baquerizo et al. 2015). Microorganisms promote the decrease of labile C compounds availability, but the increase of refractory compounds (Melillo et al. 1984, Baldwin 1999), consequently reducing the quality of POM in an opposite way than photodegradation does. Beyond climatic particularities, floodplains are highly heterogeneous systems at habitat scale (Langhans et al. 2008). The irregular distribution of riparian vegetation in floodplains configures a spatial mosaic of open- and closed-canopy areas, which markedly differ in micro-environmental conditions such as the incidence of solar radiation, soil temperature, moisture or soil nutrient content (Naiman et al. 2005). So, we could expect that the combination of climatic and habitat conditions in floodplains may control the final chemical composition of leaf litter by leading different preconditioning reactions.

So far, just a few studies have analysed the effect of terrestrial preconditioning on leaf litter processing in rivers, and the results found are not consistent among them. Meanwhile some studies show negative effects of terrestrial preconditioning on leaf litter biodegradability in rivers (Baldwin 1999, Fellman et al. 2013, Jian et al. 2016), other works show a positive influence (Pu et al. 2014, del Campo & Gómez 2016) or even, no significant effects (Dieter et al. 2011 & 2013). However, to obtain global conclusions from this compilation of works is complicated, as they studied the effect of preconditioning on the aquatic processing of DOM and POM fractions separately. In addition, these studies employed very different methodologies and conditions to simulate the terrestrial preconditioning phase. Therefore, a major effort to understand how terrestrial preconditioning modulate leaf litter processing

in rivers is still necessary to achieve a more comprehensive knowledge of fluvial ecosystems functioning.

The objective of this study was to analyse how contrasting environmental conditions in floodplains, determined by factors acting at both habitat and regional scales, alter the chemical quality of DOM and POM fractions from leaf litter inputs and their later processing in recipient streams. To do that, we exposed *Phragmites australis* leaf litter under two different habitat conditions (open- vs closed-canopy) in three floodplains with different climate (arid Mediterranean, humid Mediterranean and continental). After evaluating changes in leaf litter chemical quality through its preconditioning, we finally compared the aquatic processing of preconditioned leaf litter from the three floodplains and non-preconditioned leaves. We combined both the analysis of leaf litter decomposition in a fluvial system and their leachates biodegradation in the laboratory. We hypothesized that: (i) leaf litter preconditioning in arid and mesic floodplains would cause opposite effects on leaf litter quality and consequently on its aquatic processing because, the dominance of leaf litter photodegradation and microbial decomposition processes, respectively; (ii) differences at habitat scale would modulate the effect of such as processes, with open-canopy habitats more susceptible to be photodegradation than closed-canopy ones; and (iii) floodplain preconditioning would cause contrasting effects on the aquatic processing of DOM (leachates) and POM due to their different OM fraction size. To our knowledge, this is the first study focused on analysing how different environmental conditions during the leaf litter accumulation in floodplains can alter the chemical quality of both DOM and POM fractions derived from leaf litter inputs, and therefore modulate their fate in rivers.

4.2. Materials and methods

To approach our objective, we designed a field experiment divided into two phases. A first terrestrial, preconditioning phase, where we exposed reed leaf litter (*Phragmites australis)* to various climatic and habitat conditions in different floodplains to address the effect of contrasting environments on leaf litter chemical quality (DOM and POM fractions); and a second aquatic decomposition phase,

where we analysed the effect of previous preconditioning scenarios on the leaf litter aquatic processing by two parallel experiences: a field decomposition experiment in a river using leaf litter mesh bags, where we measured leaf litter mass loss, changes in chemical composition, microbial activity, fungal biomass and shredder density; and a laboratory assay to measure the biodegradability of leaf litter leachates, where we measured the chemical composition of leachates and the microbial metabolic activity.

4.2.1. Field experimental design and set-up

4.2.1.1. Preconditioning phase

We selected three floodplain sites differing in their bio-climatic conditions: Parra (PA), Tordera (TOR) and Demnitzer (DEM) representing a semiarid Mediterranean, humid Mediterranean and warm, humid continental floodplains, respectively (Table 4.S1). PA (Murcia, SE of Spain) is an open and wide floodplain with scarce riparian vegetation dominated by shrub species. PA experienced the most extreme climatic conditions, with the highest temperatures and the lowest relative humidity during the study period (Fig. 4S1, Table 4.1). TOR (Girona, NE of Spain) is an open floodplain dominated by shrub vegetation and isolated trees. TOR had moderate-high temperatures during the preconditioning phase and received the maximum accumulated precipitation of the tree sites (Fig. 4S1, Table 4.1). DEM (Branderburg, NE of Germany) is a forested floodplain dominated by tree species (Table 4.S1). DEM had the lowest temperatures during the study period, but a highest relative humidity (Fig. 4S1, Table 4.1). Climatic variables of each study site were obtained from the closest meteorological stations. Data from PA (Charco Taray Fortuna station) were downloaded from Agrarian Information System of Murcia (siam.imida.es), data from TOR (Santa Coloma de Farnés station) were provided by the Meteorological Service of Catalonia (METEOCAT) and data from DEM (Müncheberg station) were downloaded from Climate Data Center of Deutscher Wetterdienst (ftp-cdc.dwd.de/pub/CDC/). At each floodplain site, we selected two habitat types differing in their vegetation canopy: open-canopy habitats

consisting on areas of bare soil, totally exposed to atmospheric conditions, and closed-canopy habitats consisting on fully vegetated patches, characterized by the dominance of shaded conditions.

Before starting the preconditioning phase, we collected senescent leaf litter from *Phragmites australis* (reed) directly from standing plants in a wetland near the University of Murcia (SE Spain). Leaf litter were air-dried in dark conditions and preserved until its experimental use. For the preconditioning phase, we made open leaf litter packs consisting of 5 g of reed leaf litter tied with fishing line to a plastic mesh frame (20 x 20 cm), which were firmly anchored to the ground. Thus, leaf litter was directly exposed to both atmospheric and soil conditions of each floodplain site and habitat where they were located. Leaf litter packs were distributed into three sectors along a 100 m transect in each floodplain and split between the open- and closed-canopy habitats. In total, 108 leaf litter packs were deployed during the preconditioning phase. 18 leaf litter packs (3 floodplain sites x 3 sectors x 2 habitats) were used to analyse the effect of floodplain preconditioning on the leaf litter mass loss, chemical composition, microbial activity (extracellular enzymes) and fungal biomass (ergosterol). See Chapter II, sections 2.2.3., 2.2.4., 2.2.5.2. and 2.2.6., respectively, for method descriptions. In addition, these leaf litter packs were used to examine the chemical properties of the leaf litter leachates after the preconditioning phase. The other 90 leaf litter packs were used in the aquatic decomposition phase (see details below). At each floodplain site and habitat, we collected soil samples at the beginning of the preconditioning phase to characterize soil texture, OM content, elemental composition and gravimetric water content (GWC). See section 2.1.2. for further explanations of soil properties characterization. The preconditioning phase lasted for 105 days, from August to November 2014.

4.2.1.2. Aquatic decomposition phase

After the preconditioning phase, leaf litter packs were retrieved from floodplains; transported to the laboratory and kept in dark and cold conditions. A known mass of previously air-dried (48 h) preconditioned leaf litter from every floodplain site

and habitat was placed into coarse mesh bags (4 mm pore size). In addition, we prepared a set of mesh bags with non-preconditioned reed leaf litter, used as a control treatment. The aquatic decomposition phase was carried out from January to April 2015 in the Alharabe river, a third order stream of the Segura river catchment (38°11'31.8"N 2°03'21.1"W. Murcia, Spain). Alharabe is an open canopy river with abundant macrophytes such as *Typha latifolia* and *Potamogeton coloratus* and a riverbed substrate of pebbles and sand. All leaf litter bags were randomly distributed at three run sites along a 100-m reach in the river. Leaf litter mesh bags were tied to iron rods and then fixed on the riverbed (Fig. 2.2c). Over this period, we collected leaf litter bags on days 1, 7, 20, 48 and 90 of in-stream incubation. On each sampling date, leaf litter bags were collected and transported into plastic bags in a cooler to the laboratory. During the aquatic phase, we characterized the stream reach as indicated in section 2.1.3. The average surface discharge was 113 $l \cdot s^{-1}$ and water conductivity 800 $\mu S \cdot cm^{-1}$. Water temperatures ranged from 2.9 to 12.1 °C. Stream water was well oxygenated with an oxygen saturation about 95 % and had basic pH around 8. DIN concentration was low (421 ± 107 $\mu g \cdot l^{-1}$) and dominated by NO_3^-. Average SRP was under 1 $\mu g \cdot l^{-1}$.

4.2.2. Laboratory procedures

Once collected leaf litter from both experimental phases, were transported to the laboratory, it was washed with tap water and leaf subsamples were obtained for further processing and analyses. Five leaves were randomly selected from each leaf litter pack (preconditioning phase) or mesh bag (aquatic phase) and three sets of five leaf disks were cut using a cork borer (11 mm diameter) for further analyses described (mass loss, chemical composition, extracellular enzyme and fungal biomass) in Chapter II as commented above.

We analysed the chemical composition (i.e. DOM and nutrient concentrations) of leachates from preconditioned leaf litter in all floodplain sites and habitats, as well as from non-preconditioned leaf litter. We prepared leachates by placing 0.5 g of leaf litter in 300 mL of Milli-Q water. See section 2.3.1. for further explanations. Leachates were stored into different vials for further characterization

of chemical content (DIN, SRP and DOC), quality of DOM (absorbance and fluorescence properties) and microbial metabolic activity (R_{az}-R_{ru}). Leachate samples for DOC and nutrients were frozen at -20 °C and analysed using the same methods explained above for stream water samples. Samples for absorbance and fluorescence measurements as well as for microbial metabolic activity were stored at 4 °C until analyses, which were done within the next 3 days. From absorbance data, we computed the spectral slope for 275–295 nm (S275–295), the ratio between slopes in wavelength regions 275-295 to 350-400 (Sr) and $SUVA_{254}$, while we computed the humification index (HIX) and the fluorescence index (FI) from fluorescence data (see section 2.3.3. for further details). We estimated the microbial biodegradability of the leached DOM by measuring the microbial metabolic activity associated with the leachates. To do that, we used the R_{az}-R_{ru} chemical system as a metabolic tracer (see section 2.3.5.1. for a complete explanation). We used an aqueous extract of sediment from Alharabe river filtered by a GF/F filter as microbial inoculum.

4.2.3. Data analysis

To explore the relationship between climatic and soil properties in floodplains we performed a principal component analysis (PCA) with the habitat soil properties and the projection of climatic data from each floodplain site as supplementary variables. All variables were z-standardised prior to PCA. Note that the projection of supplementary variables allows us to graphically represent the correlation of climatic data with soil properties in the PCA biplot, but they are not used in the configuration of the PCA space.

To analyse how environmental conditions of floodplains, at both regional and habitat scales, affected to measured variables during the preconditioning phase (habitat soil descriptors, leaf litter mass loss, CBH, ergosterol and chemical composition), we applied mixed models with floodplain site (3 levels: PA, TOR, DEM) and habitat (2 levels: open- and closed-canopy) as fixed factors and sector within a floodplain as random factor. Additionally, to analyse if floodplain preconditioning altered the chemical composition of leaf litter respect to its initial characteristics, we tested for differences between preconditioned and non-

preconditioned (NP) leaf litter using the a priori contrast: $\mu_{NP} = 1/3\mu_{PA} + 1/3\mu_{TOR} + 1/3\mu_{DEM}$, i.e., the null hypothesis is that non-preconditioned leaf litter (μ_{NP}) was equal to the mean of the different preconditioned leaf litter across the three floodplain sites ($\mu_{PA} + \mu_{TOR} + \mu_{DEM}$). In both cases, post-hoc pairwise differences between levels of fixed factors were tested by Tukey's test.

To analyse the effect of different environmental conditions (at regional and habitat scale) on the chemical composition and microbial metabolic activity of leaf litter leachates we used the same approach of mixed models and contrast analyses than described before. Besides, we performed a PCA using the chemical descriptors of leachates and the projection of R_{ru} production as a supplementary variable to describe the chemical drivers of microbial metabolic activity in leachates. Finally, we used Spearman correlations to give the relationship between R_{ru} production and PCA axes.

To analyse how floodplain environmental conditions (at regional and habitat scale) affected to aquatic decomposition phase (leaf litter mass loss, shredders density, CBH, ergosterol and chemical composition), we used again the same approach of mixed models mentioned above but including the immersion time in the river (5 sampling dates) as covariable. In the case of CBH efficiency measured during the aquatic decomposition phase, we constructed an ANCOVA model using the ln of % AFDMr as response variable, the ACBH as a covariable and floodplain sites and habitats as fixed factors. Differences in all analysed parameters between preconditioned and non-preconditioned leaf litter was measured using the same contrast analyses than previously described for the preconditioning phase. Furthermore, to show graphically how differently behaved the preconditioned leaf litter regarding non-preconditioned one, we computed a preconditioning response ratio (PRR) as:

$$PRR = \ln\left(\frac{P}{NP}\right)$$

where P and NP are the values of preconditioned and non-preconditioned leaf litter, respectively, for every study variable measured during the aquatic decomposition phase. Positive values of PRR for a specific variable indicate higher values in the

preconditioned leaf litter than in the non-preconditioned one and negative values the opposite.

Finally, we explored which chemical drivers promoted aquatic decomposition of leaf litter by performing a third PCA with the chemical composition of preconditioned and non-preconditioned leaf litter at the beginning of the aquatic phase (day 0), and the projection of mass loss, fungal biomass and ACBH values at the end of the aquatic phase (day 90) as supplementary variables. Lastly, we used Spearman correlations to establish the relationship between supplementary variables and PCA axes.

Outputs of mixed models and contrast analyses are found in supplementary material (Tables S2, S3, S5 and S6). Mixed models and contrast analyses were implemented with PROC MIXED of SAS 9.4. PCAs and ANCOVA models were made with R version 3.2.1 (R Core Team 2015).

4.3. Results

4.3.1. Preconditioning phase

4.3.1.1. Comparison of environmental conditions among floodplain sites and habitats

In general, soil properties differed in a greater extent among floodplain sites than between habitats (Table 4.1, Fig. 4.S2). DEM soils had the highest values of GWC, OM, N content and N:P. PA soils highlighted by the highest C content, C:N and C:P ratios, meanwhile TOR soils showed the highest composition in P (Table 4.1, Fig. 4.S2). Mixed models revealed differences between habitats only in DEM, with higher N and P contents in soils of closed-canopy habitats (N: $F_{2,11} = 6.71$, $p = 0.012$; P: $F_{2,11} = 6.53$, $p = 0.013$. Tukey: $p < 0.05$ all) (Table 4.1). The projection of climatic data on the soil properties PCA indicated that DEM was correlated with highest relative humidity, TOR with the highest accumulated precipitation and PA with the highest temperature (Fig. 4.S2).

Table 4.1 Climate and soil properties of floodplain sites and habitat conditions during the terrestrial preconditioning phase.

	Floodplain sites								
	Parra			Tordera			Demnitzer		
Air temperature (°C)	20.9 ± 0.5 (10.8-29.52)			17.8 ± 0.4 (8.9-23.6)			11.5 ± 0.5 (-3.2-19.7)		
Relative humidity (%)	66.3 ± 1.2 (33.1-89.4)			79.4 ± 0.7 (54.1-97.4)			84.9 ± 0.8 (62.8-98.3)		
Accumulated precipitation (mm)	110.5			474.6			77.3		
	Habitats								
	Open-canopy	Closed-canopy		Open-canopy	Closed-canopy		Open-canopy	Closed-canopy	
Texture	Sandy loam	Loam		Loamy sand	Loamy sand		Loamy sand	Loamy sand	
Gravimetric water content (%)	9 ± 2.3	12.7 ± 3.1	a	17.4 ± 4.2	20.6 ± 6.3	a	35.4 ± 5.1	31.6 ± 5.9	b
Organic matter (%)	3.2 ± 0.8	2.5 ± 0.5	a	5.5 ± 0.9	10.3 ± 1.9	a	13.2 ± 5.6	17.8 ± 7.0	b
C (%)	8.0 ± 0.5	7.5 ± 0.5	a	1.5 ± 0.2	1.5 ± 0.1	b	3.5 ± 0.5	5.2 ± 0.2	c
N (%)	0.11 ± 0.02	0.09 ± 0.03	a	0.13 ± 0.01	0.12 ± 0.01	a	0.24 ± 0.04	0.36 ± 0.04	b*
P (%)	0.034 ± 0.001	0.032 ± 0.003	a	0.042 ± 0.004	0.050 ± 0.005	b	0.024 ± 0.002	0.044 ± 0.004	a*
C:N	79.2 ± 14.5	88.2 ± 13.0	a	11.8 ± 0.7	12.4 ± 0.4	b	15.0 ± 0.3	14.5 ± 0.5	b
C:P	230.0 ± 14.1	240.8 ± 31.4	a	35.4 ± 2.9	29.3 ± 1.4	b	151.6 ± 32.8	118.0 ± 5.2	c
N:P	3.1 ± 0.6	2.9 ± 0.6	a	3.0 ± 0.14	2.4 ± 0.0	a	10.1 ± 2.2	8.2 ± 0.6	b

Values are the average ± SE (n = 3). Air temperature and relative humidity include minimum and maximum values during the study period in brackets. Different letters in a row indicate significant differences among floodplain sites according to post-hoc tests after significant mixed models ($p < 0.05$). * Express significant differences between habitats, for an specific floodplain, according to mixed models ($p < 0.05$). See Table 4.S2 for statistics.

4.3.1.2. Effects of contrasting environmental conditions in floodplains on leaf litter mass loss, chemical composition, microbial activity and fungal biomass.

After 105 days of floodplain exposure, mass loss of leaf litter packs ranged from 10 to 60 % showing significant differences among floodplain sites ($F_{2,81}$ = 60.67; p < 0.001) and habitats ($F_{1, 81}$ = 4.33; p = 0.041). The highest mass loss occurred in TOR, followed by DEM and PA, whereas it was higher in the open- than in the closed-canopy habitats (Fig. 4.1a).

The comparison between preconditioned and non-preconditioned leaf litter using contrast analyses evidenced that floodplain preconditioning altered severely the chemical composition of leaf litter regarding its initial characteristics (Table 4.2, Table 4.S3). In general, the principal changes caused by the preconditioning were the increase of lignin content and the decrease of nutrients such as P, K, Mg, Na or S, but these changes varied among floodplain sites (Table 4.2).

As for soil properties, the chemical composition of preconditioned leaf litter showed major differences among floodplain sites than between habitats (Table 4.2). Either cellulose, lignin or Lig:N showed no differences among sites or between habitats (Table 4.S3). C:N, C:P and N:P revealed significant differences among floodplain sites (C:N: $F_{2,6}$ = 10.26; p = 0.012; C:P: $F_{2,6}$ = 131.75; p < 0.001; N:P: $F_{2,6}$ = 61.92; p < 0.001), showing all three ratios the highest values in leaf litter preconditioned in PA, due to their very low content in N and P in comparison with leaf litter from TOR and DEM (Table 4.2). C and P content in leaf litter showed significant differences among floodplain sites (C: $F_{2,6}$ = 8.22, p = 0.019. Tukey: p < 0.05; P: $F_{2,6}$ = 24.96, p = 0.001. Tukey: p < 0.05), mainly differing leaf litter from TOR due to their lowest C and highest P content (Table 4.2). Regarding to the effect of habitat, only N, C:N and N:P ratios showed significant differences between habitats (N: $F_{1,5}$ = 20.02, p = 0.007; C:N: $F_{1,5}$ = 13.29, p = 0.015; N:P: $F_{1,5}$ = 14.71, p = 0.003) caused by the higher N content in leaf litter from open-canopy habitats (Table 4.2).

Table 4.2 Chemical composition of non-preconditioned and preconditioned leaf litter from all floodplain site and habitats.

		Parra			Tordera			Demnitzer		
	Non-preconditioned leaf litter	Open-canopy	Closed-canopy		Open-canopy	Closed-canopy		Open-canopy	Closed-canopy	
Cellulose (%)	34.24 ± 0.88	35.73 ± 2.58	37.66 ± 2.11	a	40.57 ± 2.36	41.85 ± 0.47	a	34.73 ± 0.23	39.73 ± 2.34	a
Lignin (%)	4.43 ± 0.27	10.89 ± 0.82	11.77 ± 2.40	a	16.86 ± 2.01	11.61 ± 0.88	a	16.46 ± 0.71	14.07 ± 0.80	a
C (%)	37.88 ± 0.28	41.20 ± 0.14	41.72 ± 0.99	a	37.00 ± 1.14	40.39 ± 0.58	b	42.48 ± 2.15	42.32 ± 0.66	a
N (%)	1.26 ± 0.05	1.10 ± 0.09	0.97 ± 0.04	a*	1.66 ± 0.15	1.11 ± 0.06	a*	1.35 ± 0.08	1.22 ± 0.11	a*
P (%)	0.046 ± 0.004	0.015 ± 0.001	0.016 ± 0.001	a	0.043 ± 0.005	0.035 ± 0.002	b	0.029 ± 0.003	0.032 ± 0.003	a
Lig:N	3.61 ± 0.27	9.94 ± 0.48	11.94 ± 1.98	a	10.14 ± 0.48	10.55 ± 1.08	a	12.22 ± 0.21	11.74 ± 1.35	a
C:N	30.87 ± 1.15	37.97 ± 3.00	42.97 ± 2.05	a*	22.59 ± 1.39	36.68 ± 2.30	b*	31.71 ± 3.51	35.12 ± 2.78	b*
C:P	913 ± 76	2778 ± 160	2595 ± 43	a	870 ± 78	1146 ± 75	b	1493 ± 85	1349 ± 148	c
N:P	29.76 ± 2.58	73.41 ± 1.63	60.72 ± 3.49	a*	38.41 ± 1.26	31.24 ± 0.31	b*	47.96 ± 8.00	38.56 ± 3.49	b*

Values are the average ± SE (n = 3). Different letters in a row indicate significant differences among floodplain sites according to post-hoc tests after significant mixed models ($p < 0.05$). * Expresses significant differences between habitats according to mixed models ($p < 0.05$). See Table 4.S3 for statistics and contrast analysis tests for differences between preconditioned and non-preconditioned leaf litter.

CBH activity associated with preconditioned leaf litter showed significant differences among floodplain sites ($F_{2,6}$ = 8.59; p = 0.017), specifically due to the significantly higher values in TOR than in DEM (Tukey: p < 0.05) (Fig. 4.1b). Differences between habitats in CBH were only found in TOR according to the pairwise-comparison after the significant interaction between floodplain site and habitat ($F_{2,29}$ = 4.59; p = 0.019. Tukey: p < 0.05).

Fungal biomass in leaf litter based on ergosterol measurements showed a similar pattern than that observed for leaf litter mass loss. The highest fungal biomass was found in leaf litter preconditioned in TOR, then DEM and PA (Fig. 4.1c). Although the mixed model showed significant differences between floodplain sites ($F_{2,11}$ = 6.06; p = 0.017), post-hoc test only revealed differences between PA and TOR (Tukey: p < 0.05). Fungal biomass also showed a significant interaction between floodplain sites and habitats, with only significant differences between habitats in DEM and PA ($F_{2,6}$ = 5.81; p = 0.019. Tukey: p < 0.05).

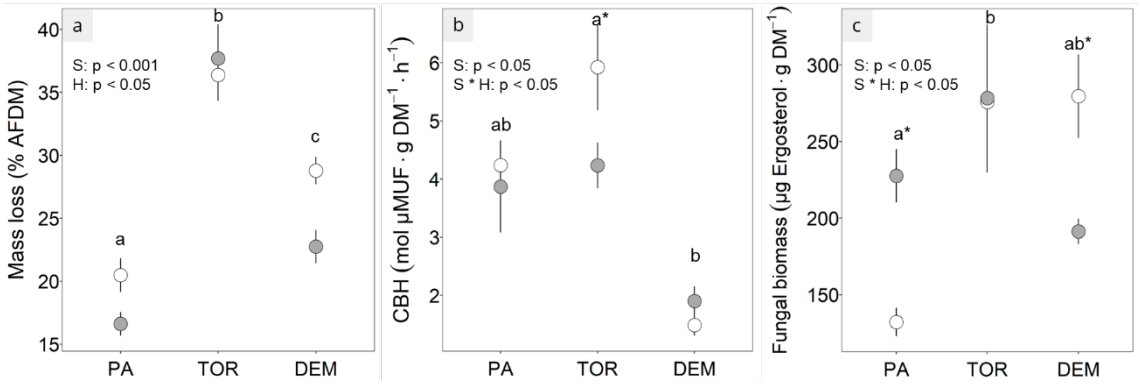

Figure 4.1 Mean values (± SE; n = 3) of mass loss expressed as percentage from initial AFDM (a), cellobiohydralase activity (CBH) (b) and fungal biomass (c) in leaf litter exposed to the preconditioning phase under open-canopy (white symbols) and closed-canopy habitats (H) (grey symbols) in the different floodplains sites (S). Panels also show p values of factors (or the interaction between factors) with significant differences based on results from mixed models. Different letters indicate significant differences among floodplain sites according to post-hoc tests (p < 0.05). * indicates significant differences between habitats according to pair-wise comparisons after a significant interaction between factors (p < 0.05). See Table 4.S3 for statistics. Floodplain sites: PA = Parra, TOR = Tordera, DEM = Demnitzer.

4.3.2. Aquatic decomposition phase

4.3.2.1. Effects of floodplains environmental conditions on leaf litter leachates chemistry and microbial metabolism

The first two axes of the PCA based on the chemical composition of leachates, explained up to 70.85 % of total variance. The PC1 (44.85% of total variance) separated leaf litter leachates from PA from the others, and was mainly related to SRP, SUVA, C:N, C:P, N:P, S275-295 and HIX (Fig. 4.2a). PC2 (26% of total variance) clearly differentiated leachates from preconditioned and non-preconditioned leaf litter. and was loaded by Sr, E2:E3, DOC, DIN and FI. Leachates of non-preconditioned leaf litter showed the highest values of FI and the highest concentration of DOC and nutrients (DIN and SRP) (Fig. 4.2a, Table 4.S4). Leachates from leaf litter preconditioned in PA were characterized by the highest values in elemental ratios, s275-296 and HIX (Fig. 4.2a, Table 4.S4), indicating a low concentration of nutrients and big, humic DOM compounds. On the contrary, leachates of leaf litter preconditioned in TOR and DEM were characterized by the highest values in Sr and E2:E3 (Fig. 4.2a, Table 4.S4), which indicates the presence of DOM compounds with low molecular weight. In agreement with the PCA output, the contrast analyses revealed significant differences between non-preconditioned and preconditioned leaf litter in the concentration of DOC and nutrients as well as in FI, SUVA, Sr and E2:E3 indexes (Table 4.S4 and Table 4.S5). Mixed models reinforced the PCA results as well and showed significant differences among floodplain sites (all $p < 0.05$, Table 4.S5), but mainly between PA and the couple DEM-TOR (Table 4.S4). Only HIX showed significant differences between habitats ($F_{1,11} = 1.96$; $p = 0.030$), with the highest values in the open-canopy ones.

Regarding the microbial metabolism associated with the leaf litter leachates, R_{ru} production rates were significantly higher in non-preconditioned than in the preconditioned leaf litter according to contrast analyses ($t_{1,15} = 22.9$; $p < 0.001$) (Fig. 4.2b). Regarding preconditioned leaf litter, leachates of leaf litter from PA showed the highest metabolic rates ($F_{2,10} = 7.35$; $p = 0.011$). No significant differences were found between floodplain habitats ($F_{1,10} = 0.20$; $p = 0.662$). R_{ru} production rates in

leachates showed a high correlation with PC2 (r = 0.92; p < 0.001) indicating a positive relationship with DOC and DIN concentration, the FI index and negatively with E2:E3 index (Fig. 4.2a).

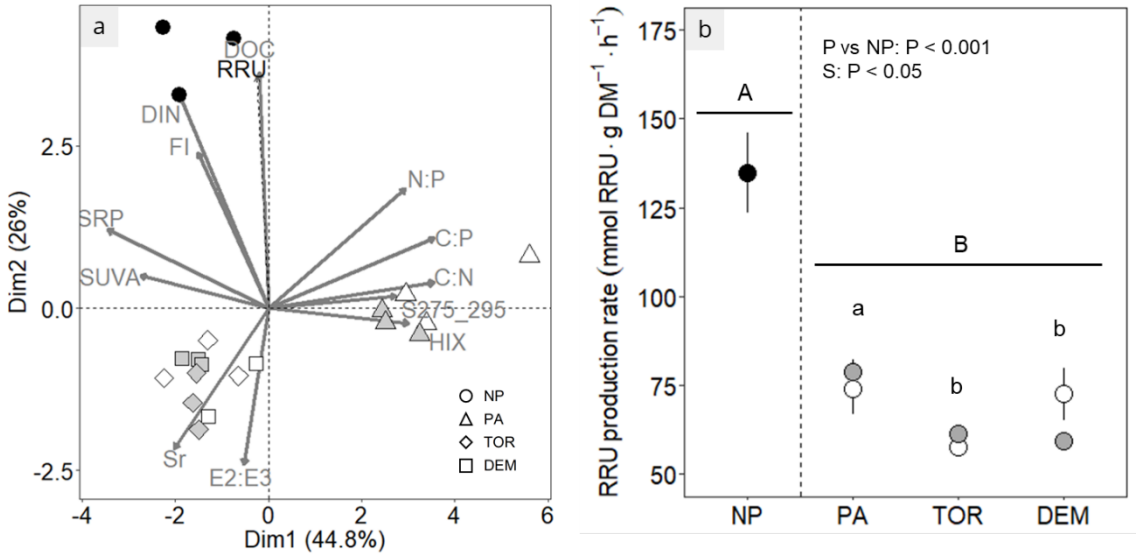

Figure 4.2 PCA biplot describing the chemical composition of leachates from non-preconditioned leaf litter (NP; black dots) and leaf litter preconditioned (P) under open-canopy (white symbols) and closed-canopy habitats (grey symbols) in the different floodplains sites (S) (a). Grey arrows represent the loading of each chemical descriptor, whereas the dotted black arrow represents the correlation of RRU (a metabolic tracer of microbial activity) with the PCA loadings. RRU production rates (mean ± SE; n = 3) from leachates coming from preconditioned and non-preconditioned leaf litter (b). Different capital letters indicate significant differences between the preconditioned (P) and non-preconditioned (NP) leaf litter according to a contrast analysis, meanwhile different small letters express significant differences among floodplain sites (S) according to post-hoc tests (p < 0.05) after significant differences in a mixed model. Floodplain sites: PA = Parra, TOR = Tordera, DEM = Demnitzer.

4.3.2.2. Effects of floodplains environmental conditions on leaf litter aquatic decomposition

Contrast analyses did not show differences in the average mass loss of preconditioned and non-preconditioned leaf litter in the stream ($t_{1,81}$ = -1.61; p =

0.112). However, pair-wise comparisons between the mass loss of non-preconditioned leaf litter and the individual floodplain sites did show strong differences for each of them ($p < 0.001$ all), indicating that the response of leaf litter mass loss in the river to floodplain preconditioning depended on the specific environmental conditions of each floodplain site (Fig. 4.3a and 4.3b). In fact, mixed models evidenced significant differences in the mass loss of leaf litter coming from the different floodplain sites ($F_{2,56} = 27.69$; $p < 0.001$. Tukey: $p < 0.05$ all), with the lowest mass loss in leaf litter from PA and the highest in TOR. On the contrary, differences in floodplain habitats did not influence leaf litter mass loss in the river (Table 4.S6).

Preconditioned and non-preconditioned leaf litter differed significantly in all measured chemical compounds, elements (except in C) and elemental ratios during their aquatic decomposition (Fig. 4S3, Table 4.S6). More striking differences between them occurred during the first 20 days of water immersion. During this time, non-preconditioned leaf litter underwent a severe drop of its N and P content that resulted in an exponential increase of its C:N, C:P and N:P ratios (Fig. S3). On the contrary, preconditioned leaf litter underwent an increase in their N content but a drop of lignin during the first 7 days in the river that resulted in a great decrease of Lignin:N and C:N ratios (Fig. 4.S3). Again, the floodplain sites conditions exerted a much higher influence on the chemical composition of preconditioned leaf litter than habitats (Table 4.S6). Specifically, the greatest differences among floodplain sites were found between PA and the couple DEM-TOR, highlighting the lowest N and P content in leaf litter from PA, and consequently the highest C:N and C:P ratios.

CBH activity did not show differences between preconditioned and non-preconditioned leaf litter according to contrast analyses ($t_{1,212} = -1.50$; $p = 0.135$). However, they showed different patterns over the aquatic phase. CBH showed much higher values in preconditioned than in non-preconditioned leaf litter during the first 20 days in the river, as evidenced by the maximum values of PRR_{CBH} during this period (Fig. 4.3c and 4.3d). From 20 days on, CBH in non-preconditioned leaf litter increased exponentially and matched CBH values in preconditioned leaf litter (Fig. 4.3c and 4.3d). In preconditioned leaf litter, CBH was significantly higher in

leaf litter from PA than in the other two floodplain sites ($F_{2,155}$ = 66.57; p< 0.001. Tukey: p < 0.001).

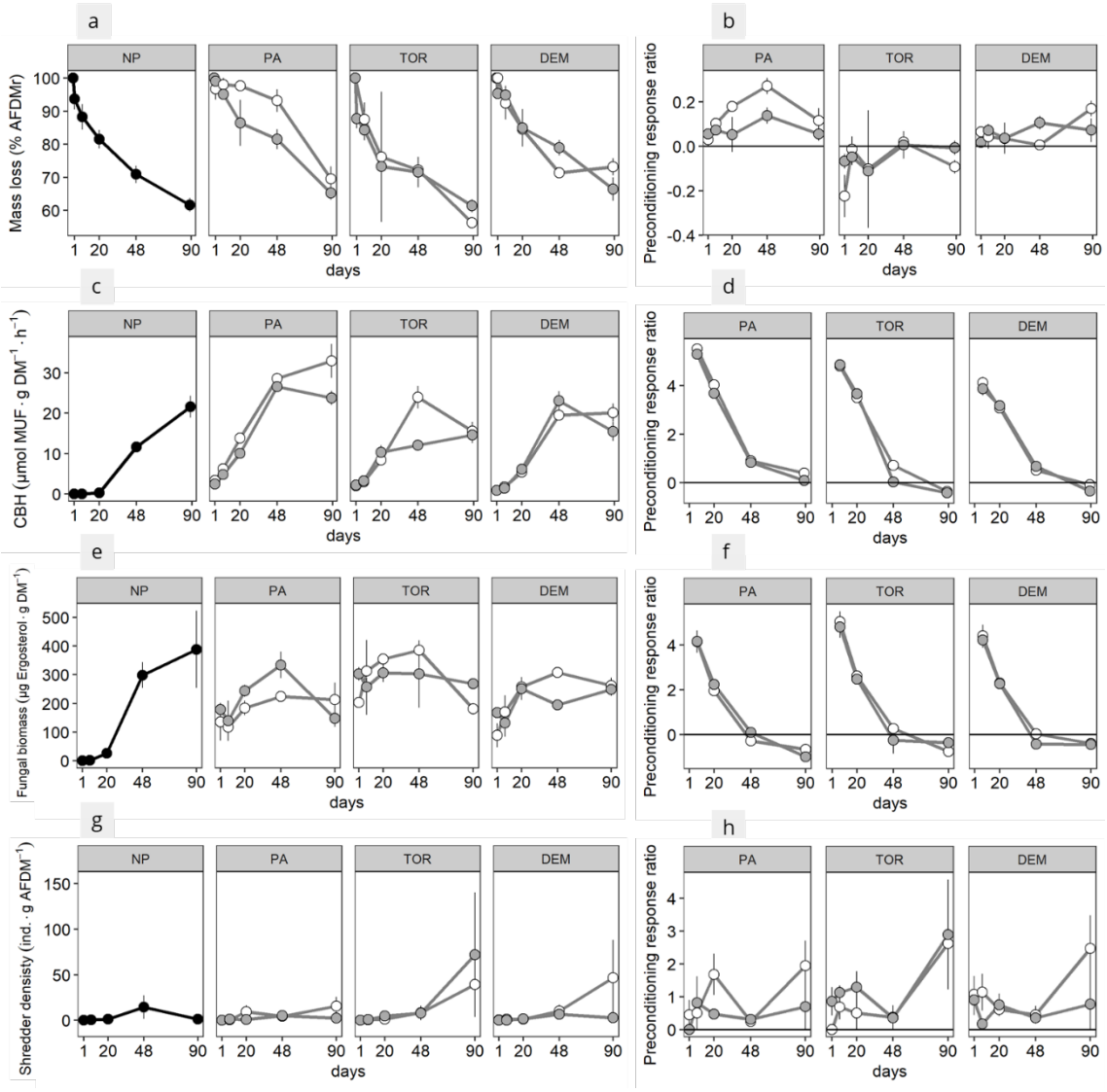

Figure 4.3 Evolution of leaf litter mass loss (a), cellobiohydrolase activity (c), fungal biomass (e) and shredder density (g) during the aquatic decomposition phase, in non-preconditioned leaf litter (NP; black dots) and leaf litter preconditioned under open-canopy (white dots) and closed-canopy habitats (grey dots) in the different floodplains sites. (b), (d), (f) and (h) show the preconditioning response ratio (PRR) for the same variables. Ratios represent the relationship between preconditioned and non-preconditioned leaf litter, so PRR > 0 means higher values of preconditioned leaf litter than the non-preconditioned one, and vice versa when PRR < 0. Dots are average values ± SE (n = 3). See Table 4.S6 for statistics. Floodplain sites: PA = Parra, TOR = Tordera, DEM = Demnitzer.

In addition, leaf litter from open-canopy habitats showed significantly higher CBH values than in closed-canopy ones ($F_{1,155}$ = 16.17; p < 0.001). Contrarily to CBH activity, CBH efficiency was significantly higher in non-preconditioned than in preconditioned leaf litter ($t_{1,94}$ = 5.10; p = 0.026) (Fig. 4.4). Considering just preconditioned leaf litter, ANCOVA model revealed significant differences in CBH efficiency among floodplain sites, but only between PA and TOR ($F_{2,94}$ = 5.94; p = 0.004), with the lowest efficiency in PA and the highest one in TOR (Fig. 4.4).

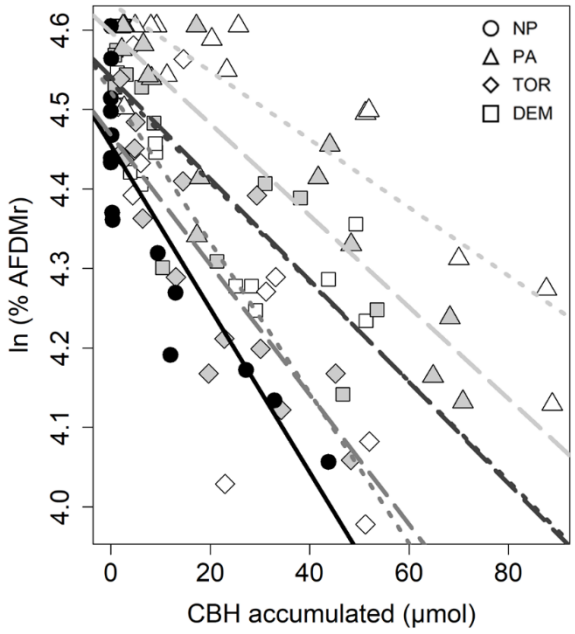

Figure 4.4 Relationships between the natural logarithm of the percentage of remaining ash free dry mass (% AFDMr) and the accumulated cellobiohydralse activity during the aquatic leaf litter decomposition. Steeper slopes mean higher enzyme efficiency (i.e., less enzyme produced to decompose a gram of leaf litter organic mass. Grey lines represent the relationships of leaf litter preconditioned under open- (white symbols) and closed-canopy habitats (grey symbols) in the different floodplain sites. The black line and symbols represent non-preconditioned leaf litter.

