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TESIS DOCTORAL

EFECTOS DE LOS CAMBIOS EN LA OCUPACIÓN DEL SUELO EN RÍOS DE MONTAÑA: UN ENFOQUE MULTINIVEL

PhD THESIS

EFFECTS OF LAND COVER CHANGE ON MOUNTAIN STREAMS: A MULTI-LEVEL APPROACH

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A mi madre

A Joseba

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Cuando llegue a Santander en 2011 tenía claro que la investigación no era para mí, que yo quería era dedicarme la gestión. Sin embargo, Pepe se cruzó en mi camino para hacerme cambiar de idea y así acabar embarcándome en esta tesis doctoral. Hoy, 5 años después, aquí estoy cerrando esta etapa, la más exigente e intensa de mi vida. Echando la vista atrás y haciendo balance, me doy cuenta de que, a pesar de momentos duros, ha merecido la pena. Esta tesis ha supuesto un reto personal en el que he aprendido a esforzarme, a centrarme en el positivo y a luchar por lo que quiero, pero sobre todo a crecer como persona. Sin embargo, todo esto no habría sido posible sin todas esas personas que de una manera u otra han estado ahí, apoyándome y formado parte de este proceso. Por ello, me gustaría dedicar un pequeño espacio de esta tesis para agradecérselo.

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Contents

Contents

Resumen

Resumen

Capítulo I. Introducción

1.1 Importancia de los ríos de cabecera

Los ríos de cabecera comprenden un grupo de ríos de pequeño tamaño que se originan donde el agua fluye sobre el terreno formando un canal discernible, lo que a menudo ocurre en áreas de montaña. Estos pequeños ríos poseen una posición estratégica en la red fluvial, ya que se encuentran conectados longitudinalmente a ríos de mayor tamaño y lateralmente a los ecosistemas terrestres adyacentes. Debido a esta estrecha conexión con los ecosistemas terrestres, los ríos de cabecera se ven fuertemente influenciados por las perturbaciones que ocurren en ellos.

1.2 Cambios en los usos y en la ocupación del suelo

Los cambios en los usos y en la ocupación del suelo son unas de las principales perturbaciones en los ecosistemas terrestres. Los cambios más dominantes en la ocupación del suelo como, por ejemplo, la expansión de las zonas urbanas, de la agricultura y de los pastizales para el consumo ganadero, así como el aumento de las plantaciones o la tala de bosques, están conduciendo a una pérdida de la vegetación natural, predominantemente bosque. Sin embargo, esta tendencia se está revirtiendo y en la actualidad, extensas áreas están siendo abandonadas en todo el mundo. La principal consecuencia del abandono del suelo es la sucesión secundaria, lo que en muchas regiones está generando un aumento de la superficie forestal. A pesar de que el abandono del suelo es un proceso que ha sido mucho menos estudiado que el aumento de las actividades agrarias y la urbanización, actualmente constituye uno de los cambios más importantes en la ocupación del suelo, el cual se espera que siga aumentando en el futuro.

1.3 Efectos de los cambios en la ocupación del suelo sobre los ecosistemas fluviales

1.3.1 Componentes físicos y químicos fluviales

La ocupación del suelo controla una gran cantidad de propiedades físicas y químicas de los ecosistemas fluviales, como: la concentración de nutrientes y carbono, el caudal y la velocidad del agua, a través de variaciones en la escorrentía durante tormentas y a la retención de agua durante épocas de caudal basal, la cantidad de radiación solar incidente o la temperatura del agua.

1.3.2 Recursos tróficos: origen, cantidad y calidad

La ocupación del suelo también ejerce una gran influencia sobre el origen, la cantidad y la calidad de la materia orgánica que constituye la fuente de energía para la biota, es decir, los recursos que sostienen las redes tróficas en los ecosistemas fluviales.

Se pueden diferenciar dos tipos de recursos tróficos en función de su origen: recursos alóctonos (importados de los ecosistemas terrestres adyacentes, como la hojarasca) y autóctonos (producidos en el ecosistema fluvial como algas bentónicas, macrófitos y fitoplancton). No obstante, algunos recursos tróficos tienen un origen mixto ya que están compuestos por materia orgánica alóctona y autóctona, como el biofilm, la materia orgánica particulada fina y gruesa o materia orgánica disuelta (MOD). En el caso de la MOD, se ha demostrado que la mayor parte de los compuestos que la forman han sido directamente lixiviados por la vegetación o restos vegetales (p. ej. hojas, ramas o frutos), o provienen de la materia orgánica acumulada en el suelo de la cuenca. Esto hace que la composición de la MOD dependa en gran medida del tipo de vegetación de la cuenca y que sea extremadamente sensible a los cambios en la ocupación de suelo. Sin embargo, hay un conocimiento muy limitado sobre el efecto que la ocupación histórica del suelo tiene sobre las propiedades de la MOD fluvial actual.

Los cambios en la ocupación del suelo pueden afectar, además de a las propiedades de la MOD, a todos los recursos tróficos disponibles para las comunidades de organismos. En este sentido, la ocupación del suelo puede cambiar la cantidad de recursos tróficos autóctonos y alóctonos y la cantidad de materia orgánica autóctona y alóctona en recursos de origen mixto. Dado que los recursos alóctonos tienen una calidad inferior a la de los recursos autóctonos, estos cambios implican una alteración simultánea de la cantidad y la calidad de los recursos tróficos. A día de hoy, la mayor parte de las investigaciones se han centrado en comprender los efectos de cambios en la cantidad de recursos o en la calidad de un único recurso, por lo que aún se desconoce cómo la estructura y la composición de la red trófica responden a cambios en la cantidad y calidad de los recursos tróficos.

1.3.3 Composición y estructura de tamaños de las redes tróficas

Los dos tipos de recursos tróficos definen a su vez dos canales de energía diferentes en las redes tróficas: el canal de energía autóctono y el canal de energía alóctono. La disponibilidad relativa de recursos autóctonos y alóctonos define la importancia de estos dos canales de energía, lo que afecta tanto a la abundancia y composición de los diferentes grupos tróficos (especialmente a los consumidores primarios como detritívoros y herbívoros) y consecuentemente a la estructura y composición de la comunidad, como al crecimiento de los organismos y, por tanto, a su tamaño corporal. Los cambios en el tamaño corporal de los organismos pueden reflejarse en la estructura de tamaños de toda la comunidad y en el espectro de tamaños. El espectro de tamaños de una comunidad informa de la eficiencia trófica (eficiencia en la transferencia de energía entre los niveles tróficos) y de la capacidad de carga del ecosistema y tiene un papel clave en el funcionamiento del ecosistema. En ausencia de perturbaciones, el espectro de tamaños tiende a ser relativamente regular y predecible. Sin embargo, puede verse alterado en comunidades sometidas a perturbaciones, pero cómo se produce esta alteración es aun altamente desconocido.

1.4 Multifuncionalidad del ecosistema

La ocupación del suelo juega un papel clave en la regulación de la multifuncionalidad del ecosistema, es decir, del conjunto de funciones que ocurren simultáneamente en un ecosistema. Desde una perspectiva energética, varias funciones informan sobre el flujo de materia y energía y la importancia de los canales de energía (autóctono y alóctono) que sostienen las redes tróficas fluviales.

Desde la perspectiva de los recursos tróficos, el crecimiento del biofilm representa la ganancia de la biomasa de productores primarios a lo largo del tiempo y define, en gran medida, la cantidad de materia orgánica disponible para las redes tróficas en canal autóctono mientras que la descomposición de la materia orgánica muestra el consumo de materia orgánica en el canal alóctono. Desde la perspectiva de la biota, las actividades enzimáticas describen la expresión de enzimas necesarias para la asimilación de nutrientes y carbono mientras que el crecimiento de los organismos describe la generación de biomasa heterotrófica. Otras funciones, como el metabolismo fluvial, pueden proporcionar una visión integradora flujo de nutrientes y materia en el ecosistema. En este sentido, el metabolismo fluvial es el resultado de la producción primaria bruta y la respiración del ecosistema y, por tanto, tiene en cuenta todos los procesos interrelacionados que fijan y mineralizan materia orgánica llevados a cabo por todos los organismos (autótrofos y heterótrofos).

La ocupación del suelo puede controlar estas funciones a través de los componentes físicos y químicos fluviales anteriormente descritos, las propiedades de la materia orgánica y la composición y tamaño corporal de los organismos que conforman las redes tróficas. Por lo tanto, para entender los mecanismos a través de los cuales los cambios en la ocupación del suelo afectan al funcionamiento de los ecosistemas fluviales, es crucial investigar cómo la ocupación del suelo controla todos estos componentes fluviales.

1.5 Objetivos de la tesis

El objetivo principal de la presente tesis es caracterizar los efectos que los cambios en la ocupación del suelo tienen sobre las vías de flujo de energía, la composición y estructura de las redes tróficas y la multifuncionalidad del ecosistema en ríos de montaña a través de componentes fluviales físicos y químicos, así como de la alteración de los recursos tróficos basales, los cuales constituyen el sustento de las redes tróficas. Los resultados de este enfoque multinivel serán altamente valiosos para diseñar soluciones que permitan mitigar los efectos de los cambios en la ocupación del suelo y conservar el funcionamiento de los ríos de cabecera y los servicios que éstos proporcionan.

Los objetivos específicos de esta tesis son:

- Analizar los efectos de la ocupación actual e histórica del suelo sobre la cantidad y composición de la materia orgánica disuelta y establecer como la distancia de un determinado tipo de ocupación al río y la topografía de la cuenca influencian dichos efectos.
- Investigar cómo la ocupación de suelo determina el tipo (alóctono vs autóctono) y la cantidad de recursos tróficos y cómo cambios en dichos recursos afectan a su asimilación por la comunidad de macroinvertebrados, a las vías de flujo de energía y a la estructura de las redes tróficas.
- Evaluar la respuesta de la estructura de tamaños de la comunidad de macroinvertebrados a las variaciones en el tipo y cantidad total de recursos tróficos, para testar si estas variaciones alteran la eficiencia trófica y la capacidad de carga del ecosistema.
- Examinar las vías abióticas y bióticas locales a través de las cuales la ocupación del suelo controla las tasas de diferentes funciones relacionadas con la energética del ecosistema y establecer el efecto de los cambios en la ocupación del suelo sobre la multifuncionalidad del ecosistema.

Capítulo II. Descripción del área de estudio, caracterización de la ocupación del suelo y selección de tramos fluviales

2.1 Área de estudio

El área de estudio se sitúa en la sección central de la Cordillera Cantábrica (norte de España), la cual alcanza los 2600 m de altitud y se expande a lo largo de más de 300 km paralela al mar Cantábrico (Océano Atlántico, Fig. 1).