Fungal biomass was significantly higher in preconditioned leaf litter than in non-preconditioned one according to the contrasts analysis ($t_{1,76}$ = -3.43; p = 0.001) (Fig. 4.3e and 4.3f). As for CBH activity, the PRR_{FB} showed the maximum values

during the first 20 days, but it decreased afterwards due to the great increase of fungal biomass in non-preconditioned leaf litter at day 48 (Fig. 4.3e and 4.3f). Fungal biomass was significantly higher in preconditioned leaf litter from TOR than in the other two sites ($F_{2,51} = 8.56$; $p < 0.001$. Tukey $p < 0.05$). No differences were found between habitats according to mixed models.

Shredder density did not show any difference between preconditioned and non-preconditioned leaf litter along the aquatic phase, although PRR ratio showed maximum values at the end of the aquatic period (Fig. 4.3g and 4.3h). No differences were found either among floodplain sites ($F_{2,51} = 1.29$; $p = 0.285$) or habitats ($F_{1,51} = 0.20$; $p = 0.660$).

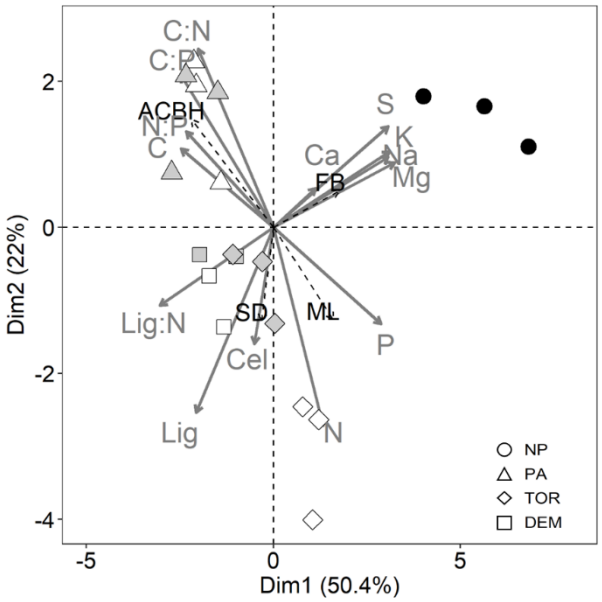

Figure 4.5 PCA biplot describing the chemical composition of non-preconditioned leaf litter (NP; black dots) and leaf litter preconditioned under open-canopy (white symbols) and closed-canopy habitats (grey symbols) in the different floodplains sites before the start of the aquatic phase. Grey arrows represent the loading of each chemical descriptor, whereas the dotted black arrows represent the correlation of mass loss (ML), accumulated cellobiohydrolase activity (ACBH), fungal biomass (FB) and shredder density (SD) after 90 days of river immersion with the PCA loadings. Chemical composition of leaf litter is shown in Table 4.2. Floodplain sites: PA = Parra, TOR = Tordera, DEM = Demnitzer.

Finally, the PCA based on the chemical composition of preconditioned and non-preconditioned leaf litter before starting the aquatic phase (carried out to analyse how chemical compositions affect aquatic processing) explained in their first two axes up to 72.4 % of total variance. The PC1 (50.4% of total variance) separated preconditioned from non-preconditioned leaf litter, and was mainly related to Lig:N, C:P, N:P, C, P, K, Mg, Na and S (Fig. 4.5). PC2 (22% of total variance) differentiated mainly leaf litter preconditioned in PA from those preconditioned in TOR and DEM, and was principally related to C:N, C:P, N and lignin. All studied descriptors of aquatic decomposition were well correlated with the PC1 or PC2, except shredder density. Both mass loss (ML), fungal biomass (FB) and ACBH were mainly correlated with PC1 although in an opposite way (r_{ML}= 0.47, $p < 0.05$; r_{FB} = 0.51, $p < 0.05$; r_{ACBH} = -0.68, p = 0.002) as indicated by their projection in the PCA as supplementary variables. C:N, C:P and N:P ratios were positively associated with ACBH, but negatively associated with mass loss (Fig. 4.5). Highest values of fungal biomass were related to the highest concentration of nutrients as P, K, Mg, Na and S in non-preconditioned leaf litter (Fig. 4.5).

4.4. Discussion

The present study demonstrates the relevance of floodplain environmental conditions on leaf litter preconditioning. Climatic and local conditions in floodplain soils modulate the contribution of leaf litter as source of energy and nutrients for rivers. Our results clearly show how the exposure of leaf litter under different conditions of soil nutrient availability and climate regulates the capacity of aquatic microbial communities to use preconditioned leaf litter through the alteration of its chemical quality.

4.4.1. Climate and soil nutrients drive the chemical and biological alteration of leaf litter accumulated in floodplains

Our selection of floodplain sites and habitats resulted basically in three floodplains sites differing in their climate and soil nutrients availability, but not differences in

soil properties of open- and closed-canopy habitats (Table 4.1). In particular, the combination of temperature, humidity and soil nutrient content at each floodplain site played a key role controlling changes occurring in leaf litter during its floodplain exposure mainly by the regulation of its microbial decomposition (Aerts 1997, Dent et al. 2006, Gavazov et al. 2014). In TOR, the high temperature, humidity and nutrient availability in soils resulted in very favourable conditions for microbial decomposition (Gavazov et al. 2014, Delgado-Baquerizo et al. 2015), fostering the highest mass loss, fungal biomass and cellobiohydrolase activity in leaf litter. On the contrary, the lower humidity and soil nutrient availability in PA (mainly in P; Table 4.1) resulted in a much lower microbial decomposition of leaf litter (Cleveland et al. 2002, Austin 2011), as evidenced by the lowest leaf litter mass loss and fungal biomass. Compared to the other two sites, leaf litter decomposition in DEM was intermediate, as expected by its higher soil humidity and nutrients content than PA, but lower ambient temperature than TOR.

Leaf litter mass loss also varied between habitats, with a significantly higher loss under open- than under closed-canopy conditions (Fig. 4.1a). Due to the apparent absence of differences in soil properties between habitats, we suggest the highest exposure of leaf litter to rain and solar radiation in open-canopy habitats could have favoured its decomposition. The higher mass loss could be caused either to the direct effect of photolysis (Austin & Vivanco 2006), or to the facilitation of microbial decomposition during wet periods by previous photodegradation (Henry et al. 2008, Wang et al. 2015, Gliksman et al. 2016).

Leaf litter decomposition in floodplains did not cause a decrease of cellulose or lignin, but a generalized increase of the lignin content in all floodplain sites and habitats (Table 4.2). This lignin increase is in line with previous works on floodplain preconditioning of OM (Baldwin 1999, Fellman et al. 2013, Jian et al. 2016). They show how preconditioned leaf litter is subjected to an increase of its recalcitrance by the accumulation of phenolic compounds, mainly by oxidative polymeric reactions occurring during the decomposition process (Baldwin 1999). The start of leaf litter decomposition during its floodplain exposure can also cause a quickly decrease of labile C compounds, such as hemicellulose, carbohydrates or amino acids by the combined action of rain leaching and microbial activity (Swift et al. 1979, Hongve et

al. 2000, O'Connell et al. 2000). Although we did not measure these compounds in the POM fraction of leaf litter, the high decrease of FI (i.e. labile DOM compounds) and specially, of DOC concentration evidenced in leachates of leaf litter exposed in all floodplains (Table 4.S4) support this idea. In conjunction, these results suggest that floodplain exposure exerts a decrease of leaf litter C quality due to the reduction of labile C compounds and the concomitant increase of recalcitrant ones.

Contrary to we expected, the higher exposure of leaf litter to solar radiation in open-canopy habitats, especially in the arid floodplain, did not translate into a lignin loss by photodegradation (Austin & Ballaré 2010) compared to closed-canopy habitats or more humid floodplains. That is, environmental differences among floodplain sites, or habitats did not translate into differences in the leaf litter C quality. However, contrasting environmental conditions of floodplain sites did affect the DOM quality of leaf litter leachates (Fig. 4.2a). Leachates from leaf litter exposed in TOR and DEM were characterized by small and highly aromatic DOM compounds, suggesting that an increased microbial decomposition could have contributed to the release of small phenolic compounds from the partially degraded leaf litter (Mastný et al. 2018). On the contrary, leachates from leaf litter exposed in PA were characterized by the dominance of big, humic DOM compounds, suggesting that conditions of high solar radiation and/or temperature could have favoured a greater humification of leaf litter (Makkar 2003, Chatani et al. 2014).

Climatic conditions, but mainly differences in soil nutrient availability among floodplains were the main drivers of the final nutrient content of leaf litter and their leachates (Hobbie & Vitousek 2000, Cleveland et al. 2002). Hence, both leaf litter exposed in PA and their leachates, showed the highest impoverishment of N and P as evidenced by the highest C:N, C:P and N:P ratios reflecting the potential soil nutrient limitation in PA (Table 4.2). In addition, the combined action of high solar radiation and heat could have increased the solubility of nutrients in arid sites and promote their loss by rain leaching (Dieter et al. 2013, del Campo & Gómez 2016). Conversely, the higher soil nutrient availability in DEM and TOR resulted in minor changes in the leaf litter nutrient content, or even in a partial N enrichment, suggesting nutrient microbial immobilization could have compensated nutrient loss by leaching specially in TOR (Parton et al. 2007). Besides to N and P, other macro

and micronutrients such as K, Mg, Na or S underwent a general depletion in leaf litter exposed in all floodplain sites, as previously showed for wood (del Campo & Gómez 2016). Our results suggest that the counterbalance between microbial immobilization and rain leaching during floodplain exposure controlled the nutrient content of leaf litter. Thus, when soil nutrient availability is high, microbial immobilization may compensate the effects of rain leaching.

4.4.2. Environmental conditions during floodplain preconditioning shape the relevance of leaf litter inputs as nutrient and energy sources for aquatic ecosystems

POM is an important source of C and nutrients for aquatic decomposers (fungi, bacteria and detritivores) but also its leachates (released DOM) fuel microbial activity (mostly bacteria) in stream and rivers. Thus, both organic matter fractions are essential for ecosystem functioning and both are affected by preconditioning as our results demonstrated.

The preconditioning of leaf litter in floodplains caused a strong drop of the potential microbial metabolic activity in leaf litter leachates, mainly associated with the loss of DOC, nutrients (mainly N) and labile C compounds as suggested by the high and positive correlation of microbial activity (R_{ru} production) with the concentration of DOC ($r = 0.90$), DIN ($r = 0.85$) and FI values ($r = 0.53$). Contrarily, R_{ru} production was inversely correlated with DOM molecular size (Sr and E2:E3: $r = -0.60$), suggesting a lower biodegradability of smaller DOM compounds as those found in leachates from leaf litter preconditioned in TOR and DEM (Amon & Benner 1996, but see van Hees et al. 2005). However, among all these factors, the DOC concentration seemed to be the main driver controlling the biodegradation of leaf litter leachates as suggested by the fact that the highest microbial activity was found in leachates of leaf litter from PA, with the lowest concentration of nutrients, but the highest concentration of DOC compared to the other two floodplain sites. Although we acknowledge that longer (>1 h) biodegradation assays should be carried out to confirm our findings, our results point out that mainly the loss of labile DOC, but also of nutrients, during leaf litter floodplain preconditioning,

would severely reduce the relevance of leachates as source of energy and nutrients for aquatic microbial communities. On the contrary, it would constitute an important resource for terrestrial microbial communities in floodplain soils, especially in arid areas or in nutrient limited soils (Cleveland et al. 2002).

Floodplain preconditioning of leaf litter also seemed to have a noticeable effect on the POM decomposition process. Our findings show severe differences in both the pathway and rate of the aquatic decomposition, between preconditioned and non-preconditioned leaf litter (Fig. 4.3). Following the common temporal decomposition pattern described in aquatic systems, non-preconditioned leaf litter underwent a strong leaching during the first 7 days of immersion that promoted the release of soluble nutrients (France et al. 1997) and dominance of mass loss by abrasion (Webster & Benfield 1986) till 20 days of aquatic immersion, when the first evidences of fungal colonization and microbial activity were observed (Fig. 4.3c and 4.3e) (Webster & Benfield 1986, Suberkropp 1998). However, the leaf litter preconditioning altered this dynamic. The previous loss of DOC and nutrients in floodplains fostered the leaching of preconditioned leaf litter to be was practically null during the first days of immersion in the river (Fig. 4.S3). Similar to have been described for preconditioned wood in arid floodplains (del Campo & Gómez 2016). On the other hand, the rapid and acute increase of microbial activity and fungal biomass in preconditioned leaf litter during the first days of immersion suggest that terrestrial microbial communities continued active and even were stimulated by the higher availability of water and nutrients (del Campo & Gómez 2016). Although, terrestrial fungi have been shown to be inhibited under aquatic conditions (Bärlocher & Boddy 2016), our results are in the line of previous works demonstrating that terrestrial fungal species can play an active role in aquatic OM decomposition (Wurzbacher et al. 2010, Voronin 2014), at least during the first days of immersion. Later, they are replaced by aquatic microbial species better adapted to the aquatic environment (Wurzbacher et al. 2010).

Preconditioned and non-preconditioned leaf litter swapped their dynamic after the first 20 days of river immersion (Fig. 4.3c, d, e and f). Whereas fungal biomass and microbial activity accelerated in non-preconditioned leaf litter, it slowed down in leaf litter coming from floodplains. This divergence in the dynamic

of the microbial decomposition could be associated with the inequality in C resources availability between non-preconditioned and preconditioned leaf litter, as it is also supported by differences in microbial decomposition efficiencies (Fig. 4.4). The more advanced stage of decomposition in the preconditioned leaf litter could have limited microbial activity and thus decreased its efficiency (Moorhead & Sinsabaugh 2006, del Campo & Gómez 2016). However, differences in the community composition of the microbial decomposers between preconditioned and non-preconditioned leaves could have influenced too. The worse adaptation of terrestrial fungi on preconditioned leaf litter to the aquatic environment, together with likely competitive inhibition between terrestrial and aquatic decomposers, could be associated with the lower decomposition efficiency observed in preconditioned leaf litter (Fukami et al. 2010, Bärlocher & Boddy 2016).

Loss of nutrients from leaf litter during its floodplain exposure had also important implications on aquatic POM decomposers. The exposure of leaf litter in TOR increased its aquatic decomposition rate we suggest because floodplain conditions kept the leaf litter nutrients content in a high level. On the contrary, preconditioning in DEM and PA decreased leaf litter aquatic decomposition rates by reducing P content in leaves, especially in PA (see Table 4.2) (see Güssewell & Gessner 2009). The importance of P in aquatic decomposition is also supported by the high correlation between leaf litter mass loss and its initial nutrient content, mainly P (Fig. 4.5). On the other hand, the highest fungal biomass in non-preconditioned leaf litter was highly correlated with its content in macro and micronutrients such as Ca, Mg, Na, K or S (Fig. 4.5) (García-Palacios et al. 2015), indicating that the loss of micronutrients by rain leaching during floodplain exposure can also mediate leaf litter decomposition in the river by limiting the activity and growth of aquatic microbial communities on leaves (Gadd 1993, Jellison et al. 1997).

In summary, our results suggest that environmental conditions of different floodplain sites, but not their habitat conditions, modulated the aquatic decomposition rate of exposed leaf litter by altering its nutrient availability. Therefore, we partially accept our first hypothesis regarding to the effect of floodplain sites, but we reject our second hypothesis because habitats did not

influence aquatic decomposition. Our results suggest that potential nutrient limitation in floodplain soils can hinder the decomposition of leaf litter, not only during its accumulation in the floodplain (Cleveland 2002, Hobbie & Vitousek 2000), but also after entering the river, translating the soil nutrient limitation across food web levels and ecosystems (Vitousek & Howarth 1991). Even so, we should not discard that changes in C quality by different preconditioning processes could have affected aquatic decomposition as well. Thus, the leaf litter humification in PA (supported by the highest humic-like DOM in leachates) could have contributed to reduce its decomposition rate (Cleveland et al. 2004). Contrarily to mass loss and fungal biomass, the highest values of microbial activity measured as accumulated cellobiohydrolase activity were found in leaf litter preconditioned in PA, highly correlating with the highest values of C:N, C:P and N:P ratios (Fig. 4.5). This positive relationship between elemental ratios and CBH clearly contradicts previous studies about stoichiometric regulation of extracellular enzymes (Sinsabaugh et al. 1993 & 1994). A plausible explanation could be in the fact that C and N allocation is enzymatically coupled, therefore N-limitation can sometimes derive in a higher expression of C degrading enzymes; however, this is not applicable for P, as this is mainly obtained from the water by phosphate-sterases (Sinsabaugh et al. 2005 & 2008). Even so, some studies in ectomycorrhizal fungi do find this response in cases of strong P-limitation (Hagerberg et al. 2003). In the end, contrary to TOR, the highest microbial activity in leaf litter preconditioned in PA translated into the lowest cellobiohydrolase efficiency, reinforcing the conception of leaf litter nutrient availability as the main regulator of aquatic decomposition in our study. However, the characterization of enzymatic capabilities related to nutrient acquisition would be necessary to confirm this assumption.

Contrary to the significant effects of floodplain preconditioning on aquatic microbial communities, it did not show any influence on shredding activity, as suggested by the absence of differences in the density of shredders between preconditioned and non-preconditioned leaf litter (Dieter et al 2011, 2013). So, although the colonization of terrestrial fungi and the advanced stage of decomposition of preconditioned leaf litter may increase the palatability of leaves

(Webster & Benfield 1986), this was not enough to increment significantly the shredder feeding preferences in our case.

4.4.3. Implications of terrestrial-aquatic interactions on C processing in rivers

The current work can serve as a cornerstone to integrate previous information about terrestrial preconditioning and elucidate its global effect on aquatic processing of OM inputs. As we initially hypothesized, our results, together with previous evidences, show how terrestrial preconditioning trigger contrasting effects on the aquatic processing of POM and DOM fractions coming from leaf litter inputs. Floodplain preconditioning caused a general negative effect on the biodegradation of leaf litter leachates, by reducing the quality of leached DOM (mainly by the accumulation of recalcitrant DOM compounds, see Baldwin 1999 and Fellman et al. 2013), or by the depletion of DOC and nutrients concentration. These changes would affect negatively to microbial metabolism in rivers as evidenced by our biodegradability assay. Nevertheless, the effects of preconditioning on leaf litter decomposition in rivers seem to absolutely depends on the environmental conditions of floodplain acting at regional scale. Our results, point out that the alteration of leaf litter nutrient content, mediated by both floodplain climate and nutrient availability in soils, determine the facilitation or the slowdown of its subsequent decomposition (Dieter et al. 2013). Previous works state that high solar radiation during POM floodplain preconditioning can facilitate the access of microorganisms to cellulosic polysaccharides through the photodegradation of lignin resulting in an aquatic decomposition increasing (Pu et al. 2014, Austin et al. 2016, del Campo & Gómez 2016), nevertheless we do not have evidence of this mechanism in this study

In summary, our findings demonstrate the importance of floodplain preconditioning modulating the fate of lateral leaf litter inputs in rivers, challenging the general assumption of leaf litter as an efficient energetic resource and nutrient source for freshwater ecosystems (Wallace et al. 1995, Bernhardt & McDowell 2008, Hagen et al. 2012). Certainly, the influence of this terrestrial process on rivers functioning would be determined partially by the proportion of leaf litter entering

the river from the floodplain respect to the total inputs. For instance, Benfield (1997) estimated that lateral leaf litter inputs can suppose up to 80% of the total inputs in North American rivers. Similarly, in rivers of arid zones, because of the absence of riparian trees, vertical inputs of leaf litter are almost null and most OM inputs come from floodplains (Jacobson 1999, Sponseller & Fisher 2006).

To continue investigating the effect of floodplain preconditioning on OM processing in rivers seems pivotal to achieve a realistic understanding of C fluxes between terrestrial and aquatic ecosystems. The study "in situ" of the effect of this preconditioned POM on ecosystem functioning it one of the future challenge to advance in this sense.

4.5. Annexes

Table 4.S1 Description of the studied floodplain sites.

Floodplain sites	Coordinates	Elevation (m a.s.l.)	Climate[a]	MAP (mm)	MAT (º C)	Lithology in the catchment	Vegetation in the floodplain
Parra	38°13'54.70"N, 1° 5'17.00"W	262	Bsh	303	20.0	Marls and limestones	*Salsola sp., Nerium oleander, Lygeum spartum*
Tordera	41°43'08.3"N, 2°33'50.6"E	150	Csb	750	16.5	Granitic	*Populus nigra*, *Alnus glutinosa, Crataegus monogyna*
Demnitzer	52°21'34.76"N, 14°11'44.02"E	40	Dfb	480	9.0	Glacial sediments	*Quercus robur, Alnus glutinosa, Carpinus betulus*

[a] Climate classification according to the Köppen-Geiger classification (Peel et al. 2007). Bsh = Hot semi-arid climate; Csb = Warm-summer Mediterranean climate; Dfb = Warm-summer humid continental climate. MAP = mean annual precipitation. MAT = mean annual temperature.

Table 4.S2 Mixed model results for soil properties of floodplain sites and habitat conditions during the preconditioning phase.

	Mixed Model[a]			
	Effect	*df*	*F*	*p*
Gravimetric water content	*Floodplain site*	2	17.89	0.003
	Habitat	1	0.12	0.735
	Floodplain site x Habitat	2	0.66	0.520
Organic matter content	*Floodplain site*	2	13.79	0.006
	Habitat	1	0.12	0.727
	Floodplain site x Habitat	2	1.35	0.267
C	*Floodplain site*	2	113.85	<0.001
	Habitat	1	1.57	0.237
	Floodplain site x Habitat	2	3.83	0.055
N	*Floodplain site*	2	54.37	<0.001
	Habitat	1	4.08	0.068
	Floodplain site x Habitat	2	6.71	0.012
P	*Floodplain site*	2	8.72	0.005
	Habitat	1	10.95	0.007
	Floodplain site x Habitat	2	6.53	0.013
C:N	*Floodplain site*	2	46.16	<0.001
	Habitat	1	0.21	0.657
	Floodplain site x Habitat	2	0.19	0.829
C:P	*Floodplain site*	2	44.61	<0.001
	Habitat	1	0.32	0.585
	Floodplain site x Habitat	2	0.61	0.562
N:P	*Floodplain site*	2	23.73	<0.001
	Habitat	1	1.14	0.308
	Floodplain site x Habitat	2	0.38	0.695

[a] Mixed Models test for differences in floodplain soil properties among floodplain sites and between habitats (both considered fixed factors). Bold values indicate significant differences ($p < 0.05$).

Table 4.S3 Mixed model and contrast results for variables measured in leaf litter after the preconditioning phase.

				Mixed Model[a]			
				Effect	*df*	*F*	*P*
Mass loss				*Floodplain site*	2	60.67	<0.001
				Habitat	1	4.33	0.041
				Floodplain site x Habitat	2	2.46	0.092
Cellobiohydrolase activity				*Floodplain site*	2	8.59	0.017
				Habitat	1	3.26	0.082
				Floodplain site x Habitat	2	4.59	0.019
Fungal biomass				*Floodplain site*	2	6.06	0.017
				Habitat	1	0.02	0.889
				Floodplain site x Habitat	2	5.81	0.019
	Contrast[b] (P vs NP)			Mixed Model[a]			
Chemical composition	*df*	*t*	*P*	Effect	*df*	*F*	*p*
Cellulose	31	5.82	0.003	*Floodplain site*	2	2.93	0.130
				Habitat	1	2.55	0.171
				Floodplain site x Habitat	2	0.42	0.678
Lignin	31	-12.44	<0.001	*Floodplain site*	2	2.43	0.168
				Habitat	1	5.48	0.066
				Floodplain site x Habitat	2	4.04	0.091
C	31	-5.89	<0.001	*Floodplain site*	2	8.22	0.019
				Habitat	1	2.58	0.169
				Floodplain site x Habitat	2	1.98	0.232
N	31	0.34	0.739	*Floodplain site*	2	4.70	0.059
				Habitat	1	20.02	0.007
				Floodplain site x Habitat	2	4.85	0.067
P	31	4.43	<0.001	*Floodplain site*	2	24.96	0.001
				Habitat	1	0.87	0.394
				Floodplain site x Habitat	2	4.20	0.085
Lig:N	31	-14.39	<0.001	*Floodplain site*	2	0.42	0.675
				Habitat	1	1.23	0.319
				Floodplain site x Habitat	2	0.63	0.569
C:N	31	-2.01	0.053	*Floodplain site*	2	10.26	0.012

	Contrast[b] (P vs NP)			Mixed Model[a]			
	df	*t*	*P*	Effect	*df*	*F*	*p*
				Habitat	1	13.29	0.015
				Floodplain site x Habitat	2	2.68	0.162
C:P	31	-8.42	<0.001	*Floodplain site*	2	131.75	<0.001
				Habitat	1	0.01	0.934
				Floodplain site x Habitat	2	2.72	0.110
N:P	31	23.99	<0.001	*Floodplain site*	2	61.92	<0.001
				Habitat	1	14.71	0.003
				Floodplain site x Habitat	2	0.42	0.665

[a] Mixed Models test for differences among floodplain sites and between habitats (both considered fixed factors) in variables measured in preconditioned leaf litter.

[b] Contrast analysis (P vs NP) test for differences between preconditioned (p) and non-preconditioned (NP) leaf litter to determine if the preconditioning altered significantly the initial chemical composition of leaf litter. Bold values indicate significant differences ($p < 0.05$).

Table 4.S4 Chemical composition of leachates from non-preconditioned and preconditioned leaf litter from all floodplain site and habitats.

	Non-preconditioned leaf litter	Parra			Tordera			Demnitzer		
		Open-canopy	Closed-canopy		Open-canopy	Closed-canopy		Open-canopy	Closed-canopy	
DOC (mg L^{-1})	104.2 ± 15.3	39.7 ± 4.8	30.4 ± 1.9	a	21.2 ± 2.2	25.6 ± 1.8	b	26.8 ± 7.7	22.7 ± 3.6	b
DIN (mg L^{-1})	1571 ± 144	290 ± 23	276 ± 23	a	620	494 ± 48	b	296	494 ± 72	b
SRP (mg L^{-1})	1.07 ± 0.07	0.15 ± 0.02	0.18 ± 0.02	a	0.79 ± 0.09	0.68 ± 0.07	b	0.72 ± 0.04	0.88 ± 0.07	b
C:N	0.067 ± 0.011	0.137 ± 0.010	0.112 ± 0.011	a	0.040	0.052 ± 0.005	b	0.065	0.046 ± 0.004	b
C:P	97.7 ± 12.4	277.8 ± 59.7	174.7 ± 7.1	a	27.9 ± 5.6	38.6 ± 6.3	b	36.9 ± 8.8	25.7 ± 3.1	c
N:P	1470 ± 60	1999 ± 287	1594 ± 168	a	924	730 ± 65	b	436	555 ± 41	c
FI	1.26 ± 0.04	1.09 ± 0.01	1.12 ± 0.02	a	1.13 ± 0.04	1.05 ± 0.01	a*	1.11 ± 0.02	1.21 ± 0.04	a*
HIX	0.68 ± 0.20	1.62 ± 0.20	1.09 ± 0.09	a*	0.86 ± 0.04	0.76 ± 0.09	b*	0.83 ± 0.13	0.70 ± 0.11	b*
SUVA (L mg^{-1} m^{-1})	2.45 ± 0.11	1.32 ± 0.18	1.28 ± 0.15	a	2.32 ± 0.50	2.33 ± 0.20	b	1.88 ± 0.77	1.75 ± 0.23	a
S275_295	-0.012 ± 0.003	-0.008 ± 0	-0.007 ± 0.001	a	-0.012 ± 0.001	-0.013 ± 0.001	b	-0.011 ± 0	-0.010 ± 0	c
Sr	0.28 ± 0.07	0.35 ± 0.02	0.23 ± 0.05	a	0.58 ± 0.08	0.58 ± 0.04	b	0.45 ± 0.04	0.39 ± 0.01	c
E2:E3	3.78 ± 0.35	4.12 ± 0.12	4.77 ± 0.23	a	4.78 ± 0.05	4.66 ± 0.24	a	5.40 ± 0.44	5.37 ± 0.40	b

Values are the average ± SE (n = 3). Different letters in a row indicate significant differences among floodplain sites according to post-hoc tests after significant mixed models ($p < 0.05$). * Expresses significant differences between habitats according mixed models ($p < 0.05$). See Table S5 for statistics.

Table 4.S5 Mixed model and contrast results for leachates chemical composition and microbial metabolic activity.

	Contrast[a] (P vs NP)			Mixed Model[b]			
	df	*t*	*p*	Effect	*df*	*F*	*p*
R_{ru}	15	10.62	<0.001	*Floodplain site*	2	7.35	0.011
				Habitat	1	0.20	0.662
				Floodplain site x Habitat	2	2.39	0.141
DOC	16	10.97	<0.001	*Floodplain site*	2	6.51	0.014
				Habitat	1	1.03	0.332
				Floodplain site x Habitat	2	1.96	0.187
DIN	13	13.99	<0.001	*Floodplain site*	2	11.97	0.004
				Habitat	1	0.16	0.701
				Floodplain site x Habitat	2	3.26	0.092
SRP	16	7.05	<0.001	*Floodplain site*	2	66.40	<0.001
				Habitat	1	0.30	0.597
				Floodplain site x Habitat	2	2.36	0.140
C:N	13	0.35	0.567	*Floodplain site*	2	59.93	<0.001
				Habitat	1	1.92	0.204
				Floodplain site x Habitat	2	3.37	0.087
C:P	16	0.00	0.965	*Floodplain site*	2	91.24	<0.001
				Habitat	1	0.86	0.374
				Floodplain site x Habitat	2	3.21	0.080
N:P	13	0.71	0.414	*Floodplain site*	2	61.71	<0.001
				Habitat	1	1.16	0.312
				Floodplain site x Habitat	2	1.92	0.20
FI	16	3.82	0.002	*Floodplain site*	2	3.48	0.067
				Habitat	1	0.54	0.478
				Floodplain site x Habitat	2	4.84	0.031
HIX	16	-1.73	0.102	*Floodplain site*	2	14.16	<0.001
				Habitat	1	6.20	0.030
				Floodplain site x Habitat	2	1.96	0.187
SUVA	16	2.14	0.048	*Floodplain site*	2	4.87	0.031
				Habitat	1	0.03	0.859
				Floodplain site x Habitat	2	0.02	0.980

	Contrast[a] (P vs NP)			Mixed Model[b]			
	df	*t*	*P*	Effect	*df*	*F*	*P*
S275_295	16	-1.14	0.273	*Floodplain site*	2	17.76	<0.001
				Habitat	1	0.32	0.583
				Floodplain site x Habitat	2	0.63	0.553
Sr	16	-2.62	0.019	*Floodplain site*	2	19.47	<0.001
				Habitat	1	2.41	0.149
				Floodplain site x Habitat	2	0.88	0.440
E2:E3	16	-3.64	0.002	*Floodplain site*	2	6.30	0.015
				Habitat	1	0.58	0.461
				Floodplain site x Habitat	2	1.36	0.296

[a] Contrast analysis (P vs NP) test for differences between preconditioned (P) and non-preconditioned (NP) leaf litter to determine if the preconditioning altered significantly the chemical composition and the potential microbial metabolic activity of leaf litter leachates compared to initial conditions (NP).

[b] Mixed Models test for differences among floodplain sites and between habitats (both considered fixed factors) for those variables measured in preconditioned leaf litter.

Bold values indicate significant differences ($p < 0.05$).

Table 4.S6 Mixed model and contrast results for variables measured during the aquatic phase.

	Contrast[a] (P vs NP)			Mixed Model[b]			
	df	*t*	*p*	Effect	*df*	*F*	*p*
Mass loss	81	-1.61	0.112	*Floodplain site*	2	27.69	<0.001
				Habitat	1	0.63	0.43
				Floodplain site x habitat	2	2.20	0.120
				Time	4	43.10	<0.001
				Floodplain site x Time	8	1.36	0.233
				Habitat x Time	4	0.69	0.599
				Floodplain site x Habitat x Time	8	1.26	0.282
Cellobiohydrolase activity	212	-1.50	0.135	*Floodplain site*	2	66.57	<0.001
				Habitat	1	16.17	<0.001
				Floodplain site x Habitat	2	6.15	0.003
				Time	5	246.15	<0.001
				Floodplain site x Time	10	8.06	<0.001
				Habitat x Time	5	2.60	0.027
				Floodplain site x Habitat x Time	10	4.27	<0.001
Fungal biomass	76	-3.43	0.001	*Floodplain site*	2	8.56	<0.001
				Habitat	1	0.07	0.786
				Floodplain site x Habitat	2	0.64	0.530
				Time	4	4.85	0.002
				Floodplain site x Time	8	0.98	0.462
				Habitat x Time	4	0.93	0.454
				Floodplain site x Habitat x Time	8	1.00	0.451
Shredder density	75	-0.88	0.384	*Floodplain site*	2	1.29	0.285
				Habitat	1	0.20	0.660
				Floodplain site x Habitat	2	1.05	0.358
				Time	4	4.69	0.003
				Floodplain site x Time	8	1.12	0.368
				Habitat x Time	4	0.09	0.984
				Floodplain site x Habitat x Time	8	0.72	0.674

	Contrast[a] (P vs NP)			Mixed Model[b]			
	df	*t*	*p*	Effect	*df*	*F*	*p*
Cellulose	94	2.13	0.036	*Floodplain site*	2	0.03	0.967
				Habitat	1	1.06	0.305
				Floodplain site x Habitat	2	0.75	0.477
				Time	1	85.23	<0.001
				Time x Floodplain site	2	0.49	0.617
				Time x Habitat	1	1.44	0.234
				Time x Floodplain site x Habitat	2	0.16	0.855
Lignin	94	-8.37	<0.001	*Floodplain site*	2	8.98	<0.001
				Habitat	1	3.76	0.056
				Floodplain site x Habitat	2	5.14	0.008
				Time	1	26.55	<0.001
				Time x Floodplain site	2	0.01	0.990
				Time x Habitat	1	0.01	0.928
				Time x Floodplain site x Habitat	2	3.91	0.024
C	94	1.54	0.126	*Floodplain site*	2	15.65	<0.001
				Habitat	1	3.76	0.056
				Floodplain site x Habitat	2	0.36	0.697
				Time	1	21.07	<0.001
				Time x Floodplain site	2	0.52	0.597
				Time x Habitat	1	0.12	0.731
				Time x Floodplain site x Habitat	2	0.92	0.404
N	94	-5.34	<0.001	*Floodplain site*	2	34.08	<0.001
				Habitat	1	15.49	<0.001
				Floodplain site x Habitat	2	7.42	0.001
				Time	1	285.04	<0.001
				Time x Floodplain site	2	3.56	0.033
				Time x Habitat	1	8.76	0.004
				Time x Floodplain site x Habitat	2	0.82	0.443
P	94	-2.87	0.005	*Floodplain site*	2	23.59	<0.001
				Habitat	1	0.15	0.701
				Floodplain site x Habitat	2	1.22	0.301

	Contrast[a] (P vs NP)			Mixed Model[b]			
	df	*t*	*p*	Effect	*df*	*F*	*p*
				Time	1	121.11	<0.001
				Time x Floodplain site	2	9.05	<0.001
				Time x Habitat	1	0.15	0.7030
Lig:N	94	-8.01	<0.001	*Floodplain site*	2	3.15	0.050
				Habitat	1	1.43	0.236
				Floodplain site x Habitat	2	0.09	0.915
				Time	5	18.32	<0.001
				Floodplain site x Time	10	1.68	0.107
				Habitat x Time	5	0.76	0.580
				Floodplain site x Habitat x Time	10	1.51	0.159
C:N	94	5.97	<0.001	*Floodplain site*	2	43.18	<0.001
				Habitat	1	15.58	<0.001
				Floodplain site x Habitat	2	5.89	0.004
				Time	1	157.01	<0.001
				Time x Floodplain site	2	6.30	0.003
				Time x Habitat	1	6.82	0.011
				Time x Floodplain site x Habitat	2	0.67	0.515
C:P	94	5.78	<0.001	*Floodplain site*	2	30.49	<0.001
				Habitat	1	0.11	0.745
				Floodplain site x Habitat	2	1.67	0.195
				Time	1	57.55	<0.001
				Time x Floodplain site	2	9.05	<0.001
				Time x Habitat	1	0.07	0.793
				Time x Floodplain site x Habitat	2	0.60	0.551
N:P	94	3.13	0.002	*Floodplain site*	2	11.72	<0.001
				Habitat	1	3.13	0.080
				Floodplain site x Habitat	2	0.18	0.833
				Time	1	15.10	<0.001
				Time x Floodplain site	2	5.26	0.007
				Time x Habitat	1	1.50	0.223
				Time x Floodplain site x Habitat	2	0.27	0.761
				Time x Floodplain site x Habitat	2	0.45	0.639

[a] Contrast analysis (P vs NP) test for differences between preconditioned (P) and non-preconditioned (NP) leaf litter during the aquatic decomposition phase.

[b] Mixed Models test for differences among floodplain sites and between habitats (both considered fixed factors) in variables measured in preconditioned leaf litter during the aquatic phase, considering the incubation time in the water as a covariable.

Bold values indicate significant differences ($p < 0.05$).

Table 4.S7 Aquatic decomposition rates of preconditioned and non-preconditioned leaf litter.

		k (dd^{-1})	R^2	p
Non-preconditioned leaf litter		$-2.54 \cdot 10^{-5}$	0.83	0.007
Precoditioned leaf liter				
Parra	Open-canopy	$-2.05 \cdot 10^{-5}$	0.94	0.001
	Closed-canopy	$-2.38 \cdot 10^{-5}$	0.94	0.001
Tordera	Open-canopy	$-2.82 \cdot 10^{-5}$	0.78	0.03
	Closed-canopy	$-2.25 \cdot 10^{-5}$	0.67	0.029
Demnitzer	Open-canopy	$-1.79 \cdot 10^{-5}$	0.54	0.058
	Closed-canopy	$-2.22 \cdot 10^{-5}$	0.90	0.003

dd-1: degree-day

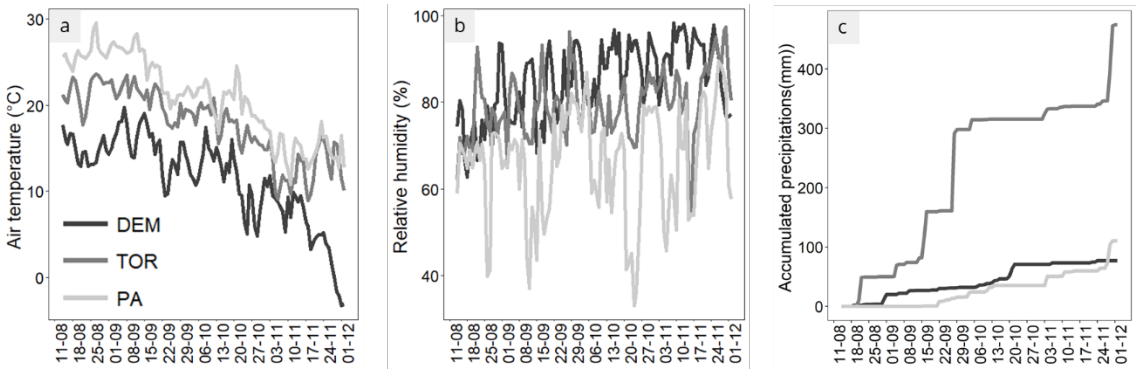

Figure 4.S1 Temporal variation in daily average of air temperature (a), daily average of relative humidity (b), and accumulated precipitation (c) during the preconditioning of leaf litter in every floodplains site (PA = Parra, TOR = Tordera, DEM = Demnitzer).

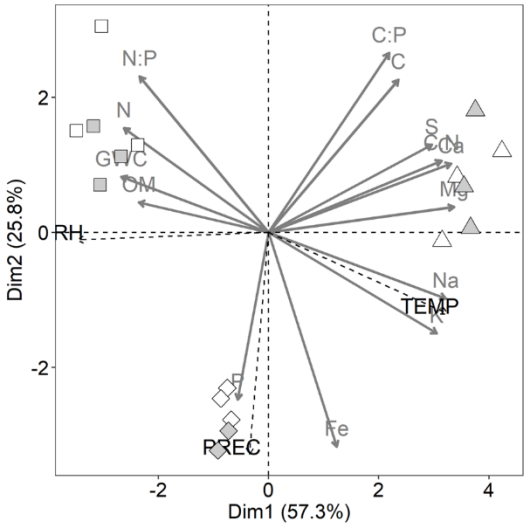

Figure 4.S2 PCA biplot describing habitat (open- and closed-canopy) soil properties and climatic conditions of every floodplain site (PA = Parra, TOR = Tordera and DEM = Demnitzer). Grey arrows represent the loading of each soil descriptor, whereas the dotted black arrows represent the correlation of climatic data with the PCA loadings. Climatic data are: air temperature (TEMP), relative humidity (RH) and accumulated precipitations (PREC). See Figure 4.2. for symbol codes explanations.

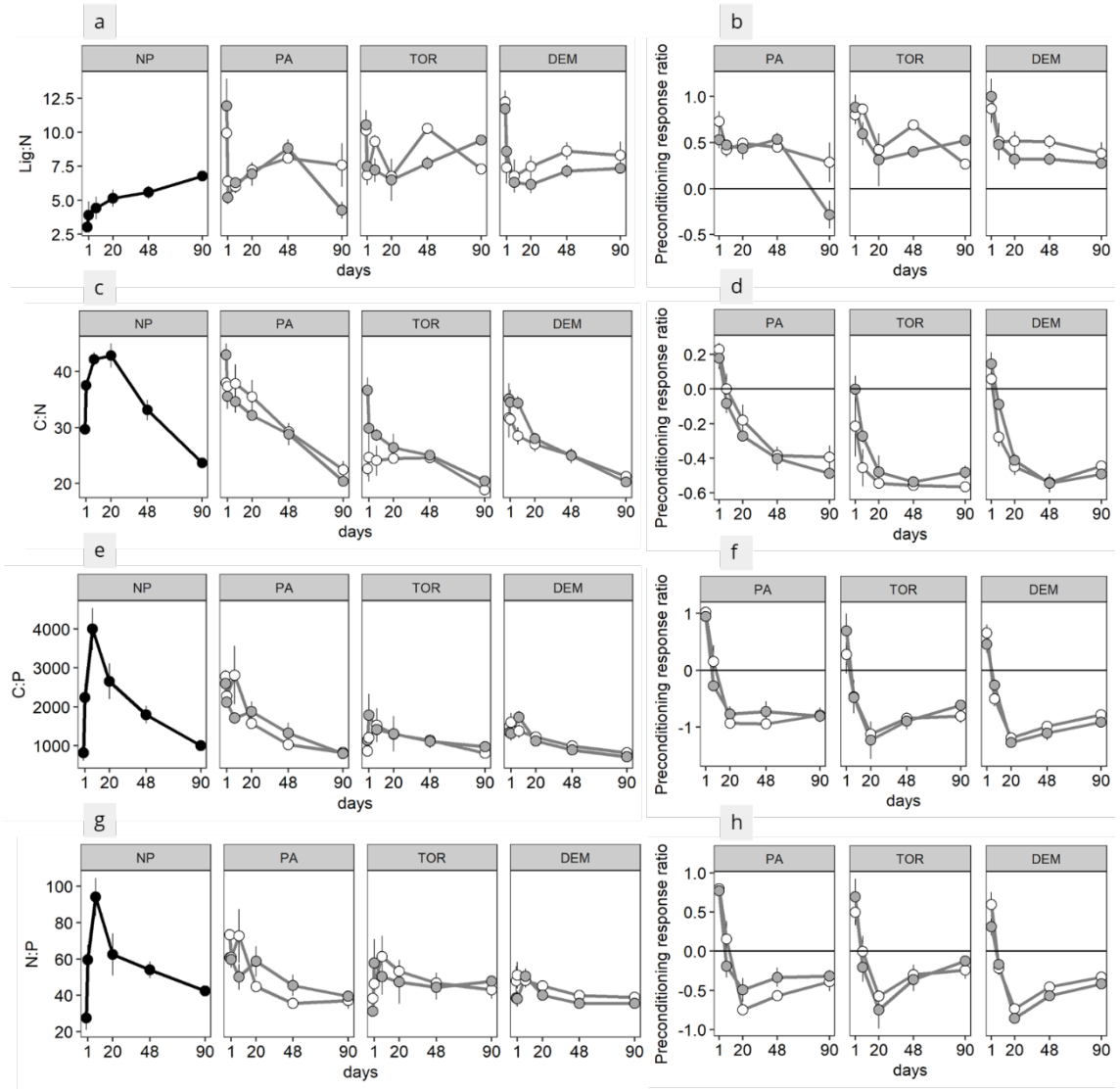

Figure 4.S3 Evolution of leaf litter Lignin:N (a), C:N (C), C:P (e) and N:P ratios (g) during the aquatic decomposition phase, in non-preconditioned leaf litter (NP; black dots) and leaf litter preconditioned under open-canopy (white dots) and closed-canopy habitats (grey dots) in the different floodplains sites. (b), (d), (f) and (h) show the preconditioning response ratio (PRR) for the same variables. Ratios represent the relationship between preconditioned and non-preconditioned leaf litter, so PRR > 0 means higher values of preconditioned leaf litter than the non-preconditioned one, and vice versa when PRR < 0. Dots are average values ± SE (n = 3). Floodplain sites: PA = Parra, TOR = Tordera, DEM = Demnitzer.

5.

Dry phase conditions prime wet-phase dissolved organic matter dynamics in intermittent rivers

Parra (open-canopy) and Demnitzer (closed-canopy) riverbeds

Abstract

During the dry phase of intermittent rivers diverse organic materials such as leaf litter or macrophytes accumulate on dry riverbeds. Together with riverbed sediments, these organic substrates are exposed to various environmental conditions that can alter their chemical composition, with potential implications for later use by heterotroph consumers when flow is re-established. Here, we investigate how different environmental conditions during the dry phase alter quantity, composition and biodegradability of dissolved organic matter (DOM) leached from dry riverbeds. To this end, we simulated the 'preconditioning' of DOM precursor materials during a dry phase of 60 days under conditions mimicking open- and closed-canopy streams. Over the whole experiment, we produced leachates for measurements of nutrients and dissolved organic carbon (DOC) concentration, DOM characterisation by absorbance and fluorescence measurements and ultrahigh-resolution mass spectrometry, and DOM biodegradability. We found that both rain and solar radiation greatly affect leached DOM quantity, composition and biodegradability. Under open-canopy conditions, sporadic rain caused the impoverishment of nutrients and DOC by leaching, while intense solar radiation and heat resulted in a drop of DOM quality and biodegradability by accelerated humification of DOM. In contrast, the preconditioning of DOM sources under closed-canopy conditions barely affected DOM quality and biodegradability due to the limitation of rain and sunlight by the forest vegetation. Our results suggest that contrasting environmental conditions during the dry phase in open and forested intermittent streams can translate into radically different DOM processing during the early wet phase.

5.1. Introduction

Dissolved organic matter (DOM) represents the most important carbon (C) source for microbial metabolism in stream and river ecosystems (Tank et al. 2010). The susceptibility of DOM to be used by microbes depends on its chemical composition (Sinsabaugh & Foreman 2003, Zhang et al. 2008), which is greatly controlled by its origin. While terrestrial DOM is believed to be mostly recalcitrant due to its humic character (Allan & Castillo 2008), DOM originating from in-stream production is regarded as highly labile and biodegradable (Bertilsson & Jones 2003). In addition, both the chemical composition and bioavailability of DOM are altered during its transport from headwaters to oceans by in-stream processes such as photodegradation and biodegradation (Moran & Zepp 1997, Sinsabaugh & Foreman 2003). Consequently, upstream processes may regulate DOM cycling in downstream ecosystems (Casas-Ruiz et al. 2017).

Particularly conspicuous habitats for en-route-transformations are intermittent river reaches, where water flow temporarily ceases before eventually complete drying (Datry et al. 2014). The attention on intermittent rivers and streams has grown greatly in the last years due to the recognition of their great proportion in the global river network (more than 50%; Datry et al. 2014) and the expectations of their increase due to global change (IPCC 2013). Several recent studies have found that flow intermittency can severely alter DOM quality and quantity through changes in the lateral and longitudinal hydrological connectivity of rivers (e.g. Vázquez et al. 2015, von Schiller et al. 2015, Casas-Ruiz et al. 2016). Of particular interest is the moment of flow resumption, when large amounts of DOM can be leached from sediments or particulate organic matter (POM) substrates accumulated on the riverbed during the dry phase, such as dead macrophytes or terrestrial leaf litter (Romaní et al. 2006b, Vázquez et al. 2015, Bianchi et al. 2017). Released nutrients and DOM can result in hot moments (McClain et al. 2003) of microbial activity after rewetting: short periods of time with intense biogeochemical processing, which can be pivotal for stream ecosystem functioning (Acuña et al. 2007, Gallo et al. 2014, Bianchi et al. 2017, Datry et al. 2018). Understanding these dynamics requires knowledge on processes occurring after complete surface water

disappearance, but mainly how environmental conditions can modulate the chemical composition of accumulated substrates, and consequently their role as DOM sources during the rewetting.

In intermittent rivers, environmental conditions during the dry phase vary greatly worldwide (Datry et al. 2014), but also among river and reaches. During drought, local conditions of solar radiation, temperature and humidity are controlled by the interaction of climate with the channel morphology and the riparian canopy structure. Individual reaches from the same river may vary from open and highly irradiated conditions due to poor riparian vegetation, to the opposite extreme of highly forested and shaded conditions. In terrestrial systems, local environmental conditions were shown to greatly affect the chemical composition of specific leaf litter species during initial degradation (Bradford et al. 2016) via controlling the relative importance of biotic and abiotic processes such as microbial decomposition and photodegradation, respectively (González-Polo & Austin 2009, Wang et al. 2014). This suggests that the incidence of solar radiation onto the riverbed may be an important factor for POM processing on dry riverbeds (Frost et al. 2005b), especially in arid systems where open river channels are common (Gómez et al. 2005). Specifically, because the potential of photodegradation to break recalcitrant compounds such as lignin, or the capacity of solar radiation and high temperatures to increase the solubility of nutrients and DOM upon rewetting (Bärlocher 1992, Austin & Ballaré 2010, del Campo & Gómez 2016).

In this study, our objective was to analyse how contrasting environmental conditions of the dry phase affect the quantity, quality and biodegradability of DOM leached from plant litter and sediments (DOM sources) when water flow returns. To this end, we exposed various DOM sources to two treatments mimicking open, highly irradiated and closed-canopy, forested streams for a dry phase of 60 days under outdoor microcosm conditions. Based on previous works on photodegradation of organic matter in terrestrial ecosystems (OM) (see Austin & Ballaré 2010 or Austin et al. 2016), we assumed solar radiation will be the main factor causing altered DOM leached from plant litter and sediments. We hypothesized that, compared to the closed-canopy treatment, the high solar radiation in the open-canopy treatment will: (i) increase the solubility of OM and therefore the yield of

leached dissolved organic carbon (DOC); (ii) cause an altered chemical composition of the leached DOM by breaking recalcitrant compounds such as lignin; and (iii) increase DOM biodegradability. Verification of these hypotheses would suggest contrasting riparian canopy cover in intermittent rivers can alter downstream DOM cycling via 'preconditioning' DOM sources during the dry phase.

5.2. Materials and Methods

5.2.1. Experimental design, setup and sampling

To address our objective, we exposed various types of plant litter and streambed sediments (hereafter referred to as "DOM sources") to two treatments mimicking contrasting environmental conditions typically occurring on natural dry riverbeds: open and closed vegetation canopy (hereafter referred to as "open-canopy" and "closed-canopy" treatments) for 60 days from July to September of 2015. These treatments were carried out in outdoor microcosm installed in a facility of the University of Murcia (Spain). The open-canopy treatment was performed in an open area, totally exposed to solar radiation, whereas the closed-canopy treatment was made in a holm oak woodland area, where the shading was intense and no light influence microcosms (Fig. 5.1).

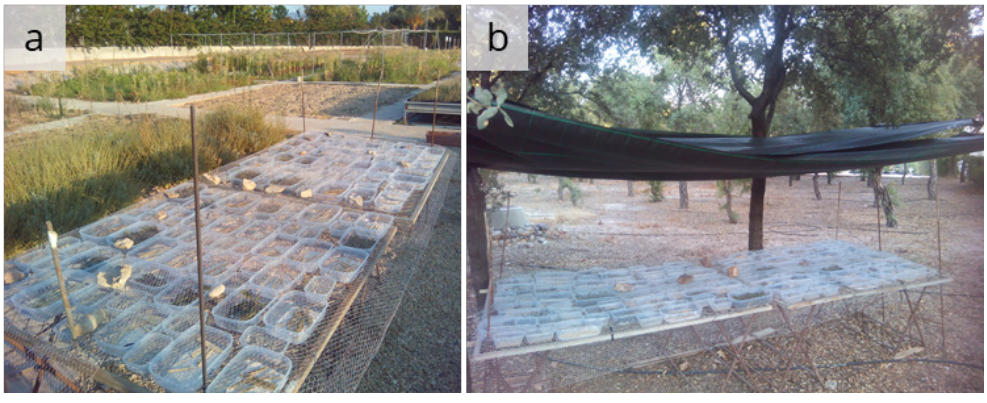

Figure 5.1 Open-canopy (a) and closed-canopy (b) outdoor treatments where microcosms (plastic tanks) were exposed during the simulated dry phase.

We used three plant litter types and sediments from three different streams as DOM sources. Plant litter types consist of leaves of a submerged macrophyte (*Potamogeton coloratus*) and leaf litter of common reed (*Phragmites australis*) in two stages (freshly fallen and partially decomposed after 21 days of stream immersion). We only regard these six DOM sources as distinct, exemplary sources of DOM in an intermittent stream. The three types of plant litter differed in terms of C:P and N:P stoichiometry, and lignin concentration (Table 5.1). The three streambed sediments were physically similar, but differed in % OM and C:N stoichiometry (Table 5.1). All DOM sources were collected from streams of the Segura River Basin (Region of Murcia, Southeast of Spain) located in an arid Mediterranean climate area (Peel et al. 2007). Reed leaf litter was obtained directly from standing plants in an intermittent stream (Mula River) poised to dry. Half of the reed leaves was air-dried and kept dark until experimental use (hereafter referred to as reed), the other half was submerged in the perennial Alharabe stream in the form of leaf packs for 21 days prior to washing with tap water and air-drying in dark conditions (hereafter referred to as pre-decomposed reed). Fresh leaves of *Potamogeton coloratus* were collected in Alharabe stream, washed with tap water and air-dried in dark conditions until experimental use. Superficial streambed sediments (5 cm depth) were collected in three first order perennial streams: Alharabe (ALH), Arroyo Blanco (AB) and Polladas (PO). In each stream, sediment samples were collected at several points along a reach of 50-100 m avoiding anoxic zones, homogenized, and maintained in wet, fresh and cool conditions until the experiment started for 24 h. Aliquots of each DOM source were put into transparent acrylic glass tanks (24 x 16 x 6 cm) with perforations at the bottom to allow drainage in case of rain. Plant litter was deposited on a thin layer of silica sand. Tanks containing sediments were filled with wet sediments up to a height of 2 cm and 300 mL of stream water (i.e. from the same stream where sediments were obtained) to ensure similar initial humidity conditions. Next, sediments were allowed to drain freely at identical, shaded environmental conditions until all of them were dry after two days. A total of 180 tanks (i.e., microcosm units) containing plant litter or sediments were split into two groups and distributed between the open- and closed-canopy treatments. On five sampling dates (0, 15, 25, 45 and 60 days after the

experiment start) we randomly retrieved three tanks (as replicate samples) of each DOM source and treatment. After sampling, dry sediment and plant litter were kept dark in plastic bags pending preparation of leachates.

Table 5.1 Initial chemical composition of plant litter types and chemical and physical features of sediments used in the experiment as DOM sources.

	Plant litter types		
	Macrophyte (*Potamogeton coloratus*)	Reed (*Phragmites australis*)	Pre-decomposed reed (*Phragmites australis*)
C:N	13. 55 ± 0.24	29.78 ± 1.05	32.64 ± 0.53
C:P	193 ± 9	568 ± 68	1639 ± 154
N:P	14.26 ± 0.71	19.12 ± 2.34	50.36 ± 5.02
Lignin (%)	9.88 ± 0.59	4.62 ± 0.32	8.32 ± 0.74
Cellulose (%)	21.23 ± 0.55	31.79 ± 0.69	34.63 ± 0.55
	Sediments		
	Arroyo Blanco (AB)	Alharabe (ALH)	Polladas (PO)
OM (%)	1.06 ± 0.07	1.03 ± 0.09	2.86 ± 0.34
C:N	107.7 ± 10.8	327.0 ± 107.8	60.4 ± 3.9
Texture	sandy loam	sandy loam	sandy loam
Bulk density* (g cm^{-3})	3.04 ± 0.13	2.77 ± 0.05	2.57 ± 0.09
GWC* (%)	0.60 ± 0.09	0.33 ± 0.03	0.62 ± 0.07

* Values of bulk density and gravimetric water content (GWC) were obtained from completely drained sediments.

Air temperature, air humidity and solar radiation (measured as illuminance) were recorded in each treatment area in hourly intervals using dataloggers (H08-003-02, Hobo H8 Family, U.S.A). In addition, the ultraviolet (UV) spectrum (UV-A, UV-B, UV-C) was characterized at midday on specific days using a quantum-

photo radiometer datalogger (DO 9721, Delta Ohm, Italy). The main difference between treatments was the much higher intensity of solar radiation (both illuminance and UV spectrum) and temperature in the open-canopy treatment compared to the closed-canopy one (Table 5.S1). On the contrary, humidity was higher in the closed-canopy treatment (Table 5.S1).

5.2.2. Leaching assays, samplings and DOM chemical characterization

We prepared aqueous leachates from all DOM sources (plant litter and sediments) following methods explained in Chapter II (section 2.31.) Vials for DOC and spectroscopic (absorbance and fluorescence) measurements were stored in darkness at 4 °C pending analysis within 7 days. Vials for analyses of nutrients and mass-spectrometry were frozen at -20 °C. DOC analyses and spectroscopic measurements were carried out for all sampling dates, analyses of nutrients just for 0, 25 and 60 days. DOC, SRP, NO_3^-, NH_4^+ and DIN were measured as indicated in section 2.3.2.

Spectroscopic characterization of leachates is explained in section 2.3.3. PARAFAC modelling identified 7 components (Fig. 5S1). Based on comparison with the literature, components C2, C4, C5 and C6 were identified as humic-like compounds, while components C1, C3 and C7 were identified as protein-like compounds.

Mass spectrometry (FT-ICR-MS, section 2.3.4.) was limited to the 3 types of plant litter sampled at time 0 (i.e. prior to any treatment) and after 60 days of both treatments. To facilitate the interpretation of main FT-ICR-MS results, we synthetized the molecular group information selecting the four molecular groups with higher differences among treatments (Table 5.4): small (C<15) polycyclic condensed aromates (hereafter, referred as SPCA), oxygen-rich polyphenols including polycyclic condensed aromates and aliphatic chains (hereafter, referred as polyphenols), lignin products characterized by unsaturated aliphatic chains with phenolics and aromatic structures (hereafter referred as lignin), and unsaturated oxygen-poor aliphates (hereafter referred as aliphatic compounds).

5.2.3. Biodegradation assays

Similar to mass spectrometry, measurements of bioavailability and DOC decay rates were limited to plant litter at time 0 and after 60 days of both treatments. See section 2.3.5.2. in Chapter II for a complete explanation. Surface water from a non-impacted stream (Löcknitz, Brandenburg, Germany) filtered by GF/F filters was used as a common microbial inoculum for all leachates samples.

5.2.4. Data analyses

We used a principal component analysis (PCA) performed on spectroscopic (absorbance, fluorescence) data of all samples collected during the dry phase period to explore the changes of chemical composition of leached DOM caused by open- and closed-canopy treatments. Before running the PCA, fluorescence intensities of PARAFAC components were normalized by the sum of absolute intensities of all components. All variables were z-standardised prior to PCA for dimensional homogenization. For each DOM source and treatment, the chemical changes occurring during the dry phase were depicted by drawing arrows from the average PCA scores at day 0 to the ones at day 60. To analyse these changes, both the length (as Euclidean distance along all PCs) and the angle of the arrows (in the plane of the first two PCs taking PC1 as the reference) were computed as proxies for the overall magnitude and the pathway of compositional change. The longer the distance between PCA scores, the stronger is the change in DOM composition, while differences in the angle of arrows indicate that DOM was transformed in different ways. Both the lengths and the angles of the arrows were checked for differences among DOM sources (6 levels: 3 types of plant litter and 3 streambed sediments) and among treatments (2 levels: open- and closed-canopy) using two-way ANOVA (2W-ANOVA). Tukey test and pair-wise comparisons of least-squares means (in case of significant interactions between DOM source and treatment) were used as post-hoc tests.

For a deeper analysis of the temporal changes of chemical composition of DOM promoted by treatments during the dry phase, we modelled changes in the scores of the first two PCA axes using generalized additive models (GAM). In the same manner, we modelled the changes in DOC and nutrient leaching yields along the dry phase. GAMs were selected to deal with non-linear responses of study variables along the dry phase (Zuur et al. 2009). Dry phase duration was used as a covariate, DOM source (6 levels) and treatment (2 levels) as fixed factors. An initial model, which included all main factors (i.e., *duration*, *DOM source*, *treatment*) and first-order interactions between variable pairs, was reduced stepwise using the Akaike Information Criteria (Sakamoto et al. 1986). GAMs were constructed using penalised regression splines with smoothing parameters selected by the generalized cross validation (GCV) criteria (Craven & Whaba 1978). Thin-plate regression splines were used as smoothing basis. The GAMs used here have two parts: the smoother term that regulates the covariate (*"s(duration)"*) and the parametric part that shapes the fixed factors *("+ DOM source*" and/or *"+ treatment*") (see Table 2 for a further explanation). A significant interaction between the covariate and one of the fixed factors in the smoother term ("*s(duration x DOM source)*" or "*s(duration x treatment)*") means that the resulting smoothed curve is influenced by the fixed factor. In other words, in such a model the temporal pattern of the data is co-determined by the fixed factor. On the contrary, the parametric part of the GAM does not influence the temporal distribution of data, it either adds or subtracts a constant value to the smoothed curve, either increasing or decreasing the magnitude of the analysed variable.

To test for compositional changes of DOM over the dry phase based on the FT-ICR-MS data, we ran 2W-ANOVAs using as fixed factor the DOM source (3 levels: 3 types of plant litter) and the treatment (3 levels: non-treated, open- and closed-canopy), followed by Tukey or pair-wise comparisons (in case of significant interaction between DOM source and treatment) for the following responses: the average raw intensity of the different molecular groups, the average DBE, the average molecular mass and molecular richness. Additionally, we checked agreement of the average molecular mass from FT-ICR-MS with optical indices of molecular mass using Spearman correlation. For further analyses, we computed Bray-Curtis

dissimilarity based on log-transformed relative intensity data for (i) a graphical representation of sample similarity using non-metric multidimensional scaling (nMDS), and (ii) an assessment of the variation (i.e., multivariate dispersion) of chemical composition of leachates from all types of plant litter in non-treated state and after 60 days of both treatments.

DOC decay rates in the biodegradation assays were computed from exponential fits of DOC over incubation time. 2W-ANOVA was again used to check for differences in the %BDOC and the decay rate of BDOC using the types of plant litter (3 levels) and treatments (3 levels: non-treated, open- and closed-canopy) as fixed factors.

All data analyses were carried out in R version 3.2.1 (R Core Team 2015) using the packages "*mgcv*" (Wood 2011), vegan (Oksanen et al. 2017) and "*MASS*" (Venables & Ripley 2002).

5.3. Results

5.3.1. DOC and nutrient leaching yields

At day 0, the various DOM sources produced extremely different initial DOC concentration in leachates. Initial DOC yield was 10^2-10^3 times greater from plant litter than from sediments, but it also differed by factors 10-15 and 4-8 across the three litter types and sediments, respectively. The DOC concentration in leachates from plant litter or sediment was very variable over the dry phase, especially from 25-60 days (Fig. 5.2). At 25 days we observed a general acute drop of DOC yield from most of the DOM sources, followed by a rather steady increase. GAM modelling identified that DOC temporal patterns depended on DOM source, while the magnitude of DOC yield was affected by DOM source and treatment (Table 5.3).

Similar to DOC, initial nutrient yields were 10-100 times higher from plant litter than from streambed sediments. Leaching yields of nutrients (DIN and SRP) showed a general decrease over the dry phase (Fig. 5.S2 and 5.S3). This trend with time was stronger for DOM sources collected from the open-canopy treatment than

for those coming from the closed-canopy one, which barely varied between day 0 and 60. Consequently, closed-canopy treatment resulted in leachates with higher nutrient concentrations (both DIN and SRP) at the end of the dry phase. As for DOC, GAM modelling identified temporal patterns in leaching yields of nutrients over the dry phase to be dependent on DOM source, whereas the differences in the magnitude of yields were dependent on both DOM source and treatment (Table 5.3).

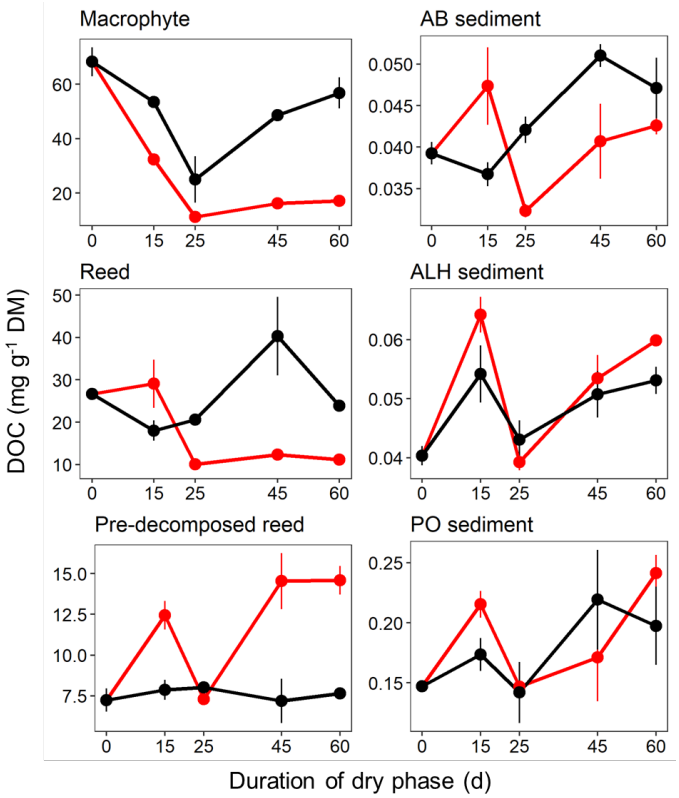

Figure 5.2 Temporal changes in DOC concentration (mean ± SE, n = 3) for the different plant litter (left panels) and sediments (right panels) subjected for both open- (red lines) and closed-canopy (black lines) treatments.

5.3.2. Chemical composition of DOM

The first two axis of the PCA based on DOM quality descriptors of leachates captured up to 59 % of total variance, with a similar distribution between them (PC1: 30.1 %, PC2: 28.9 %) (Fig. 5.3a). PC1 was mainly explained by FI, C1, and C7 (indicators of protein-like compounds) and by HIX, C2 and C6 (indicators of humic-like compounds). PC1 separated leachates by treatments. Leachates from non-treated DOM sources or the closed-canopy treatment were gathered in the negative part of the PC1 due to their higher values of protein-like indicators (Fig.

5.3a and 5.3b), whereas leachates from the open-canopy treatment were located in the positive part of the PC1 because of their higher loadings of humic-like signatures. PC2 was mainly explained by E_2:E_3, Sr, $S_{275\text{-}295}$ (indicators of DOM molecular mass) and $SUVA_{254}$ (indicator of aromaticity). PC2 differentiated leachates from the distinct DOM sources, separating mainly sediment from plant litter leachates along the axis (Fig. 5.3b).

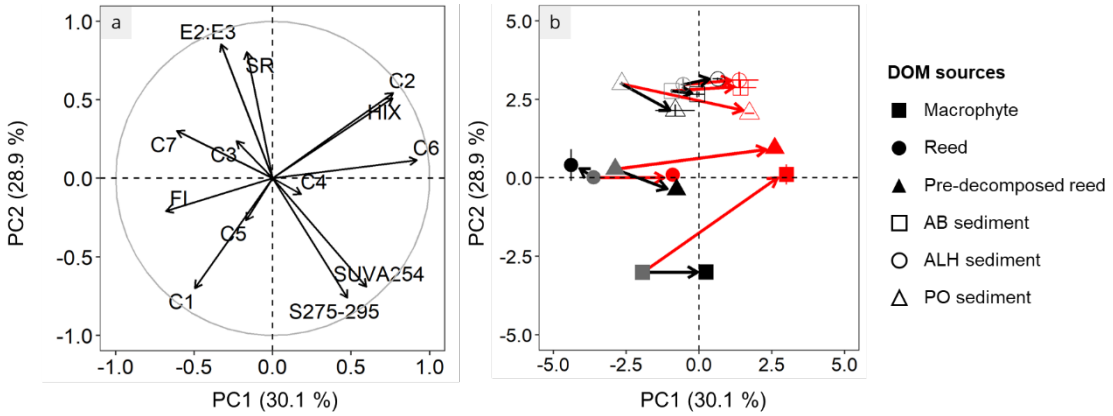

Figure 5.3 PCA based on spectroscopic measurements of leachates from all DOM sources and treatments obtained during the dry phase and in the biodegradation assay (a). Changes in DOM chemical composition over the dry phase (b). Lengths of arrows indicate the magnitude of shift in DOM composition of leachates.

PC2 configured a gradient of DOM molecular mass and aromaticity from the positive to the negative part (Fig. 5.3a). This interpretation of PC2 was confirmed by its significant, negative correlation (r_s = -0.69, p <0.001) with the average molecular mass determined by mass spectrometry for a subset of samples.

The dry phase caused a general increase of humic-like signatures in the leached DOM of all DOM sources, however this humification was significantly much stronger in the open-canopy treatment than in the closed-canopy one (Fig. 5.3b, Table 5.2). Although this trend was clear, DOM sources influenced the magnitude and direction of chemical transformations of leachates as evidenced by the strong interactions between DOM sources and treatments revealed by 2W-AOV (magnitude: $F_{5,24}$ = 2.67, p < 0.05; direction ($F_{5,23}$ = 12.56, p < 0.001).

In agreement with PCA results, GAM modelling identified temporal patterns in PC1 scores (continuous increase of humic-like signatures in the open-canopy treatment over time, see Fig. 5.4) as dependent on treatment, while its magnitude was dependent on both treatment and DOM source (Table 5.3). In contrast, GAM modelling identified only DOM source as a factor controlling both the temporal pattern and differences in magnitude in PC2 scores (Table 5.3).

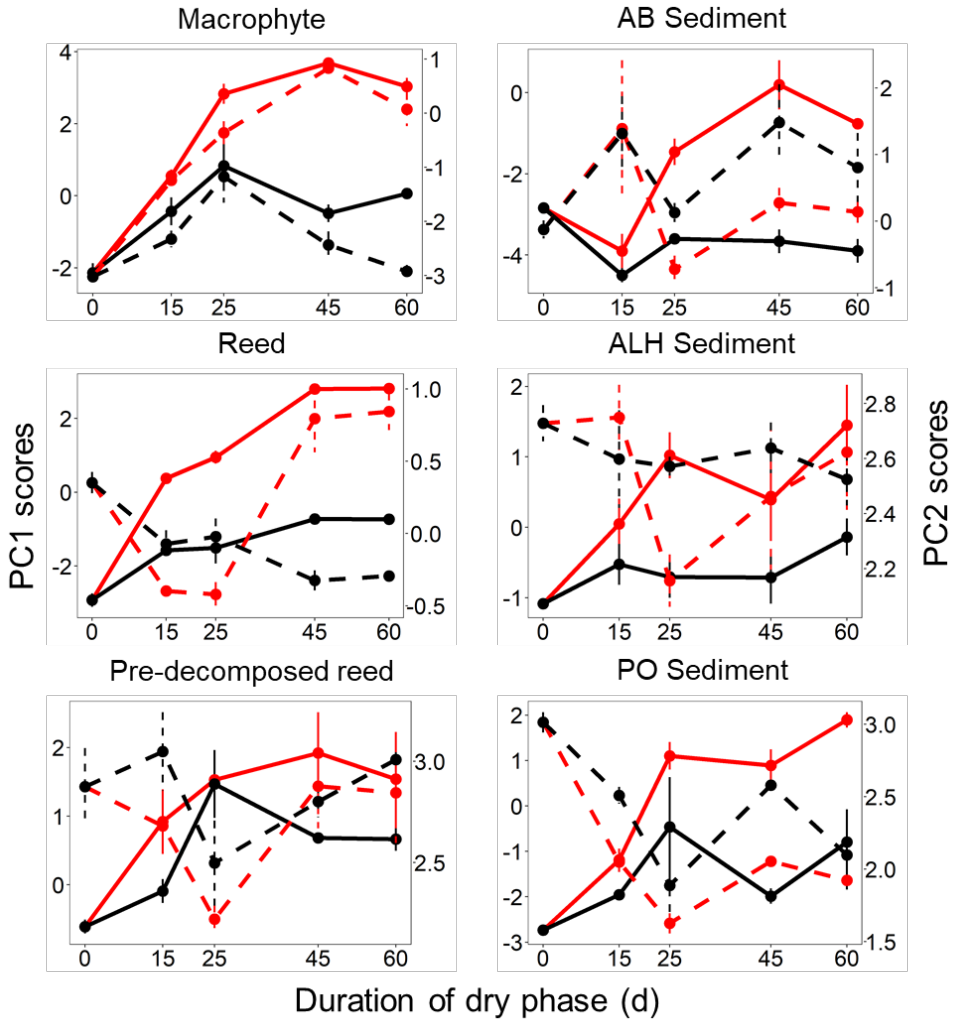

Figure 5.4 Temporal changes in PC1 scores (solid lines) and PC2 scores (dashed lines) (mean ± SE, n = 3) for the different DOM sources (plant litter on the left side, sediments on the right) subjected to open-canopy (red lines) and closed-canopy treatments (black lines).

Table 5.2 Characterization of the magnitude and direction of changes in chemical composition of DOM in leachates over the dry phase. Magnitude of compositional change was computed as the Euclidean distance between PCA scores at day 0 and day 60 of every DOM source and treatments. For the first two PCs this measure is indicated as an arrow in Figure 5.3b. The direction was computed as the angle between these arrows and the X-axis of the PCA space. Negative values indicate angles in clockwise direction and positive values indicate angles in counter-clockwise direction (with theoretical maxima of 180 and -180°). Values are equal to means ± SE (n = 3). For each DOM source, superscripted letters indicate significant differences between treatments according to post-hoc tests ($p < 0.07$ if marked with "*").

	Magnitude (Euclidean distance)	Direction (Angle degrees)
Macrophyte		
Closed-canopy	3.20 ± 0.41[a]	-5.42 ± 2.13[a]
Open-canopy	6.45 ± 0.23[b]	28.07 ± 0.96[b]
Reed		
Closed-canopy	2.86 ± 0.39[a]	-32.91 ± 4.95[a]
Open-canopy	3.79 ± 0.17[a]	3.71 ± 4.12[b]
Pre-decomposed reed		
Closed-canopy	3.16 ± 0.29[a]	-23.71 ± 2.08[b]
Open-canopy	5.87 ± 0.05[b]	1.76 ± 0.80[a]
AB sediment		
Closed-canopy	1.51 ± 0.09[a]	-16.64 ± 5.86[a]
Open-canopy	3.18 ± 0.20[b]	-12.70 ± 4.09[a]
ALH sediment		
Closed-canopy	1.70 ± 0.06[a]	11.22 ± 8.97[a]
Open-canopy	3.01 ± 0.32[b]	-14.63 ± 3.60[b]
PO sediment		
Closed-canopy	2.55 ± 0.43[a]	-29.10 ± 1.18[a]
Open-canopy	5.34 ± 0.10[b]	-19.04 ± 0.35[a]

Table 5.3. Most parsimonious GAM models selected by the Akaike information criterion for various responses describing leachate properties during the dry phase. Terms used in models are *duration* (dry phase duration), *DOM source* (plant litter types and sediments), *treatment* (open-canopy and closed-canopy 'preconditioning' treatments). See *Methods* for variable explanation; *df*: degrees of freedom; ΔAIC: difference to second most parsimonious model; GCV: generalized cross validation.

Variable	Best model	*df*	ΔAIC	GCV	Deviance explained
DOC	s(*duration* x *DOM source*) + *DOM source* + *treatment*	19.7	10.8	0.081	97%
DIN	s(*duration* x *DOM source*) + *DOM source* + *treatment*	15.9	8.1	0.006	86%
SRP	s(*duration* x *DOM source*) + *DOM source* + *treatment*	15.9	10.5	0.182	98%
PC1	s(*duration* x *treatment*) + *DOM source* + *treatment*	15.0	6.4	0.870	81%
PC2	s(*duration* x *DOM source*) + *DOM source*	20.1	24.4	0.100	84%

5.3.2.1. FT-ICR-MS results

FT-ICR-MS results revealed significantly higher raw intensity values of SPCA, polyphenols and lignin in leached DOM from plant litter subjected to the open-canopy treatment than those from non-treated and the closed-canopy treatment ($F_{2,18} = 22.7$, $p < 0.001$; $F_{2,18} = 117$, $p < 0.001$; $F_{2,18} = 18.9$, $p < 0.001$, respectively. Table 5.4). On the contrary, aliphatic compounds showed a strong interaction between plant litter type and treatment ($F_{4,18} = 124$, $p < 0.001$); whereas the open-canopy treatment caused higher intensity in leachates from the macrophyte and reed compared to the other two treatments, but not for the pre-decomposed reed (Table 5.4). Counts had the exactly same pattern than raw intensity, whereas both relative intensity and relative counts were more variable, reflecting complicated interactions between plant litter types and treatments (Table 5.S2). 2W-ANOVA indicated significant interactions between plant litter types and treatment for average

molecular mass, molecular richness and average DBE (degree of unsaturation) ($F_{4,18}$ = 31.32, $p < 0.001$; $F_{4,18}$ = 51.47, $p < 0.001$, $F_{4,18}$ = 50.04, $p < 0.001$; respectively). All three indicators showed maximum values in leachates from plant litter subjected to the open-canopy treatment (Fig. 5.5).