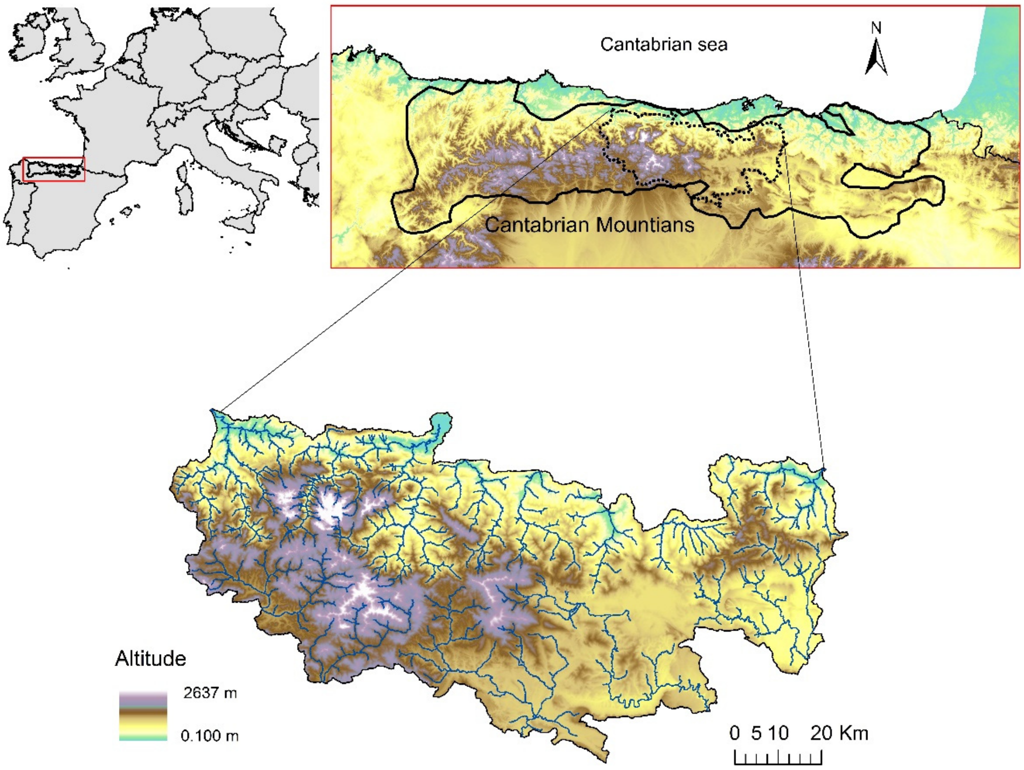

Figura 1 – Mapa del área de estudio situada la Cordillera Cantábrica, norte de España.

2.1.1 Geomorfología y geología

La Cordillera Cantábrica se formó por dos grandes orogenias: la Orogenia Herciniana, en el Carbonífero tardío, y la Orogenia Alpina, en el Cretácico superior. Durante la Orogenia Alpina, los sedimentos acumulados a lo largo del tiempo (p. ej. cuarcitas, areniscas, lutitas, conglomerados, capas de carbón y calizas) se comprimieron, plegaron y fracturaron para formar las montañas que actualmente encontramos en la Cordillera Cantábrica. Tras esta orogenia, la topografía fue modelada por procesos exógenos, incluyendo una serie de períodos glaciales que dejaron una clara huella en el relieve. Debido a estos procesos erosivos, en la actualidad, los sedimentos más resistentes (p. ej. calizas, rocas calcáreas) pueden encontrarse en las zonas más altas de la cordillera mientras que los sedimentos más blandos (p. ej. areniscas, rocas silíceas) se erosionaron por la acción de ríos y glaciares y aparecen predominantemente en los fondos de valle.

2.1.2 Clima

La Cordillera Cantábrica posee un clima único para la latitud sur en la que se ubica, pudiéndose diferenciar en ella dos zonas climáticas distintas: la vertiente atlántica y la vertiente mediterránea. La vertiente atlántica es estrecha y empinada, con solo unas pocas decenas de kilómetros (40 - 50 km) entre el mar y la divisoria de aguas, situada a 2600 m a.s.l.. En esta vertiente, las precipitaciones son abundantes (precipitación media anual de 1315 ± 200 mm) y las temperaturas frescas (temperatura media anual de 8,8 ± 1,8 °C). Por el contrario, la vertiente mediterránea se caracteriza por una menor pendiente, debido a un descenso de altitud mucho menor (1500 m) en una distancia mucho mayor (cientos de kilómetros), así como por un clima continental sub-mediterráneo. En esta vertiente, las temperaturas son más elevadas y las precipitaciones más escasas que la vertiente atlántica (la temperatura media anual es 10.2 ± 2.2 ° C y la precipitación media anual 990 ± 230 mm) pero presentan un mayor contraste entre verano e invierno.

2.1.3 Socioeconomía

En la Cordillera Cantábrica, la agricultura, la silvicultura y la ganadería (principalmente trashumante) han sido los pilares de la economía desde la Edad Media, teniendo especial relevancia la minería a partir de la segunda mitad del siglo XIX. En las últimas décadas, la Cordillera Cantábrica ha experimentado: un fuerte desarrollo de las actividades terciarias, la despoblación de los municipios rurales hacia las zonas urbanas (principalmente ubicados en municipios costeros), el abandono de las actividades agrícolas tradicionales y la reorientación de la economía agrícola, la disminución de la explotación minera y la desaparición de la trashumancia. Esto ha llevado a la re-naturalización del paisaje en algunas zonas, con un importante avance de las masas forestales. Por ello, el paisaje actual de la Cordillera Cantábrica se caracteriza por la alternancia de: antiguos pastizales mantenidos a lo largo del tiempo para el pastoreo, bosques maduros, jóvenes bosques secundarios en pastizales y zonas agrícolas abandonadas, y grandes áreas urbanas, localizadas principalmente a lo largo de la costa.

2.1.4 Flora y fauna

La Cordillera Cantábrica representa la frontera norte-sur y este-oeste en la distribución de muchas especies y comunidades de flora y fauna.

La Cordillera Cantábrica, gracias a su gran heterogeneidad ambiental, posee la mayor riqueza florística de las montañas europeas, con un alto porcentaje de endemicidad. La vegetación se caracteriza por una mezcla de vegetación caducifolia y esclerófila de climas templados, representada por: hayas (*Fagus sylvatica* L.), robles (*Quercus petraea* Matt. Lieb. y *Quercus robur* L.) y abedules (*Betula alba* L.) en las zonas más frías y húmedas de la vertiente atlántica, mientras que las zonas más cálidas y secas de la vertiente mediterránea están dominadas por especies de roble más mediterráneas (*Quercus pyrenaica* Willd. y *Quercus rotundifolia* Lam.). La vegetación arbustiva muestra un gradiente similar que varía desde comunidades semiáridas mezcladas con pastizales anuales y cultivos en el sureste, hasta comunidades de orla de bosque en las áreas más septentrionales y occidentales.

La Cordillera Cantábrica también concentra una de las mayores riquezas de vertebrados de la Península Ibérica. La Cordillera Cantábrica está habitada por 18 especies de anfibios, 22 especies de reptiles, 190 especies de aves nidificadoras y 67 especies de mamíferos de las 22, 35, 263 y 67 especies que se pueden encontrar en la Península Ibérica, respectivamente. Sus ecosistemas fluviales albergan numerosas especies de peces, entre los que destacan: el salmón (*Salmo salar*), la trucha (*Salmo trutta*), el foxino (*Phoxinus vigerri*), la anguila (Anguilla anguilla) y la lamprea marina (*Petromyzon marinus*), y numerosas familias de invertebrados bentónicos.

2.1.5 Ecosistemas acuáticos

La Cordillera Cantábrica comprende una amplia red fluvial así como numerosos lagos y lagunas originados durante los períodos glaciales. Los ríos se ubican tanto en la vertiente atlántica (desembocan en el océano Atlántico) como en la vertiente mediterránea y desembocan en el mar Mediterráneo (río Ebro) o el océano Atlántico (río Duero). Los ríos de la vertiente atlántica son cortos y empinados y se caracterizan por un régimen de caudal regular con ausencia de sequías estivales mientras que ríos de la vertiente mediterránea son más largos y tendidos y se caracterizan por un régimen de caudal muy irregular, con inundaciones estacionales y fuertes sequías estivales.

2.2 Caracterización de la ocupación del suelo

Dado que el objetivo principal de esta tesis es comprender los efectos de los cambios sobre la ocupación del suelo, en el área de estudio se caracterizaron tanto la ocupación actual del suelo (año 2009) como la ocupación histórica (año 1984).

La información de la ocupación del suelo se obtuvo a partir de una clasificación supervisada de imágenes de satélite. Dos imágenes Landsat TM, descargadas del Servicio Geológico de Estados Unidos para el área de estudio en los años 1984 y 2009, se corrigieron radiométricamente y atmosféricamente. Posteriormente, se aplicó una clasificación por píxel, utilizando un algoritmo de máxima verosimilitud sobre una combinación de información espectral obtenida de las imágenes satelitales y topográfica obtenida de un modelo digital de elevación. El algoritmo de máxima verosimilitud asignó píxeles a una determinada clase de ocupación del suelo (es decir: bosques, pastos, matorrales, suelo urbano o agrícola, afloramientos rocosos o masas de agua) basándose en aquella clase con la mayor probabilidad de ocurrencia en cada píxel.

2.3 Selección de tramos fluviales

La información relativa a la ocupación del suelo se integró en una red sintética fluvial previamente delineada para el área de estudio, lo cual permitió establecer, para cada tramo fluvial, el porcentaje de cada clase de ocupación del suelo tanto en la cuenca drenante como en un búfer de 200 m a lo largo esta. En cada tramo fluvial se calculó el cambio en ocupación del suelo entre los años 2009 y 1984 para considerar la historia de la ocupación del suelo.

En base a la ocupación del suelo obtenida, se seleccionaron 31 tramos fluviales (Fig. 2) a lo largo de un gradiente de cobertura forestal actual. Para maximizar el efecto del gradiente forestal, se seleccionaron tramos fluviales sin vertidos u otras grandes fuentes de contaminación y con una ocupación mínima de suelo urbano y agrícola en la cuenca drenante (< 1% del área de la cuenca).

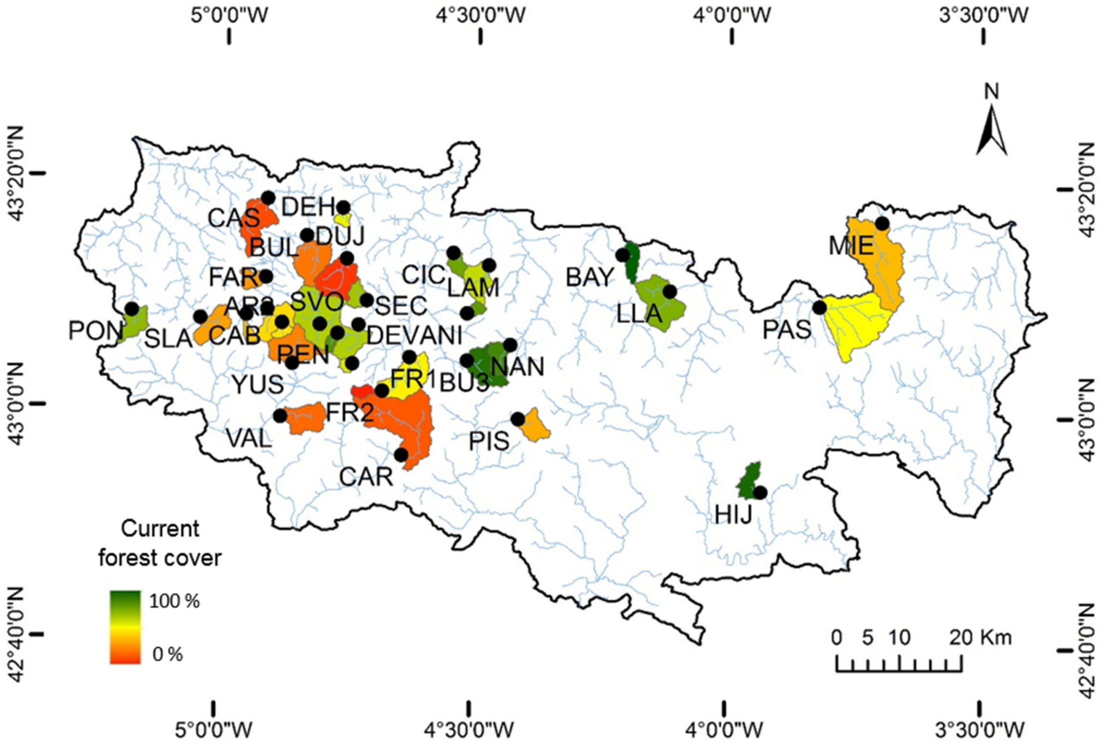

Figura 2 - 31 tramos fluviales de estudio seleccionados en base al porcentaje de cobertura forestal en la cuenca drenante. El color de la cuenca indica el porcentaje de cobertura forestal actual.