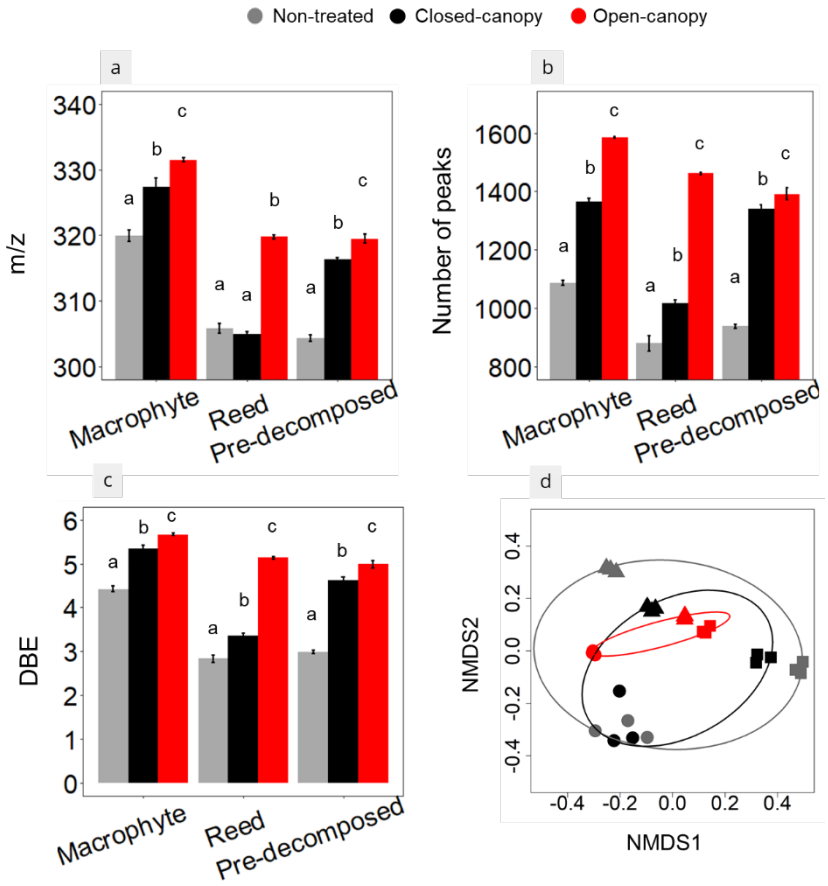

Figure 5.5 Results from FT-ICR-MS: Average molecular mass of assigned sum formulas (a), molecular richness (b), and average degree of unsaturation (as double-bond-equivalents, DBE) (c) (mean ± SE, n = 3) of leachates from not-treated plant litter and those subjected to open-canopy and closed-canopy treatments. Letters indicate significant differences between treatments according to post-hoc tests. (d) nMDS based on Bray-Curtis dissimilarity computed from log-transformed relative intensities of assigned formulas. Symbol and colour code as in Figure 5.3b.

The nMDS based on Bray-Curtis dissimilarity showed a clear separation of plant litter types before the dry phase, as reflected by the maximum dissimilarity among leachates of the non-treated DOM sources (Fig. 5.5d). However, the dry

phase treatments significantly reduced the chemical dissimilarity among plant litter leachates (permutational test on dispersion: $F_{2,24} = 6.2$, $p = 0.005$; Fig. 5.5d), especially in the case of the open-canopy treatment, which fostered the maximum chemical homogeneity among leachates.

Table 5.4. Raw intensity values (mean x 10^7 ± SE, n = 3) of the molecular groups identified by FT-ICR-MS for plant litter leachates. SPCA: small polycyclic condensed aromates. For each plant litter type, the superscripted letters indicate significant differences between treatments according to post-hoc tests p <0.07 if marked with "*").

	SPCA	Polyphenols	Lignin	Aliphatic compounds
Macrophyte				
Non-treated	6.7[a] (25.0)	7.6[a] (22.2)	827[a] (26)	893[a] (24)
Closed-canopy	5.2[a] (23.3)	8.7[a] (11.9)	981[a] (20)	1470[b] (20)
Open-canopy	9.8[b] (15.7)	17.7[b] (17)	1210[b] (12)	3550[c] (6)
Reed				
Non-treated	1.8[a] (90.8)	0.7[a] (23.1)	369[a] (14)	173[a] (11)
Closed-canopy	1.1[a] (36.6)	2[a] (4.3)	474[a] (4)	211[a] (12)
Open-canopy	3[b] (23.5)	9.4[b] (32.2)	912[b] (28)	700[b] (16)
Pre-decomposed reed				
Non-treated	0[a]	0.4[a] (15.8)	288[a] (10)	2020[a] (9)
Closed-canopy	0.6[a] (32.3)	2.6[a] (26.7)	469[a] (10)	1370[b] (4)
Open-canopy	3.6[b] (13.6)	14.5[b] (9.5)	629[b] (23)	533[c] (29)

5.3.3. DOM biodegradation

2W-ANOVA of % BDOC and DOC decay rates showed significant interactive effects of plant litter types and treatment (including non-treated plant litter) on

biodegradation (% BDOC: $F_{4,18} = 3.24$, $p < 0.05$; decay rates: $F_{4,18} = 3.77$, $p < 0.05$). For all plant litter leachates, the open-canopy treatment reduced DOC bioavailability and DOC decay rates by about 50% compared to leachates of non-treated plant litter (Table 5.5). Conversely, closed-canopy treatment barely affected either BDOC or DOC decay rates of plant litter leachates. Pair-wise post-hoc tests for % BDOC and decay rates only identified significant differences ($p < 0.05$) among the open-canopy treatment and the other two in reed and pre-decomposed reed (Table 5.5).

Table 5.5. Percentage of BDOC (mean ± SE, n = 3) at the end of the biodegradation assay (8 days) and DOC decay rates (mean ± SE, n = 3) for plant litter leachates. Individual decay rates were computed for each replicate bioassay and then averaged. For each plant litter type, superscripted letters indicate significant differences between treatments according to post-hoc tests ($p < 0.07$ if marked with "*").

	% BDOC	DOC decay rate (d^{-1})
Macrophyte		
Non-treated	34.31 ± 1.68^{a}	-0.046 ± 0.002^{a}
Closed-canopy	32.35 ± 1.27^{a}	-0.044 ± 0.004^{a}
Open-canopy	20.15 ± 1.60^{b*}	-0.029 ± 0.001^{a}
Reed		
Non-treated	62.83 ± 9.25^{a}	-0.120 ± 0.025^{a}
Closed-canopy	71.17 ± 0.99^{a}	-0.143 ± 0.005^{a}
Open-canopy	38.13 ± 4.44^{b}	-0.058 ± 0.008^{b}
Pre-decomposed reed		
Non-treated	65.18 ± 3.76^{a}	-0.100 ± 0.010^{a}
Closed-canopy	56.97 ± 3.84^{a}	-0.086 ± 0.005^{a}
Open-canopy	28.80 ± 1.48^{b}	-0.039 ± 0.002^{b}

5.4. Discussion

Dryness itself is likely the most prominent control on ecological processes in intermittent streams. Yet, our study clearly showed the importance of locally differentiated environmental conditions during a dry phase. Open- and closed-canopy treatments indeed shaped the quality of leachates from various DOM sources, but – contrary to our expectations – high solar radiation in open-canopy treatment resulted in a notable decrease of leachate DOM quality towards increased humification and consequently lower DOM biodegradability. In contrast, the exclusion of direct sunlight by the forest vegetation in the closed-canopy treatment affected DOM quality and biodegradability only marginally. Consequently, we have to reject our initial predictions about the positive effect of solar radiation on DOM quality and biodegradability.

5.4.1. The interaction of solar radiation and rains during the dry phase triggers a heterogeneous alteration of DOC and nutrient leaching yields of DOM sources

Our experiment compared leachates from various DOM sources exposed to the natural environment under a closed Holm oak canopy and in an open non-vegetated area. The existence of a closed canopy mainly implies differences in solar radiation but can also reduce the temperature and mediate the effects of sporadic rain events during summer. Our data on DOC and nutrient concentrations in leachates throughout the 60-day dry phase bears testimony to both sunlight- and rain-associated effects. During the first 15 days, high solar radiation increased DOC concentrations in leachates from the open-canopy relative to the closed-canopy treatment. This could be owed to increased solubility of DOC from plant litter and sediments caused by photodegradation and high temperatures (Bärlocher 1992, Gallo et al. 2009). Then, at day 25, which was immediately preceded by a short storm (5 mm in 1 h), we observed markedly lower leaching of DOC and nutrients, especially in open-canopy treatment. This impoverishment of nutrients and DOC

could be associated with preceding leaching by rain and/or transiently supported microbial activity (Bechtold et al. 2003, Schrumpf et al. 2006). On the contrary, under canopy cover, the vegetation protection may have alleviated DOC and nutrient leaching by rain. Then, as no further rainfall occurred, DOC and nutrient yield in leachates may again have increased in open-canopy treatment due to continued photodegradation and heat driving higher solubility. In the end, what is leachable from DOM sources under open-canopy conditions is very variable and depends on the preceding rain and sunlight history. Our results highlight the importance of accounting for such short and sporadically occurring summer storms when trying to understand consequences for DOC and nutrient yield from precursor material on dry riverbeds. After a long uninterrupted dry phase, reestablishment of flowing water by heavy rain will flush DOM and nutrients into downstream aquatic ecosystems and trigger hot moments of microbial activity with consequences for C processing and CO_2 emissions (Gallo et al. 2014, Bianchi et al. 2017, Datry et al. 2018). In contrast, the interruption of a long dry phase by sporadic, small rain events will sequentially impoverish leachates from dry riverbeds, especially in exposed open-canopy areas, with obviously diminished implications for downstream ecosystems at later complete flow resumption (see Muñoz et al. 2018).

Our results also point to the great variability in DOM and nutrient yield in leachates from various DOM sources due to their differences in initial chemical composition and origin (Wieder et al. 2008, Fellman et al. 2013). Although there are obvious, strong differences between plant litter and sediment with regard to leachate yields, both can represent important sources of DOC and nutrients for rivers and streams (Strauss & Lamberti 2002). The role of dry riverbed sediments as source of DOC and nutrients (Arce et al. 2014, Merbt et al. 2016, Bianchi et al. 2017) cannot be undervalued, especially when we consider the large surface area that dry sediments may occupy in Mediterranean and arid zones (Datry et al. 2014).

5.4.2. Solar radiation and heat cause altered composition and decreased bioavailability of leached DOM from dry riverbeds

DOM composition as captured by PC1 showed a consistent increase of humification of leached DOM in all DOM sources subjected to the open-canopy treatment over the whole dry phase (Fig. 5.3a and 5.4). This finding largely agrees with similar previous studies, such as those by Baldwin (1999), Fellman et al. (2013) or Jian et al. (2016), where showed the ageing of leaf litter by its exposure in floodplains over long time spans of several months to shift leachate DOM towards increased aromaticity, higher humification and lower biodegradability. Our results of FT-ICR-MS and optical analyses demonstrate that plant litter and dry riverbed sediments exposed under intense solar radiations are subject to a similar effect already over a much shorter time span, suggesting that photodegradation accelerates the ageing of DOM sources on dry riverbeds under open-canopy conditions. In our case, for DOM sources *preconditioned* under open-canopy conditions, the increase of recalcitrant, humic-like compounds in leached DOM was caused by the great accumulation of lignin-like compounds, polyphenols and polycyclic condensed aromatic structures. A possible explanation for such an increase could be a boosted oxidative polymerization of simple phenolic compounds as a consequence of the intense heat and ultraviolet radiation (see Goering & van Soest 1970, Makkar 2003). Baldwin (1999) described similar polymerization of phenolic compounds during terrestrial decomposition of plant litter in a floodplain. Moreover, the increase of DOM molecular mass (Fig. 5.5a) in leachates of all plant litter types preconditioned under open-canopy conditions supports this idea. Further, our results and others (Baldwin 1999, Jian et al. 2016) suggest that these polymerized phenolic compounds are easily leached, thereby increasing average recalcitrance of leachate DOM. Conversely, open-canopy treatment caused the depletion of presumably labile, protein-like compounds. This could be attributed to microbial degradation stimulated by the rain event just before day 25 (Jian et al. 2016). Additionally, the loss of protein-like fluorescence signal in leachates may have occurred due to a selective photodegradation of amino acids with aromatic structures such as tyrosine or tryptophan (Stedmon & Markager 2005, Boreen et al. 2008). In the end, independent

of whether these various reactions happened in concert or isolation, these processes caused a profound compositional change of leachate DOM over the dry phase, with maximum molecular diversity reached under highly irradiated conditions (Fig. 5.5b). Interestingly, this molecular diversification happened in concert with chemical homogenization among plant litter types (Fig. 5.5d). This indeed suggests that OM processing by photodegradation and heat under highly irradiated conditions are able to transform DOM into a poorly biodegradable resource for later aquatic consumers, independently of their initial chemical composition.

However, contrary to our results, there are also numerous studies showing positive effects of photodegradation on OM biodegradability in terrestrial and aquatic habitats. For instance, Henry et al. (2008) or Austin et al. (2016) evidenced that photodegradation of lignin in leaf litter on soils can favour its later microbial decomposition by facilitating the access of decomposers to cellulose. Similarly, Moran & Zepp (1997) and Fellman et al. (2013) showed how photodegradation of DOM in stream water increased its bioavailability for microbial consumers by breaking apart large and recalcitrant structures into smaller, more available molecules. The main reasoning to justify seemingly contradiction between our study and those previously mentioned is that our work focuses on how the chemical composition of DOM is affected by *preconditioning* conditions experienced by its *particulate* DOM source, rather than DOM itself. In contrast, the previous studies address the direct effect of photodegradation of either plant litter fibres in terrestrial ecosystems, or aquatic DOM in the aquatic ones. Therefore, we suggest that, depending on the hydrological phase, photodegradation plays a two-sided role in DOM processing in intermittent streams; (1) with a positive effect on aquatic biodegradation of DOM if sunlight acts on DOM in the water column during the aquatic phase; or (2) with a negative effect if sunlight acts on plant litter and riverbed sediments as DOM precursor materials during the dry phase.

The closed-canopy treatment had a much weaker effect on the composition and bioavailability of leached DOM (Fig. 5.3); both remained almost unaltered when compared to non-treated plant litter (Table 5.2 and 5.5). This suggests that ageing of OM is much slower in the absence of intense solar radiation and/or heat, canopy cover may effectively preserve DOM precursor materials. Previous studies in a

forested intermittent stream have indeed shown how leaf litter accumulated on the riverbed during the dry phase produces a great input of highly bioavailable DOM for aquatic microbial communities at flow resumption (Romaní et al. 2006b, Vázquez et al. 2015). Therefore, riparian vegetation canopy could be considered as a strong regulator of DOM quality and biodegradability due to its protective action against sunlight in intermittent rivers.

The differences in the chemical quality and bioavailability of DOM leached from precursor material from open and forested environments has important implications for its microbial processing and consequently stream functioning after the rewetting. Low-quality leachates from plant litter treated under the open-canopy conditions were hardly biodegradable in the 8-day timeframe, likely due to the scarcity of labile compounds, the high concentration of humic-like compounds (Cleveland et al. 2004, Hur et al. 2013) and notably increased overall molecular diversity with diluted individual structures. In contrast, high-quality leachates from plant litter preconditioned under closed-canopy conditions experienced fast biodegradation. Leachates from non-treated plant litter behaved similarly, which reinforces the idea that leachate DOM has constantly high chemical quality and bioavailability over the dry phase as long as solar radiation cannot affect its DOM sources, for instance due to protection by riparian vegetation or high stream banks.

5.5. Conclusions

In summary, our results suggest different pathways of DOM processing in open and forested intermittent streams during dry and aquatic phases. We have demonstrated plant litter or sediments during the dry phase not to be affected by simply the dry phase itself but rather by very local conditions, specifically protection from solar radiation and sporadic rain by riparian vegetation. While open intermittent streams produce DOM of low quality and bioavailability, mainly as a consequence of solar radiation (Fig. 5.6a), forested intermittent streams protect DOM precursor material from sunlight and rain and consequently produce highly biodegradable DOM that is available for downstream systems when flow is re-established (Fig. 5.6b).

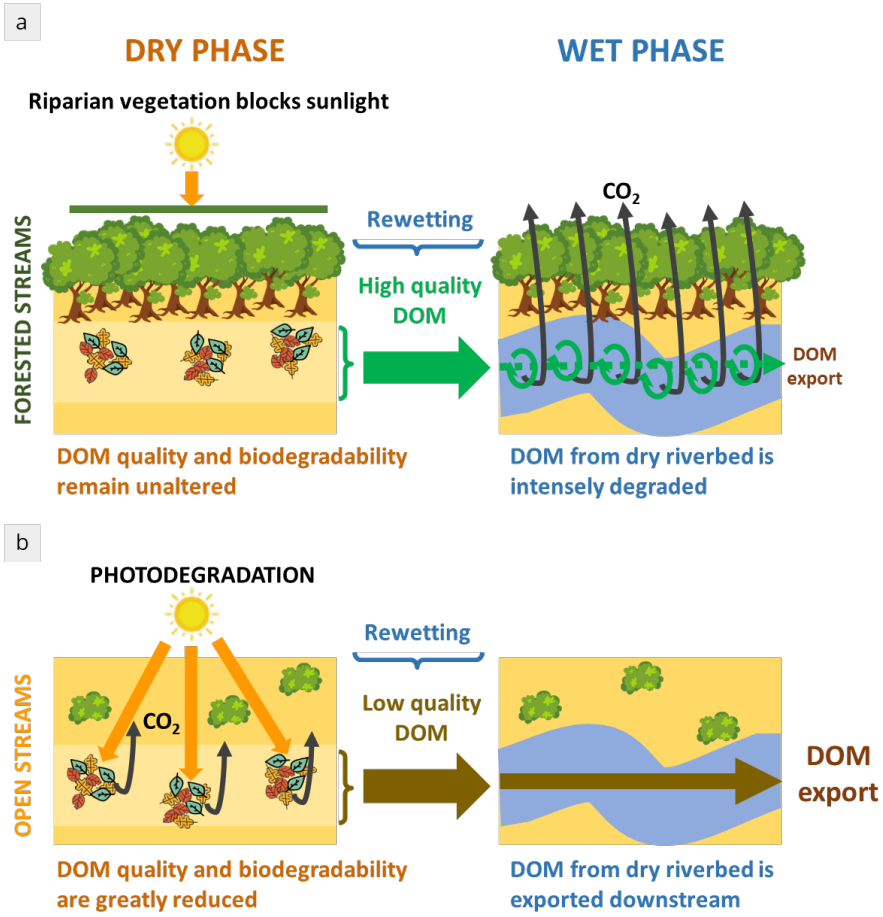

Figure 5.6 Conceptual scheme showing differences in the processing of DOM coming from dry riverbeds in a forested intermittent stream (a); and an open intermittent stream (b), both during dry and wet phase.

This translates to an increased export of recalcitrant DOM from open intermittent streams (Fig. 5.6a), with potentially noticeable implications for C cycling in far-downstream receptor systems (Hladyz et al. 2011, Bianchi et al. 2017). With respect to carbon, open intermittent streams are thus rather active reactors during the dry phase, but conservative "pipes" in the aquatic phase. In contrast, forested intermittent streams are less active reactors when dry, but strongly fuel the longitudinal bioreactor of a re-established aquatic continuum with DOM supporting faster uptake, microbial respiration and outgassing of CO_2 (Fig. 6b; see Romaní et al. 2006b, Vázquez et al. 2015). However, we recognized that these conclusions cannot be drawn to landscape scale without previously checking these hypotheses under natural conditions in open and forested intermittent rivers, as our

microcosm design could have overemphasized the effect of solar radiation on leached DOM from substrates accumulated on dry riverbeds.

On the other hand, the here presented landscape-scale reasoning only pertains to DOM leached from the riverbeds, whose fraction in the total budget of organic matter in intermittent streams is quite unknown to date. However, under the assumption that our results for open intermittent streams might represent the common DOM dynamics of little investigated streams in arid regions, whether these systems act as C pipes or bioreactors becomes increasingly important, especially when taking into account predictions of expanding arid lands and deforestation driven by climate and global change (Reynolds et al. 2007, Bonan 2008). The rather plausible near-future shift from forested to open stream conditions in many regions because of the loss of riparian forests could cause notable changes in the C cycling mainly through the severe reduction of DOM biodegradability, that underscores the necessity to protect and conserve these habitats in order to avoid the alteration of river networks functioning.

5.6. Acknowledgments

We thank Thorsten Dittmar's lab at the University of Oldenburg for assistance and support with FT-ICR-MS analysis. We also thank Barbara Behounek and the Chemical Lab of the IGB-Berlin for their assistance with laboratory analyses as well as the SEAF of the University of Murcia with the experimental set-up. This research was funded by the project 19525/PI/14 from Seneca Foundation -Agency for Science and Technology in the Region of Murcia. R. del Campo was funded by a PhD contract (FPU R-269/2014) from the University of Murcia and was also supported by a grant for a short-term scientific mission to Berlin, within the COST Action CA15113 (SMIRES, Science and Management of Intermittent Rivers and Ephemeral Streams, www.smires.eu), funded by COST (European Cooperation in Science and Technology). The authors declare no conflict of interest.

5.7. Annexes

Table 5.S1 Daytime environmental variables (mean ± SE) in the experimental areas used for treatment of DOM sources.

	Closed-canopy (forested area)	Open-canopy (open area)
Air temperature (°C)	32.62 ± 0.07	39.25 ± 0.14
RH (%)	53.76 ± 0.27	38.56 ± 0.31
Illuminance (lx)	453 ± 14	37329 ± 564
UVA (W m^{-2})	0.124 + 0.013	9.56 ± 0.62
UVB (W m^{-2})	0.004 + 0.000	0.366 ± 0.027
UVC (W m^{-2})	0	0.061 + 0.005

Table 5.S2 Average values of relative intensity (Rel. Int.), counts (Cou.) and relative counts (Rel. Cou.) (CV in brackets, n = 3) of molecular groups identified by FT-ICR-MS for plant litter leachates. SPCA: small polycyclic condensed aromates.

	SPCA			Polyphenols			Lignin			Aliphatic compounds		
	Rel. Int.	Cou.	Rel. Cou.	Rel. Int.	Cou.	Rel. Cou.	Rel. Int.	Cou.	Rel. Cou.	Rel. Int.	Cou.	Rel. Cou.
Macrophyte												
Non-treated	0.23 (21.4)	6 (11)	0.5 (20)	0.27 (28.0)	9 (13)	0.9 (11)	29 (12.8)	446 (4)	41.1 (2.4)	30.9 (16.5)	261 (4)	24 (5)
Closed-canopy	0.12 (17.7)	6 (11)	0.4 (0)	0.20 (10.7)	8 (8)	0.6 (0)	22 (3.1)	551 (2)	40.4 (0.7)	34.6 (30.0)	374 (2)	27.4 (3)
Open-canopy	0.18 (9.1)	7 (0)	0.4 (0)	0.32 (10.8)	12 (0)	0.8 (0)	22 (4.9)	580 (0)	36.6 (0.3)	65.3 (0.8)	501 (0)	31.6 (0.3)
Reed												
Non-treated	0.09 (86.3)	2 (35)	0.2 (50)	0.03 (5.9)	3 (22)	0.3 (33)	19 (5.0)	321 (7)	36.6 (1.6)	8.9 (9.7)	191 (5)	21.8 (0.5)
Closed-canopy	0.06 (39.0)	3 (22)	0.3 (33)	0.11 (2.4)	6 (19)	0.6 (17)	27 (3.8)	389 (2)	38.3 (1.0)	12.0 (13.3)	218 (2)	21.5 (0.5)
Open-canopy	0.12 (8.0)	8 (8)	0.5 (0)	0.36 (13.1)	12 (5)	0.8 (0)	35 (8.4)	546 (1)	37.4 (0.8)	27.5 (8.7)	393 (1)	26.9 (1.5)
Pre-decomposed reed												
Non-treated	0	0	0	0.02 (21.3)	2 (35)	0.2 (50)	10 (2.8)	363 (3)	38.7 (2.1)	71.7 (2.5)	320 (1)	34.1 (1.5)
Closed-canopy	0.03 (31.0)	3 (33)	0.2 (50)	0.12 (25.3)	7 (16)	0.5 (20)	21 (7.9)	525 (2)	39.2 (0.5)	61.4 (4.5)	411 (0)	30.7 (2.3)
Open-canopy	0.22 (12.2)	7 (0)	0.5 (0)	0.89 (18.5)	12 (0)	0.9 (0)	37 (1.2)	541 (3)	38.9 (0.3)	31.4 (8.6)	397 (1)	28.5 (1.4)

.

Table 5.S3 Compilation of 2W-ANOVA results from the analysis of the magnitude and the direction of changes in DOM chemical composition of leachates during the dry phase and the biodegradation assay, as well as the %BDOC and DOC decay rate.

Dry phase				
Variable	Effect	*df*	*F*	*P*
Magnitude (Euclidean distance)	*Treatment*	1	81.70	< 0.001
	DOM source	5	13.87	< 0.001
	Treatment x DOM source	5	2.67	0.050
Direction (Angle degree)	*Treatment*	1	20.38	< 0.001
	DOM source	5	12.56	< 0.001
	Treatment x DOM source	5	12.56	< 0.001
Biodegradation assay				
Variable	Effect	*df*	*F*	*P*
% BDOC	*Treatment*	2	38.26	< 0.001
	DOM source	2	40.99	< 0.001
	Treatment x DOM source	4	3.24	0.036
DOC decay rate	*Treatment*	2	22.76	< 0.001
	DOM source	2	34.06	0.009
	Treatment x DOM source	4	3.77	0.021

Table 5.S4 Compilation of 2W-ANOVA results for FT-ICR-MS data. SPCA: small polycyclic condensed aromates.

Integrative results				
	Effect	*df*	*F*	*P*
Molecular mass	*Treatment*	2	297	< 0.001
	DOM source	2	471	< 0.001
	Treatment x DOM source	4	31.32	< 0.001
Molecular richness	*Treatment*	2	1000	< 0.001
	DOM source	2	198	< 0.001
	Treatment x DOM source	4	51.47	< 0.001
DBE	*Treatment*	2	605.48	< 0.001
	DOM source	2	346.97	< 0.001
	Treatment x DOM source	4	50.04	< 0.001
Raw intensity				
	Effect	*df*	*F*	*P*
SPCA	*Treatment*	2	82.3	< 0.001
	DOM source	2	22.7	< 0.001
	Treatment x DOM source	4	2.0	0.14
Polyphenols	*Treatment*	2	47.76	< 0.001
	DOM source	2	117.06	< 0.001
	Treatment x DOM source	4	2.43	0.085
Lignin	*Treatment*	2	33.46	< 0.001
	DOM source	2	18.90	< 0.001
	Treatment x DOM source	4	0.77	0.56
Aliphatic compounds	*Treatment*	2	214.3	< 0.001
	DOM source	2	35.8	< 0.001
	Treatment x DOM source	4	123.8	< 0.001

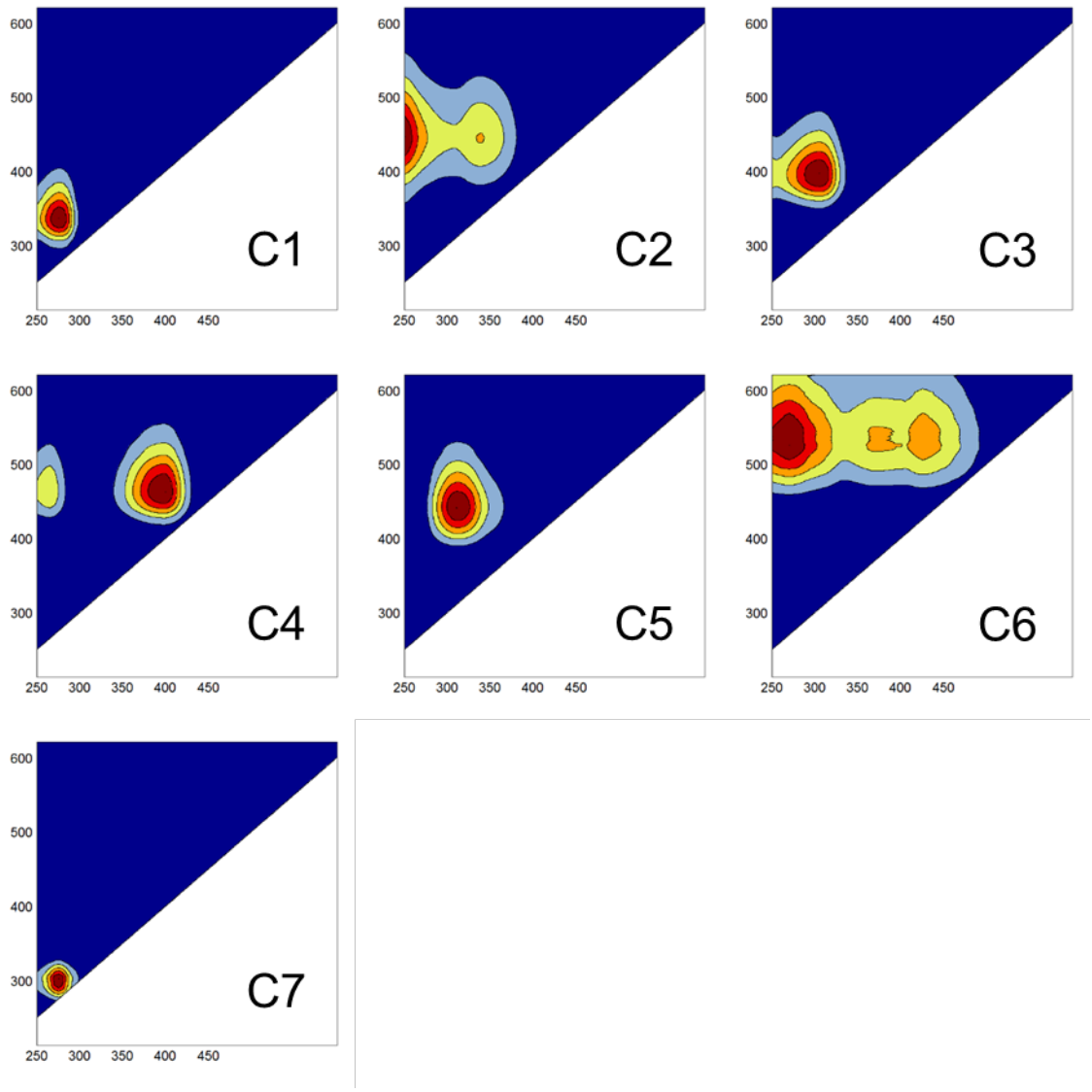

Figure 5.S1 Seven PARAFAC components identified from PARAFAC model.

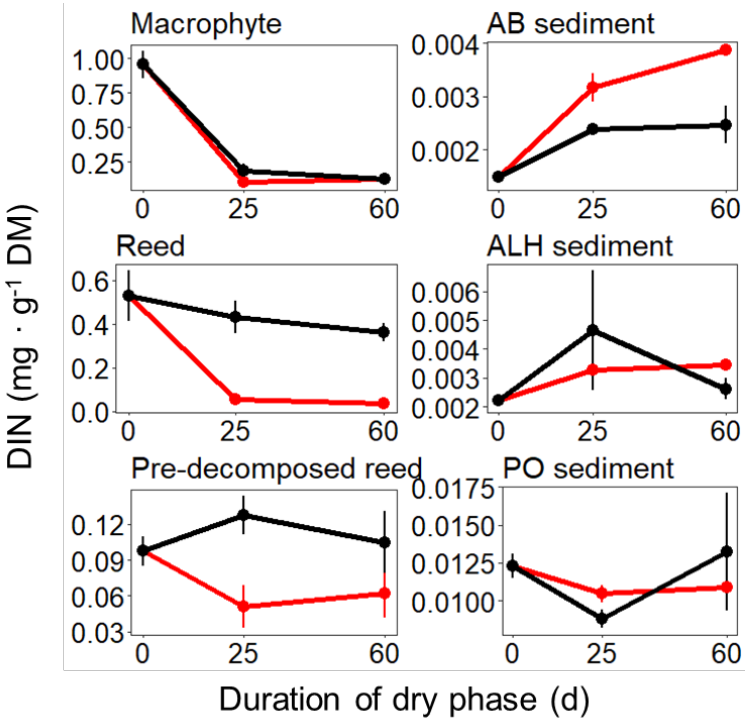

Figure 5.S2 Temporal changes in DIN concentration (mean ± SE, n = 3) for the different DOM sources (plant litter on the left side, sediments on the right) subjected to open-canopy (red lines) and closed-canopy treatments (black lines).

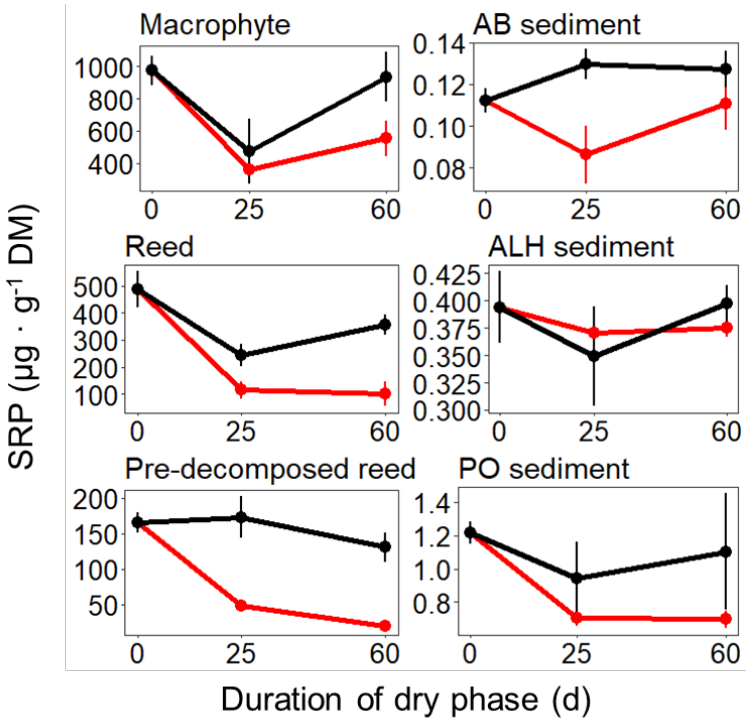

Figure 5.s3 Temporal changes in SRP concentration (mean ± SE, n = 3) for the different DOM sources (plant litter on the left side, sediments on the right) subjected to open-canopy (red lines) and closed-canopy treatments (black lines).

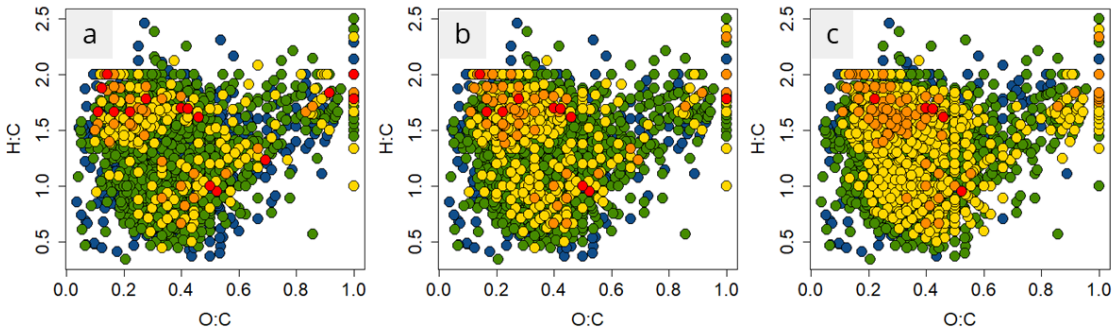

Figure 5.S4 Additional FT-ICR-MS results: Van Krevelen graphs for non-treated plant litter (a), plant litter subjected to the closed-canopy (b) and the open-canopy (c) treatments. Warmer colors indicate molecules with higher raw intensity, molecules with higher intensity are plotted over molecules with lower intensity.

6.

Flow intermittence alters carbon processing in rivers through the chemical diversification of leaf litter

Mosaic of terrestrial and aquatic habitats in intermittent rivers.

Abstract

In many intermittent rivers the dry phase is associated with large amounts of leaf litter accumulating in a great variety of habitats, e.g. open and sun-exposed zones, isolated and stagnant pools, or areas subjected to periodic wet/dry cycles. This diversity of environmental conditions promotes various changes in the chemical quality of leaf litter. At later rewetting, large amounts of leaves with diverse preconditioning histories are mixed and transported downstream, where they likely represent an important resource for decomposer communities. Here, we investigate whether the high diversity of preconditioning situations of leaf litter typically found in intermittent riverbeds during the dry phase has implications for its subsequent aquatic decomposition. We addressed this question by (1) preconditioning single species (alder) of leaf litter under 7 laboratory treatments mimicking the diversity of conditions of the aquatic-terrestrial habitat mosaic of intermittent rivers in non-flowing phases, (2) assorting preconditioned leaf litter to mixtures of increasing richness of preconditioning treatments, and (3) measuring their decomposition by microbes and shredders in a perennial stream. The different preconditioning treatments resulted in a diversification of the chemical composition of leaves as measured by elemental analysis and Fourier-transform infrared spectroscopy (FTIR). Increasing richness of preconditioning treatments in litter mixtures caused an increase of aquatic decomposition by microorganisms and shredders. This was generally achieved through complementarity effects among resource fractions, which allowed the decomposer community to optimize acquisition of nutrients and labile C compounds. Unique features of intermittent river functioning, such as this alteration of aquatic decomposition as a result of chemical diversity, present new challenges for future C cycling models that should integrate intermittent rivers in larger scale modelling efforts.

6.1. Introduction

The decomposition of riparian leaf litter is an essential ecosystem process in rivers, supporting heterotrophic food webs and allowing the cycling of carbon (C) and nutrients (Webster & Benfield 1986, Gessner et al. 2010). At river network scale, decomposition is intertwined with downstream transport (Battin et al. 2008, Raymond et al. 2016). The dominance of one or another depends on retention mechanisms, intimately related to river size, and hydrological dynamics (Larrañaga et al. 2003, Sponseller & Fisher 2006, Cordova et al. 2008). An extreme scenario is epitomized by intermittent rivers, especially those in the upper part of the catchments, which receive the bulk of terrestrial leaf litter input mainly in temperate regions (Wipfli et al. 2007). From a biogeochemical perspective, intermittent rivers are considered as pulsed bioreactors where the alternation of dry and wet periods controls the processing of OM via repeated cycles of accumulation, transport and decomposition along the river network (Larned et al. 2010, Datry et al. 2018). During the dry phase, the cessation of water flow is thought to halt decomposition by limiting decomposer and detritivore activity (Corti et al. 2011, Foulquier et al. 2015) while simultaneously promoting the accumulation of diverse organic substrates in dry riverbeds, but mostly riparian leaf litter (Sanpera-Calbet et al. 2016, Datry et al. 2018).

This simple model, however, is challenged by the fact that the fragmentation of surface flow during the drying phase prompts the emergence of a shifting mosaic of terrestrial and aquatic habitats (Stanley et al. 1997), where leaf litter can remain, exposed to diverse environmental conditions (Larned et al. 2010, Datry et al. 2014). Such diversity of conditions includes highly irradiated dry riverbed areas, isolated pools with high temperature, cold pools connected to hyporheic flow, wet and shaded remnant sediments or even areas subjected to recurrent wet-dry cycles. This environmental heterogeneity can promote the chemical differentiation of the accumulated leaf litter through particular abiotic and biotic factors in a process so called “preconditioning”, with important consequences for later aquatic decomposition after flow resumption (Dieter et al. 2011, 2013, Abril et al. 2016, Datry et al. 2018). For instance, the exposure of leaf litter to intense solar radiation

on dry riverbeds can cause an increase of its biodegradability through the reduction of lignin by photodegradation (Austin & Ballaré 2010, Austin et al. 2016), while leaf litter in stagnant pools can reduce its biodegradability by the leaching of labile compounds and the accumulation of phenols (Dieter et al. 2011 & 2013).