De los 31 ríos seleccionados, en el capítulo III únicamente se consideraron 24 ríos, puesto que en 2 ríos la MOD podría no estar afectada por la ocupación del suelo de la cuenca dada su cercana ubicación al manantial y las muestras tomadas en otros 5 ríos mostraron un severo grado de contaminación. En los capítulos IV y V se seleccionaron únicamente 10 ríos debido a las limitaciones metodológicas que supone la determinación de la biomasa de los macroinvertebrados, mientras que en el capítulo VI se consideraron los 31 ríos seleccionados.

Capítulo III. Ausencia de legado de los cambios en la ocupación del suelo en la materia orgánica disuelta fluvial

La MOD representa la mayor fuente de carbono orgánico en los ecosistemas fluviales. Se ha demostrado que la mayor parte de la MOD fluvial es de origen terrestre, por lo que la mayoría de compuestos de la MOD son directamente lixiviados por la vegetación o restos vegetales (p. ej. hojas, ramas o frutos), o provienen de la materia orgánica acumulada en el suelo de la cuenca. Esto hace que la composición de la MOD dependa en gran medida del tipo de vegetación presente en la cuenca y que sea extremadamente sensible a cambios en la ocupación de suelo. El abandono del territorio vinculado al cese de la agricultura y a la reducción del pastoreo es uno de los principales cambios en la ocupación del suelo en muchas regiones de montaña. Este abandono del territorio está dando lugar a procesos de sucesión secundaria y, consecuentemente, a un cambio en el tipo de vegetación: el desplazamiento de arbustos y pastizales por el bosque autóctono. Estos cambios en la ocupación del suelo tienen un gran efecto sobre las propiedades de la vegetación y del suelo de la cuenca. Sin embargo, la transformación de la vegetación ocurre en una escala temporal mucho menor que la transformación de la materia orgánica del suelo. Aunque esto sugiere que ocupación histórica del suelo podría tener un fuerte efecto sobre las propiedades de la MOD fluvial actual, aún hay un conocimiento muy limitado sobre cómo estas se ven afectadas. Por ello, el objetivo del capítulo III fue analizar los efectos de la ocupación actual e histórica del suelo sobre la cantidad y composición de la MOD así como determinar cómo la distancia de un determinado tipo de ocupación al río y la topografía de la cuenca influencian dichos efectos. Para ello, se analizó la cantidad y la composición de la MOD fluvial mediante análisis espectroscópicos, cromatográficos y de espectrometría de masas.

Los resultados obtenidos mostraron una la dominancia de compuestos de origen terrestre en todos los ríos estudiados. Sin embargo, el grado de cobertura forestal y la pendiente de la zona ripiara determinaron en gran medida la cantidad y la composición de la MOD. Una mayor cobertura forestal se relacionó con una mayor cantidad de MOD de origen terrestre más oxigenada y ligeramente más aromática. Esto se asoció con una mayor proporción de taninos y compuestos aromáticos policíclicos y una menor proporción de lignina y compuestos derivados de la vegetación o restos vegetales o procedentes de suelos jóvenes, tales como compuestos alifáticos pobres en oxígeno (Fig. 3). La presencia de laderas más tendidas en la zona riparia se asoció con una mayor cantidad de MOD, caracterizada por la dominancia de compuestos húmicos y aromáticos altamente descompuestos (compuestos fenólicos y aromáticos policíclicos) y una menor proporción de lignina y compuestos recientes y poco descompuestos producidos tanto en el sistema fluvial como procedentes de la vegetación (p. ej. carbohidratos, ácidos grasos y proteínas; Fig. 4).

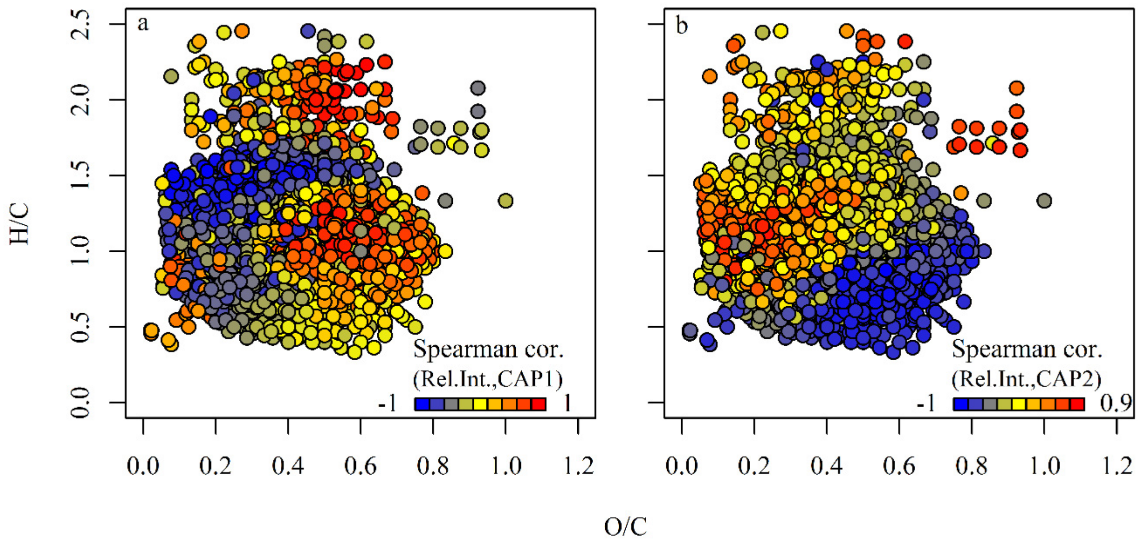

Figura 3 – Los diagramas de Van Krevelen muestran los cambios en la composición de la MOD asociados a los dos ejes canónicos definidos por (a) el grado de cobertura forestal actual en un búfer de 200 m y (b) la pendiente de las laderas en el búfer de 200 m. Cada círculo representa una molécula y su color indica la correlación entre la intensidad relativa de la molécula con los ejes canónicos para todos los ríos muestreados. Las moléculas rojas/azules aumentan/disminuyen en intensidad relativa con la cobertura forestal y la pendiente de las laderas.

Sin embargo, no se observó ningún efecto de la ocupación histórica del suelo sobre la composición de la MOD. Esto, posiblemente, se deba a una rápida recuperación de la vegetación tras abandono del suelo junto a un rol más dominante de la vegetación, en comparación con la materia orgánica del suelo, a la hora de definir las propiedades de la MOD fluvial en caudal basal.

Capítulo IV. Influencia de la ocupación del suelo sobre la estructura de las redes tróficas de macroinvertebrados y las vías de flujo de energía

Comprender cómo los diferentes recursos sustentan las redes tróficas es fundamental para aumentar nuestro conocimiento sobre la estructura de las redes tróficas y las vías de flujo de energía en los ecosistemas fluviales. En pequeños ríos de montaña, las redes tróficas se sustentan tanto por recursos autóctonos (producción primaria fluvial) como por recursos alóctonos (materia orgánica procedente del ecosistema terrestre) y la importancia relativa estos dos tipos de recursos depende en gran medida de la ocupación del suelo de la cuenca. En el capítulo IV se investigó cómo la ocupación del suelo de la cuenca determina la cantidad y el tipo (autóctonos vs alóctonos) de recursos tróficos disponibles y cómo cambios en los recursos afectan al tipo de recurso asimilado por la comunidad de macroinvertebrados, a las vías de flujo de energía y a la estructura de la red trófica en ríos de montaña. Para ello, se determinó la biomasa de los distintos tipos de recursos y grupos tróficos de macroinvertebrados (detritívoros, herbívoros, omnívoros y carnívoros) y se analizaron isótopos estables (δ^2H y $\delta^{15}N$) en todos ellos.

Los resultados obtenidos en este capítulo mostraron que el tipo de ocupación del suelo determina el tipo de recursos tróficos disponibles para la comunidad de macroinvertebrados, a pesar de la dominancia de recursos alóctonos en todos los ríos estudiados. Los ríos dominados por pastos se caracterizaron por una mayor proporción de recursos autóctonos (p. ej. mayor biomasa de macroalgas y biofilm con un mayor contenido de clorofila *a*) mientras que los ríos con un mayor grado de cobertura forestal mostraron una mayor proporción de recursos alóctonos (p. ej. madera y hojarasca). Sin embargo, se observó que la cantidad de recursos alóctonos no solo depende del tipo de ocupación del suelo, sino también de la pendiente de la cuenca, ya que pendientes más tendidas limitan el transporte de hojarasca de las laderas al río. Además, las redes tróficas mostraron una gran dependencia en el tipo y la cantidad de recursos tróficos disponibles. Los recursos alóctonos fueron el principal sustento de la comunidad de macroinvertebrados en cuencas altamente forestadas, mientras los recursos autóctonos fueron su principal sustento en ríos que drenan cuencas dominadas por pastos y/o zonas arbustivas. La respuesta a la variación en el tipo de recurso difirió entre grupos tróficos, ya que los detritívoros mostraron una asimilación fija de recursos alóctonos independiente de la cantidad de recursos disponible, mientras que los omnívoros asimilaron mayoritariamente el recurso más dominante (Fig. 4).

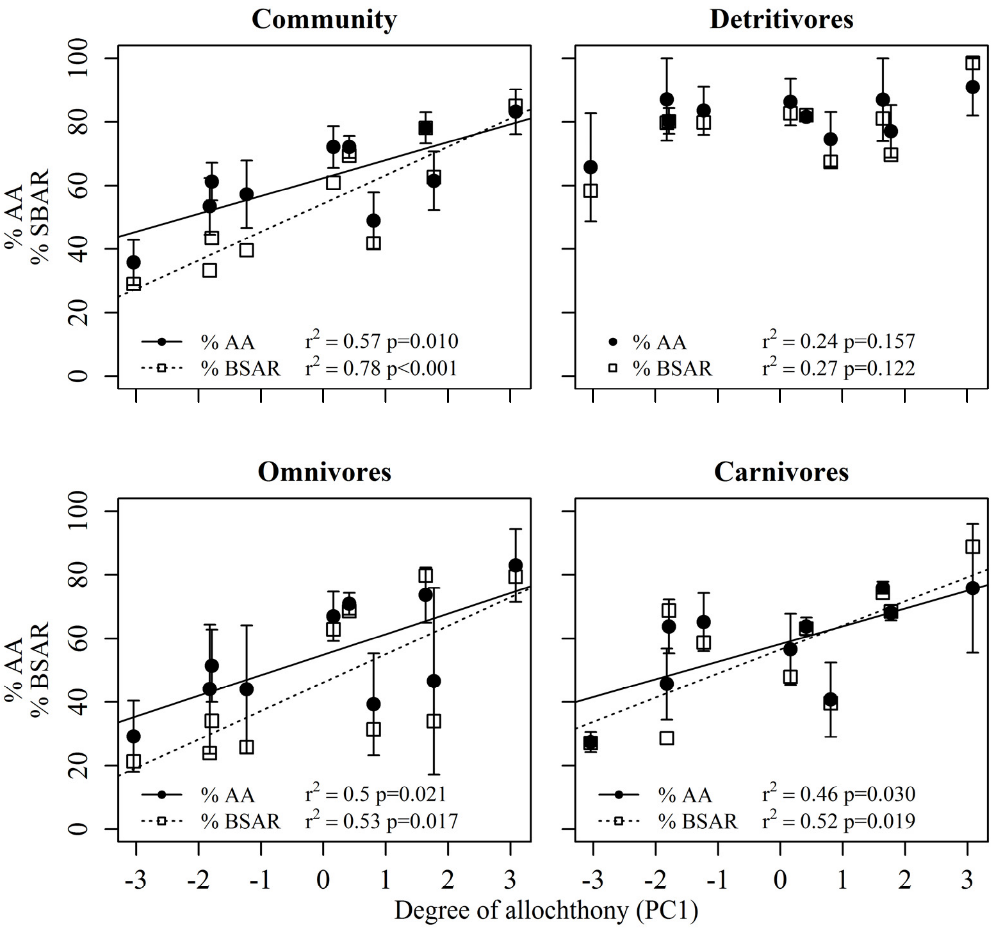

Figura 4 – Porcentaje medio (± desviación estándar) de la asimilación alóctona (●, ecuación superior) y del porcentaje de biomasa sustentada por recursos alóctonos (□; ecuación inferior) respecto al grado de aloctonía (PC1) para la toda la comunidad de macroinvertebrados y para detritívoros, omnívoros y carnívoros. Las líneas de regresión significativas

La diferente respuesta de detritívoros y omnívoros al aumento de la cobertura forestal se tradujo en un aumento en la biomasa de detritívoros y una conservación de la biomasa de omnívoros, lo que resultó en el aumento de la asimilación de recursos alóctonos en carnívoros. Estas variaciones en los distintos grupos tróficos se vieron reflejadas en la estructura de la red trófica, ya que la biomasa de la comunidad se distribuyó de forma más equitativa entre los organismos que la componen con el aumento de la cobertura forestal en la cuenca (Fig. 5).

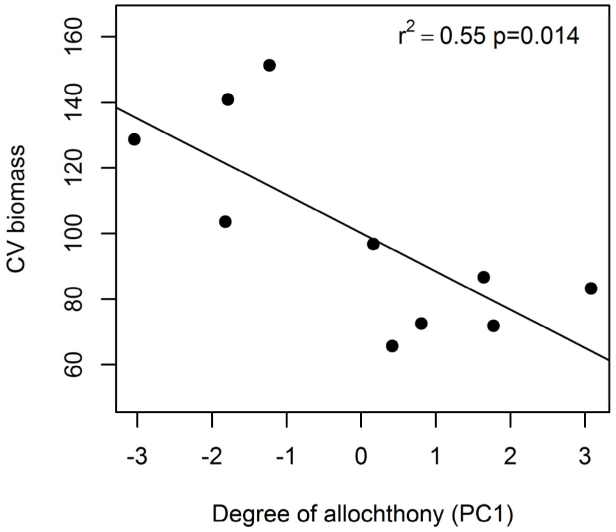

Figura 5 – Relación entre el coeficiente de variación (CV) de la biomasa de macroinvertebrados y el grado de aloctonía (PC1).

Capítulo V. Efectos de la pérdida de bosque sobre la estructura de tamaños de la comunidad de macroinvertebrados fluviales

Como se evidencia el capítulo IV, los cambios en la ocupación del suelo son claves a la hora de determinar el tipo de recursos tróficos disponibles para la comunidad de macroinvertebrados en ríos de montaña. En la actualidad, los efectos de los cambios en el tipo de recursos se han estudiado principalmente en la estructura y composición de las comunidades fluviales. Sin embargo, se ha prestado mucha menos atención a su efecto sobre la estructura de tamaños de los organismos las componen. En el caso de la pérdida de cobertura forestal, predecir la respuesta de la estructura de tamaños de la comunidad de macroinvertebrados a los cambios en los recursos tróficos se ve fuertemente obstaculizada por cómo la calidad y labilidad de los recursos y la estrategia de vida de los organismos afectan a su tamaño corporal. Por ello, en el capítulo V se analizó el efecto de los cambios en el tipo y cantidad de recursos tróficos debido a la pérdida de cobertura forestal sobre el espectro de tamaños de la comunidad de macroinvertebrados fluviales para comprender si eficiencia trófica (pendiente del espectro de tamaños) y la capacidad de carga del ecosistema (intersección del espectro de tamaños) se ven alteradas por cambios en los recursos. Además, se investigó cómo responde la estructura de tamaños a variaciones en el tipo y cantidad de recursos tróficos: mediante cambios en la composición taxonómica (es decir, a través de cambios en riqueza de taxones o de un reemplazo taxonómico), en la distribución de los tamaños corporales o mediante una combinación de ambos. Para ello, se construyeron espectros del tamaño de la comunidad de macroinvertebrados y se determinó la riqueza de taxones, la densidad, biomasa y tamaño corporal de toda la comunidad, así como de cada grupo trófico (detritívoros, herbívoros, omnívoros y carnívoros).

Los resultados obtenidos mostraron que la pendiente del espectro de tamaños se mantuvo a pesar de los cambios en el tipo y cantidad de recursos tróficos (Fig. 6). Esto indica una regulación interna de la estructura de tamaños de la comunidad para mantener la eficiencia trófica (pendiente del espectro de tamaños) ajustando únicamente su capacidad de carga (intersección del espectro de tamaños) a la cantidad total de recursos disponibles mediante variaciones en la abundancia de organismos. Este ajuste de la comunidad se produjo mediante la sustitución de organismos detritívoros por omnívoros. Sin embargo, los cambios en la estructura de tamaños en detritívoros y omnívoros se produjeron a través de diferentes mecanismos. La respuesta de los omnívoros fue numérica (aumento en densidad y, por consiguiente, en biomasa), no relacionada con el tamaño corporal o la composición taxonómica, y principalmente en los organismos de gran tamaño. Por el contrario, los detritívoros mostraron una reducción en el tamaño corporal (pero no en biomasa total o densidad) con la pérdida de cobertura forestal, debido a un reemplazo taxonómico de tricópteros de gran tamaño

(Odontoceridae, Limnephilidae y Beraeidae) por organismos de pequeño tamaño (p.ej. Elmidae).

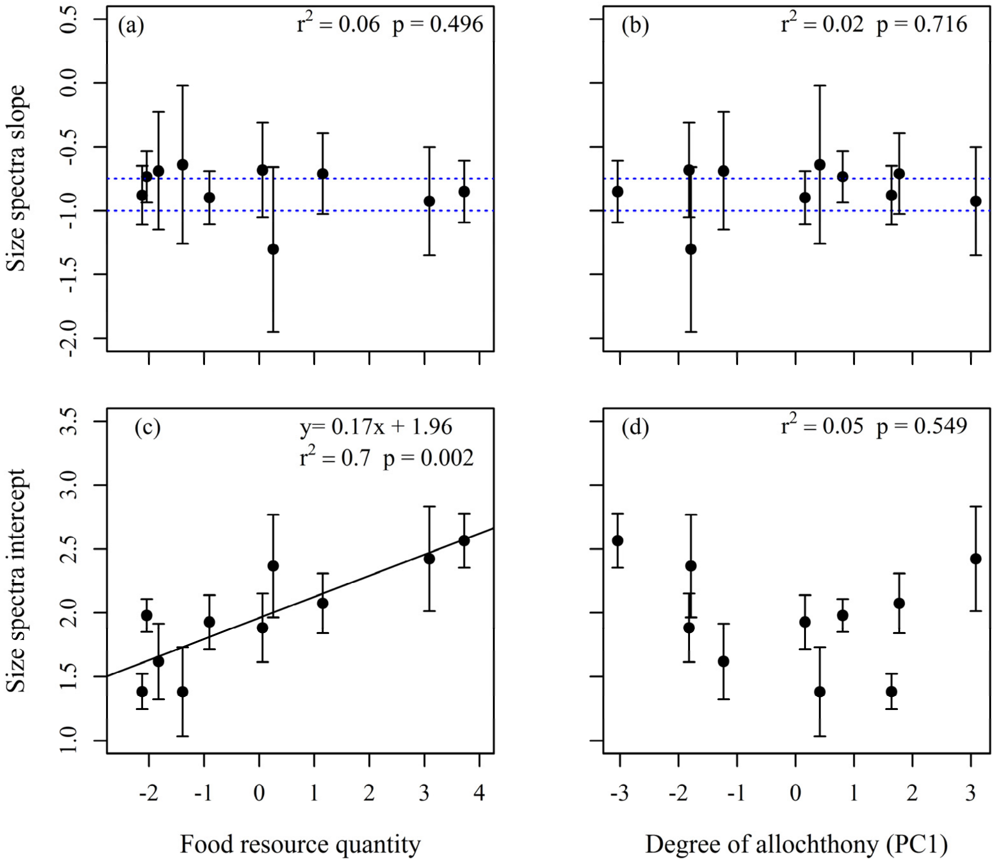

Figura 6 – Regresiones lineales entre la cantidad total de recursos tróficos (a, c) y el grado de aloctonía (PC1; b, d) y la pendiente (a, b) y la intersección (c, d) de los espectros de tamaño de la comunidad de macroinvertebrados. Se muestran los intervalos de confianza (5% - 95%) para las pendientes y las intersecciones y las líneas y ecuaciones de regresión únicamente para las relaciones significativas (*p* < 0.05). Las líneas punteadas azules muestras los valores de la pendiente - 0.75 y - 1.

Capítulo VI. Influencia del bosque sobre la multifuncionalidad de los ecosistemas fluviales

Los cambios en la ocupación del suelo, a través de la alteración de la hidrología, la vegetación, la erosión del suelo o la cobertura del dosel arbóreo, pueden tener grandes efectos sobre numerosos factores ambientales y componentes del ecosistema, incluida la temperatura del agua, la intensidad de la luz, la concentración de nutrientes, las propiedades de la materia orgánica o la composición de la comunidad de macroinvertebrados. Todos estos factores controlan múltiples funciones del ecosistema que ocurren simultáneamente en los ecosistemas fluviales (multifuncionalidad del ecosistema). Sin embargo, la forma en que estos factores, que actúan a escala local, interactúan para determinar las tasas de funcionamiento y cómo estas interacciones se ven influenciadas por otros factores distales (p. ej. el clima, la altitud, la geología), sigue siendo poco conocida. Por ello, el objetivo principal del capítulo VI fue examinar las rutas abióticas y bióticas a escala local mediante las que la ocupación del suelo determina las tasas de funciones relacionadas con la energía del ecosistema (p. ej. descomposición de la madera, crecimiento del biofilm, la producción primaria y la respiración ecosistémica) en ríos de montaña (Fig. 7). Además, se investigaron los efectos de los cambios en la ocupación del suelo sobre la multifuncionalidad del ecosistema fluvial y las interacciones directas e indirectas entre la ocupación del suelo y factores a escala de cuenca (p. ej. geología y área de la cuenca).