The re-establishment of water flow reconnects dry tributaries to the river network and prompts the mixing and downstream transport of variously preconditioned leaf litter, till they are retained and let to decomposed as diversified litter packs (Corti & Datry 2012). Thus, at river networks scale, the hydrological dynamics of intermittent rivers could suppose a powerful mechanism of chemical diversification with unknown consequences for decomposition dynamics and C biogeochemistry at this larger spatial scale.

Under aquatic conditions, decomposition is mainly controlled by chemical and physical traits of leaf litter species (García-Palacios et al. 2015, Boyero et al. 2017). Some species are easily decomposed due to availability of labile C compounds and/or nutrients, however other species are hardly decomposed because their high concentration in recalcitrant lignin or polyphenols (Talbot & Treseder 2012). Besides differences among individual leaf litter species, the mixing of various species can trigger non-additive effects on decomposition (Gessner et al. 2010, Handa et al. 2014), meaning that decomposition of mixtures is either above or below the decomposition expected from each induvial species (Gartner & Cardon 2004, Lecerf et al. 2011). Positive effects of litter diversity on decomposition are often attributed to fungi-driven nutrient transfer or synergistic interactions among leaf components with contrasting chemical qualities (Hättenschwiler et al. 2005, Gessner et al. 2010), while negative effects are usually associated with the inhibition of decomposer activity by secondary metabolites (Chomel et al. 2016). Although the presence or absence of particular chemical traits in litter mixtures can influence their decomposition (Schindler & Gessner 2009, Bruder et al. 2014), last works point out chemical or functional diversity in mixtures as responsible for governing decomposition rates (Meier & Bowman 2008, Lecerf et al. 2011, Stoler et al. 2016). In these cases, higher chemical divergence in mixtures accelerates decomposition owing to the greater diversity and availability of essential nutrients and C compounds for decomposer metabolism (Gessner et al. 2010).

Despite the abundance of studies evidencing the relevance of biodiversity on ecosystem functioning (Hooper et al. 2005, Hector 2011), our understanding of bio or chemical diversity effects on important ecological processes such as decomposition is still incomplete, even more at larger spatial scales than forest soils or river reaches. The hydrological dynamic of intermittent rivers provides plausible scenarios to study diversity effects in natural landscapes. But also, accounting for dry-phase associated diversity effects seems critical to achieve mechanistic understanding and realistic modelling capacity for C fluxes in river networks (Datry et al. 2018). A pending and necessary challenge to assume, considering that intermittent rivers represent over half of the global river networks and they are expected to keep increasing due to global change (Datry et al. 2014, IPCC 2013).

The objectives of this study are: (i) to address the potential role of environmental heterogeneity during the dry phase of intermittent rivers as a driving force of chemical diversification of leaf litter; (ii) to analyse the effect of increasing diversity of preconditioned leaf litter on aquatic decomposition after flow resumption. To do that, we first simulated the preconditioning of a single leaf litter species (*Alnus glutinosa*) under various habitat conditions typically found during the dry phase of intermittent rivers. Then, we measured the decomposition of leaf litter mixtures with increasing number of preconditioning situations. In analogy to effects of leaf species diversity on decomposition, we hypothesize that the increase of chemical diversity in mixtures of preconditioned leaves will accelerate decomposition in aquatic conditions.

6.2. Materials and methods

6.2.1. Experimental set-up

To address our objectives, we carried out an experiment with two phases. First, we simulated in the laboratory various preconditioning situations typically found in the terrestrial-aquatic mosaic of habitats during the dry phase of intermittent rivers to then, combine leaves from the different preconditioning treatments to make mixtures of increasing treatment richness (combinations of 1, 2, 4 and 6 treatments).

We analysed leaf chemistry after preconditioning by a range of techniques to describe elemental content of C and nutrients and the macromolecular composition by Fourier-transformed infrared spectroscopy (FTIR). In the second phase, we measured the decomposition by microbial decomposers and detritivores of leaf litter subjected to single preconditioning treatments and also the different mixtures under natural aquatic conditions in a river. We used fine and coarse mesh bags, where we measured the fungal biomass and the shredder density, respectively. Complementary, we ran a parallel respiration assay in the laboratory to analyse the effect of mixing on microbial metabolism.

6.2.2. Leaf litter preconditioning and preparation of mixtures

Approximately, 1 kg of air-dried of alder leaves (see section 2.2.2 for further details) was distributed into the 7 laboratory treatments (see Table 6.1 for detailed explanations and Fig. 6.1 for pictures of the treatments).

Once leaf litter preconditioning was completed, we prepared litter bags containing single treatments (7 treatments x 4 replicates) and mixtures of leaves of increasing treatment richness in all possible combinations of 2, 4, and 6 treatments. This design resulted in 4 richness levels comprising a total number of 91 bags (28 single-treatments + 21 2-treatment combinations + 35 4-treatment combinations + 7 6-treatment combinations) for each mesh size. Each litterbag consisted of 12 leaves enclosed in either coarse (8 mm) or fine (0.5 mm) plastic mesh (15 x 15 cm). In mixtures, the 12 leaves were partitioned equally among the component treatments. All leaves were scanned prior to bag assembly to measure treatment-specific leaf areas by digital image analysis using ImageJ software (https://imagej.nih.gov/ij/) and compute dry mass (DM). For this, we established relationships of leaf area to DM (48h, 105 °C) for every treatment from 20 leaves.

Table 6.1 Summary of the preconditioning treatments used in the study to mimic the terrestrial-aquatic habitat mosaic of intermittent rivers in non-flowing phases.

Treatment	Riverbed habitats during the dry phase	Laboratory simulation		Physicochemical conditions in aquatic habitats
T1	Stagnant pool subjected to anoxia and no light	Container filled with Spring Volvic where we added 8 mg · mg DO^{-1} of Na_2SO_3 to make anoxic conditions. The container was kept at dark conditions and room temperature		T = 24.6 °C DO = 0.15 mg L^{-1} pH = 5.5 Cond = 1650 µS cm^{-1}
T2	Dry riverbed exposed to intense solar radiation	Irradiation for 12 h/day with a UV lamp (Cosmedico Arimed B6, Osram Biolux 965; with 31% UVB of total UV) at room temperature		
T3	Disconnected pool with warm and eutrophic water supporting algal growth	Both pools were simulated in two different aquariums, filled with Spring Volvic water and stones with biofilm from the Löcknitz river. Both aquariums were continuously illuminated and oxygenated with air-bubbling	Kept at room temperature. Nutrient addition: 0.6 g L^{-1} of $NaNO_3$ and 0.3 g L^{-1} of KH_2PO_4	T = 25.1°C DO = 6.72 mg L^{-1} pH = 7.66 Cond = 800 µS cm^{-1}
T4	Pool connected to hyporheic flow paths with cold and oligotrophic water supporting limited algal growth		Kept in a fridge. No nutrient addition	T = 15.3 °C DO = 9.45 mg L^{-1} pH = 7.94 Cond = 925 µS cm^{-1}
T5	Shaded riverbed areas with high moisture content	Container with soil from the floodplain of the Löcknitz river watered with 500 mL every 4 days and kept at room temperature.		
T6	Zones subjected to wet/dry cycles associated with fluctuating water levels caused by rain events	Alternating T2 and T3 every 7 days		
T7	Vertical inputs of leaf litter entering the river days before flow resumption	Air-dried leaves kept at room temperature and dark conditions		

T: water temperature, DO: dissolved oxygen, Cond: water conductivity. The duration of preconditioning treatments were 21 days, except for T2 and T6, which extended for 60 days, since terrestrial decomposition processes occur at a longer time-scales than aquatic ones.

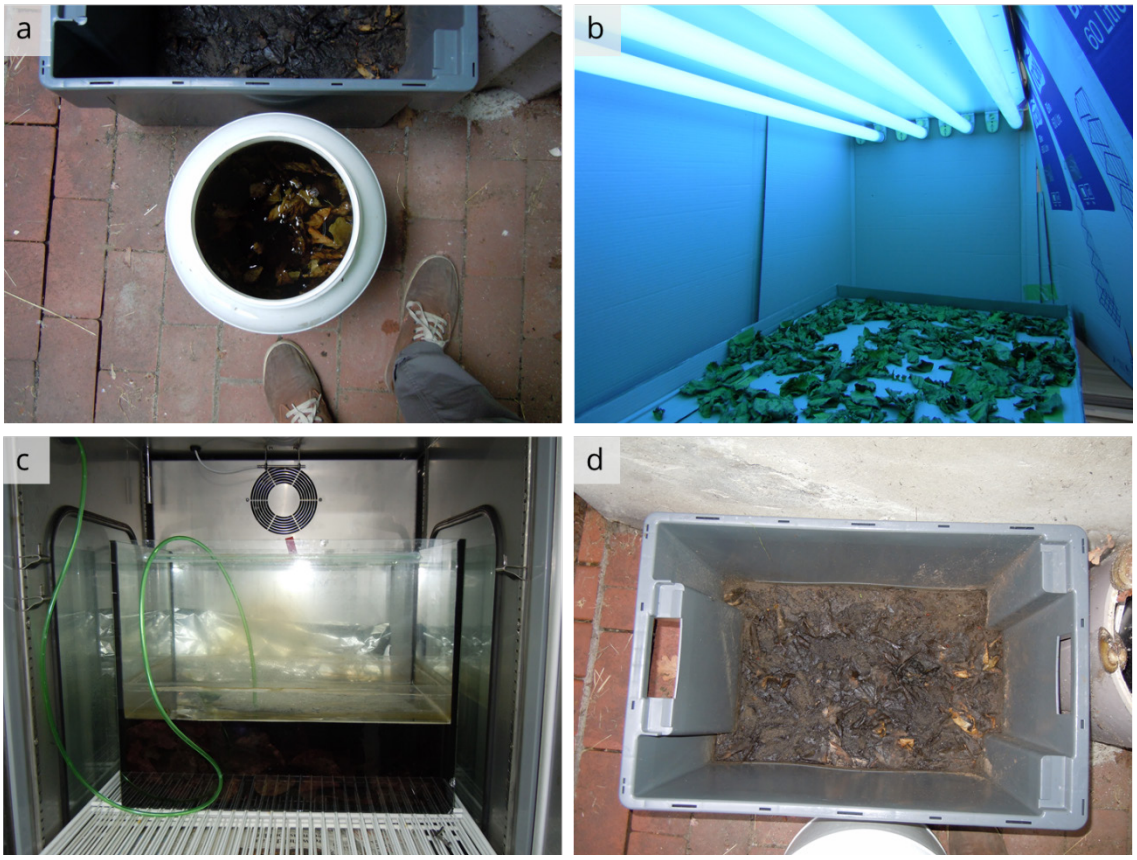

Figure. 6.1 Laboratory treatments simulating preconditioning situations typically found in the terrestrial and aquatic habitat mosaic of intermittent rivers during the dry phase: Stagnant pool subjected to anoxia or T1 (a); dry riverbed exposed to intense solar radiation or T2 (b); cold pool connected with oligotrophic water or T4 (c); shaded riverbed areas with high moisture content or T5 (d). Pictures by Roland Corti.

6.2.3. Analysis of leaf litter chemical quality and calculation of chemical diversity of mixtures

Following preconditioning sub-samples of all treatments were freeze-dried, ground using a ball mill and analysed for C, N, P, Ca, Mg and K using standard procedures explained in Chapter II (section 2.2.4.). Macromolecular organic C moieties were analysed by Fourier-transform infrared spectroscopy (FTIR; see Chapter II, section 2.2.4.1).

We combined information from FTIR peaks with the elemental composition of leaf litter in C and nutrients to perform a single principal component analyses

(PCA), where we condensed the information of C biochemistry and nutrient composition of leaf litter in a single analysis that serves as a proxy of the chemical composition of single preconditioning treatments through the average score of each single treatment in the first two PCA axes. All chemical variables were z-standardised prior to PCA. Then, we calculated the mean PCA scores of mixtures using community-weighted means (CWMs), which is computes as the average of each trait (here, the PCA scores) weighed by the relative abundance of each treatment in the mixture (Lavorel et al. 2008):

$$\mathrm{CWM} = \sum_{i=1}^{n} \rho_i \times \mathrm{PC}_i$$

We computed Rao's quadratic entropy (RaoQ; Laliberté & Legendre 2010) as a proxy of the chemical diversity of leaf litter mixtures according to Stoler et al. (2016). RaoQ is a functional diversity measure that indicates the mean functional distance among a group of species weighted by their relative abundance. In our case, RaoQ is based on the mean Euclidean distance of the chemical traits of the treatments present in a mixture. To reduce the number of traits, we used the scores of the first two PCA axes as chemical traits to compute the RaoQ value of every mixture as follows (Stoler et al. 2016):

$$\mathrm{RaoQ} = \sum_{i=1}^{R} \sum_{j=1}^{R} \rho_i \rho_j d_{ij}$$

where d_{ij} is defined as the Euclidean distance between treatments i and j included in a set of R treatments, being ρ the relative abundance of each treatment. RaoQ was calculated using the package FD (Laliberté & Legendre 2010) in R 3.2.1 (R Core Team 2015).

6.2.4. Aquatic decomposition experiment

To measure the aquatic decomposition of leaf litter mixtures and single treatments, we incubated all litterbags in the Löcknitz River (Brandenburg, Germany) for 23 days at the end of August in 2014. Löcknitz is a forested, 3rd-order lowland river in the Elbe catchment. Litterbags were tied to iron rods and fixed on the riverbed along

four randomly selected reaches of 50 m with running water and homogeneous substrate, depth and flow conditions. During incubation the water temperature oscillated between 13 and 17 ºC, average dissolved oxygen concentration was always higher than 6.5 mg L^{-1}, conductivity and pH averaged 560 µS cm^{-1} and 7.5, respectively. Litterbags were retrieved from the river in a unique sampling date when ~50% of initial litter mass was lost in an extra set of bags installed for weekly monitoring of mass loss. Once retrieved, litterbags were carried to the laboratory in dark and cold conditions. We used methods explained in Chapter II to analyse mass loss (section 2.2.3.), fungal biomass in leaves from fine mesh bags (section 2.2.6.) and the shredder density in coarse mesh bags (section 2.2.7). Mass loss was expressed in percentage as the difference between initial and final %AFDMr.

6.2.5. Respiration assay

Parallel to the aquatic decomposition experiment, we measured oxygen consumption rates on different leaf mixtures and single treatments as a proxy for microbial metabolism (see Chapter II, section 2.2.5.3.).

6.2.6. Data analysis

To analyse the response of leaf litter decomposition to the mixing of different preconditioned leaf litter along the gradient of increasing treatment richness we built generalized additive models for location scale and shape (GAMLSS) for all the study variables (mass loss, fungal biomass, shredder density and microbial respiration) using the treatment richness (1, 2, 4, 6 treatments) as explanatory variable. We used GAMLSS as this approach allow us to model the influence of treatment richness not only in the average values (µ) of the response variable, but also in its variance (σ), which is clearly useful in cases of heteroscedasticity as we expected for our dataset based on previous works (see Lecerf et al 2007). We also applied GAMLSS to test for the relationship between chemical diversity in mixtures and treatment richness.

We estimated expected values (E) of all study variables from the observed values (O) in single treatments decomposed alone (i.e. monocultures). In a mixture,

the expected value was computed as the average of observed values in component treatments divided by the number of treatments in the mixture. We compared observed and expected values to asses for non-additive effects of mixing. For this, we checked for significant differences between observed and expected values using paired *t* test (Gartner & Cardon 2004). Non-additive effects were considered synergistic when observed values were significantly higher than expected ones, but antagonistic when the expected values were higher.

In addition, we estimated the functional diversity response (FDR) of every study variable in mixtures as the relative deviation between observed (O) and expected (E) values (Gartner & Cardon 2004):

$$FDR = (O - E) / E$$

Finally, we used general linear models (GLM) to explain the FDR in mixtures by their chemical composition and/or their chemical diversity. To do that, we used GLM that included RaoQ (chemical diversity), PC1 and PC2 (chemical composition of mixtures) as predictors. We built an initial model that included all main effects and first-order interactions between RaoQ and each PCA axis and then selected the most parsimonious models using multi-model inference approach (Grueber et al. 2011, Feld et al. 2016) using the R package MuMIm (Bartón 2016). This approach allows us to select a list of "top models" instead of focussing on a single best model. This decision is often used when there is not an overwhelming support for choosing a single model based on information criteria such as AIC (Sakamoto et al. 1986). Doing this we avoid missing important predictors included in alternative models with similar AIC values than the estimated best model (Grueber et al. 2011, Feld et al. 2016). In our case, we selected as "top models" those with delta AICc < 2 to the best model. Finally, we generated an averaged model from the top model set to obtain a robust, weighted mean for each predictor coefficient and its errors based on AIC weights (Grueber et al. 2011, Feld et al. 2016). Model averaging was made using the natural method (see Burnham & Anderson 2002 for further details). All explanatory variables were z-standardized to have scaled and comparable average predictor coefficients (Grueber et al. 2011). From the outputs of the averaged model, we evaluated the effect of chemical diversity and chemical composition in mixtures on FDR by: (i) using the magnitude and direction of the averaged predictor

coefficients, (ii) checking whether the 95% confidence intervals spanned zero (i.e. significant effects of predictors) and (iii) computed the relative variable importance (RVI) of each predictor as the sum of the weights of all models containing this predictor (see Burnham & Anderson 2002). RVI ascertains the contribution of each predictor to the averaged ranging from 0 to 1.

6.3. Results

6.3.1. Chemical diversification of leaf litter under diverse preconditioning situations

The PCA based on the chemical traits of leaf litter clearly separated the various preconditioning treatments (Fig. 6.2a). PC1 (44.5 % of the total variance) principally separated treatments T2 and T7 with the highest abundance of carbohydrates from T3 and T6, which had the highest contents of nutrients N and P and FTIR-peaks indicating the presence of lignin-like and phenolic-like compounds. PC2 (28.2% of total variance) was mainly formed by the abundance of structural polysaccharides such as cellulose, and separated T1 from the other treatments. T4 and T5 were characterized by intermediate values of nutrients and structural C compounds. As expected, GAMLSS identified a significant increase of the average and a reduction of the variance of chemical diversity with increasing treatment richness in mixtures (Fig. 6.2b), meaning that leaf litter mixtures of 2-treatment combination had a lower average diversity but a higher variability than 6-treatment combinations.

6.3.2. Effect of preconditioning treatment richness on the aquatic decomposition of leaf litter mixtures

The increase of the treatment richness in mixtures caused a generalized, significant increase in the average of observed values of leaf litter mass loss in coarse and fine mesh bags, the microbial metabolism, the fungal biomass, and the shredder density (Fig. 6.3a and 6.4). On the contrary, the increase of treatment richness only caused a significant reduction of the variance of mass loss in fine mesh bags and ergosterol

(Fig. 6.4). In contrast to observed values, expected values of mass loss did not respond to treatment richness in their mean but showed a decrease of the variance (Fig. 6.3b). This pattern was identical for all other study variables (data not shown).

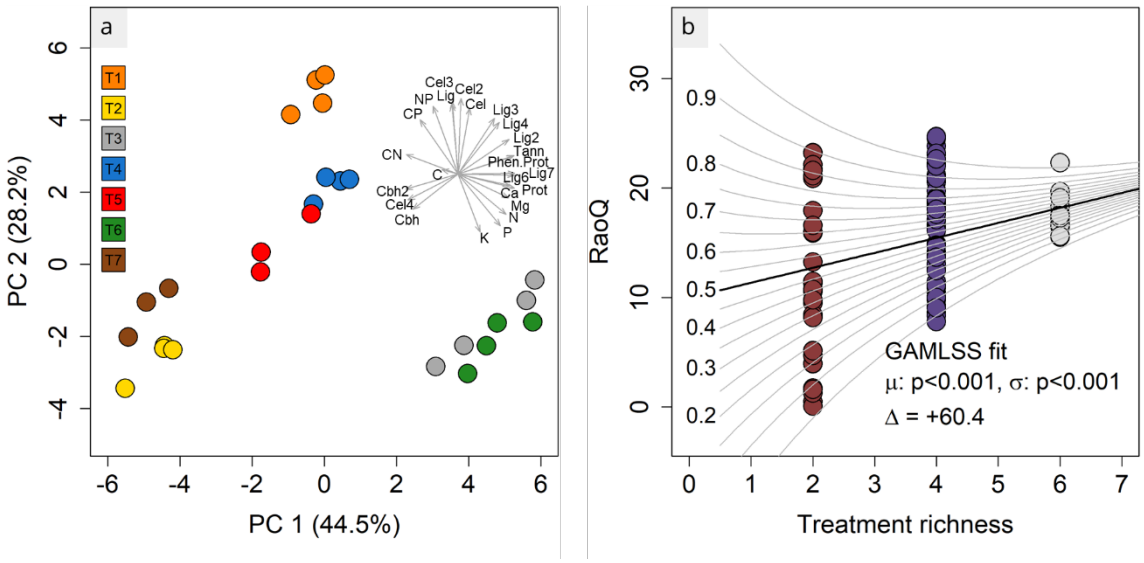

Figure 6.2 PCA based on the chemical quality of leaf litter after its preconditioning under different treatments (see Table 6.1 for code explanation) (a). Change of the chemical diversity (RaoQ) in mixtures along the treatment richness gradient (b). GAMLSS identified a significant increase of the average (μ, black line) and a significant reduction of the variance (σ, grey lines) of chemical diversity with increasing richness. Different colours indicate different levels of treatment richness in mixtures.

The increase of the treatment richness in mixtures caused a generalized, significant increase of the average of observed values of leaf litter mass loss in coarse and fine mesh bags, the microbial metabolism, the fungal biomass, and the shredder density (Fig. 6.3a and 6.4). On the contrary, the increase of treatment richness in leaf litter mixtures only originated a significant reduction of the variance of mass loss in fine mesh bags and ergosterol (Fig. 6.4a & 6.4c). In contrast to observed values, expected values of mass loss did not respond to treatment richness in their mean but showed a decrease of the variance (Fig. 6.3b). This pattern was identical for all other study variables (data not shown).

The increase in the average of observed values in mixtures promoted positive, non-additive effects in all the mentioned variables as shown by significant differences between observed and expected values by paired *t* test (Fig. 6.S1).

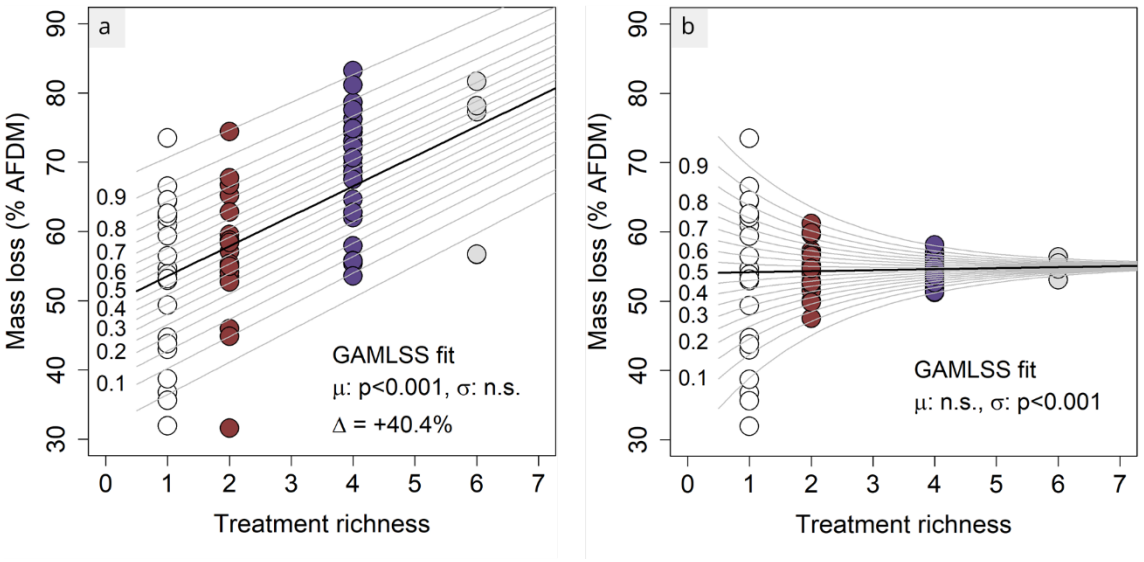

Figure 6.3 Observed (a) and expected (b) values of mass loss in coarse mesh bags in single treatments and mixtures along the treatment richness gradient. GAMLSS identified a significant increase of the average (μ, black line) but no change in the variance (σ, grey lines) of observed values of mass loss with increasing richness, while there was no change in average but a decrease in variance of the expected values. Different colours indicate different levels of treatment richness in mixtures.

6.3.3. Effect of chemical composition and chemical diversity of leaf litter mixtures on functional diversity response

According to model averaging results, chemical diversity (estimated through RaoQ) was the most important predictor for FDR for all the study variables (except mass loss in fine mesh bags). This was supported for all the three lines of evidences obtained from model averaging. Chemical diversity had the highest averaged coefficients and RVI values for all study variables (Fig. 6.5 and 6.S2). Except for shredder density and mass loss in fine mesh bags, the confidence intervals of chemical diversity separated from 0 in all the other study variables, indicating significant positive effect of chemical diversity on FDR in all of them (Fig. 6.5).

The chemical composition of mixtures (estimated through the average score of PC1 and PC2) also had an important influence on microbial decomposition as indicated by the significant positive effect of PC2 on mass loss in fine mesh bags and microbial metabolism, and the negative effect of PC1 on fungal biomass (confidence intervals excluded 0) (Fig. 6.5).

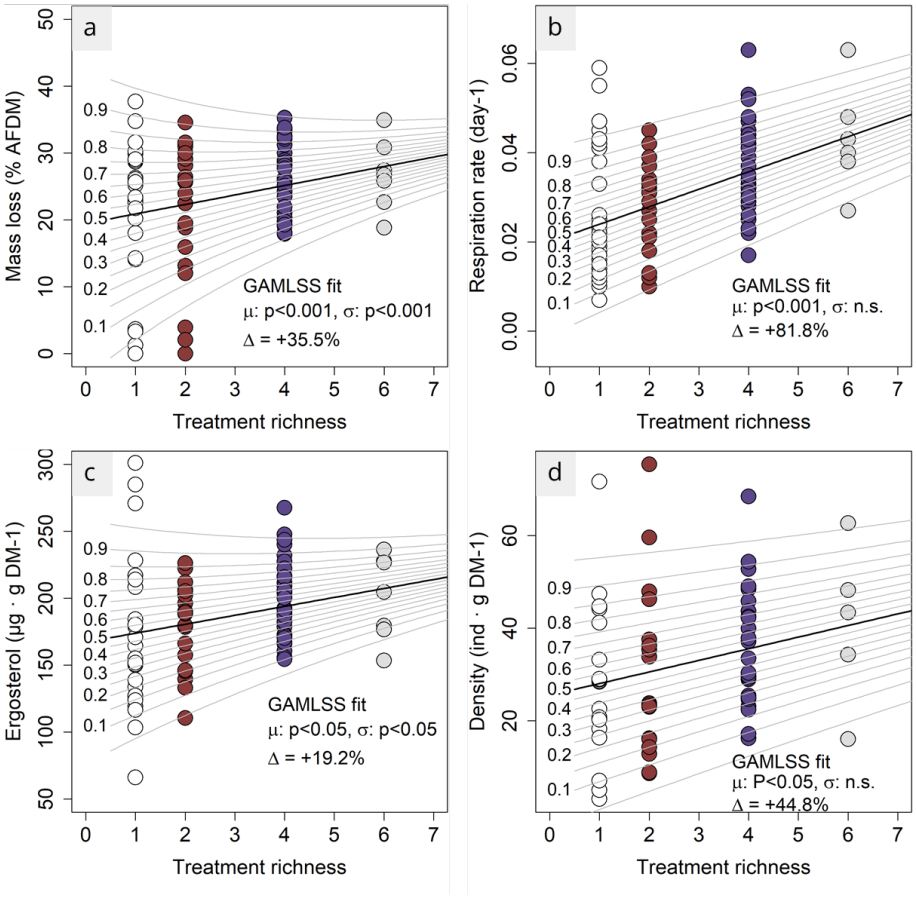

Figure 6.4 Observed mass loss in fine mesh bags (a), microbial metabolism (b), fungal biomass in leaf litter (c) and shredder density (d) in single treatments and mixtures along the treatment richness gradient. GAMLSS identified a significant increase of the average (μ, black line) for all four variables with increasing richness, but a decrease in the variance (σ, grey lines) only for mass loss in fine mesh bags and ergosterol. Different colours indicate different levels of treatment richness in mixtures.

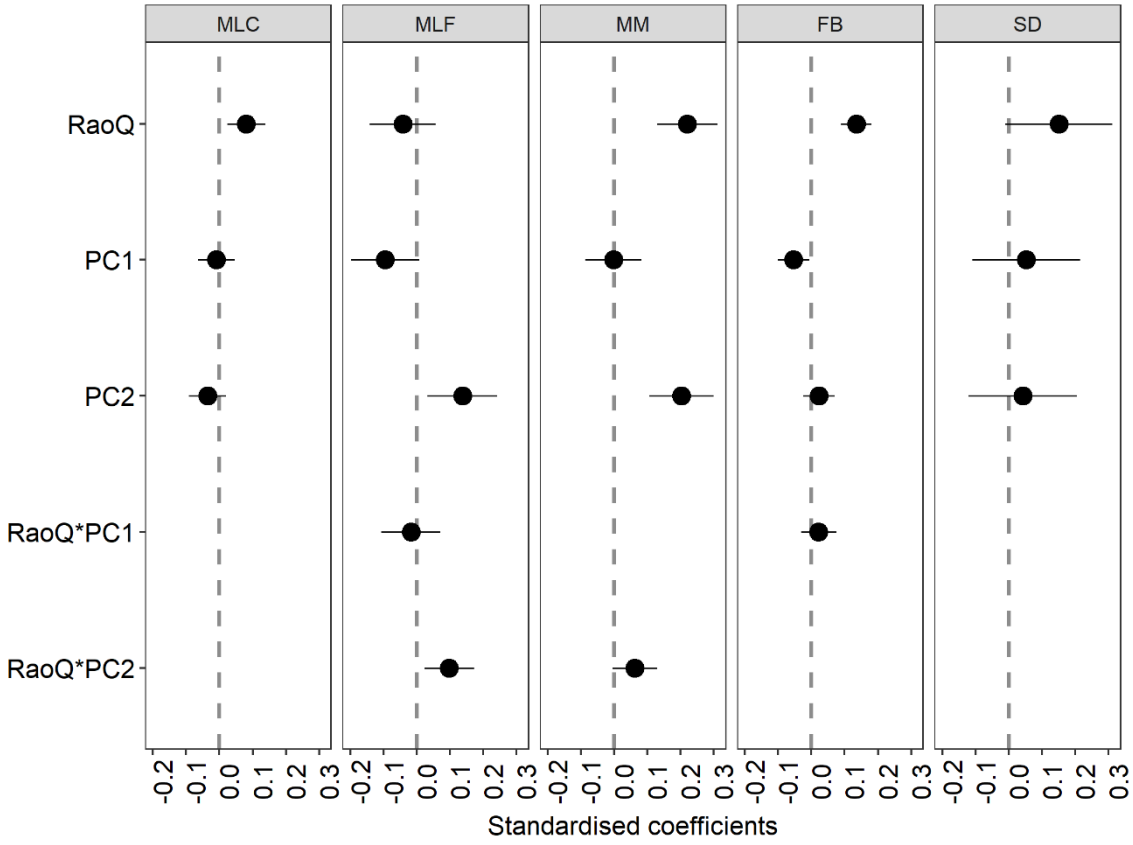

Figure 6.5 95% confidence intervals for standardised predictor coefficients from averaged models with a ΔAICc < 2 used to analyse the effect of chemical diversity (RaoQ) and chemical composition (PCA scores) on the functional diversity response (FDR) of mixtures for all study variables. A confidence interval excluding 0 indicates a significant effect on the respective functional diversity response. Predictors without dot were not retained in the final averaged model. MLC = mass loss in coarse mesh bags; MLF = mass loss in fine mesh bags; MM = microbial metabolism; FB = fungal biomass; SD = shredder density.

Table 6.2 Relative variable importance (RVI) for predictors obtained after model averaging. RVI ascertains the contribution of each predictor to the averaged model ranging from 0 to 1 (null to maximum contribution). See Figure 6.5 for acronyms.

	MLC	MLF	MM	FB	SD
RaoQ	1	1	1	1	0.8
PC1	0.17	1	0.2	1	0.2
PC2	0.29	1	1	0.28	0.18
RaoQ*PC1	0	0.29	0	0.25	0
RaoQ*PC2	0	1	0.76	0	0

6.4. Discussion

6.4.1. The dry phase of intermittent rivers drives the chemical diversification of accumulated leaf litter

In intermittent rivers, the cyclic contraction and expansion of water flow creates a great diversity of terrestrial and aquatic habitats across the river network, which are continuously shifting over the hydrological year (Stanley et al. 1997, Datry et al. 2014). The drying period is the moment of maximum spatial habitat heterogeneity in intermittent rivers, as the fragmentation of water flow fosters the emergence of flowing, non-flowing and dry habitats. In addition, the fragmentation of flow halts the longitudinal transport of OM along the river network and promotes its accumulation under a high diversity of terrestrial and aquatic habitat conditions (Datry et al. 2017 & 2018), which control the preconditioning of this material previous to flow resumption (Dieter et al. 2011 & 2013, Abril et al. 2016)

As we expected, our results demonstrated that contrasting environmental conditions during leaf litter preconditioning drives a chemical diversification of the leaf litter retained in the different dry-phase habitats. This was evidenced by the separation of preconditioning treatments in the PCA based on the chemical composition of preconditioned leaf litter (Fig. 6.2a). The separation of treatments along the PC1 was associated with the degree of decomposition of leaf litter, which greatly varied between terrestrial and aquatic habitats (Langhans et al. 2008, Abril et al. 2016). Thus, leaf litter preconditioned under terrestrial conditions (T2: leaf litter exposed to UVB radiation and T7: recently fallen leaves from riparian vegetation) kept a high content of labile C compound such as carbohydrates because of the limitation of microbial activity by water scarcity (Gavazov et al. 2014). Conversely, leaves in aquatic habitats, especially in pools with high temperature and nutrient concentration (T3 and T6), were subjected to an intense decomposition (Fernandes et al. 2014), with the consequent consumption of carbohydrates and the increase of nutrients and phenols by microbial immobilization (Melillo et al 1984, Webster & Benfield 1986). Besides, other processes different to microbial decomposition affected leaf litter during preconditioning, for instance in stagnant pools (T1), where

the acid and anoxic conditions favoured the increase of the proportion of structural polysaccharides such as cellulose, probably by the leaching of other more soluble C compounds, as previously evidenced by Dieter et al (2011 & 2013). Although we recognize the limitations of our study because of the simulation of the preconditioning treatments under laboratory conditions, our results challenge the generalized assumption of the dry phase of intermittent rivers as a simple static phase and point out its relevance as powerful mechanism of OM chemical diversification along river networks.

6.4.2. The mixing of diverse preconditioned leaf litter accelerates decomposition after flow resumption

The re-establishment of water flow reconnects dry tributaries to the river network and prompts the mixing and downstream transport of variously preconditioned leaf litter, till they are retained and let to decomposed as diversified litter packs (Corti & Datry 2012). Under the new fully aquatic conditions, our findings suggest that mixing of diversely preconditioned would accelerate the decomposition rates though positive non-additive effects in a similar way than mixtures of riparian leaf litter species (Gartner & Cardon 2004, Gessner et al. 2010, Lecerf et al. 2011). The increase in the treatment richness accelerated the mass loss in leaf litter mixtures both in coarse and fine mesh bags (Fig. 6.3a and 6.4a), but also it stimulated shredder density (measured in coarse mesh bags), fungal biomass (measured in fine bags) and microbial metabolism (analysed in an independent respiration assay) (Fig. 6.4). These results demonstrated that the diversity of preconditioning situations during the dry phase enhanced the action of both microbial decomposers and detritivores involved in the aquatic decomposition of our mixtures (Handa et al. 2014). In addition, the higher regression slopes for mass loss in coarse mesh bags (Δ = +40.4 %) than in fine ones (Δ = +35.5 %), but also in shredder density (Δ = +44.8) than in fungal biomass (Δ = +19.2), suggest that shredder action fostered an acceleration of mixtures decomposition mediated by preconditioning diversity (Lecerf et al. 2011, Handa et al. 2014).

Besides the positive effect of treatment richness of leaf litter mixtures on aquatic decomposition, it caused a decrease of the variability in fungal biomass and mass loss in fine mesh bags (Fig. 6.4a & 6.4c). This decrease in the variability of both variables measured in mixtures along the treatment richness gradient responds to the "portfolio effect" demonstrated in previous studies such as Dang et al. (2005) or Lecerf et al. (2007). This effect is derived from statistical and biological mechanisms. It emerges as the influence of species with extreme values is dampened as the richness of species increases in leaf litter mixtures. The reduction of variability in fungal-mediated decomposition, but not in shredder density or mass loss in coarse mesh bags is a striking result. It may suggest that preconditioning diversity not only increases microbial decomposition rates, but also its reliability by reducing its natural variability (Dang et al. 2005). This would not be the case of detritivore-mediated decomposition, where preconditioned leaf litter with extreme values can still cause an important influence on the final decomposition rate.

There are a great variety of mechanisms which can explain the diversity effect shown by leaf litter mixtures on decomposition (Gessner et al. 2010, Hättenschwiler et al. 2005). Usually, mechanisms behind the diversity effect are partitioned in complementarity and selection effects (Loureau & Hector 2001). Complementarity effects derived from the interaction among leaf litter species composing a mixture, whereas selection effects emerge when a specific species imposes its characteristics over the rest of the species in the mixture. The most common example of selection effect found on decomposition is the feeding preference of decomposers, but specially detritivores by leaf litter species rich in labile C compounds or nutrients (Sanpera-Calbet et al. 2009, Tonin et al. 2017). Complementarity effects are denominated as synergistic or antagonistic depending on interactions among leaf litter species trigger a positive or negative response on decomposition. Synergistic interactions can arise from facilitation reactions among species with contrasting chemical quality. An example is the increase of mixtures decomposition by the active transfer of nutrients from nutrient rich to nutrient poor leaves mediated by fungi (Handa et al. 2014, Tonin et al. 2017). Another type of synergistic interactions among leaf litter species is resource partitioning. There are many cases. For instance, the mixing of different leaf litter species with distinct forms and textures promotes

an increase of the structural heterogeneity of the mixture, which in turn increase the availability of niches for shredders (Kominoski et al. 2009, Sanpera-Calbet et al 2009). Another case, leaf litter mixtures with high chemical diversity allow consumers to meet their stoichiometric needs (Frost et al. 2005a, Frainer et al. 2015a), or complementary C compounds they require for metabolism (Gessner et al. 2010).

In this study, we could not partition the diversity effect caused by preconditioned leaves mixing into their complementarity and selection effect because of our experimental design (see Loreau & Hector 2001). However, the high relationship between the increase of treatment richness and chemical divergence in mixtures (estimated by RaoQ index) (Fig. 6.2b) suggest that complementarity was the main factor promoting the accelerated decomposition rates during the aquatic phase (Lecerf et al. 2011, Stoler et al 2016). Not only that, the results of model averaging indicated that chemical diversity was the main factor explaining the functional diversity response in mass loss in coarse mesh bags, microbial metabolism, fungal biomass and shredder density (although marginally significant this last one) (Fig. 6.5). Although we cannot elucidate which specific mechanism is behind the chemical diversity effect of leaf litter mixture on decomposition, we suggest chemical diversity would have enhanced the activity of microbial communities and shredders by the facilitation of acquisition of all essential resources for their growth and metabolism such as nutrients, labile C compounds like carbohydrates, or long-lasting resources like cellulose (Gessner et al. 2010, Handa et al. 2014).