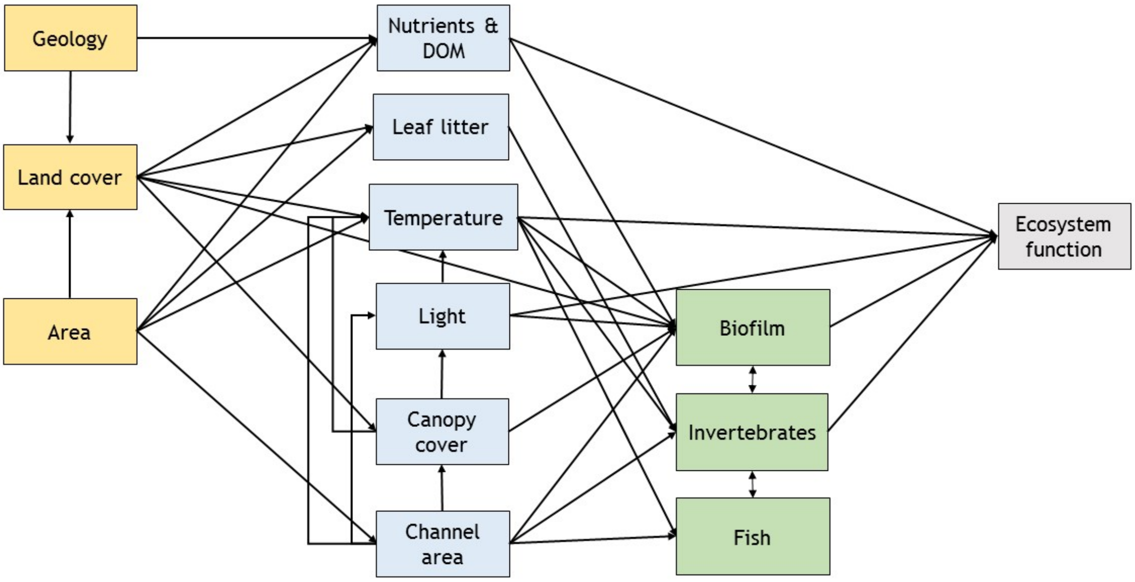

Figura 7 – Diagrama conceptual que describe los factores a escala de cuenca (amarillo oscuro) y a escala local tanto abióticos (azul) como bióticos (verde) que pueden influir en las 5 funciones ecosistémicas fluviales consideradas en este estudio.

Los resultados mostraron cómo la cobertura forestal controla las funciones del ecosistema, principalmente a través de dos factores abióticos: la temperatura mínima del agua en el caso de la descomposición de la madera y la disponibilidad de luz en el caso del crecimiento del biofilm, la producción primaria y la respiración ecosistémica (Fig. 8). Además, los resultados obtenidos revelaron una fuerte interacción entre el grado de cobertura forestal y el área de la cuenca, que definió el nivel de cobertura del dosel arbóreo sobre el río y, por lo tanto, a través de la disponibilidad de luz, las tasas de aquellas funciones que son controladas por organismos autótrofos (el crecimiento del biofilm y la producción primaria). Esta interacción se reflejó en la multifuncionalidad del ecosistema, que experimentó una variación superior al 50% en los ríos estudiados y fue el resultado del incremento de la descomposición de la madera y la disminución de la producción primaria con el aumento de la cobertura forestal y el incremento del crecimiento del biofilm y la producción primaria con área de la cuenca.

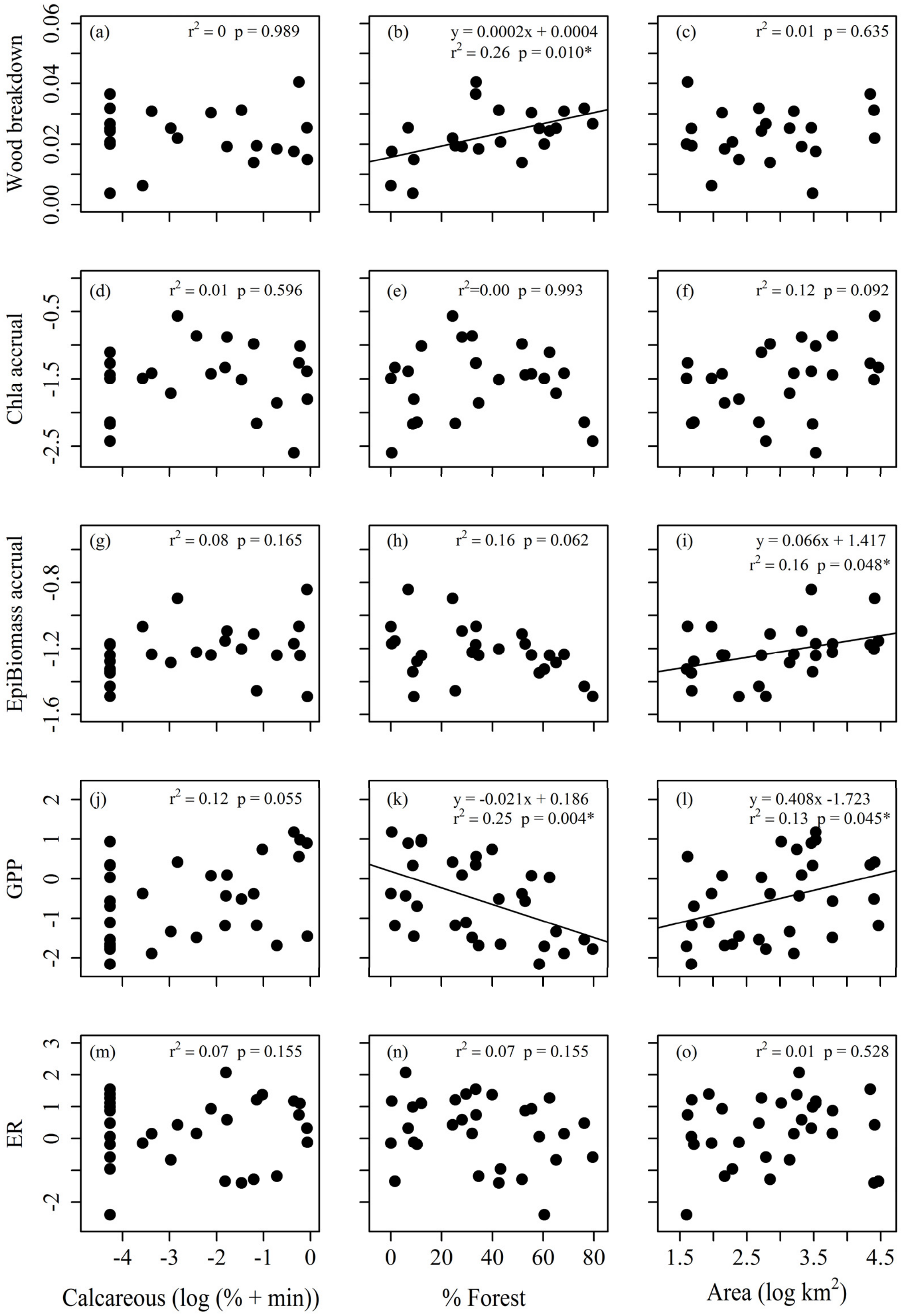

Figura 8- Análisis de regresión lineal entre el porcentaje de rocas calcáreas (a, d, g, l, m), el porcentaje de bosque (b, e, h, k, n) y el área de la cuenca (e, f, i, l, o) y las funciones ecosistémicas: descomposición de madera (d^{-1}; a, b, c), tasa de acumulación de clorofila *a* en biofilms (Chla accrual, mg $Chla.m^{-2}.d^{-1}$; d, e, f), tasa de acumulación de carbono epilítico en biofilms (EpiBiomass accrual; mg

AFDM.m^{-2}.d^{-1}; g, h, i), producción primaria bruta (GPP, g O_2.m^{-2}.d^{-1}; j, k, l) y respiración del ecosistema (ER, g O_2.m^{-2}.d^{-1}; m, n, o). * Regresiones lineales significativas ($p < 0.05$). Se muestran las líneas y ecuaciones de regresión únicamente para las relaciones significativas ($p < 0.05$).

Capítulo VII. Conclusiones generales y futuras líneas de investigación

7.1 Conclusiones generales

En general, podemos concluir que los cambios en la ocupación del suelo tienen un gran efecto sobre las vías de flujo de energía, la composición y la estructura de tamaños de las redes tróficas así como en la multifuncionalidad de los ecosistemas fluviales, a través de la alteración del origen, la cantidad y la calidad de los recursos tróficos que sustentan las comunidades de organismos y los componentes físicos y químicos.

A continuación, se describen las conclusiones de cada uno de los capítulos de esta tesis:

Capítulo III. Ausencia de legado de los cambios en la ocupación del suelo en la materia orgánica disuelta fluvial

- A pesar del dominio de los compuestos terrestres en la MOD en ríos de cabecera, la ocupación del suelo determinó la cantidad y composición de MOD. En las cuencas más forestadas, la MOD fue principalmente de origen terrestre, más oxigenada y ligeramente más aromática, lo que se asoció con el aumento de taninos y compuestos aromáticos policíclicos y la disminución de lignina y compuestos procedentes de suelos jóvenes y de la vegetación como compuestos alifáticos pobres en oxígeno. Por lo tanto, la composición de la vegetación adyacente al río fue el principal factor que definió las propiedades de la MOD fluvial, lo que evidenció una baja conexión hidrológica entre la cuenca y el río en caudal basal.
- La pendiente en la zona riparia influyó fuertemente en las propiedades de la MOD que alcanza el ecosistema fluvial. Una mayor cantidad de MOD, caracterizada por el dominio de compuestos aromáticos y húmicos, pero con una menor proporción de lignina y compuestos derivados de la vegetación o producida en el ecosistema fluvial, estuvo presente en aquellos ríos que drenan cuencas con suaves pendientes en la zona riparia. Este efecto se asoció a una mayor acumulación y degradación de la materia orgánica en los suelos de la cuenca y a un mayor tiempo de residencia del agua en ellos.
- La ocupación histórica del suelo no mostró ningún efecto sobre las propiedades de la MOD fluvial, lo que apunta a una ausencia de legado de los cambios en la ocupación del suelo en caudal basal. Esto podría asociarse a la rápida recuperación de la vegetación tras el abandono de la cuenca junto a la dominancia de la vegetación, y no de la materia orgánica del suelo, para definir las propiedades de la MOD en caudal basal.

Capítulo IV. Influencia de la ocupación del suelo sobre la estructura de las redes tróficas de macroinvertebrados y las vías de flujo de energía

- La ocupación del suelo determinó el tipo de recursos tróficos disponibles para la comunidad de macroinvertebrados fluviales, a pesar del dominio de recursos alóctonos en ríos de montaña. Los ríos dominados por pastos se caracterizaron por una mayor proporción de recursos autóctonos mientras que los ríos forestados mostraron una mayor proporción de recursos alóctonos. Sin embargo, la cantidad de recursos alóctonos también dependió de la pendiente de la cuenca ya que pendientes más tendidas limitan el transporte de hojarasca desde las laderas al río.
- El tipo de recursos que los macroinvertebrados asimilan cuando estos varían con los cambios en la ocupación del suelo difirió entre grupos tróficos. Los organismos detritívoros mostraron una asimilación fija de recursos alócotonos independiente de la cantidad de recursos disponibles mientras que la asimilación de los organismos omnívoros se ajustó al recurso más dominante. Esto demuestra que el modo de alimentación es un rasgo clave a la hora de determinar la capacidad de adaptación de los organismos a las variaciones en los recursos tróficos.
- La ocupación del suelo determinó, a través de la alteración del tipo y la cantidad de recursos, el tipo de recursos asimilados a nivel de comunidad y las vías de flujo de energía. La asimilación de recursos alóctonos y la biomasa sustentada por recursos alóctonos aumento con el porcentaje de bosque en la cuenca. Sin embargo, la asimilación media de un recurso difirió considerablemente de la biomasa sustentada por éste, siendo ésta más autóctona es ríos dominados por recursos autóctonos. Esto demuestra que el tipo de recursos asimilados por los organismos solo proporciona una medida de la preferencia por el recurso. Por ello, es necesario combinar medidas de asimilación de recursos y biomasa de organismos para establecer las vías de flujo de energía.

Capítulo V. Efectos de la pérdida de bosque sobre la estructura de tamaños de la comunidad de macroinvertebrados fluviales

- Las pendientes de los espectros de tamaño de la comunidad de macroinvertebrados permanecieron invariables a la alteración del tipo y la cantidad total de recursos tróficos debido a los cambios en la ocupación del suelo. Por el contrario, la intersección del espectro aumentó con la cantidad de recursos tróficos pero no respondió a los cambios en el tipo de recurso. Esto muestra que la comunidad de macroinvertebrados de ríos de cabecera regula su estructura de tamaños para mantener la eficiencia trófica, ajustando su capacidad de carga a la cantidad total de recursos tróficos.

- La regulación de la estructura de tamaños de la comunidad se logró a través de la sustitución de organismos detritívoros por omnívoros ya que los detritívoros dominaron en ríos forestados mientras que los omnívoros dominaron en ríos dominados por pastos.
- Los mecanismos que rigen la respuesta de la estructura de tamaños de la comunidad de macroinvertebrados a las alteraciones del tipo de recursos difirió entre grupos tróficos. La estructura de tamaños de organismos omnívoros se modificó únicamente por cambios en la distribución del tamaño corporal mientras que en detritívoros se modificó a través de cambios en el tamaño corporal y la composición taxonómica. Esto indica que la regulación interna de la estructura de tamaños se debe a una interacción entre la estrategia de vida (estrategia K vs r), la cual determina si la respuesta afecta al número de individuos o a su tamaño corporal y el modo de alimentación (capacidad para adaptarse a los recursos disponibles), el cual establece si estos cambios se ven acompañados por variaciones en la composición taxonómica.

Capítulo VI. Influencia del bosque sobre la multifuncionalidad de los ecosistemas fluviales

- La ocupación del suelo controló funciones relacionadas con la energética del ecosistema a través de las vías abióticas, especialmente a través de la temperatura mínima del agua en el caso de la descomposición de madera y de la disponibilidad de luz en el caso del crecimiento de biofilm, la producción primaria y la respiración del ecosistema. La dominancia de estos factores abióticos superó por completo la influencia de los factores bióticos sobre las funciones ecosistémicas.
- La ocupación del suelo mostró una gran interacción con el área de la cuenca ya que ambos establecieron el nivel cobertura del dosel arbóreo y, por tanto, la disponibilidad de luz. Concretamente, la disponibilidad de luz disminuyó a mayor niveles de cobertura del dosel arbóreo el cual a su vez aumentó con el porcentaje de bosque en la cuenca y disminuyó con el área de la cuenca.
- Los cambios en la ocupación del suelo y el área de la cuenca llevaron a una variación de un 50% de la multifuncionalidad del ecosistema. Esta variación fue el resultado del aumento en la descomposición de madera y la disminución de la producción primaria con el aumento en la cobertura forestal y el aumento en el crecimiento del biofilm y la producción primaria con área le cuenca.
- La interacción entre la ocupación del suelo y el área de la cuenca evidenció la dominancia de la ocupación del suelo a la hora de controlar el funcionamiento del ecosistema en pequeños ríos de cabecera en zonas montañosas.

7.2 Futuras líneas de investigación

De acuerdo con los objetivos establecidos en esta tesis, se han identificado importantes consecuencias de los cambios en la ocupación del suelo en ríos de montaña. Además, se ha revelado la existencia de lagunas de conocimiento en las que la investigación futura debería centrarse. A continuación describimos algunas de estas líneas de investigación.

- La ausencia de un efecto de la ocupación del suelo histórica sobre la MOD fluvial podría estar relacionada con la similitud de la materia orgánica del suelo, lo cual no fue analizado en esta tesis. Por ello, las futuras investigaciones debería centrarse en entender cómo los procesos de sucesión secundaria afectan a las propiedades de la materia orgánica de los diferentes horizontes del suelo. Además, es necesario considerar que diferentes caudales activan diferentes vías de flujo del agua a través de diferentes horizontes del suelo y solo algunos horizontes pueden diferir en la composición de la materia orgánica con los cambios en la ocupación del suelo. Por tanto, también sería necesario analizar la variación en las propiedades de la MOD a lo largo del año. Esto permitiría comprender si existe un efecto del legado de la ocupación del suelo histórica y los mecanismos mediante los cuales los cambios en la ocupación del suelo determinan las características de la MOD fluvial.
- La variación en la composición de MOD con la ocupación del suelo actual observada en esta tesis implica un cambio en la calidad y la labilidad de la MOD (los compuestos tienen una estructura molecular diferente y pueden tener una resistencia diferente a la degradación biológica). Dado que la MOD es la fuente más importante de energía y carbono para bacterias heterótrofas, es necesario entender cómo la composición de la MOD afecta a su utilización por microorganismos (p. ej. a la biodisponibilidad) y las consecuencias para el funcionamiento del ecosistema (p. ej. respiración microbiana).
- Los resultados de isótopos estables evidenciaron que los organismos omnívoros asimilaron una amplia variedad de recursos tróficos. Los omnívoros poseen varios modos de alimentación, lo que les permite ingerir diversos recursos. Sin embargo, para lograr una asimilación efectiva de estos recursos, los omnívoros necesitarían tener una microbiota intestinal más diversa que otros grupos tróficos con un comportamiento de alimentación más estricto (p. ej. organismos detritívoros). Las investigaciones futuras deberían por tanto centrarse en caracterizar la microbiota intestinal de macroinvertebrados e investigar cómo ésta varía entre organismos con una alimentación estricta frente a organismos con alimentación flexible. Además, la asimilación de recursos con diferente calidad puede afectar al crecimiento del organismo. Por lo tanto, también es necesario investigar cómo la producción secundaria de macroinvertebrados varía con la asimilación de diferentes recursos alimenticios, es

decir, desde una alimentación predominantemente autóctona hasta completamente alóctona.

- Esta tesis solo describe la importancia de los recursos tróficos en el momento más autóctono del año. Sin embargo, los recursos tróficos y la biomasa de macroinvertebrados varían a lo largo del año. Por lo tanto, es necesario investigar cómo las vías de flujo de energía varían con los cambios en la ocupación del suelo anualmente para comprender completamente la importancia de los diferentes recursos tróficos. Esto podría lograrse combinando la disponibilidad de recursos tróficos con medidas de asimilación y estimaciones de producción secundaria a lo largo del año.
- Los resultados obtenidos en esta tesis evidenciaron un ajuste de la estructura de tamaños de la comunidad de macroinvertebrados para mantener la eficiencia trófica en respuesta a la alteración de los recursos tróficos. Sin embargo, cómo la estructura de tamaños de una comunidad responde a las perturbaciones es, a día de hoy, muy poco conocida y en gran parte, contradictoria. Por lo tanto, es necesario comprender cómo los espectros de tamaño de diferentes comunidades de organismos se comportan en gradientes ambientales naturales y éstas responden a diferentes tipos de perturbaciones para desarrollar un marco teórico más sólido que permita predecir valores de eficiencia trófica e identificar patrones de respuesta a perturbaciones. Esto también permitiría determinar si los resultados obtenidos en esta tesis se observan en otros tipos de perturbaciones.
- El canal de energía dominante en las redes tróficas de ríos de cabecera se vio modificado con las variaciones en la ocupación del suelo (es decir, el canal de energía autóctona domina en ríos dominados por pastos los mientras que el canal de energía alóctona ríos forestados). Dado que estos canales de energía tienen diferentes propiedades (p. ej. estrategias de vida: K vs r), las redes tróficas podrían responder de manera diferente a las perturbaciones en términos de resistencia, resilencia o tiempos de recuperación. Por lo tanto, futuras investigaciones deberían centrarse en identificar cómo las redes tróficas dominadas por canal de energía autóctono o por el canal de energía alóctono responden a perturbaciones similares (p. ej. inundaciones).
- Los efectos de los cambios en la ocupación del suelo sobre la multifuncionalidad de los ecosistemas se han caracterizado por funciones que describen las dinámicas de la materia orgánica y la energía en el ecosistema, esencialmente, los dos canales de energía que sustentan las redes tróficas fluviales. Por lo tanto, investigar cómo otras funciones del ecosistema, como las actividades exoenzimáticas, la absorción de nutrientes, la producción secundaria o la emergencia de insectos, se ven afectadas por cambios en la ocupación del suelo proporcionaría una perspectiva más amplia de los efectos que estos cambios producen sobre el funcionamiento del ecosistema.

Chapter I

Introduction and background to the research

Chapter I. Introduction and background to the research

1.1 Importance of headwater mountain stream ecosystems

Headwater streams comprise a group of small size streams, from order 1 to 3 (defined by the Strahler classification ;Strahler, 1957), which begging where water flowing overland first coalesces to form a discernible channel, often in mountain areas (Vannote *et al.*, 1980). These streams, despite accounting for only 0.0001% of the water on Earth, occupy 0.1% of Earth's surface (Wetzel, 2001) and represent more than 85% of the global river channel length (Leopold, Wolman & Miller, 1965; Downing *et al.*, 2012).

Headwater streams are complex and dynamic ecosystems characterized by steep slopes, high speed of water flow, low or very low temperatures (Lowe & Likens, 2005) and high habitat diversity. Moreover, they are inhabited by wide diversity of organisms (Dudgeon *et al.*, 2006) including headwater-specialist species of aquatic invertebrates, amphibians and fish, shelter numerous ecosystem processes and provide humans with multiple and essential goods and services such as water supply and purification, food resources, hydropower or recreation (Revenga *et al.*, 2000).

Headwater streams also held a strategic position due to the hierarchical nature of fluvial networks. These streams are laterally closely connected to the adjacent terrestrial ecosystems and longitudinally to downstream rivers (Allan & Castillo, 2007), to which they serve as source of water, sediments, organic matter, energy and organisms (Alexander *et al.*, 2007; Freeman, Pringle & Jackson, 2007). This intimate connection between headwater streams and terrestrial ecosystems makes them strongly subjected to disturbances occurring in terrestrial ecosystems, and their relative isolation, small size and unidirectional and linear nature, exacerbates their vulnerability (Perkins *et al.*, 2010). Consequently, almost any disturbance in terrestrial ecosystems has the potential to have strong effects on the functions and services these headwater ecosystems provide, and on downstream rivers.