Even so, we cannot dismiss the influence of specific chemical composition of preconditioned leaf litter types on the decomposition of mixtures. There are two main reasons. First, model averaging evidenced significant effects of chemical composition (PCA scores) on functional diversity response of mixtures (Fig. 6.5). The averaged model for mass loss in fine mesh bags was mainly explained by the chemical composition of mixtures, not by the chemical diversity (Bruder et al. 2014, Frainer et al. 2015b). This is also logic, as the positive, significant effect of PC2 scores on mass loss in fine mesh bags and microbial metabolism could indicate a higher microbial activity associated with mixtures with higher proportion of cellulose (see Fig. 6.2a) (Talbot & Treseder 2012). On the other hand, the negative effect of PC1

on mass loss in fine mesh bags could be associated with a negative effect of high concentration of lignin and phenolic compounds on the decomposition of mixtures (Talbot & Treseder 2012, Chomel et al. 2016), or conversely, a higher decomposition of leaf litter mixtures containing higher concentration of carbohydrates (i.e. preconditioned leaf litter types with negative loadings of PC1) (Stoler et al. 2016). The other plausible reason explaining why composition can have an important influence on our results is related to our experimental design. According to our aims, we made leaf litter mixtures by mixing randomly different preconditioned leaf litter types, but with unknown chemical quality. We were not interested in mixing leaves with different functional traits (e.g. nutrient rich with nutrient poor species), but to know if heterogeneous preconditioning situations could promote chemical diversity. As a consequence, our random and "blind" design did not ensure a balanced representation of chemical traits in leaf litter mixtures (see Stoler et al. 2016). Therefore, our mixtures of leaf litter could be partially biased by preconditioned leaf litter types with similar chemistry. In the end, the chemical diversity of our leaf litter mixtures (estimated through the mean PCA scores of each mixture using RaoQ) was slightly correlated with PC2 scores ($r = -0.26$, $p = 0.006$), but not with PC1 ($r = 0.01$, $p = 0.680$). In other words, our chemical diversity was biased to some extent by the chemical composition of leaf litter reflected by PC2 scores. However, this bias did not translate into a collinearity problem between predictors in selected top models as evidenced by VIF values (< 3 for all predictors and models).

In addition to the chemical diversity or composition of preconditioned leaf litter mixtures, other factors such as the number of food web levels composing the decomposer community could explain the acceleration of the decomposition rates in the river (Gessner et al. 2010, Handa et al. 2014). A direct evidence of this would be the higher effect of treatment richness in mixtures on mass loss in coarse, than in fine mesh bags, due to inclusion of shredders (Srivastava et al. 2009). Another potential factor influencing decomposition o leaf litter mixtures could be the composition of microbial communities (Fukami et al. 2010, Gessner et al. 2010). During the preconditioning of leaf litter under diverse terrestrial and aquatic conditions, abiotic conditions could have favoured the growth of different fungal and bacterial species on leaves. For instance, Dieter et al. (2013) showed how acid

and anoxic conditions of stagnant pools can foster the development of different fungal communities on leaves accumulated there, compared to leaves preconditioned under solar radiation or non-preconditioned leaves. So, potential diversity of microbial decomposer communities might have contributed to increase the decomposition of mixtures due to a higher spectrum of different exoenzyme that can degrade a higher variety of compounds in leaf litter (Gessner et al. 2010), although we do not have direct evidences to prove this idea, and genetic and molecular analysis must be done to corroborate it.

6.4.3. Intermittent streams as diversity hotspots in river networks

Although intermittent rivers have been much less studied than perennial ones, there is a growing body of evidences highlighting the potential role of intermittent rivers in C fluxes in river networks (Datry et al. 2018). This is mainly because the great accumulation of OM during their dry phase triggers hot moments of microbial activity during flow resumption associated with the release of large amounts of nutrients, particulate and dissolved organic matter from riverbeds (Mertbt et al. 2016, Bianchi et al. 2017, Datry et al. 2018). Indeed, last evidences provided by Datry et al. (2018) estimate than the inclusion of intermittent and ephemeral rivers in C cycling models would increase annual estimates of global CO_2 emissions from streams and rivers by 7-152%. All these studies evidence that hot moments associated with flow resumption in intermittent rivers are leaded by OM quantity, but they make no reference about the potential role of OM chemical quality as a modulator of such microbial activity. Complementarily, our results suggest that flow re-establishment in intermittent rivers triggers not only a pulse of OM, but a pulse of chemical diversity, which is transported downstream the river network, and consequently may alter OM processing. To accept this hypothesis, the environmental heterogeneity, emerged by the mosaic of terrestrial and aquatic habitats with flow fragmentation, needs to be maintained for most of the dry phase time till flow resumption. However, it is well known that the temporal pattern of drying of intermittent rivers greatly varies among years, across bioclimatic regions or depending on catchment hydrology (Stanley et al. 1997). For instance, in arid

sites, riverbeds completely dry up after a few days from the beginning of flow fragmentation due to the high summer temperatures (Guerrero 2002, Gómez et al. 2005). Consequently, in these rivers the short lifespan of the terrestrial-aquatic habitat mosaic could not lead to such chemical diversification of leaf litter.

So far, the theoretical framework of OM dynamics in river networks have considered only the importance of the transport and the aquatic processing of OM, considering its retention periods out of the water biogeochemically inactive (Larned et al. 2010). Our results demonstrate that the dry phase can be an active period of chemical alteration of OM. Furthermore, they suggest that, depending on the temporal drying pattern of the river network, spatial environmental heterogeneity of riverbeds can emerge as a mechanism of chemical diversification (see Fisher et al. 2007). The downstream transport of chemically diversified leaf litter could have important implications in OM fluxes along the river network, accelerating the processing rates and thus decreasing the spiral length for complete OM mineralization.

Due to the experimental character of our study we realise the necessity of corroborating these assumptions under natural conditions in the field in future studies, as well as consider the influence of other factors acting at catchment scale such as land use or vegetation types (Laudon & Sponseller 2017). However, our results, together with the expectations of future prevalence of intermittent rivers in fluvial networks, reinforce the potential relevance of these systems in global C cycling and the necessity of integrating them in larger scale modelling efforts.

6.5. Annexes

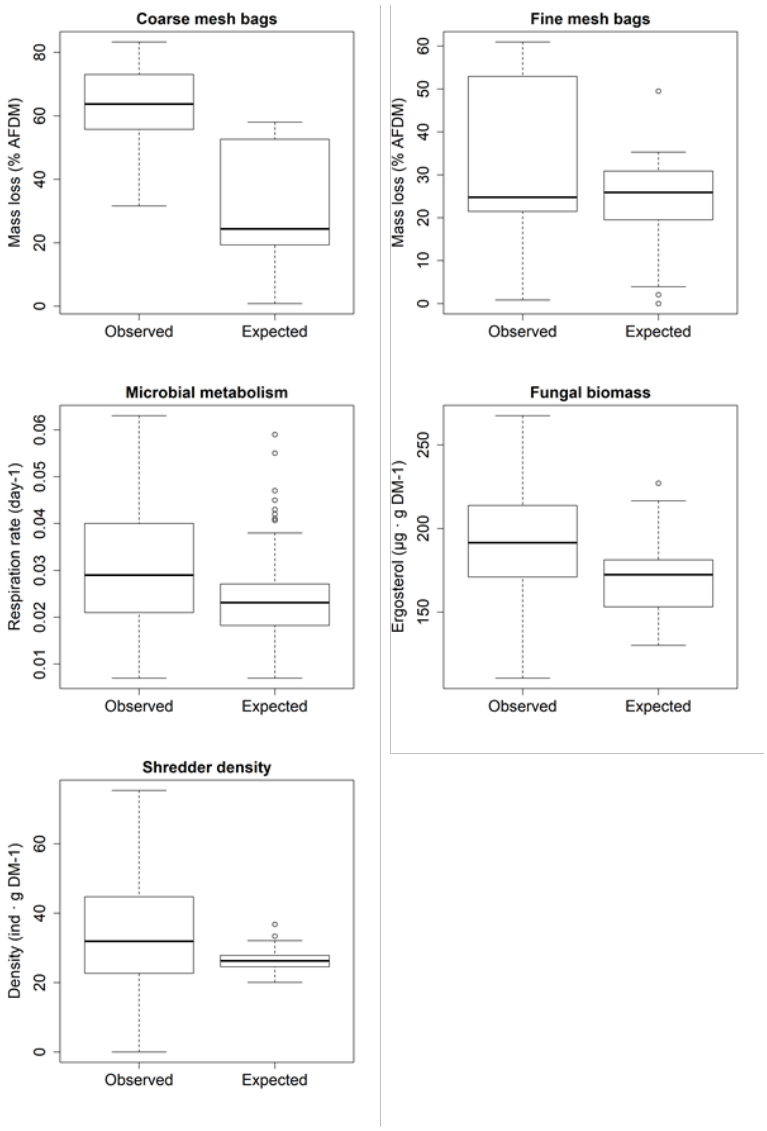

Figure 6.S1 Differences between observed and expected values in leaf litter mixtures. All comparisons revealed significant differences by paired *t* tests.

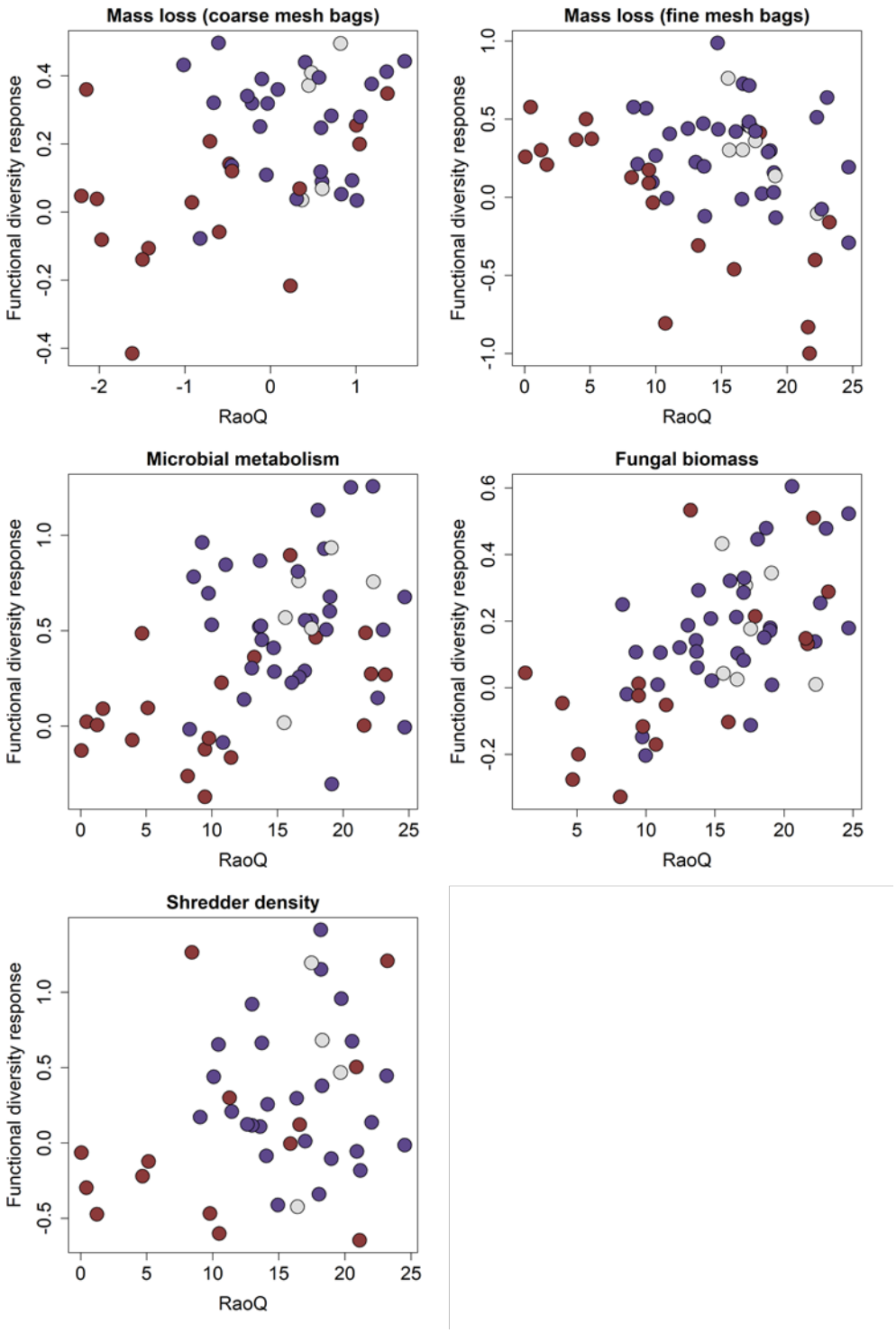

Figure 6.S2 Relationship between functional diversity response (FDR) of all measured variables and chemical diversity (RaoQ). Different colours indicate different levels of treatment richness in leaf litter mixtures: combinations of 2 treatments (dark red), 4 treatments (purple) and 6 treatments (grey).

7.

General discussion: Challenging classic paradigms about organic matter processing in fluvial ecosystems

Chícamo (arid stream) and Demnitzer (forested stream)

Despite the relevance of terrestrial-aquatic interactions on ecosystem functioning and C fluxes in rivers has been well recognized for so long (e.g. Junk et al. 1989), the study of the influence of processes occurring in the terrestrial ecosystems on aquatic OM processing has contrarily been neglected during all this time. Even when some studies have pointed out the potential underestimation of floodplain inputs to OM budgets in rivers (Tank et al. 2010). One of the main reasons behind this could be that OM decompositions studies have been traditionally carried out in headwaters-forested streams, mainly from temperate and humid areas, where vertical OM inputs from the riparian vegetation dominate lateral inputs from floodplain soils (Wallace et al. 1995, Tank et al. 2010). Moreover, in areas where lateral inputs can really exceed vertical ones, such as in arid regions (Jacobson et al. 1999), the dominance of the autotrophic sources of energy over the allochthonous ones (Bunn et al. 2003, Velasco et al. 2003, Brett et al. 2017) have downplayed the role of OM decomposition as an important process for ecosystem functioning. But probably, the most important factor could be the traditional assumption of the period of OM retention and accumulation in either floodplain soils or dry riverbeds, just as a static retention phase (Webster 2007, Larned et al. 2010).

Far from supporting this hypothesis, the results obtained in this dissertation highlight the terrestrial phase of OM processing in fluvial ecosystems as an active biogeochemical period, pivotal for its subsequent aquatic biodegradation. This general discussion focuses on the main processes involved in the chemical alteration of OM during its terrestrial exposure and how they modify its aquatic processing pathways. Although hypothetically, the results discussed here challenge classic concepts in OM biogeochemistry in fluvial ecosystems.

7.1. Understanding intermittent rivers from floodplain ecology concepts

Over the last years, with the aim of fostering the scientific interest in intermittent rivers, freshwater ecologists have highlighted their rather unique functioning. Nevertheless, we usually forget that many concepts used for intermittent river ecology, at least for OM processing, are based on floodplain – river systems (see Larned et al. 2010); such as the idea of the shifting mosaic of habitats (Langhans et

al. 2006) or the pulsed longitudinal bioreactor concept (Battin et al. 2008).The interaction between rivers and floodplains is mainly spatially constrained, whereas in intermittent streams the interaction between terrestrial and aquatic conditions is basically, temporally constrained (occurrence of wet and dry phases). Thus, the main difference between them is that OM retention, and consequently its preconditioning, is usually much longer in floodplains than in dry riverbeds subject to seasonal flow resumption. On the contrary, biotic and abiotic mechanisms behind terrestrial preconditioning in floodplain soils and dry riverbeds are basically the same. Therefore, considering that the current efforts in intermittent river ecology focus on upscaling and integrating the acquired knowledge to larger scale, we think the knowledge generated from floodplain ecology it would allow us to achieve this goal more easily.

7.2. The relevance of allochthonous POM in aquatic food webs not only depends on its initial chemical quality

Results of Chapters III and IV allow us to compare the effect of terrestrial preconditioning on two POM sources with very different initial quality: wood (Chapter III) and leaf litter (Chapter IV). Despite of the much lower chemical quality (as defined by its C:N and Lignin:N ratios) of wood than of leaf litter, both organic substrates underwent similar changes during their floodplain exposure (general decrease of nutrients and labile C compounds and colonization by terrestrial microbes) and consequently they were subjected to similar decomposition pathways in the river, with an accelerated microbial decomposition during the first days of aquatic immersion, but a decrease at later stages. These results challenge general assumptions about allochthonous POM inputs as energy and nutrient sources in fluvial ecosystems and conversely, they point out that terrestrial preconditioning reduces the “lifespan” of both leaf litter and wood as relevant resources for aquatic microorganisms. This finding would be especially striking for wood, as it is traditionally considered as a long-lasting resource (Díez et al. 2002, Tank et al. 2010). Therefore, after its preconditioning, POM may shift from an energy resource to a physical substrate for the development of microbial

communities metabolising DOC from the water column once the labile C resources of preconditioned POM are exhausted (Pastor et al. 2014.).

7.3. Nutrient leaching *vs* immobilization: the importance of soil-OM nutrient balance for terrestrial and aquatic microorganisms

One of the most important result from Chapter IV is that the counterbalance in leaves between nutrient immobilization or their loss by rain leaching during the terrestrial phase seems to be a key factor modulating its later microbial decomposition in the river. The dominance of one process over the other depends on environmental conditions during the terrestrial exposure of OM. When moisture and especially nutrients availability in soils facilitate microbial activity during the terrestrial phase, nutrients can be strongly immobilized in OM (Parton et al. 2007). Under these circumstances, a positive balance between immobilization vs leaching contribute to maintain a high nutrient content in preconditioned OM, and thus allowing its transference to the aquatic microbial communities (the case of leaves preconditioned in TOR in Chapter IV). On the contrary, when floodplain conditions do not favour microbial immobilization because of the scarcity of water and nutrient (the case of leaves from PA in Chapter IV), the abiotic release of nutrients by leaching dominates the balance and results in the exhaustion of nutrients in preconditioned OM. In arid floodplains, the nutrient depletion is maximum, as high temperatures and solar radiation further increase OM solubility (Bärlocher 1992, Gallo et al. 2009). Under these circumstances, nutrients from OM are not transferred to aquatic communities in the river but to terrestrial microorganisms present in floodplain soils, where they can be an important resource together with the leached DOC (Kalbitz et al. 2000, Cleveland et al. 2002).

In dry riverbeds the rain leaching of POM may mobilize nutrients and soluble C to surrounding sediments, where the biotic demand is diminished during drying. There, the combined increase of nutrient, C and water availability could explain isolated pulses of high microbial activity in sediments observed in dry rivers after short rain events (Arce et al. 2014, Timoner et al. 2014, Muñoz et al. 2018).

7.4. Elucidating the multiple role of solar radiation in OM fluxes in fluvial ecosystems

Within this dissertation we have talked about a positive (Chapter III) and a negative effect (Chapter V) of photodegradation on the OM biodegradability. Although this could sound contradictory at first glance, our results demonstrate that the effect of photodegradation depends on the fraction of OM (POM or DOM) affected by solar radiation. In Chapter III, photodegradation affected positively to wood biodegradability (POM fraction) by the decrease of its lignin content (see Austin & Ballaré 2010, Austin et al. 2016). Conversely, we detected a negative effect in Chapter V. However, the photodegradation effect was not measured on the POM substrates themselves (either plant litter or sediments), but on the DOM leached after preconditioning. In this case, the negative effect on leachates biodegradability was due to the accumulation of recalcitrant compounds such as polyphenols in them (see Baldwin 1999, Fellman et at l. 2013). We suggest the simplest explanation for this double role of photodegradation on POM/DOM biodegradability is that photodegradation breaks partially lignin and big polyphenols polymers in plant litter (increasing litter bioavailability) but causing the release of simple phenol monomers or smaller polymers (with low bioavailability), which are highly soluble in water (see Chatani et al. 2014). This hypothesis is partially supported by FT-ICR-MS results in Chapter V. These results show how the exposure of the macrophyte and reed leaf litter to intense solar radiation caused a huge release of aliphatic C compounds in leachates, we hypothesize by the photochemical breaking of big condensed aromatic structures naturally present in plant litter (see Table 5.4) (Stubbins et al. 2010).

Although they can seem opposite processes, solar radiation can break molecules but also polymerize them (Table 7.1) (see Chatani et al. 2014). In fact, results from Chapter V showed that. In addition to the mentioned photochemical breaking, solar radiation conducted the accumulation of big DOM compounds such as polyphenols and condensed aromatic structures in leachates (Table 5.4.), likely by the oxidative polymerization of simple phenolic compounds. This was also evidenced by the increase of DOM molecular mass in leachates (Fig. 5.5a).

Furthermore, previous studies show how polyphenol polymerization from simple aromatic compounds can be boosted by light (Chatani et al. 2014) but also by high temperatures (Goering & van Soest 1970, Makkar 2003). This suggests that in arid places, the effect of solar radiation on OM chemistry cannot be separated from the effect of high temperatures, and even we can confuse both. We suggest that solar radiation (together with its associated heat), can have multiple effects on OM fluxes in fluvial ecosystems, which will depend on the target OM fraction and the hydrological phase (Table 7.1.).

To elucidate the relative contribution of radiation and temperature, factorial laboratory experiments with all the possible combinations of light exposure and temperature should be made in the future.

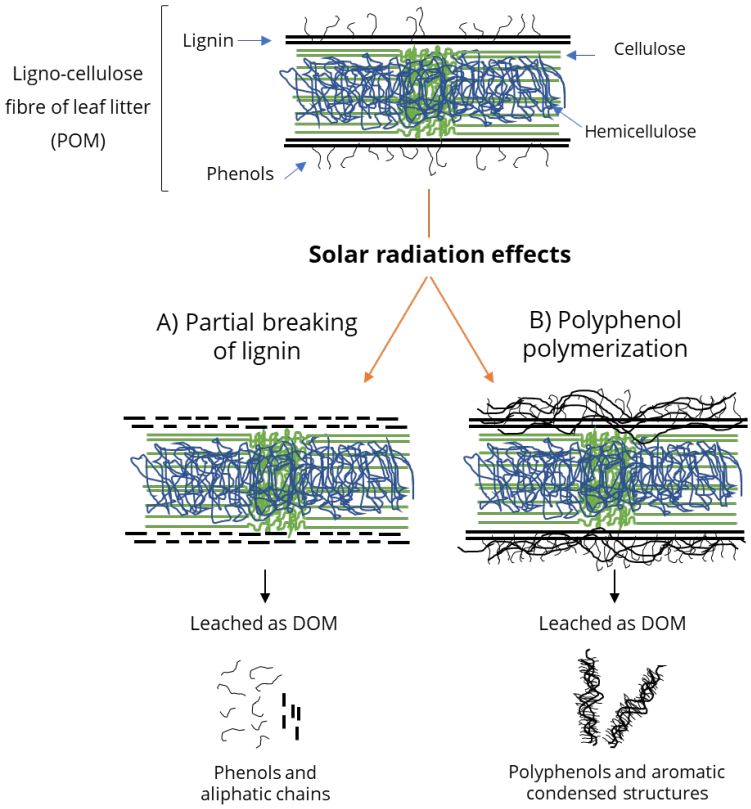

Figure 7.1 Simplified diagram showing potential effects of solar radiation (photodegradation + heat) on the chemical composition of POM and DOM fractions from leaf litter. Modified from Lee et al. 2014.

Table 7.1 Multiple effects and underlying causes of solar radiation on OM fluxes in fluvial ecosystems.

Hydrological phase	OM fraction	Effect on biodegradability	Underlying cause
Terrestrial	POM	+	Partial breaking of lignin enhances the access to cellulose by microorganisms
	DOM	-	Accumulation of humic, recalcitrant compounds in leachates
Aquatic	POM	+	Photodegradation of lignin*
	DOM	+	Breaking big aromatic molecules into smaller and more labile compounds

* So far as we know, there have been no other works showing a photo-facilitation of microbial decomposition in POM substrates exposed to light in aquatic systems, but there are evidences supporting the positive effects of photodegradation on mass and lignin loss (see Anesio et al. 1999 and Liu et al. 2016b).

7.5. Spatial heterogeneity along the dry phase modulates OM chemistry in intermittent rivers

Results from Chapters V and VI demonstrate the relevance of environmental conditions of dry riverbeds as modulators of OM chemical quality, but also the importance of environmental homogeneity or heterogeneity (see Fisher et al. 2007). Chapter V shows how different OM substrates (macrophytes, leaf litter and sediments) exposed on dry riverbeds to intense solar radiation and high temperatures undergoes a homogenization of the chemical quality of their leachates, independently of their origin (Fig. 5.5d). Such homogenization did not happen under shaded conditions. On the contrary, results from Chapter VI point out that the same leaf litter species preconditioned under heterogeneous habitat conditions experiences a great chemical diversification. The combination of these results suggests that, under the influence of an intense abiotic or biotic factor affecting OM,

uniform environmental conditions can homogenize OM quality, but heterogeneous conditions diversify it.

Environmental heterogeneity in intermittent rivers is maximised at the starting of the drying phase, when surface flow disconnects. At that moment, diverse aquatic and terrestrial habitats emerge in riverbeds (isolated or connected pools, irradiated or shaded areas, zones exposed to wet and dry cycles, etc.) not only through drying reaches, but also across tributaries into the same river network (Fig. 7.2). However, the temporal patterns of such heterogeneity along the river networks vary, mainly depending on climatic conditions. For instance, in humid Mediterranean areas, where evapotranspiration is not so high, habitat heterogeneity can be maintained over the whole dry phase until flow resumption (Fig. 7.2.). On the contrary, in arid and semiarid intermittent rivers, the extremely high summer temperatures promote a quick "evaporation" of habitats heterogeneity in days. Passing then to a more homogeneous and usually constant conditions of intense solar radiation along the river network till rewetting (Guerrero 2002). Therefore, diverse drying pattern in intermittent rivers (from different bioregions or with distinct hydrological dynamics) could shape the OM chemical quality in very different ways, and consequently its later processing when flow is re-established (Fig. 7.2.).

In the light of the results of Chapter V and VI, it is worth noting that the increase of temperature, evapotranspiration rates and solar radiation expected to occur under the climate change scenario (IPCC 2013), and mostly in Mediterranean areas, could notably affect the role of OM inputs in aquatic ecosystems. Similarly, our findings also could have implication in river management as riparian alteration and river bed homogenization are some of the common impacts of human activities in streams and rivers.

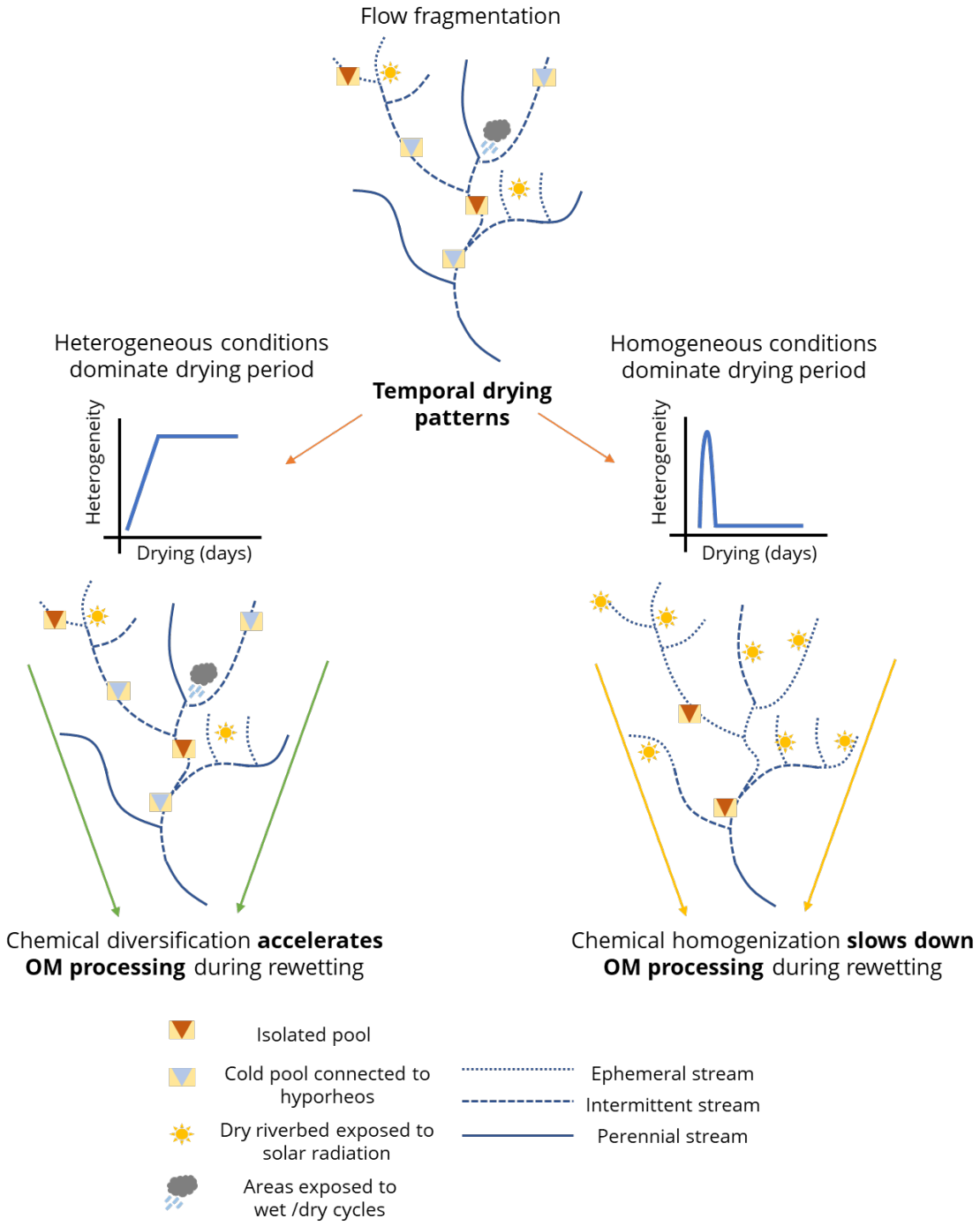

Figure 7.2 Diagram showing the drying temporal patterns in two intermittent river networks in a humid Mediterranean (left) and an arid region (right) and their consequences on the chemical composition of OM accumulated during the dry phase and the posterior OM processing during rewetting.

7.6. For a conceptual model of OM dynamics in arid rivers integrating terrestrial-aquatic biogeochemical interactions

POM dynamics in river networks of temperate and humid areas has been well known for so long (Webster et al. 1999, Battin et al. 2008). In these systems, most of the POM enters headwaters streams mainly as leaf litter from riparian canopy, where it is retained and decomposed till transported downstream, mainly by flood events. This cycle is repeated again and again until POM processing is complete. Larned et al. (2010), adapted this conceptual model from Battin et al. (2008) to intermittent rivers, including the idea that the dry phase halts decomposition and promotes the retention of POM on riverbeds till flow is re-established and the decomposition cycle starts again that is, as a pulse bioreactor (Larned et al. 2010). In intermittent arid rivers, POM dynamics does not differ from this model except: (i) woody litter can replace leaf litter as the main POM source, and (ii) the POM input, does not occur in forested headwaters, but diffusely all along the river network (Jacobson et al. 1999, Sponseller & Fisher 2006). POM processing is especially dependent on floods in arid rivers (Sponseller et al. 2013). This water pulses transport and relocate POM downstream in floodplains (Jacobson et al. 1999, Sponseller & Fisher 2006), at the same time that decomposition is reactivated.

Results obtained within this dissertation helps to complete these conceptual models with the biogeochemical processing of POM during its terrestrial-aquatic interaction. Within this thesis, we have seen that in arid, and likely in nutrient-poor floodplains, abiotic processes dominate over biotic ones during the preconditioning of OM (photodegradation > microbial activity; leaching > immobilization). As a result, remaining labile resources (nutrients and C) in preconditioned POM are quickly exhausted by aquatic microorganisms during the first days of immersion in the river. This, together with the initial low quality of wood, may cause the dominance of POM transport along the river network over the active, biotic processing (Fig. 7.3.). Consequently, POM stock would be merely mobilized along the river network in arid rivers, without significant losses, as lateral inputs from floodplains would counterbalance the low POM losses by biotic or abiotic processing.

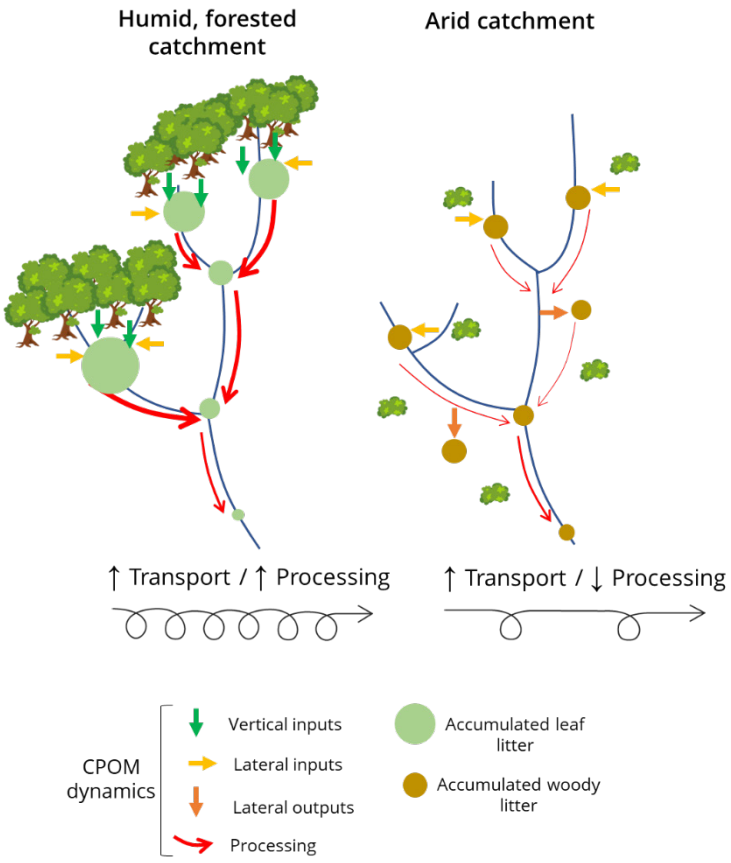

Figure 7.3 Hypothesized river networks showing differences in the POM dynamics in a humid, forested catchment (left) and an arid one (right). The size of circles represents the amount of POM accumulated in each location of the river network. The thickness of red arrows indicates the balance between POM processing and downstream transport (as thicker the arrows, the higher the processing). Note that differences are exacerbated according to processes and OM substrate dominating in each catchment type.

On the contrary, the propitious environmental conditions in more humid areas would promote the dominance of POM biotic processing during both the terrestrial and the aquatic phases. Therefore, in such ecosystems, POM entering as leaf litter bulks in headwaters, would be rapidly processed as they are transported downstream, consequently reducing POM stocks along the river network (Fig. 7.3).

Finally, it is worth noting that both hypothesized conceptual models of POM dynamics in humid and arid catchments explained above are largely in line with results from Chapter V about the processing of DOM leached from dry riverbeds in forested and arid rivers. This Chapter mainly underpins how intense solar radiation

and temperature in arid intermittent rivers can drastically reduce the biodegradability of DOM leached from dry riverbed, and thus foster its transport downstream, instead of its processing. Contrary to the above, this Chapter suggests that the riparian vegetation in forested intermittent rivers "protects" POM accumulated in dry riverbeds from the negative influence of solar radiation on its chemistry, thus keeping a high biodegradability of their leachates. Therefore, the conjunction of results of POM and DOM processing within this dissertation reinforces the idea of arid rivers as "passive pipes" of C, against the "active bioreactor" role assigned to forested rivers in humid regions.

Although we realise that these conceptual models lacks of essential determinants of OM fluxes such as land uses and vegetation types within the catchment (Laudon & Sponseller 2017), our results clearly differentiate the barely known OM dynamics in arid fluvial systems from those more temperate. Therefore, and considering the current expansion of arid lands as a result on-going global change (Reynolds et al. 2007), to continue investigating OM fluxes in arid systems is key for understand how this future scenario could affect C budget in fluvial ecosystems and, at the same time, adapting the future management of fluvial ecosystems worldwide.

7.7. Next steps and future directions

Although this dissertation underpins the relevance of terrestrial-aquatic interactions on OM processing and dynamics in fluvial ecosystems, three main handicaps remain unresolved over these compilations of works: (a) understanding the effect of OM preconditioning on the whole ecosystem functioning, principally after the rewetting of intermittent rivers; and (b) the upscaling of terrestrial-aquatic interaction to catchment level. As reiterated previously, allochthonous OM is not only a resource for decomposer communities in rivers, but also a source of nutrients and C for the rest of river food webs components. To address that, we have already performed a BACI (before-after, control-impact) experiment "in situ" where we analysed the functional river response to the immersion of a high bulk of leaves with contrasting chemical quality as result of different preconditioning stories.

The other important missing point in this thesis is to contextualize the effect of preconditioned OM (from floodplains and from dry riverbeds) in river networks, as well as the main controlling drivers. To achieve this, experiments at sub-catchment level have been designed to test for the contribution of OM accumulated on dry headwater tributaries to OM fluxes and metabolism downstream when tributary and main channel are connected.

8. Conclusions

Organic matter accumulated in terrestrial-aquatic interfaces

Chapter III. Exposure of wood in floodplains affects its chemical quality and its subsequent breakdown in streams

- Abiotic factors during the exposure or "preconditioning" of wood in arid floodplains altered severely its chemical composition mainly by (i) the depletion of soluble nutrients such as P or K through rain leaching and (ii) the reduction of lignin content by photodegradation.
- Because of the lignin reduction together with the colonization by terrestrial fungi, preconditioned wood underwent a different decomposition pattern regarding non-preconditioned wood, with an early and short pulse of microbial decomposition during the first week of wood immersion in rivers, but a decrease afterwards.
- Wood preconditioning in floodplains would change its role from a long-lasting resource for freshwater food webs, to a short-term resource, due to the reduction of nutrients and C availability for heterotrophic aquatic communities.

Chapter IV. Linking terrestrial and aquatic carbon processing: Environmental conditions of floodplains control the fate of leaf litter inputs in rivers

- Environmental conditions (mainly climate and soil nutrient content) drove differently the chemical alteration of leaf litter during its accumulation in floodplain habitats, but did not affect the chemical composition of their leachates, which undergo a generalized depletion of nutrients and labile DOC independently of floodplain conditions.
- Terrestrial preconditioning promoted a great depreciation of leaf litter leachates as an energetic and nutrient source for aquatic heterotrophic communities, but had a variable effect on leaf litter decomposability depending on previous preconditioning history.
- The net balance between the release of nutrients by rain leaching and nutrient microbial immobilization during leaf litter exposure in floodplains arose as the main driver of later aquatic microbial decomposition

Chapter V. Dry phase conditions prime wet-phase dissolved organic matter dynamics in intermittent rivers

- Solar radiation and heat promoted the solubilization of DOC and nutrients from DOM sources (leaf litter, macrophytes or sediments) accumulated in dry riverbeds, consequently favoring its later release during a storm event. These results suggest that sporadic rains during the dry phase can generate short pulses of nutrient and DOC to surrounding dry sediments, which can derive in hot moments of microbial activity under conditions of moisture availability.
- The combination of heat and solar radiation under open-canopy conditions resulted in a high accumulation of aromatic and recalcitrant DOM from all DOM sources, independently of their origin. Therefore, OM accumulated in open-canopy intermittent rivers are subjected to both processes: (i) a high decrease of their leached DOM biodegradability, and (ii) to chemical homogenization.
- The exposure of DOM sources under closed-canopy conditions promoted the maintaining of the high quality and biodegradability of their leachates during their dry phase.
- Results from this chapter identify the canopy cover of riverbeds as a major modulator of the biodegradability of leachates from OM accumulated during the dry phase, and consequently of their aquatic processing after the re-establishment of river surface flow.

Chapter VI. Flow intermittence alters carbon processing in rivers through the chemical diversification of leaf litter

- Flow fragmentation can drive the emergence of a heterogeneous mosaic of terrestrial and aquatic habitats in intermittent rivers during the drying phase.
- The spatial heterogeneity of environmental conditions along reaches and across the river network results in multiple preconditioning situations, which

ultimately promote the chemical diversification of leaf litter accumulated along the intermittent riverbeds.

- During flow resumption, the diversified leaf litter is mixed and transported downstream, where chemical diversity become the main driver of decomposition rates. Chemical divergence of leaf litter mixtures promoted an acceleration of decomposition as a result of synergistic reactions among leaf litter components, which stimulated microbial and detritivores action.

Chapter VII. General implications

- The dry phase of intermittent rivers and periods of OM accumulation on floodplain soils are not static, but active periods of chemical alteration of OM.
- Contrasting effects of terrestrial preconditioning on OM biodegradability in arid and humid regions can lead to great differences in OM processing at river network scale between both regions.
- Results of this dissertation highlight the relevance of incorporating terrestrial-aquatic interactions in current models of OM fluxes in fluvial ecosystems. Otherwise, models could be overestimating the capacity of rivers to degrade allochthonous OM inputs, since (i) they are partially processed during its terrestrial exposure in the floodplains or dry riverbeds before acceding the water column, and (ii) this preconditioning alters severely its later processing in rivers.
- Future research about the implications of terrestrial-aquatic interactions on complete aquatic food webs and OM fluxes at catchment scale are necessary to contextualize the real implications of findings described within this dissertation at larger temporal and spatial scales in river networks.

References

Abril, M., I. Muñoz, and M. Menéndez. 2016. Heterogeneity in leaf litter decomposition in a temporary Mediterranean stream during flow fragmentation. Science of the Total Environment 553:330-339.

Abril, M., I. Muñoz, J. P. Casas-Ruiz, L. Gómez-Gener, M. Barceló, F. Oliva, and M. Menéndez. 2015. Effects of water flow regulation on ecosystem functioning in a Mediterranean river network assessed by wood decomposition. Science of The Total Environment 517:57–65.

Acuña, V., A. Giorgi, I. Muñoz, F. Sabater, and S. Sabater. 2007. Meteorological and riparian influences on organic matter dynamics in a forested Mediterranean stream. Journal of the North American Benthological Society 26:54-69.

Acuña, V., and K. Tockner. 2010. The effects of alterations in temperature and flow regime on organic carbon dynamics in Mediterranean river networks. Global Change Biology 16:2638-2650.

Acuña, V., T. Datry, J. Marshall, D. Barceló, C. N. Dahm, A. Ginebreda, G. McGregor, S. Sabater, K. Tockner, and M. A. Palmer. 2014. Why should we care about temporary waterways? Science 343:1080.

Aerts, R. 1997. Climate, Leaf Litter Chemistry and Leaf Litter Decomposition in Terrestrial Ecosystems: A Triangular Relationship. Oikos 79:439-449.

Aerts, R. 2006. The freezer defrosting: global warming and litter decomposition rates in cold biomes. Journal of Ecology 94:713-724.

Aitkenhead-Peterson, J. A., W. H. McDowell, and J. C. Neff. 2003. Sources, Production, and Regulation of Allochthonous Dissolved Organic Matter Inputs to Surface Waters. Pages 25-70 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Allan, J. D., and M. M. Castillo. 2007. Detrital energy sources. Pages 135-161 *in* J. D. Allan and M. M. Castillo, editors. Stream Ecology: Structure and function of running waters. Springer Netherlands, Dordrecht.

Amon, R. M. W., and R. Benner. 1996. Bacterial utilization of different size classes of dissolved organic matter. Limnology and Oceanography 41:41-51.

Anesio Alexandre, M., J. Tranvik Lars, and W. Granéli. 1999. Production of inorganic carbon from aquatic macrophytes by solar radiation. Ecology 80:1852-1859.

APHA. 2005. Standard Methods for the Examination of Water and Wastewater. American Public Health Association. 21st edition, Washington DC.

Araujo, P. I., L. Yahdjian, and A. T. Austin. 2012. Do soil organisms affect aboveground litter decomposition in the semiarid Patagonian steppe, Argentina. Oecologia 168:221-230.

Arce, M. I., M. d. M. Sánchez-Montoya, M. R. Vidal-Abarca, M. L. Suárez, and R. Gómez. 2014. Implications of flow intermittency on sediment nitrogen availability and processing rates in a Mediterranean headwater stream. Aquatic Sciences 76:173-186.

Arroita, M., I. Aristi, L. Flores, A. Larrañaga, J. Díez, J. Mora, A. M. Romaní, and A. Elosegi. 2012. The use of wooden sticks to assess stream ecosystem functioning: Comparison with leaf breakdown rates. Science of The Total Environment 440:115–122.

Aufdenkampe Anthony, K., E. Mayorga, A. Raymond Peter, M. Melack John, C. Doney Scott, R. Alin Simone, E. Aalto Rolf, and K. Yoo. 2011. Riverine coupling of biogeochemical cycles between land, oceans, and atmosphere. Frontiers in Ecology and the Environment 9:53-60.

Austin, A. T. 2011. Has water limited our imagination for aridland biogeochemistry? Trends in Ecology & Evolution 26:229 - 235.

Austin, A. T., and C. L. Ballaré. 2010. Dual role of lignin in plant litter decomposition in terrestrial ecosystems. PNAS 107:4618-4622.

Austin, A. T., and L. Vivanco. 2006. Plant litter decomposition in a semi-arid ecosystem controlled by photodegradation. Nature 442:555.

Austin, A. T., M. S. Méndez, and C. L. Ballaré. 2016. Photodegradation alleviates the lignin bottleneck for carbon turnover in terrestrial ecosystems. Proceedings of the National Academy of Sciences 113:4392-4397.

Baldwin, D. S. 1999. Dissolved organic matter and phosphorus leached from fresh and 'terrestrially' aged river red gum leaves: implications for assessing river–floodplain interactions. Freshwater Biology 41:675-685.

Bärlocher, F. 1992. Effects of drying and freezing autumn leaves on leaching and colonization by aquatic hyphomycetes. Freshwater Biology 28:1-7.

Bärlocher, F. 2005. Leaf Mass Loss Estimated by Litter Bag Technique. Pages 37-42

in M. A. S. Graça, F. Bärlocher, and M. O. Gessner editors. Methods to Study Litter Decomposition. Springer Netherlands, Dordrecht.

Bärlocher, F., and B. Kendrick. 1974. Dynamics of the fungal population on leaves in a stream. Journal of Ecology 62:761-791.

Bärlocher, F., and L. Boddy. 2016. Aquatic fungal ecology – How does it differ from terrestrial? Aquatic Fungi 19:5-13.

Bartón, K. 2016. MuMIn: Multi-Model Inference. R Package Version1.15.6., https://cran.r-project.org/web/packages/MuMIn/index.html.

Battin, T. J., L. A. Kaplan, J. D. Newbold, and S. P. Hendricks. 2003. A mixing model analysis of stream solute dynamics and the contribution of a hyporheic zone to ecosystem function. Freshwater Biology 48:995-1014.

Battin, T. J., L. A. Kaplan, S. Findlay, C. S. Hopkinson, E. Marti, A. I. Packman, J. D. Newbold, and F. Sabater. 2008. Biophysical controls on organic carbon fluxes in fluvial networks. Nature Geoscience 1:95-100.

Bechtold, J. S., R. T. Edwards, and R. J. Naiman. 2003. Biotic versus hydrologic control over seasonal nitrate leaching in a floodplain forest. Biogeochemistry 63:53-72.

Beer, C., M. Reichstein, E. Tomelleri, P. Ciais, M. Jung, N. Carvalhais, C. Rödenbeck, M. A. Arain, D. Baldocchi, G. B. Bonan, A. Bondeau, A. Cescatti, G. Lasslop, A. Lindroth, M. Lomas, S. Luyssaert, H. Margolis, K. W. Oleson, O. Roupsard, E. Veenendaal, N. Viovy, C. Williams, F. I. Woodward, and D. Papale. 2010. Terrestrial Gross Carbon Dioxide Uptake: Global Distribution and Covariation with Climate. Science 329:834.

Bell, D. T., and S. K. Sipp. 1975. The Litter Stratum in the Streamside Forest Ecosystem. Oikos 26:391-397.

Bell, D. T., F. L. Johnson, and A. R. Gilmore. 1978. Dynamics of Litter Fall, Decomposition, and Incorporation in the Streamside Forest Ecosystem. Oikos 30:76-82.

Benfield, E. F. 1997. Comparison of litterfall input to streams. Journal of the North American Benthological Society 16:104-108.

Berg, B., and C. McClaugherty. 2003. Plant litter. Springer-Verlag Berlin Heidelberg.

Berggren, M., and P. A. del Giorgio. 2015. Distinct patterns of microbial metabolism associated with riverine dissolved organic carbon of different source and quality. Journal of Geophysical Research: Biogeosciences 120:989-999.

Bernal, S., D. von Schiller, F. Sabater, and E. Martí. 2013. Hydrological extremes modulate nutrient dynamics in mediterranean climate streams across different spatial scales. Hydrobiologia 719:31-42.

Bernhardt, E. S., and W. H. McDowell. 2008. Twenty years apart: Comparisons of DOM uptake during leaf leachate releases to Hubbard Brook Valley streams in 1979 versus 2000. Journal of Geophysical Research: Biogeosciences 113.

Bertilsson, S., and J. B. Jones Jr. 2003. Supply of dissolved organic matter to aquatic ecosystems: Autochthonous sources. Pages 3-24 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Bianchi, T. S., D. Butman, P. A. Raymond, N. D. Ward, R. J. S. Kates, K. W. Flessa, H. Zamora, A. R. Arellano, J. Ramirez, and E. Rodriguez. 2017. The experimental flow to the Colorado River delta: Effects on carbon mobilization in a dry watercourse. Biogeosciences 122:607-627.

Blair, J. M. 1988. Nutrient release from decomposing foliar litter of three tree species with spicial reference to calcium, magnesium and potassium dynamics. Plant and Soil 110:49-55.

Bonan, G. B. 2008. Forests and climate change: Forcings, feedbacks, and the climate benefits of forests. Science 320:1444.

Boreen, A. L., B. L. Edhlund, J. B. Cotner, and K. McNeill. 2008. Indirect photodegradation of dissolved free amino acids: The contribution of singlet oxygen and the differential reactivity of DOM from various sources. Environmental Science & Technology 42:5492-5498.

Box, G. E. P. 1954. Some Theorems on Quadratic Forms Applied in the Study of Analysis of Variance Problems, I. Effect of Inequality of Variance in the One-Way Classification. The Annals of Mathematical Statistics 25:290-302.

Boyero, L., M. A. S. Graça, A. M. Tonin, J. Pérez, A. Swafford, V. Ferreira, A. Landeira-Dabarca, M. Alexandrou, M. O. Gessner, B. G. McKie, R. J. Albariño, L. A. Barmuta, M. Callisto, J. Chará, E. Chauvet, C. Colón-Gaud, D. Dudgeon, A. C. Encalada, R. Figueroa, A. S. Flecker, T. Fleituch, A.

Frainer, J. F. G. Jr., J. E. Helson, T. Iwata, J. Mathooko, C. M'Erimba, C. M. Pringle, A. Ramírez, C. M. Swan, C. M. Yule, and R. G. Pearson. 2017. Riparian plant litter quality increases with latitude. Scientific Reports 7.

Boyero, L., R. G. Pearson, C. Hui, M. O. Gessner, J. Pérez, M. A. Alexandrou, M. A. S. Graça, B. J. Cardinale, R. J. Albariño, M. Arunachalam, L. A. Barmuta, A. J. Boulton, A. Bruder, M. Callisto, E. Chauvet, R. G. Death, D. Dudgeon, A. C. Encalada, V. Ferreira, R. Figueroa, A. S. Flecker, J. F. Gonçalves, J. Helson, T. Iwata, T. Jinggut, J. Mathooko, C. Mathuriau, C. Erimba, M. S. Moretti, C. M. Pringle, A. Ramírez, L. Ratnarajah, J. Rincon, and C. M. Yule. 2016. Biotic and abiotic variables influencing plant litter breakdown in streams: a global study. Proceedings of the Royal Society B: Biological Sciences 283.

Bradford, M. A., B. Berg, D. S. Maynard, W. R. Wieder, and S. A. Wood. 2016. Understanding the dominant controls on litter decomposition. Journal of Ecology 104:229-238.

Brandt, L. A., C. Bohnet, and J. Y. King. 2009. Photochemically induced carbon dioxide production as a mechanism for carbon loss from plant litter in arid ecosystems. Journal of Geophysical Research: Biogeosciences 114:G02004.

Brandt, L. A., J. Y. King, S. E. Hobbie, D. G. Milchunas, and R. L. Sinsabaugh. 2010. The role of photodegradation in surface litter decomposition across a grassland ecosystem precipitation gradient. Ecosystems 13:765-781.

Bruder, A., E. Chauvet, and M. O. Gessner. 2011. Litter diversity, fungal decomposers and litter decomposition under simulated stream intermittency. Functional Ecology 25:1269-1277.

Bruder, A., M. H. Schindler, M. S. Moretti, and M. O. Gessner. 2014. Litter decomposition in a temperate and a tropical stream: the effects of species mixing, litter quality and shredders. Freshwater Biology 59:438-449.

Bruno, D., O. Belmar, D. Sánchez-Fernández, and J. Velasco. 2014. Environmental determinants of woody and herbaceous riparian vegetation patterns in a semi-arid mediterranean basin. Hydrobiologia 730:45-57.

Burnham, K. P., and D. R. Anderson. 2002. Model Selection and Multimodel Inference: A Practical Information–Theoretic Approach. Springer, New

York.

Casas-Ruiz, J. P., J. Tittel, D. von Schiller, N. Catalán, B. Obrador, L. Gómez-Gener, E. Zwirnmann, S. Sabater, and R. Marcé. 2016. Drought-induced discontinuities in the source and degradation of dissolved organic matter in a Mediterranean river. Biogeochemistry 127:125-139.

Casas-Ruiz, J. P., N. Catalán, L. Gómez-Gener, D. von Schiller, B. Obrador, D. N. Kothawala, P. López, S. Sabater, and R. Marcé. 2017. A tale of pipes and reactors: Controls on the in-stream dynamics of dissolved organic matter in rivers. Limnology and Oceanography 62:S85-S94.

Catalán, N., R. Marcé, D. N. Kothawala, and L. J. Tranvik. 2016. Organic carbon decomposition rates controlled by water retention time across inland waters. Nature Geoscience 9:501.

Cebrian, J. 1999. Patterns in the Fate of Production in Plant Communities. The American Naturalist 154:449-468.

Chatani, S., C. J. Kloxin, and N. Bowman. 2014. The power of light in polymer science: photochemical processes to manipulate polymer formation, structure, and properties. Polymer Chemistry 5:2187-2201.

Cheever, B. M., and J. R. Webster. 2014. Effects of consumers and nitrogen availability on heterotrophic microbial activity during leaf decomposition in headwater streams. Freshwater Biology 59:1768-1780.

Chomel, M., M. Guittonny-Larchevêque, C. Fernandez, C. Gallet, A. DesRochers, D. Paré, G. Jackson Benjamin, and V. Baldy. 2016. Plant secondary metabolites: a key driver of litter decomposition and soil nutrient cycling. Journal of Ecology 104:1527-1541.

Claret, C., and A. J. Boulton. 2003. Diel variation in surface and subsurface microbial activity along a gradient of drying in an Australian sand-bed stream. Freshwater Biology 48:1739-1755.

Cleveland, C. C., A. R. Townsend, and S. K. Schmidt. 2002. Phosphorus Limitation of Microbial Processes in Moist Tropical Forests: Evidence from Short-term Laboratory Incubations and Field Studies. Ecosystems 5:0680-0691.

Cleveland, C. C., J. C. Neff, A. R. Townsend, and E. Hood. 2004. Composition, dynamics, and fate of leached dissolved organic matter in terrestrial

ecosystems: Results from a decomposition experiment. Ecosystems 7:175-285.

Cleveland, C. C., S. C. Reed, A. B. Keller, D. R. Nemergut, S. P. O'Neill, R. Ostertag, and P. M. Vitousek. 2014. Litter quality versus soil microbial community controls over decomposition: a quantitative analysis. Oecologia 174:283-294.

Cole, J. J., Y. T. Prairie, N. F. Caraco, W. H. McDowell, L. J. Tranvik, R. G. Striegl, C. M. Duarte, P. Kortelainen, J. A. Downing, J. J. Middelburg, and J. Melack. 2007. Plumbing the Global Carbon Cycle: Integrating Inland Waters into the Terrestrial Carbon Budget. Ecosystems 10:172-185.

Confederación Hidrográfica del Segura. 2007. Estudio general sobre la demarcación hidrográfica del Segura. Ministry of Environment, Murcia, Spain.

Cordova, J. M., E. J. Rosi-Marshall, J. L. Tank, and G. A. Lamberti. 2008. Coarse particulate organic matter transport in low-gradient streams of the Upper Peninsula of Michigan. Journal of the North American Benthological Society 27:760-771.

Cornut, J., V. Ferreira, A. L. Gonçalves, E. Chauvet, and C. Canhoto. 2015. Fungal alteration of the elemental composition of leaf litter affects shredder feeding activity. Freshwater Biology:n/a-n/a.

Corti, R., and T. Datry. 2012. Invertebrates and sestonic matter in an advancing wetted front travelling down a dry river bed (Albarine, France) - Portail documentaire. Freshwater Science 31:1187-1201.

Corti, R., T. Datry, L. Drummond, and S. T. Larned. 2011. Natural variation in immersion and emersion affects breakdown and invertebrate colonization of leaf litter in a temporary river. Aquatic Sciences 73:537-550.

Craven, P., and G. Wahba. 1978. Smoothing noisy data with spline functions. Numerische Mathematik 31:377-403.

Cummins, W. T. 1974. The importance of different energy sources in freshwater ecosystems. Pages 50-54 *in* D. E. Reichle, J. F. Franklin, and D. W. Goodall, editors. Productivity of world ecosystems. Proceedings of the National Academy of Sciences, Washington D.C.

Dang C. K., E. Chauvet, and M. O. Gessner. 2005. Magnitude and variability of

process rates in fungal diversity-litter decomposition relationships. Ecology Letters 8:1129-1137.

Danger, M., M. O. Gessner, and F. Bärlocher. 2015. Ecological stoichiometry of aquatic fungi: current knowledge and perspectives. Fungal Ecology.

Datry, T., A. Foulquier, R. Corti, D. von, Schiller, K. Tockner, C. Mendoza-Lera, J., C. Clement, M. O. Gessner, M. Moleon, R. Stubbington, B. Gucker, R. Albarino, D., C. Allen, F. Altermatt, M. I. Arce, S. Arnon, D. Banas, A. Banegas-Medina, E., Beller, M. L. Blanchette, J. F. Blanco-Libreros, J. J. Blessing, I. G. Boechat, K. S. Boersma, M. T. Bogan, N. Bonada, N. R. Bond, K. C. B. Barria, A., Bruder, R. M. Burrows, T. Cancellario, C. Canhoto, S. M. Carlson, S., Cauvy-Fraunie, N. Cid, M. Danger, T. B. d. Freitas, A. M. D. Girolamo, B. E, d. La, R. d. Campo, V. D. Diaz-Villanueva, F. Dyer, A. Elosegi, E. Faye, C., Febria, B. Four, S. Gafny, S. D. Ghate, R. Gomez, L. Gomez-Gener, M. A. S., Graca, S. Guareschi, F. Hoppeler, J. Hwan, J. I. Jones, S. Kubheka, A. Laini, S. D. Langhans, C. Leigh, C. J. Little, S. Lorenz, J. C. Marshall, E. Martin, A., R. McIntosh, E. I. Meyer, M. Miliša, M. C. Mlambo, M. Morais, N. Moya, P. M., Negus, D. K. Niyogi, A. Papatheodoulou, I. Pardo, P. Pařil, S. U. Pauls, V., Pešić, M. Polašek, C. T. Robinson, P. Rodriguez-Lozano, R. J. Rolls, M. M., Sanchez-Montoya, A. Savić, O. Shumilova, K. R. Sridhar, A. L. Steward, R., Storey, A. Taleb, A. Uzan, R. V. Vorste, N. J. Waltham, C. Woelfle-Erskine, D. Zak, C. Zarfl, and A. Zoppini. 2018. A global analysis of terrestrial plant litter dynamics in non-perennial waterways. *Nature Geoscience*.

Datry, T., R. Corti, C. Claret, and M. Philippe. 2011. Flow intermittence controls leaf litter breakdown in a French temporary alluvial river: the "drying memory". Aquatic Sciences 73:471-483.

Datry, T., R. Corti, J. Heino, B. Hugueny, R. J. Rolls, and A. Ruhí. 2017. Habitat Fragmentation and Metapopulation, Metacommunity, and Metaecosystem Dynamics in Intermittent Rivers and Ephemeral Streams. Pages 377-403 *in* T. Datry, N. Bonada, and A. Boulton, editors. Intermittent Rivers and Ephemeral Streams: Ecology and Managment. Academic Press.

Datry, T., S. T. Larned, and K. Tockner. 2014. Intermittent rivers: A challenge for

freshwater ecology. BioScience 64:229-235.

De Haan, H., and T. De Boer. 1987. Applicability of light absorbance and fluorescence as measures of concentration and molecular size of dissolved organic carbon in humic Lake Tjeukemeer. Water Research 21:731-734.

del Campo, R., and R. Gómez. 2016. Exposure of wood in floodplains affects its chemical quality and its subsequent breakdown in streams. Science of The Total Environment 543:652-661.

del Giorgio, P. A., and J. Davis. 2003. Patterns in Dissolved Organic Matter Lability and Consumption across Aquatic Ecosystems. Pages 399-424 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Delgado-Baquerizo, M., P. García-Palacios, R. Milla, A. Gallardo, and F. T. Maestre. 2015. Soil characteristics determine soil carbon and nitrogen availability during leaf litter decomposition regardless of litter quality. Soil Biology & Biochemistry 81:134-142.

Dent, D. H., R. Bagchi, D. Robinson, N. Majalap-Lee, and D. F. R. P. Burslem. 2006. Nutrient fluxes via litterfall and leaf litter decomposition vary across a gradient of soil nutrient supply in a lowland tropical rain forest. Plant and Soil 288:197-215.

Dieter, D., D. von Schiller, E. M. García-Roger, M. M. Sánchez-Montoya, R. Gómez, J. Mora-Gómez, F. Sangiorgio, J. Gelbrecht, and K. Tockner. 2011. Preconditioning effects of intermittent stream flow on leaf litter decomposition. Aquatic Sciences 73:599-609.

Dieter, D., K. Frindte, A. Krüger, and C. Wurzbacher. 2013. Preconditioning of leaves by solar radiation and anoxia affects microbial colonisation and rate of leaf mass loss in an intermittent stream. Freshwater Biology 58:1981-1931.

Díez, J., A. Elosegi, E. Chauvet, and J. Pozo. 2002. Breakdown of wood in the Agüera stream. Freshwater Biology 47:2205-2215.

Dighton, J., M. Mascarenhas, and G. A. Arbuckle-Keil. 2001. Changing resources: assessment of leaf litter carbohydrate resource change at a microbial scale of resolution. Soil Biology & Biochemistry 33:1429-1432.

Dirks, I., Y. Navon, D. Kanas, R. Dumbur, and J. M. Grünzweig. 2010. Atmospheric

water vapor as driver of litter decomposition in Mediterranean shrubland and grassland during rainless seasons. Global Change Biology 16:2799-2812.

Döll, P., and H. M. Schmied. 2012. How is the impact of climate change on river flow regimes related to the impact on mean annual runoff? A global-scale analysis. Environmental Research Letters 7:14-37.

Duarte, S., C. Pascoal, A. Alves, A. Correia, and F. Cássio. 2010. Assessing the dynamic of microbial communities during leaf decomposition in a low-order stream by microscopic and molecular techniques. Microbiological Research 165:351–362.

Duarte, S., J. Mora-Gómez, M. Romaní Anna, F. Cássio, and C. Pascoal. 2017. Responses of microbial decomposers to drought in streams may depend on the environmental context. Environmental Microbiology Reports 9:756-765.

Duboc, O., F. Zehetner, I. Djukic, M. Tatzber, T. W. Berger, and M. H. Gerzabek. 2012. Decomposition of European beech and Black pine foliar litter along an Alpine elevation gradient: Mass loss and molecular characteristics. Geoderma 189-190:522-531.

Elosegi, A., J. Díez, and J. Pozo. 2007. Contribution of dead wood to the carbon flux in forested streams. Earth Surface Processes and Landforms 32:1219-1228.

Enríquez, S., C. M. Duarte, and K. Sand-Jensen. 1993. Patterns in decomposition rates among photosynthetic organisms: the importance of detritus C:N:P content. Oecologia 94:457-471.

FAO. 2006. Guidelines for soil description, Rome.

Feld, C. K., P. Segurado, and C. Gutiérrez-Cánovas. 2016. Analysing the impact of multiple stressors in aquatic biomonitoring data: A 'cookbook' with applications in R. Science of the Total Environment 573:1320-1339.

Fellman Jason, B., E. Hood, and G. M. Spencer Robert. 2010. Fluorescence spectroscopy opens new windows into dissolved organic matter dynamics in freshwater ecosystems: A review. Limnology and Oceanography 55:2452-2462.

Fellman, J. B., K. C. Petrone, and P. F. Grierson. 2013. Leaf litter age, chemical quality, and photodegradation control the fate of leachate dissolved organic

matter in a dryland river. Journal of Arid Environments 89:30–37.

Feng, X., K. M. Hills, A. J. Simpson, J. K. Whalen, and M. J. Simpson. 2011. The role of biodegradation and photo-oxidation in the transformation of terrigenous organic matter. Organic Geochemistry 42:262-274.

Fernandes, I., S. Seena, C. Pascoal, and F. Cássio. 2014. Elevated temperature may intensify the positive effects of nutrients on microbial decomposition in streams. Freshwater Biology 59:2390-2399.

Findlay, S. 2003. Bacterial Response to Variation in Dissolved Organic Matter. Pages 363-379 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Fischer, H. 2003. The Role of Biofilms in the Uptake and Transformation of Dissolved Organic Matter. Pages 285-313 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Fisher, S. G., and G. E. Likens. 1973. Energy flow in Bear Brook, New Hampshire: an integrative approach to stream ecosystem metabolism. Ecological Monographs 43:421:439.

Fisher, S. G., J. B. Heffernan, R. A. Sponseller, and J. R. Welter. 2007. Functional ecomorphology: Feedbacks between form and function in fluvial landscape ecosystems. Geomorphology 89:84-96.

Foereid, B., J. Bellarby, W. Meier-Augenstein, and H. Kemp. 2010. Does light exposure make plant litter more degradable? Plant and Soil 333:275-285.

Foulquier, A., J. Artigas, S. Pesce, and T. Datry. 2015. Drying responses of microbial litter decomposition and associated fungal and bacterial communities are not affected by emersion frequency. Freshwater Science 34:1233-1244.

Frainer, A., J. Jabiol, M. O. Gessner, A. Bruder, E. Chauvet, and G. McKie Brendan. 2015a. Stoichiometric imbalances between detritus and detritivores are related to shifts in ecosystem functioning. Oikos 125:861-871.

Frainer, A., M. S. Moretti, W. Xu, and M. O. Gessner. 2015b. No evidence for leaf-trait dissimilarity effects on litter decomposition, fungal decomposers, and nutrient dynamics. Ecology 96:550-561.

France, R., H. Culbert, C. Freeborough, and R. Peters. 1997. Leaching and early mass loss of boreal leaves and wood in oligotrophic water. Hydrobiologia

345:209-214.

Frost, P. C., J. H. Larson, L. E. Kinsman, G. A. Lamberti, and S. D. Bridgham. 2005b. Attenuation of ultraviolet radiation in streams of northern Michigan. Journal of the North American Benthological Society 24:246-255.

Frost, P. C., M. A. Evans-White, Z. V. Finkel, T. C. Jensen, and V. Matzek. 2005a. Are you what you eat? Physiological constraints on organismal stoichiometry in an elementally imbalanced world. Oikos 109:18-28.

Fukami, T., I. A. Dickie, J. Paula Wilkie, B. C. Paulus, D. Park, A. Roberts, P. K. Buchanan, and R. B. Allen. 2010. Assembly history dictates ecosystem functioning: evidence from wood decomposer communities. Ecology Letters 13:675-684.

Gadd G, M. 1993. Interactions of fungip with toxic metals. New Phytologist 124:25-60.

Gallo, E. L., K. A. Lohse, C. M. Ferlin, T. Meixner, and P. D. Brooks. 2014. Physical and biological controls on trace gas fluxes in semi-arid urban ephemeral waterways. Biogeochemistry 121:189-207.

Gallo, M. E., A. Porras-Alfaro, K. J. Odenbach, and R. L. Sinsabaugh. 2009. Photoacceleration of plant litter decomposition in an arid environment. Soil Biology & Biochemistry 41:1433-1441.

García-Palacios, P., E. A. Shaw, H. Wall Diana, and S. Hättenschwiler. 2016. Temporal dynamics of biotic and abiotic drivers of litter decomposition. Ecology Letters 19:554-563.

García-Palacios, P., G. McKie Brendan, T. Handa Ira, A. Frainer, and S. Hättenschwiler. 2015. The importance of litter traits and decomposers for litter decomposition: a comparison of aquatic and terrestrial ecosystems within and across biomes. Functional Ecology 30:819-829.

Gartner, T. B., and Z. G. Cardon. 2004. Decomposition dynamics in mixed-species leaf litter. Oikos 104:230-246.

Gavazov, K., R. Mills, T. Spiegelberger, J. Lenglet, and A. Buttler. 2014. Biotic and Abiotic Constraints on the Decomposition of Fagus sylvatica Leaf Litter Along an Altitudinal Gradient in Contrasting Land-Use Types. Ecosystems 17:1326-1337.

German, D. P., M. N. Weintraub, A. S. Grandy, C. L. Lauber, Z. L. Rinkes, and S. D. Allison. 2011. Optimization of hydrolytic and oxidative enzyme methods for ecosystem studies. Soil Biology & Biochemistry 43:1387-1397.

Gessner, M. O. 2005. Ergosterol as a measure of fungal biomass.*in* M. Graça, F. Bärlocher, and M. O. Gessner, editors. Methods to Study Litter Decomposition. A Practical Guide. Springer, The Netherlands.

Gessner, M. O., and E. Chauvet. 1994. Importance of Stream Microfungi in Controlling Breakdown Rates of Leaf Litter. Ecology 75:1807-1817.

Gessner, M. O., C. M. Swan, C. K. Dang, B. G. McKie, R. D. Bardgett, D. H. Wall, and S. Hättenschwiler. 2010. Diversity meets decomposition. Trends in Ecology & Evolution 25:372-380.

Gillian, A., and H. Duncan. 2001. Development of a sensitive and rapid method for the measurement of total microbial activity using fluorescein diacetate (FDA) in a range of soils. Soil Biology & Biochemistry 33:943-951.

Gliksman, D., A. Rey, R. Seligmann, R. Dumbur, O. Sperling, Y. Navon, S. Haenel, P. De Angelis, A. Arnone John, and M. Grünzweig José. 2016. Biotic degradation at night, abiotic degradation at day: positive feedbacks on litter decomposition in drylands. Global Change Biology 23:1564-1574.

Goering, H., and P. Van Soest. 1970. Forage fiber analyses (apparatus, reagents, procedures, and some applications). Agriculture Research Service, United States Department of Agriculture, Washington, DC.

Golladay, S. W., and R. L. Sinsabaugh. 1991. Biofilm development on leaf and wood surfaces in a boreal river. Freshwater Biology 25:437-450.

Gómez, R., A. D. Asencio, J. M. Picon, R. Del Campo, M. I. Arce, M. Del Mar Sanchez-Montoya, M. L. Suarez, and M. R. Vidal-Abarca. 2015. The effect of water salinity on wood breakdown in semiarid Mediterranean streams. Science of the Total Environment 541:491-501.

Gómez, R., I. Hurtado, M. L. Suárez, and M. R. Vidal-Abarca. 2005. Ramblas in south-east Spain: threatened and valuable ecosystems. Aquatic Conservation: Marine and Freshwater Ecosystems 15:387-402.

Gómez, R., V. García-García, M. R. Vidal-Abarca, and M. L. Suárez. 2009. Effect of intermittency on N spatial variability in an arid Mediterranean stream.

Journal of The North American Benthological Society 28:572-583.

González-Pinzón, R., R. Haggerty, and D. Myrold. 2012. Measuring aerobic respiration in stream ecosystems using the resazurin-resorufin system. Journal of Geophysical Research 117:G00N06.

González-Polo, M., and A. T. Austin. 2009. Spatial heterogeneity provides organic matter refuges for soil microbial activity in the Patagonian steppe, Argentina. Soil Biology and Biochemistry 41:1348–1351.

Gosz, J. R., G. E. Likens, and F. H. Bormann. 1973. Nutrient release from decomposing leaf and branch litter in the Hubbard Brook Forest, New Hampshire. Ecological Monographs 43:173-191.

Graça, M. A. S. 2001. The Role of Invertebrates on Leaf Litter Decomposition in Streams – a Review. International Review of Hydrobiology 86:383-393.

Graça, M. A. S., and J. M. Poquet. 2014. Do climate and soil influence phenotypic variability in leaf litter, microbial decomposition and shredder consumption? Oecologia 174:1021-1032.

Graça, M. A. S., V. Ferreira, C. Canhoto, A. C. Encalada, F. Guerrero-Bolaño, K. M. Wantzen, and L. Boyero. 2015. A conceptual model of litter breakdown in low order streams. International Review of Hydrobiology 100:1-12.

Grueber C, E., S. Nakagawa, J. Laws R, and G. Jamieson I. 2011. Multimodel inference in ecology and evolution: challenges and solutions. Journal of Evolutionary Biology 24:699-711.

Guerrero, C. 2002. Patrones ecológicos y respuesta de la comunidad de macroinvertebrados acuáticos al estiaje. El caso del río Chícamo (SE de España). University of Murcia.

Gulis, V., and K. Suberkropp. 2003. Interactions between stream fungi and bacteria associated with decomposing leaf litter at different levels of nutrient availability. Aquatic Microbial Ecology 30:149-157.

Gulis, V., K. Suberkropp, and A. D. Rosemond. 2008. Comparison of Fungal Activities on Wood and Leaf Litter in Unaltered and Nutrient-Enriched Headwater Streams ▿ . Appl Environ Microbiol 74:1094-1101.

Güsewell, S., and M. O. Gessner. 2009. N : P ratios influence litter decomposition and colonization by fungi and bacteria in microcosms. Functional Ecology

23:211-219.

Hagen, E. M., K. E. McCluney, K. A. Wyant, C. U. Soykan, A. C. Keller, K. C. Luttermoser, E. J. Holmes, J. C. Moore, and J. L. Sabo. 2012. A meta-analysis of the effects of detritus on primary producers and consumers in marine, freshwater, and terrestrial ecosystems. Oikos 121:1507-1515.

Hagerberg, D., G. Thelin, and H. Wallander. 2003. The production of ectomycorrhizal mycelium in forests: Relation between forest nutrient status and local mineral sources. Plant and Soil 252:279-290.

Haggerty, R., A. Argerich, and E. Martí. 2008. Development of a "smart" tracer for the assessment of microbiological activity and sediment-water interaction in natural waters: The resazurin-resorufin system. Water Resources Research 44.

Handa, I. T., R. Aerts, F. Berendse, M. P. Berg, A. Bruder, O. Butenschoen, E. Chauvet, M. O. Gessner, J. Jabiol, M. Makkonen, B. G. McKie, B. Malmqvist, E. T. H. M. Peeters, S. Scheu, B. Schmid, J. v. Ruijven, V. C. A. Vos, and S. Hättenschwiler. 2014. Consequences of biodiversity loss for litter decomposition across biomes. Nature 509:218-221.

Hastie, T. J., and R. J. Tibshirani. 1990. Generalized Additive Models. CRC press, New York, U.S.A.

Hättenschwiler, S., A. V. Tiunov, and S. Scheu. 2005. Biodiversity and Litter Decomposition in Terrestrial Ecosystems. Annual Review of Ecology, Evolution, and Systematics 36:191-218.

Hector, A. 2011. Diversity favours productivity. Nature 472:45.

Heffernan, J. B., and R. A. Sponseller. 2004. Nutrient mobilization and processing in Sonoran Desert riparian soils following artificial re-wetting. Biogeochemistry 70:117-134.

Helms, J. R., A. Stubbins, J. D. Ritchie, E. C. Minor, D. J. Kieber, and K. Mopper. 2008. Absorption spectral slopes and slope ratios as indicators of molecular weight, source, and photobleaching of chromophoric dissolved organic matter. Limnology and Oceanography 53:955-969.

Henry, H. A. L., K. Brizgys, and C. B. Field. 2008. Litter decomposition in a California annual grassland: interactions between photodegradation and

litter layer thickness. Ecosystems 11:545-554.

Hernes, P. J., R. G. M. Spencer, R. Y. Dyda, A. T. O'Geen, and R. A. Dahlgren. 2017. The Genesis and Exodus of Vascular Plant DOM from an Oak Woodland Landscape. Frontiers in Earth Science 5.

Hirabayashi, Y., R. Mahendran, S. Koirala, L. Konoshima, D. Yamazaki, S. Watanabe, H. Kim, and S. Kanae. 2013. Global flood risk under climate change. Nature Climate Change 3:816-821.

Hladyz, S., S. C. Watkins, K. L. Whitworth, and D. S. Baldwin. 2011. Flows and hypoxic blackwater events in managed ephemeral river channels. Journal of Hydrology 401:117-125.

Hobbie, S. E., and P. M. Vitousek. 2000. Nutrient Limitation of Decomposition in Hawaiian Forests. Ecology 81:1867-1877.

Homyak, P. M., R. D. Yanai, D. A. Burns, R. D. Briggs, and R. H. Germain. 2008. Nitrogen immobilization by wood-chip application: Protecting water quality in a northern hardwood forest. Forest Ecology and Management 255:2589–2601.

Hongve, D., P. A. W. Van Hees, and U. S. Lundström. 2000. Dissolved components in precipitation water percolated through forest litter. European Journal of Soil Science 51:667-677.

Hooper, D. U., F. S. Chapin, J. J. Ewel, A. Hector, P. Inchausti, S. Lavorel, J. H. Lawton, D. M. Lodge, M. Loreau, S. Naeem, B. Schmid, H. Setälä, A. J. Symstad, J. Vandermeer, and D. A. Wardle. 2005. Effects of biodiversity on ecosystem functioning: A consensus of current knowledge. Ecological Monographs 75:3-35.

Hotchkiss, E. R., R. O. Hall Jr, R. A. Sponseller, D. Butman, J. Klaminder, H. Laudon, M. Rosvall, and J. Karlsson. 2015. Sources of and processes controlling CO2 emissions change with the size of streams and rivers. Nature Geoscience 8:696.