1.2 Land use – land cover change

Land use – land cover change is the principal disturbance in the terrestrial ecosystems, which, in concert with climate change, constitutes one of the major environmental threats to fluvial ecosystem conservation and stability (Millennium Ecosystem Assessment, 2005).

To date, the extension of human settlements and the consequent increase in urbanization (Geist & Lambin, 2002), the expansion of land management activities including farming, agriculture and pasturelands for cattle grazing and the increment of plantations and clear-cutting for obtaining wood and wood-derived products (J. Brandt, 1999; Bürgi, Hersperger & Schneeberger, 2004) have been considered the most dominant changes in land cover. These land cover changes have resulted in the transformation of more than 43% of the of Earth's terrestrial surface (Daily, 1995) and led to the loss of natural vegetation, most predominantly forest, as demonstrated by the loss of 2.3 million km^2 of forest from 2000 to 2012 (Millennium Ecosystem Assessment, 2005). Indeed, 24% of the terrestrial surface is currently occupied by cultivated systems (Millennium Ecosystem Assessment, 2005), 5% by urban lands (Paul & Meyer, 2001) and more than 3451 million hectares by pasturelands (Klein Goldewijk et al., 2011). However, forest loss has exhibited diverse pace over time and among regions (Pinto-Correia & Kristensen, 2013). Deforestation has historically been much more intensive in temperate regions than in the tropics, being Europe the continent with the smallest fraction of its original forests remaining (70% of the original temperate forests had disappeared by 1950; Millennium Ecosystem Assessment, 2005). At present, forest loss is more accentuated in the tropics, where it has increased by 62% in the first decade of the millennium compared to the 1990s. (Kim, Sexton & Townshend, 2015).

Although the expansion of land management activities and urbanization is the most recognized land cover change, this tendency is being reverted since mid of the twentieth century and large areas of land are being abandoned worldwide, but especially in temperate regions. In Europe, the hotspots of land abandonment are located in Eastern Europe (Estel *et al.*, 2015), in the Mediterranean (Sluiter & De Jong, 2007) and in mountain regions (MacDonald *et al.*, 2000). Particularly, the abandonment of mountain areas already affects the 30% of the Carpathian Mountains (Griffiths *et al.*, 2013), almost the 70% of the eastern Alps (Tasser *et al.*, 2007) and more than 90% of the Spanish Pyrenees (García-Ruiz & Lana-Renault, 2011).

Land abandonment has been motivated by several drivers including the decline in population density in rural areas, the low productivity of some mountain regions (Duarte, Jones & Fleskens, 2008), the forces of global markets or the effect of the Common Agricultural Policy and governmental initiatives, which subsidized some crops to the detriment of others (Lasanta *et al.*, 2000). The main ecological consequence of land abandonment is vegetation secondary succession processes (Pugnaire *et al.*, 2006). Secondary succession involves the increase in vegetation density and the progressive rise in the complexity of plant associations (García-Ruiz & Lana-Renault, 2011), which in many areas, has led to the increase in forest cover as shown by the gain of more than 0.8 million km^2 of forest cover worldwide from 2000 to 2012 (Hansen *et al.*, 2013). This is a clear evidence that land abandonment is a global phenomenon (Munroe *et al.*, 2013) which is, in fact, forecasted to continue in the future (e.g., Rounsevell *et al.*, 2006; Pointereau *et al.*, 2008). Thus, despite land abandonment being overlooked in the face of land management activities and urbanization, it constitutes, in conjunction with them, a major current and future land cover change.

1.3 Impacts of land cover changes in stream ecosystems

1.3.1 Physical and chemical fluvial components

Land cover has shown to affect a great number of physical and chemical properties of fluvial ecosystems. For example, land cover influences nutrients, carbon and sediment yield into the streams through the alteration of soil organic matter content, and soil erosion and particle detachment (Vallejo et al., 2012). Land cover also determines stream flow and water velocity primarily because variations in vegetation composition and density affect rainfall interception, water runoff and infiltration during severe rainstorms and water yield during low flows (Robinson, Gannon & Schuch, 1991; Gallart *et al.*, 2002; Belmar *et al.*, 2018). Moreover, canopy cover on the stream section, which varies with vegetation composition (i.e., herbaceous vs arboreal vegetation), controls the incident solar radiation (Monteith & Unsworth, 1990) and the convective heat losses from stream water (Dugdale *et al.*, 2018) highly influencing the amount of solar radiation that reaches the stream surface and the water temperature.

1.3.2 Food resources: origin, quantity and quality

Land cover exerts a strong influence on the origin, quantity and quality of the organic matter that constitutes the source of energy for the stream biota, this is, the food resources that sustain the food webs in fluvial ecosystems (Allan, 2004).

Two types of food resources can be differentiated depending on their origin: allochthonous and autochthonous food resources (Fig. 1.1). Allochthonous food resources are imported from adjacent terrestrial ecosystems and comprise the leaves, twigs, flowers and wood (Allan & Castillo, 2007) that enter the stream directly via litter fall or indirectly through lateral pathways driven by wind or surface run-off (Cummins, 1974). In headwater streams, leaf litter is the most abundant food resource.

Autochthonous food resources are in-stream produced from solar energy and inorganic carbon by autotrophic organisms. Autochthonous food resources comprise benthic algae, macrophytes and phytoplankton. In headwater streams, phytoplankton or macrophytes are often absent or of negligible biomass and benthic algae constitute the most dominant autochthonous food resource. Benthic algae appear predominantly in biofilms because the presence of macroalgae or filamentous algae in non-polluted mountain streams is restricted by environmental factors characteristic of these streams (e.g., low nutrient concentration, low water temperature, turbulent hydraulic conditions; Goldman & Carpenter, 1974; Horner & Welch, 1981; Hill & Knight, 1988). Biofilms are complex matrixes that bind together algae, bacteria, fungi, detrital particles, exudates, exoenzymes, and metabolic products and organic compounds including proteins or nucleic acids and humic compounds (Sinsabaugh & Foreman, 2003). Therefore, biofilms are not only composed of autochthonous organic matter, but of a mixture of autochthonous and allochthonous organic matter and can be considered as food resources of mixed origin. Alike biofilms, other food resources such as fine particulate organic matter (organic matter > 0.5µm and < 1mm) and coarse particulate organic matter (organic matter > 1mm and < 100 mm), which are mainly generated from the breakdown of the larger food resources including leaves, twigs and algae (Petersen & Cummins, 1974) or dissolved organic matter (DOM), have a mixed origin (Fig. 1.1).

DOM is the largest carbon pool in freshwater ecosystems (Battin *et al.*, 2009) and it constitutes the most important energy and carbon source for heterotrophic bacteria (Meyer *et al.*, 1988), despite being rarely consumed by macroinvertebrate or fish (Eggert & Wallace, 2007; Karlsson, 2007). DOM is composed of a complex mixture of allochthonous organic matter from the degradation products of terrestrial plant organic matter and autochthonous organic matter produced by the autotrophic organisms (extracellular release) or by predatory grazing, cell death and senescence and viral lysis. Nevertheless, most DOM has shown to be of terrestrial origin, especially in headwater streams (Raymond & Bauer, 2001; Benner et al., 2004; Jaffé et al., 2013). This suggests that most of the compounds in DOM leach directly from vegetation components (e.g., leaves, twigs, fruits; Kaplan & Newbold, 1993) or from organic matter accumulated in soils (Fiebig, Lock & Neal, 1990). Although changes in land cover strongly affect both vegetation and soil properties (e.g., Vallejo et al., 2012), the transformation of soil organic matter characteristics occurs over longer time scales than transformations of vegetation composition (Trumbore, 2009). This suggests that historic land cover may have strong effects on fluvial DOM properties, even long after the land cover changed. However, how past land cover affects DOM properties is poorly understood and requires an in-depth characterization of the multiple DOM compounds.

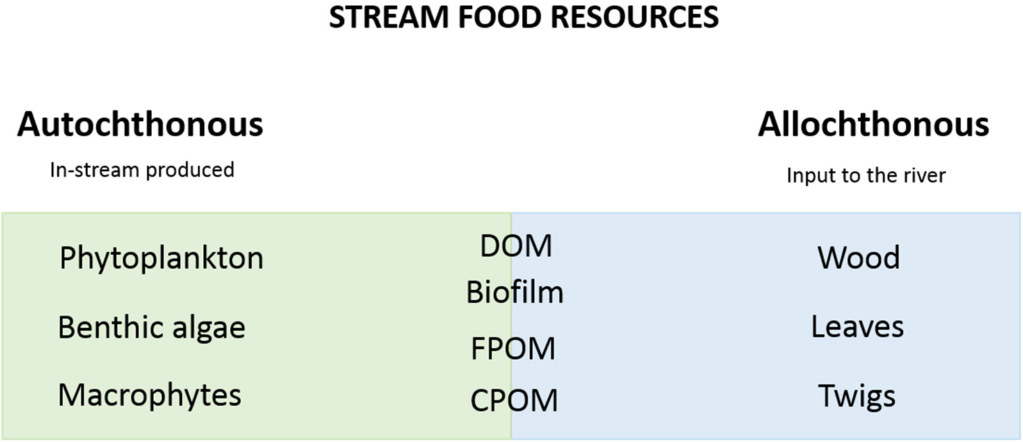

Figure 1.1 - Stream food webs are sustained by two types of food resources according to their origin: allochthonous food resources imported from adjacent terrestrial ecosystems and autochthonous in-stream produced food resources. However, some food resources are composed of both autochthonous and allochthonous organic matter and can be considered to have a mixed origin.* DOM = dissolved organic matter, FPOM = fine particulate organic matter (organic matter > 0.5µm and < 1mm), CPOM = coarse particulate organic matter (organic matter > 1mm and < 100 mm)

Land cover change can, not only affect DOM properties, but all food resources available for stream communities. Land cover can alter both the relative quantity of allochthonous and autochthonous food resources in opposed directions (decline of leaf litter quantity while increase of algae biomass or vice versa) and the relative content of autochthonous and allochthonous organic matter in food resources with mixed origin (e.g., relative abundance of algae in biofilms). These changes in food resources not only imply a change in the quantity of autochthonous vs allochthonous food resources or autochthonous vs allochthonous organic matter in mixed origin food resources, but a simultaneous alteration of quality. Quality is defined by the food resource chemical composition (i.e., carbon to nutrient ratio, fatty acid content or the structure of carbon molecules). Autochthonous organic matter has a higher quality than the allochthonous organic matter (Thorp & Delong, 2002), as this encompasses heterogeneous refractory organic compounds of high molecular weight (McDonald, Bishop et al. 2004) and low quality (Kaplan and Newbold 1995). Moreover, quality defines food resource palatability and lability, properties that highly determine their processing and incorporation into food webs (Lau, Leung & Dudgeon, 2008; Twining *et al.*, 2016a b). Nevertheless, food resource processing and incorporation into food webs depends on both food resource quantity and quality (Marcarelli *et al.*, 2011) and to date, most studies have focused either on resource quantity (e.g., Hawkins and Sedell 1981, Delong and Brusven 1998, Rosi-Marshall and Wallace

2002, Collins et al. 2016) or on a single resource quality (essentially leaf litter; Larrañaga et al. 2009). Thus, how food web structure and composition respond to changes in food resource quantity and quality is still unclear and could have profound implications for river functioning.