Hur, J., M.-H. Lee, H. Song, and M. A. Schlatman. 2013. Microbial transformation of dissolved organic matter from different sources and its influence on disinfection byproduct formation potentials. Environmental Science and Pollution Research 20:4176-4187.

IPCC. 2013. Climate change 2013: The physical science basis. Contribution of working group I to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, USA.

Jacobson, P. J., K. M. Jacobson, P. L. Angermeier, and S. C. Don. 1999. Transport, retention and ecological significance of woody debris within a large ephemeral river. Journal of the North American Benthological Society 18:429-444.

Jaffé, R., D. McKnight, N. Maie, R. Cory, W. H. McDowell, and J. L. Campbell. 2008. Spatial and temporal variations in DOM composition in ecosystems: The importance of long-term monitoring of optical properties. Journal of Geophysical Research: Biogeosciences 113.

Jellison, J., J. Connolly, B. Goodell, B. Doyle, B. Illman, F. Fekete, and A. Ostrofsky. 1997. The role of cations in the biodegradation of wood by the brown rot fungi. International Biodeterioration & Biodegradation 39:165–179.

Jian, Q., T. H. Boyer, X. Yang, B. Xia, and X. Yang. 2016. Characteristics and DBP formation of dissolved organic matter from leachates of fresh and aged leaf litter. Chemosphere 152:335-344.

Kalbitz, K., S. Solinger, J. H. Park, B. Michalzik, and E. Matzner. 2000. Controls on the dynamics of dissolved organic matter in soils: a review. Soil Science 165.

Kaplan, L. A., T. N. Wiegner, J. D. Newbold, P. H. Ostrom, and H. Gandhi. 2008. Untangling the complex issue of dissolved organic carbon uptake: a stable isotope approach. Freshwater Biology 53:855-864.

Karlsson, O. M., J. S. Richardson, and P. M. Kiffney. 2005. Modelling organic matter dynamics in headwater streams of south-western British Columbia, Canada. Ecological Modelling 183:463-476.

King, J. Y., L. A. Brandt, and E. C. Adair. 2012. Shedding light on plant litter decomposition: advances, implications and new directions in understanding the role of photodegradation. Biogeochemistry 111:57-81.

Koch, B. P., and T. Dittmar. 2006. From mass to structure: an aromaticity index for high-resolution mass data of natural organic matter. Rapid Communications

in Mass Spectrometry 20:926–932.

Koch, B. P., T. Dittmar, M. Witt, and G. Kattner. 2007. Fundamentals of molecular formula assignment to ultrahigh resolution mass data of natural organic matter. Analytical Chemistry 79:1758-1763.

Kominoski John, S., and M. Pringle Catherine. 2009. Resource–consumer diversity: testing the effects of leaf litter species diversity on stream macroinvertebrate communities. Freshwater Biology 54:1461-1473.

Kuehn, K. A. 2015. Lentic and lotic habitats as templets for fungal communities: traits, adaptations, and their significance to litter decomposition within freshwater ecosystems. Fungal Ecology.

Kujawinski, E. B. 2002. Electrospray Ionization Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (ESI FT-ICR MS): Characterization of Complex Environmental Mixtures. Environmental Forensics 3:207-216.

Laliberté, E., and P. Legendre. 2010. A distance-based framework for measuring functional diversity from multiple traits. Ecology 91:299-305.

Lambert, R. L., G. E. Lang, and W. A. Reiners. 1980. Loss of mass and chemical change in decaying boles of a subalpine balsam fir forest. Ecology 61:1460–1473.

Lambert, T., S. Bouillon, F. Darchambeau, C. Morana, F. A. E. Roland, J.-P. Descy, and A. V. Borges. 2017. Effects of human land use on the terrestrial and aquatic sources of fluvial organic matter in a temperate river basin (The Meuse River, Belgium). Biogeochemistry 136:191-211.

Lammers, K., G. Arbuckle-Keil, and J. Dighton. 2009. FT-IR study of the changes in carbohydrate chemistry of three New Jersey pine barrens leaf litters during simulated control burning. Soil Biology and Biochemistry 41:340-347.

Langhans, S. D., and K. Tockner. 2006. The role of timing, duration, and frequency of inundation in controlling leaf litter decomposition in a river-floodplain ecosystem (Tagliamento, northeastern Italy). Oecologia 147:501-509.

Langhans, S. D., S. D. Tiegs, M. O. Gessner, and K. Tockner. 2008. Leaf-decomposition heterogeneity across a riverine floodplain mosaic. Aquatic Sciences 70:337-346.

Langhans, S. D., S. D. Tiegs, U. Uehlinger, and K. Tockner. 2006. Environmental

heterogeneity controls organic-matter dynamics in river-floodplain ecosystems. Polish Journal of Ecology Vol. 54:675-680.

Langhans, S. D., U. Richard, J. Rueegg, U. Uehlinger, P. Edwards, M. Doering, and K. Tockner. 2013. Environmental heterogeneity affects input, storage, and transformation of coarse particulate organic matter in a floodplain mosaic. Aquatic Sciences 75:335-348.

Larned, S. T. 2000. Dynamics of coarse riparian detritus in a Hawaiian stream ecosystem: a comparison of drought and post-drought conditions. Journal of the North American Benthological Society 19:215-234.

Larned, S. T., T. Datry, D. B. Arscott, and K. Tockner. 2010. Emerging concepts in temporary-river ecology. Freshwater Biology 55:717-738.

Larrañaga, S., J. R. Díez, A. Elosegi, and J. Pozo. 2003. Leaf retention in streams of the Agüera basin (northern Spain). Aquatic Sciences 65:158-166.

Laudon, H., and A. Sponseller Ryan. 2017. How landscape organization and scale shape catchment hydrology and biogeochemistry: insights from a long-term catchment study. WIREs Water 5:e1265.

Lavorel, S., K. Grigulis, S. McIntyre, S. G. Williams Nick, D. Garden, J. Dorrough, S. Berman, F. Quétier, A. Thébault, and A. Bonis. 2007. Assessing functional diversity in the field – methodology matters! Functional Ecology 22:134-147.

Leberfinger, K., I. Bohman, and J. Herrmann. 2010. Drought impact on stream detritivores: experimental effects on leaf litter breakdown and life cycles. Hydrobiologia 652:247-254.

Lecerf, A., G. Marie, S. Kominoski John, J. LeRoy Carri, C. Bernadet, and M. Swan Christopher. 2011. Incubation time, functional litter diversity, and habitat characteristics predict litter-mixing effects on decomposition. Ecology 92:160-169.

Lecerf, A., G. Risnoveanu, C. Popescu, M. O. Gessner, and E. Chauvet. 2007. Decomposition of diverse litter mixtures in streams. Ecology 88:219-227.

Lee, H. V., S. B. A. Hamid, and S. K. Zain. 2014. Conversion of Lignocellulosic Biomass to Nanocellulose: Structure and Chemical Process. The Scientific World Journal 2014:20.

Leigh, C., A. J. Boulton, J. L. Courtwright, K. Fritz, C. L. May, R. H. Walker, and

T. Datry. 2016. Ecological research and management of intermittent rivers: an historical review and future directions. Freshwater Biology 61:1181-1199.

Lesaulnier, C. C., C. W. Herbold, C. Pelikan, D. Berry, C. Gérard, X. L. Coz, S. Gagnot, J. Niggemann, T. Dittmar, G. A. Singer, and A. Loy. 2017. Bottled aqua incognita: Microbiota assembly and dissolved organic matter diversity in natural mineral waters. Microbiome 5.

Lin, Y., S. D. Karlen, J. Ralph, and J. Y. King. 2018. Short-term facilitation of microbial litter decomposition by ultraviolet radiation. Science of the Total Environment 615:838-848.

Liu, D., K. M. Keiblinger, S. Leitner, A. Mentler, and S. Zechmeister-Boltenstern. 2016a. Is there a convergence of deciduous leaf litter stoichiometry, biochemistry and microbial population during decay? Geoderma 272:93-100.

Liu, X., Q.-H. Huang, H.-L. Jiang, and N. Song. 2016b. Effects of visible light radiation on macrophyte litter degradation and nutrient release in water samples from a eutrophic shallow lake. Chemistry and Ecology 32:961-975.

Loreau, M., and A. Hector. 2001. Partitioning selection and complementarity in biodiversity experiments. Nature 412:72.

Makkar, H. P. S. 2003. Treatment of plant material, extraction of tannins, and an overview of tannin assays presented in the manual. Pages 43-48 *in* H. P. S. Makkar, editor. Quantification of tannins in tree and shrub foliage: A laboratory manual. Springer Netherlands, Dordrecht.

Marín-Spiotta, E., K. E. Gruley, J. Crawford, E. E. Atkinson, J. R. Miesel, S. Greene, C. Cardona-Correa, and R. G. M. Spencer. 2014. Paradigm shifts in soil organic matter research affect interpretations of aquatic carbon cycling: transcending disciplinary and ecosystem boundaries. Biogeochemistry:1-19.

Marschner, B., and K. Kalbitz. 2003. Controls of bioavailability and biodegradability of dissolved organic matter in soils. Geoderma 113:211-235.

Martin, F., C. Delaruelle, and J. L. Hilbert. 1990. An improved ergosterol assay to estimate fungal biomass in ectomycorrhizas. Mycological Research 94:1059-1064.

Mastný, J., E. Kaštovská, J. Bárta, A. Chroňáková, J. Borovec, H. Šantrůčková, Z. Urbanová, K. R. Edwards, and T. Picek. 2018. Quality of DOC produced

during litter decomposition of peatland plant dominants. Soil Biology and Biochemistry 121:221-230.

McClain, M. E., E. W. Boyer, C. L. Dent, S. E. Gergel, N. B. Grimm, P. M. Groffman, S. C. Hart, J. W. Harvey, C. A. Johnston, E. Mayorga, W. H. McDowell, and G. Pinay. 2003. Biogeochemical hot spots and hot moments at the interface of terrestrial and aquatic ecosystems. Ecosystems 6:301-312.

McDowell, W. H., and S. G. Fisher. 1976. Autumnal Processing of Dissolved Organic Matter in a Small Woodland Stream Ecosystem. Ecology 57:561-569.

McKnight, D. M., E. W. Boyer, P. K. Westerhoff, P. T. Doran, T. Kulbe, and D. T. Andersen. 2001. Spectrofluorometric characterization of dissolved organic matter for indication of precursor organic material and aromaticity. Limnology and Oceanography 46:38-48.

Meier, C. L., and W. D. Bowman. 2008. Links between plant litter chemistry, species diversity, and below-ground ecosystem function. Proceedings of the National Academy of Sciences 105:19780-19785.

Melillo, J. M., R. J. Naiman, J. D. Aber, and A. E. Linkins. 1984. Factors controlling mass loss and nitrogen dynamics of plant litter decaying in Northern streams. Bulletin of Marine Science 35:341-356.

Melillo, J. M., R. J. Naiman, J. D. Aber, and K. N. Eshleman. 1983. The influence of substrate quality and stream size on wood decomposition dynamics. Oecologia 58:281-285.

Merbt, S. N., L. Proia, J. I. Prosser, E. Martí, E. O. Casamayor, and D. von Schiller. 2016. Stream drying drives microbial ammonia oxidation and first-flush nitrate export. Ecology 97:2192-2198.

Moorhead, D. L., and R. L. Sinsabaugh. 2000. Simulated patterns of litter decay predict patterns of extracellular enzyme activities. Applied Soil Ecology 14:71-79.

Moorhead, D. L., and R. L. Sinsabaugh. 2006. A theoretical model of litter decay and microbial interaction. Ecological Monographs 76:151-174.

Mora, J. 2014. Leaf litter decomposition in Mediterranean streams: microbial processes and responses to drought under current global change scenario.

University of Girona.

Moran, M. A., and J. S. Covert. 2003. Photochemically Mediated Linkages between Dissolved Organic Matter and Bacterioplankton. Pages 243-262 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Moran, M. A., and R. G. Zepp. 1997. Role of photoreactions in the formation of biologically labile compounds from dissolved organic matter. Limnology and Oceanography 42:1307-1316.

Morin, P. J. 1998. Realism, precision, and generality in experimental ecology. Pages 50-70 *in* W. J. Resetarits and J. Bernardo, editors. Experimental ecology: issues and perspectives. Oxford University Press, Oxford, United Kingdom.

Muñoz, I., M. Abril, P. Casas-Ruiz Joan, M. Casellas, L. Gómez-Gener, R. Marcé, M. Menéndez, B. Obrador, S. Sabater, D. Schiller, and V. Acuña. 2018. Does the severity of non-flow periods influence ecosystem structure and function of temporary streams? A mesocosm study. Freshwater Biology 63:613-625.

Naiman, R. J., J. S. Bechtold, D. C. Drake, J. J. Latterell, T. C. O'Keefe, and E. V. Balian. 2005. Origins, Patterns, and Importance of Heterogeneity in Riparian Systems. Pages 279-309 *in* G. M. Lovett, M. G. Turner, C. G. Jones, and K. C. Weathers, editors. Ecosystem Function in Heterogeneous Landscapes. Springer New York, New York, NY.

Nikolcheva, L. G., and F. Barlocher. 2004. Seasonal and substrate preferences of fungi colonizing leaves in streams: traditional versus molecular evidence. Environmental Microbiology 7:270-280.

Nikolcheva, L. G., T. Bourque, and F. Bärlocher. 2005. Fungal diversity during initial stages of leaf decomposition in a stream. Mycological Research 109:246–253.

O'Connell, M., D. S. Baldwin, A. I. Robertson, and G. Rees. 2000. Release and bioavailability of dissolved organic matter from floodplain litter: influence of origin and oxygen levels. Freshwater Biology 45:333-342.

Oksanen, J., F. G. Blanchet, M. Friendly, R. Kindt, P. M. Legendre, D., P. R. Minchin, O. Gavin, G. L. Simpson, P. Solymos, M. H. H. Stevens, E. Szoecs, and H. Wagner. 2017. Vegan: Community ecology package. R package

version 2.4-3.

Parikh, S. J., K. W. Goyne, A. J. Margenot, F. N. D. Mukome, F. J. Calderón, and D. L. Sparks. 2014. Soil Chemical Insights Provided through Vibrational Spectroscopy. Pages 1-148 *in* D. L. Sparks, editor. Advances in Agronomy. Academic Press.

Parton, W., W. L. Silver, I. C. Burke, L. Grassens, M. E. Harmon, W. S. Currie, J. Y. King, E. C. Adair, L. A. Brandt, S. C. Hart, and B. Fasth. 2007. Global-Scale Similarities in Nitrogen Release Patterns During Long-Term Decomposition. Science 315:361-364.

Pastor, A., Z. Compson, P. Dijkstra, J. Riera, E. Martí, F. Sabater, B. Hungate, and J. Marks. 2014. Stream carbon and nitrogen supplements during leaf litter decomposition: contrasting patterns for two foundation species. Oecologia 176:1111-1121.

Payne, T. G., A. D. Southam, T. N. Arvanitis, and M. R. Viant. 2009. A signal filtering method for improved quantification and noise discrimination in fourier transform ion cyclotron resonance mass spectrometry-based metabolomics data. Journal of the American Society for Mass Spectrometry 20:1087-1095.

Peel, M. C., B. L. Finlayson, and T. A. McMahon. 2007. Updated world map of the Köppen-Geiger climate classification. Hydrology and Earth System Sciences 11:1633-1644.

Petersen, R. C., and K. W. Cummins. 1974. Leaf processing in a woodland stream. Freshwater Biology 4:345-368.

Pozo, J., E. González, J. R. Díez, J. Molinero, and A. Elósegui. 1997. Inputs of particulate organic matter to streams with different riparian vegetation. Journal of the North American Benthological Society 16:602-611.

Pu, G., J. Du, X. Ma, Y. Lv, Y. Jia, X. Jia, and X. Tian. 2014. Contribution of ambient atmospheric exposure to Typha angustifolia litter decomposition in aquatic environment. Ecological Engineering 67:144-149.

R Core Team. 2015. R: A language and environment for statistical computing.

Rabeni, C. F., and S. M. Hoel. 2000. The importance of woody debris to benthic invertebrates in two Missouri prairie streams. Pages 1499-1502 *in* 27th

Congress of the International-Association-of-Theoretical-and-Applied-Limnology, Dublin, Ireland.

Raymond, P. A., and J. E. Bauer. 2000. Bacterial consumption of DOC during transport through a temperate estuary. Aquatic Microbial Ecology 22:1-12.

Raymond, P. A., J. E. Saiers, and W. V. Sobczak. 2016. Hydrological and biogeochemical controls on watershed dissolved organic matter transport: pulse-shunt concept. Ecology 97:5-16.

Raymond, P. A., J. Hartmann, R. Lauerwald, S. Sobek, C. McDonald, M. Hoover, D. Butman, R. Striegl, E. Mayorga, C. Humborg, P. Kortelainen, H. Durr, M. Meybeck, P. Ciais, and P. Guth. 2013. Global carbon dioxide emissions from inland waters. Nature 503:355-359.

Reynolds, J. F., D. M. Smith, E. F. Lambin, B. L. Turner, M. Mortimore, S. P. J. Batterbury, T. E. Downing, H. Dowlatabadi, R. J. Fernández, J. E. Herrick, E. Huber-Sannwald, H. Jiang, R. Leemans, T. Lynam, F. T. Maestre, M. Ayarza, and B. Walker. 2007. Global desertification: Building a science for dryland development. Science 316:847-851.

Riedel, T., and T. Dittmar. 2014. A method detection limit for the analysis of natural organic matter via Fourier transform ion cyclotron resonance mass spectrometry. Analytical Chemistry 86:8376-8382.

Robertson, G. P., D. C. Coleman, C. S. Bledsoe, and P. Sollins. 1999. Standard Soil Methods for Long-Term Ecological Research.

Romaní, A. M., E. Vázquez, and A. Butturini. 2006b. Microbial availability and size fractionation of dissolved organic carbon after drought in an intermittent stream: Biogeochemical link across the stream–riparian interface. Microbial Ecology 52:501-512.

Romaní, A. M., H. Fischer, C. Mille-Lindblom, and L. J. Tranvik. 2006a. Interactions of Bacteria and Fungi on Decomposing Litter: Differential Extracellular Enzyme Activities. Ecology 87:2559-2569.

Romaní, A. M., S. Amalfitano, J. Artigas, S. Fazi, S. Sabater, X. Timoner, I. Ylla, and A. Zoppini. 2013. Microbial biofilm structure and organic matter use in mediterranean streams. Hydrobiologia 719:43-58.

Romero, L. M., T. J. Smith, and J. W. Fourqurean. 2005. Changes in mass and

nutrient content of wood during decomposition in a south Florida mangrove forest. Journal of Ecology 93:618-631.

Rutledge, S., I. Campbell David, D. Baldocchi, and A. Schipper Louis. 2010. Photodegradation leads to increased carbon dioxide losses from terrestrial organic matter. Global Change Biology 16:3065-3074.

Sabater, S., X. Timoner, C. Borrego, and V. Acuña. 2016. Stream Biofilm Responses to Flow Intermittency: From Cells to Ecosystems. Frontiers in Environmental Science 4.

Safriel, U., Z. Adeel, D. Niemeijer, J. Puigdefabregas, R. White, R. Lal, M. Winslow, J. Ziedler, S. Prince, E. Archer, C. King, B. Shapiro, K. Wessels, T. T. Nielsen, B. Portnov, I. Reshef, J. Thornell, E. Lachman, and D. McNab. 2005. Dryland systems. Pages 625–658 *in* R. Hassan, R. Scholes, and N. Ash, editors. Ecosystems and human well-being, current state and trends. Island Press, Washington.

Sakamoto, Y., M. Ishiguro, and G. Kitagawa. 1986. Akaike information criterion statistics. D. Reidel Publishing Company.

Salinas, M. J., and J. J. Casas. 2007. Riparian vegetation of two semi-arid Mediterranean rivers: Basin-scale responses of woody and herbaceous plants to environmental gradients. Wetlands 27:831-845.

Sánchez–González, A., M. Chapela–Lara, E. Germán–Venegas, R. Fuentes-García, F. d. Río-Portilla, and C. Siebe. 2017. Changes in quality and quantity of soil organic matter stocks resulting from wastewater irrigation in formerly forested land. Geoderma 306:99-107.

Sánchez-Montoya, M. M., D. von Schiller, G. G. Barberá, A. M. Díaz, M. I. Arce, R. del Campo, and K. Tockner. 2018. Understanding the effects of predictability, duration, and spatial pattern of drying on benthic invertebrate assemblages in two contrasting intermittent streams. PLOS ONE 13:e0193933.

Sanpera-Calbet, I., A. Lecerf, and E. Chauvet. 2009. Leaf diversity influences in-stream litter decomposition through effects on shredders. Freshwater Biology 54:1671-1682.

Sanpera-Calbet, I., V. Acuña, A. Butturini, R. Marcé, and I. Muñoz. 2016. El Niño

southern oscillation and seasonal drought drive riparian input dynamics in a Mediterranean stream. Limnology and Oceanography 61:214-226.

Santschi, F., I. Gounand, E. Harvey, and F. Altermatt. 2017. Leaf litter diversity and structure of microbial decomposer communities modulate litter decomposition in aquatic systems. Functional Ecology 32:522-532.

Savory, J. J., N. K. Kaiser, A. M. McKenna, F. Xian, G. T. Blakney, R. P. Rodgers, C. L. Hendrickson, and A. G. Marshall. 2011. Parts-per-billion Fourier transform ion cyclotron resonance mass measurement accuracy with a "walking" calibration equation. Analytical Chemistry 83:1732-1736.

Schade, J. D., and S. G. Fisher. 1997. Leaf litter in a Sonoran Desert stream ecosystem. Journal of the North American Benthological Society 16:612-626.

Schindler, M. H., and M. O. Gessner. 2009. Functional leaf traits and biodiversity effects on litter decomposition in a stream. Ecology 90:1641-1649.

Schrumpf, M., W. Zech, J. Lehmann, and H. V. C. Lyaruu. 2006. TOC, TON, TOS and TOP in rainfall, throughfall, litter percolate and soil solution of a montane rainforest succession at Mt. Kilimanjaro, Tanzania. Biogeochemistry 78:361-387.

Seifert, A.-G., V.-N. Roth, T. Dittmar, G. Gleixner, L. Breuer, T. Houska, and J. Marxsen. 2016. Comparing molecular composition of dissolved organic matter in soil and stream water: Influence of land use and chemical characteristics. Science of The Total Environment 571:142-152.

Servais, P., A. Anzil, and C. Ventresque. 1989. Simple method for determination of biodegradable dissolved organic carbon in water. Applied and Environmental Microbiology 55:2732-2734.

Shearer, C. A., E. Descals, B. Kohlmeyer, J. Kohlmeyer, L. Marvanová, D. Padgett, D. Porter, H. A. Raja, J. P. Schmit, H. A. Thorton, and H. Voglymayr. 2007. Fungal biodiversity in aquatic habitats. Biodiversity and Conservation 16:49-67.

Siebers Andre, R., E. Pettit Neil, G. Skrzypek, B. Fellman Jason, S. Dogramaci, and F. Grierson Pauline. 2015. Alluvial ground water influences dissolved organic matter biogeochemistry of pools within intermittent dryland streams. Freshwater Biology 61:1228-1241.

Sinsabaugh, R. L., and C. M. Foreman. 2003. Integrating dissolved organic matter metabolism and microbial diversity: An overview of conceptual models. Pages 425-454 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Sinsabaugh, R. L., and D. L. Moorhead. 1994. Resource allocation to extracellular enzyme production: A model for nitrogen and phosphorus control of litter decomposition. Soil Biology and Biochemistry 26:1305-1311.

Sinsabaugh, R. L., C. L. Lauber, M. N. Weintraub, B. Ahmed, S. D. Allison, C. Crenshaw, A. R. Contosta, D. Cusack, S. Frey, M. E. Gallo, T. B. Gartner, S. E. Hobbie, K. Holland, B. L. Keeler, J. S. Powers, M. Stursova, C. Takacs-Vesbach, M. P. Waldrop, M. D. Wallenstein, D. R. Zak, and L. H. Zeglin. 2008. Stoichiometry of soil enzyme activity at global scale. Ecology Letters 11:1252-1264.

Sinsabaugh, R. L., M. E. Gallo, C. Lauber, M. P. Waldrop, and D. R. Zak. 2005. Extracellular Enzyme Activities and Soil Organic Matter Dynamics for Northern Hardwood Forests receiving Simulated Nitrogen Deposition. Biogeochemistry 75:201-215.

Sinsabaugh, R. L., M. M. Carreiro, and D. A. Repert. 2002. Allocation of extracellular enzymatic activity in relation to litter composition, N deposition, and mass loss. Biogeochemistry 60:1-24.

Sinsabaugh, R. L., R. K. Antibus, A. E. Linkins, C. A. McClaugherty, L. Rayburn, D. Repert, and T. Weiland. 1992. Wood decomposition over a first-order watershed: Mass loss as a function of lignocellulase activity. Soil Biology and Biochemistry 24:743-749.

Sponseller, R. A., and S. G. Fisher. 2006. Drainage Size, Stream Intermittency, and Ecosystem Function in a Sonoran Desert Landscape. Ecosystems 9:344-356.

Sponseller, R. A., J. B. Heffernan, and S. G. Fisher. 2013. On the multiple ecological roles of water in river networks. Ecosphere 4:17.

Srivastava Diane, S., J. Cardinale Bradley, L. Downing Amy, J. E. Duffy, C. Jouseau, M. Sankaran, and P. Wright Justin. 2009. Diversity has stronger top-down than bottom-up effects on decomposition. Ecology 90:1073-1083.

Stanley, E. H., S. G. Fisher, and N. B. Grimm. 1997. Ecosystem Expansion and

Contraction in StreamsDesert streams vary in both space and time and fluctuate dramatically in size. BioScience 47:427-435.

Stedmon, C. A., and R. Bro. 2008. Characterizing dissolved organic matter fluorescence with parallel factor analysis: a tutorial. Limnology and Oceanography: Methods 6:572-579.

Stedmon, C. A., and S. Markager. 2005. Tracing the production and degradation of autochthonous fractions of dissolved organic matter by fluorescence analysis. Limnology and Oceanography 50:1415-1426.

Stoler, A. B., D. J. Burke, and R. A. Relyea. 2016. Litter chemistry and chemical diversity drive ecosystem processes in forest ponds. Ecology 97:1783-1795.

Strauss, E. A., and G. A. Lamberti. 2002. Effect of dissolved organic carbon quality on microbial decomposition and nitrification rates in stream sediments. Freshwater Biology 47:65-74.

Stubberfield, L. C. F., and P. J. A. Shaw. 1990. A comparison of tetrazolium reduction and FDA hydrolysis with other measures of microbial activity. Journal of Microbiological Methods 12:151-162.

Stubbins, A., R. G. M. Spencer. H. Chen, P. G. Hatcher, K. Mopper, P. J. Hernes, V. L. Mwamba, A. M. Mangangu, J. N. Wabakanghanzi, and J. Six. 2010. Illuminated darkness: Molecular signatures of Congo River dissolved organic matter and its photochemical alteration as revealed by ultrahigh precision mass spectrometry. Limnology and Oceanography 55:1467-1477.

Stursova, M., and R. L. Sinsabaugh. 2008. Stabilization of oxidative enzymes in desert soil may limit organic matter accumulation. Soil Biology and Biochemistry 40:550–553.

Suberkropp, K. 1998. Microorganisms and organic matter processing. Pages 120-143 *in* R. J. Naiman and R. E. Bilby, editors. River Ecology and Management. Springer-Verlag, New York.

Suberkropp, K., and E. Chauvet. 1995. Regulation of Leaf Breakdown by Fungi in Streams: Influences of Water Chemistry. Ecology 76:1433-1445.

Suberkropp, K., G. L. Godshalk, and M. J. Klug. 1976. Changes in the Chemical Composition of Leaves During Processing in a Woodland Stream. Ecology 57:720-727.

Sun, L., E. M. Perdue, J. L. Meyer, and J. Weis. 2003. Use of elemental composition to predict bioavailability of dissolved organic matter in a Georgia river. Limnology and Oceanography 42:714-721.

Swift, M. J., O. W. Heal, and J. M. Anderson. 1979. Decomposition in terrestrial ecosystems. Blackwell Scientific, Oxford.

Talbot, J. M., and K. K. Treseder. 2012. Interactions among lignin, cellulose, and nitrogen drive litter chemistry—decay relationships. Ecology 93:345-354.

Tank, J. L., and M. J. Winterbourn. 1995. Microbial activity and invertebrate colonisation of wood in a New Zealand forest stream. New Zealand Journal of Marine and Freshwater Research 30:271-280.

Tank, J. L., E. J. Rosi-Marshall, N. A. Griffiths, S. A. Entrekin, and M. L. Stephen. 2010. A review of allochthonous organic matter dynamics and metabolism in streams. Journal of the North American Benthological Society 29:118-146.

Tant, C. J., A. D. Rosemond, A. S. Mehring, K. A. Kuehn, and J. M. Davis. 2015. The role of aquatic fungi in transformations of organic matter mediated by nutrients. Freshwater Biology 60:1354-1363.

Tatzber, M., M. Stemmer, H. Spiegel, C. Katzlberger, G. Haberhauer, A. Mentler, and H. Gerzabek Martin. 2007. FTIR-spectroscopic characterization of humic acids and humin fractions obtained by advanced NaOH, Na4P2O7, and Na2CO3 extraction procedures. Journal of Plant Nutrition and Soil Science 170:522-529.

Throop, H. L., and S. R. Archer. 2009. Resolving the Dryland Decomposition Conundrum: Some New Perspectives on Potential Drivers. Pages 171-194 *in* U. Lüttge, W. Beyschlag, B. Büdel, and D. Francis, editors. Progress in Botany. Springer Berlin Heidelberg, Berlin, Heidelberg.

Tonin, A. M., L. Boyero, S. Monroy, A. Basaguren, J. Pérez, R. G. Pearson, B. J. Cardinale, J. F. Gonçalves, and J. Pozo. 2017. Stream nitrogen concentration, but not plant N-fixing capacity, modulates litter diversity effects on decomposition. Functional Ecology 31:1471-1481.

Treplin, M., and M. Zimmer. 2012. Drowned or Dry: A Cross-Habitat Comparison of Detrital Breakdown Processes. Ecosystems 15:477-491.

van Hees, P. A. W., D. L. Jones, R. Finlay, D. L. Godbold, and U. S. Lundström.

2005. The carbon we do not see—the impact of low molecular weight compounds on carbon dynamics and respiration in forest soils: a review. Soil Biology and Biochemistry 37:1-13.

Vázquez, E., E. Ejarque, I. Ylla, A. M. Romaní, and A. Butturini. 2015. Impact of drying/rewetting cycles on the bioavailability of dissolved organic matter molecular-weight fractions in a Mediterranean stream. Freshwater Science 34:263-275.

Vázquez, E., S. Amalfitano, S. Fazi, and A. Butturini. 2011. Dissolved organic matter composition in a fragmented Mediterranean fluvial system under severe drought conditions. Biogeochemistry 102:59-72.

Velasco, J., A. Millan, M. R. Vidal-Abarca, M. L. Suarez, C. Guerrero, and M. Ortega. 2003. Macrophytic, epipelic and epilithic primary production in a semiarid Mediterranean stream. Freshwater Biology 48:1408-1420.

Venables, W. N., and B. D. Ripley. 2002. Modern applied statistics with S. Springer, New York, U.S.A.

Vidal-Abarca, M. R., M. L. Suárez, and L. Ramírez-Díaz. 1992. Ecology of Spanish semiarid streams. Limnética 8:151-160.

Vidal-Abarca, M. R., M. L. Suárez, C. Guerrero, J. Velasco, J. L. Moreno, A. Millán, and A. Perán. 2001. Dynamics of dissolved and particulate organic carbon in a saline and semiarid stream of southeast Spain (Chicamo stream). Hydrobiologia 455:71-78.

Vitousek, P. M., and R. W. Howarth. 1991. Nitrogen limitation on land and in the sea: How can it occur? Biogeochemistry 13:87-115.

von Schiller, D., D. Graeber, M. Ribot, X. Timoner, V. Acuña, E. Martí, S. Sabater, and K. Tockner. 2015. Hydrological transitions drive dissolved organic matter quantity and composition in a temporary Mediterranean stream. Biogeochemistry 123:429-446.

von Schiller, D., V. Acuña, D. Graeber, E. Martí, M. Ribot, S. Sabater, X. Timoner, and K. Tockner. 2011. Contraction, fragmentation and expansion dynamics determine nutrient availability in a Mediterranean forest stream. Aquatic Sciences 73:485.

von Wachenfeldt, E., and L. J. Tranvik. 2008. Sedimentation in Boreal Lakes—The

Role of Flocculation of Allochthonous Dissolved Organic Matter in the Water Column. Ecosystems 11:803-814.

Voronin, L. V. 2014. Terrigenous micromycetes in freshwater ecosystems (review). Inland Water Biology 7:352-356.

Wagener, S. M., M. W. Oswood, and J. P. C. Schimel. 1998. Rivers and soils: Parallels in carbon and nutrient processing. BioScience 48:104-108.

Wall, D. H., M. A. Bradford, M. G. St John, J. A. Trofymow, V. Behan-Pelletier, D. E. Bignell, J. M. Dangerfield, W. J. Parton, J. Rusek, W. Voigt, V. Wolters, H. Z. Gardel, F. O. Ayuke, R. Bashford, O. I. Beljakova, P. J. Bohlen, A. Brauman, S. Flemming, J. R. Henschel, D. L. Johnson, T. H. Jones, M. Kovarova, J. M. Kranabetter, L. Kutny, K. C. Lin, M. Maryati, D. Masse, A. Pokarzhevskii, H. Rahman, M. G. SabarÁ, J. A. Salamon, M. J. Swift, A. Varela, H. L. Vasconcelos, D. White, and X. Zou. 2008. Global decomposition experiment shows soil animal impacts on decomposition are climate-dependent. Glob Chang Biol 14:2661-2677.

Wallace, J. B., M. R. Whiles, S. Eggert, T. F. Cuffney, G. J. Lugthart, and K. Chung. 1995. Long-term dynamics of coarse particulate organic matter in three Appalachian mountain streams. Journal of the North American Benthological Society 14:217-232.

Wang, J., L. Liu, X. Wang, and Y. Chen. 2015. The interaction between abiotic photodegradation and microbial decomposition under ultraviolet radiation. Global Change Biology 21:2095–2104.

Wang, J., Y. You, Z. Tang, S. Liu, and O. J. Sun. 2014. Variations in leaf litter decomposition across contrasting forest stands and controlling factors at local scale. Journal of Plant Ecology 8:261–272.

Wang, L., and W. D'Odorico. 2008. Decomposition and Mineralization. Pages 838-844 *in* S. E. Jørgensen and B. D. Fath, editors. Encyclopedia of Ecology. Elsevier, Oxford.

Webster, J. R., and E. F. Benfield. 1986. Vascular plant breakdown in freshwater ecosystems. Annual Review of Ecology and Systematics 17:567-594.

Webster, J. R., E. F. Benfield, T. P. Ehrman, M. A. Schaeffer, J. L. Tank, J. J. Hutchens, and D. J. D'Angelo. 1999. What happens to allochthonous

material that falls into streams? A synthesis of new and published information from Coweeta. Freshwater Biology 41:687-705.

Weishaar, J. L., G. R. Aiken, B. A. Bergamaschi, M. S. Fram, R. Fujii, and K. Mopper. 2003. Evaluation of specific ultraviolet absorbance as an indicator of the chemical composition and reactivity of dissolved organic carbon. Environmental Science & Technology 37:4702-4708.

Wetzel, R. G. 2003. Dissolved Organic Carbon: Detrital Energetics, Metabolic Regulators, and Drivers of Ecosystem Stability of Aquatic Ecosystems1. Pages 455-477 *in* S. E. G. Findlay and R. L. Sinsabaugh, editors. Aquatic Ecosystems. Academic Press, Burlington.

Whitford, W., and E. L. Wade. 2002. Decomposition and nutrient cycling. Pages 235-274 *in* W. Whitford and E. L. Wade, editors. Ecology of Desert Systems. Academic Press, London.

Wickings, K., A. S. Grandy, C. Reed Sasha, and C. Cleveland Cory. 2012. The origin of litter chemical complexity during decomposition. Ecology Letters 15:1180-1188.

Wickland, K. P., G. R. Aiken, K. Butler, M. M. Dornblaser, R. G. M. Spencer, and R. G. Striegl. 2012. Biodegradability of dissolved organic carbon in the Yukon River and its tributaries: Seasonality and importance of inorganic nitrogen. Global Biogeochemical Cycles 26.

Wieder, W. R., C. C. Cleveland, and A. R. Townsend. 2008. Tropical tree species composition affects the oxidation of dissolved organic matter from litter. Biogeochemistry 88:127-138.

Wiegner, T. N., L. A. Kaplan, S. E. Ziegler, and R. H. Findlay. 2015. Consumption of terrestrial dissolved organic carbon by stream microorganisms. Aquatic Microbial Ecology 75:225-237.

Winterbourn, M. J. 1976. Fluxes of litter falling into a small beech forest stream. New Zealand Journal of Marine and Freshwater Research 10:399-416.

Wipfli, M. S., J. S. Richardson, and R. J. Naiman. 2007. Ecological Linkages Between Headwaters and Downstream Ecosystems: Transport of Organic Matter, Invertebrates, and Wood Down Headwater Channels1. JAWRA Journal of the American Water Resources Association 43:72-85.

Wood, S. N. 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. Journal of the Royal Statistical Society: Series B (Statistical Methodology) 73:3-36.

Woodward, G., M. O. Gessner, P. S. Giller, V. Gulis, S. Hladyz, A. Lecerf, B. Malmqvist, B. G. McKie, S. D. Tiegs, H. Cariss, M. Dobson, A. Elosegi, V. Ferreira, M. A. S. Graça, T. Fleituch, J. O. Lacoursière, M. Nistorescu, J. Pozo, G. Risnoveanu, M. Schindler, A. Vadineanu, L. B. M. Vought, and E. Chauvet. 2012. Continental-Scale Effects of Nutrient Pollution on Stream Ecosystem Functioning. Science 336:1438.

Wurzbacher, C. M., F. Bärlocher, and H. P. Grossart. 2010. Fungi in lake ecosystems. Aquatic Microbial Ecology 59:125-149.

Wymore, A. S., Z. G. Compson, W. H. McDowell, J. D. Potter, B. A. Hungate, T. G. Whitham, and J. C. Marks. 2015. Leaf-litter leachate is distinct in optical properties and bioavailability to stream heterotrophs. Freshwater Science 34:857-866.

Timoner, X., V. Acuña, L. Frampton, P. Pollard, S. Sabater, and S. E. Bunn. 2014. Biofilm functional responses to the rehydration of a dry intermittent stream. Hydrobiologia 727:185-195.

Yates, C. A., P. J. Johnes, and R. G. M. Spencer. 2016. Assessing the drivers of dissolved organic matter export from two contrasting lowland catchments, U.K. Science of The Total Environment 569-570:1330-1340.

Zimmer, M. 2008. Detritus. Pages 903-911 *in* S. E. Jørgensen and B. D. Fath, editors. Encyclopedia of ecology. Elsevier, Oxford.

Zsolnay, A., E. Baigar, M. Jimenez, B. Steinweg, and F. Saccomandi. 1999. Differentiating with fluorescence spectroscopy the sources of dissolved organic matter in soils subjected to drying. Chemosphere 38:45-50.

Zuur, A. F., E. N. Ieno, N. J. Walker, A. A. Saveliev, and G. M. Smith. 2009. Things are not always linear: Additive modelling. Pages 35-69 *in* A. F. Zuur, E. N. Ieno, N. Walker, A. A. Saveliev, and G. M. Smith, editors. Mixed effects models and extensions in ecology with R. Springer New York, New York, NY.