1.3.3 Food web composition and size structure

River food webs are composed of wide variety of organisms including macroinvertebrates (i.e., insects, crustaceans and mollusks), amphibians and fish, and non-aquatic organism closely linked to fluvial ecosystems such as mammals like otters or desmans and birds like dippers and herons.

The two types of food resources define two different energy channels in food webs (Fig. 1.2): autochthonous and allochthonous energy channels. The organisms that conform each of these energy channels can be identified by their feeding mode and the type of food resource they rely on. The autochthonous energy channel is conformed by herbivores, which mainly forage on autochthonous food resources (i.e., algae and biofilm) whereas the allochthonous energy channel, often referred to as the detrital energy channel, is conformed by detritivores, which feed on allochthonous food resources. These energy channels are coupled by omnivores, organism that do not show a clear preference in the food type ingested, and carnivores, which prey on other organisms (Rooney et al., 2006).

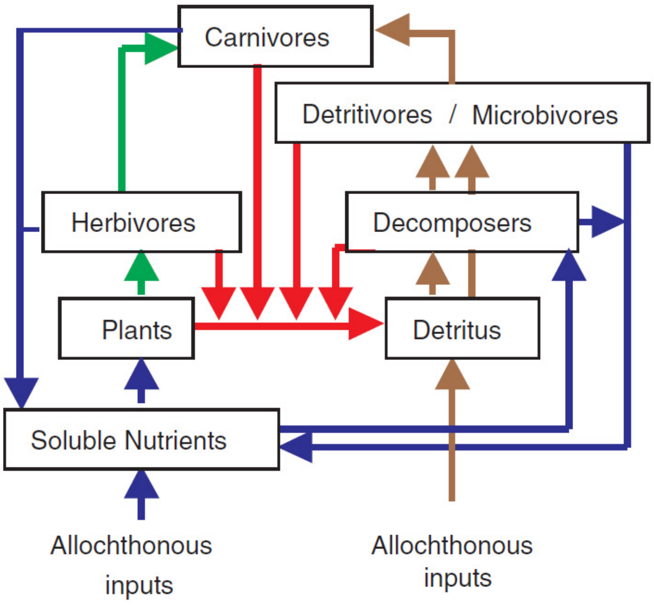

Figure 1.2 – Description of the energy channels in stream food webs. Green arrows represent the flow of matter through the autochthonous energy channel while brown arrows represent the allochthonous (detrital) energy channel. Red arrows depict the matter flow to the detritus pool that results from

death of all living organisms and from unassimilated prey. Blue arrows signify the mineralization and immobilization of soluble nutrients. Figure extracted from Moore *et al.* (2004).

The relative availability of autochthonous and allochthonous food resources defines the importance of these two energy channels. Therefore, variations in food resources often affect the abundance and composition of different feeding groups (particularly, primary consumers as detritivores and herbivores), and consequently the community structure and composition (Minshall, 1967; Cummins, 1974; Doisy & Rabeni, 2001; Baumgartner & Robinson, 2017). Nevertheless, changes in food resource quantity and quality can also affect organism growth and be reflected in organism body size (e.g., Dell *et al.*, 2015).

The prediction of size responses to changes in food resources is strongly hampered by discrepancies on how resource quality and lability and organism life strategy affect body size. Energy channels not only differ in the properties of the food resources themselves (quality and lability), but are hypothesized to be structurally and functionally different, providing the basis for the life history characteristics (i.e., life strategy) of the organisms that conform each of these energy channels (Rooney & McCann, 2012). This suggests that, as allochthonous food resources have lower quality than the autochthonous ones (Thorp & Delong, 2002), smaller organisms might be expected in ecosystems dominated by allochthonous resources because nutritional constraints to secondary production might limit organism growth (McNeely, Finlay & Power, 2007). However, as allochthonous energy channel is less competitive than the autochthonous one because it is donor-controlled (Polis and Strong 1996), the dominant life strategy in this energy channel is characterized by a slower growth and longer reproduction cycles (k strategy taxa; MacArthur and Wilson 1967), what might result in larger body sizes. On the contrary, the autochthonous energy channel is hypothesized to be a more competitive environment as food resource abundance is consumer density – dependent (Hill & Knight, 1987) what might favor a life strategy of growing and reproducing fast and lead to smaller body sizes. Size responses in predators, are even more difficult to predict as they depend on the effect that the food resource change has on primary consumers.

Changes in individual body size can be upscaled and reflected in the size structure of the entire community. At a community, the number of organisms is shown to decline with body size, and relationship of the abundance of organisms with their body size is represented by the community size spectrum (Fig. 1.3). The size spectrum intercept informs of the ecosystem carrying capacity, this is, the total organismal abundance that can be supported in the ecosystem (Gaedke, 1993; Daan *et al.*, 2005; Sweeting *et al.*, 2009), while the size spectrum slope describes the efficiency in energy transference among trophic levels (Woodward *et al.*, 2005; Andersen, Beyer & Lundberg, 2009).

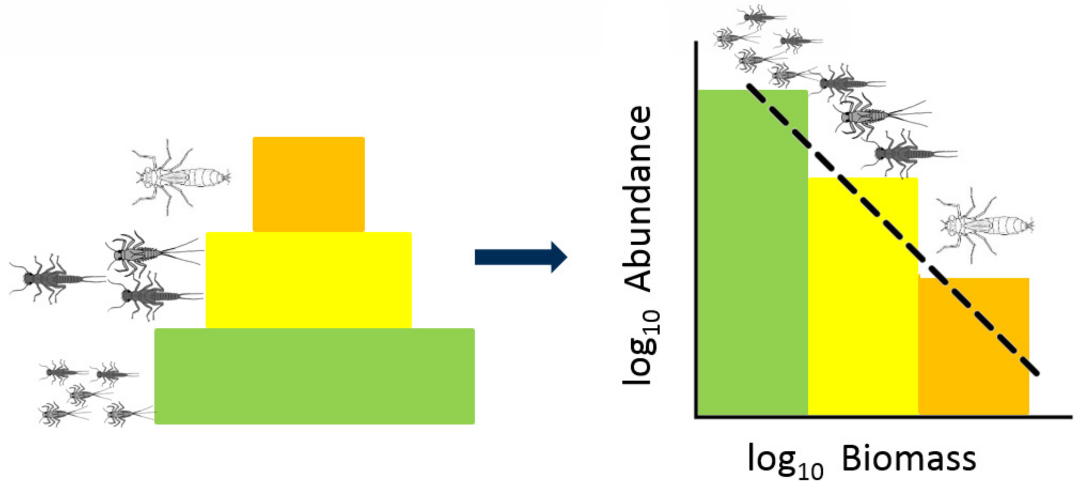

Figure 1.3 - Size spectrum represents the distribution of the abundance of organisms in relation to their body size. The size spectrum intercept informs of the total organismal abundance that can be supported in in an ecosystem (ecosystem carrying capacity) while the size spectrum slope is strongly related to the efficiency in energy transference among trophic levels.

In absence of disturbances, the size spectrum is relatively regular and predictable (e.g., Sheldon et al. 1972) because communities tend to be trophically efficient (Kerr, 1974; Jennings & Mackinson, 2003). However, how size spectrum responds to changes in land cover is not been investigated. Although size spectrum slope has been considered to be relatively stable, even in communities subjected to disturbances because of compensatory regulation mechanisms of the community size structure (Klug et al., 2000; Downing et al., 2008), recent investigations suggest that it may be altered by disturbances. Some studies (e.g., Petchey & Belgrano 2010) have theorized that in disturbed ecosystems the size spectra slope increases, whereas shallower size spectra slopes have been predicted in ecosystems subsidized by allochthonous food resources (Trebilco et al., 2013). Understanding how food webs are size structured and respond to disturbances is of critical importance given that trophic dynamics underpin the ecological functioning and evolution of biological communities, and inform of the ecosystem stability (Perkins *et al.*, 2014). Hence, it is necessary to understand whether land cover changes modify size spectra through the alteration of organism size structure or, on the contrary, the organism size structure is altered and adjusted to maintain the size spectrum and the efficiency in energy transference.

1.3.4 Ecosystem multifunctionality

Land cover plays a key role in regulating the ecosystem multifunctionality, this is, the set of functions that simultaneously occur in an ecosystem. From an ecosystem energetics perspective, functions that describe variations in food resources or the organism enzymatic activity and growth, inform of the organic matter cycling in the ecosystem and the importance of the energy channels (autochthonous and allochthonous) that sustain the river food webs.

From the food resources perspective, biofilm growth represents the gain of primary producers biomass over time (Tank et al., 2010) and highly defines the organic matter available for food webs within the autochthonous energy channel. In contrast, the organic matter decomposition shows the consumption of food resources in the allochthonous energy channel (Gessner, Chauvet & Dobson, 1999). Organic matter decomposition can be analyzed from the decomposition rate of coarse (organic matter > 1mm) and fine organic matter by both microbial decomposers and animal detritivores over time or from the DOM uptake and degradation by microbial heterotrophs. From the organism perspective, enzymatic activity describes the expression of enzymes such as lipase, leucine-aminopeptidase, ß-glucosidase, ß-xylosidase or alkaline phosphatase that are involved in the breakdown of large macromolecules into soluble monomers that can be taken up and metabolized by prokaryotic and eukaryotic cells, including bacteria, fungi, algae and protozoa (Sinsabaugh & Follstad Shah, 2012; Arnosti *et al.*, 2014). Organism growth, which can be analyzed by measuring macroinvertebrates and fish secondary production, describes the generation of heterotrophic biomass through time (Huryn & Wallace, 2000).

Other functions such as ecosystem metabolism can provide an integrative view of nutrient and organic matter cycling in the ecosystem as it encompasses all the interrelated processes that fix and mineralize organic matter of all autotrophic and heterotrophic organisms in the ecosystem (Hall et al., 2016). Indeed, ecosystem metabolism is the result of gross primary production (GPP), i.e. the synthesis of new organic matter by autotrophic organism, and ecosystem respiration (ER), i.e. the transformation of organic matter into inorganic carbon by autotrophic and heterotrophic organism for obtaining energy (Hall et al., 2016).

Land cover can control these functions through the described physical and chemical fluvial components, organic matter properties, organism body size and community composition. For instance, light controls biofilm growth and GPP as it stimulates algae growth (Steinman, 1992; Hill & Dimick, 2002) and enhances algae primary production (Hill, Mulholland & Marzolf, 2001). Temperature controls all ecosystem functions mediated by organisms since it is a strong determinant of metabolic activity (Brown *et al.*, 2004). Moreover, changes on DOM composition can affect ER (Fuß *et al.*, 2017), detritivore organisms such as shredders control the decomposition of coarse organic matter (Anderson & Sedell, 1979) while organism body size is a driver of metabolism or feeding rate (Brown and Maurer 2007). Thus, exploring how land cover controls physical and chemical fluvial components, organic matter origin, quantity and quality, organism body size and community composition, and the role of these in defining ecosystem multifunctionality is crucial to unravel the mechanisms through which land cover changes affect fluvial ecosystem functioning.

1.4 Objectives of the thesis

The main objective of the present thesis is to characterize the effects of land cover change on mountain stream energy flow pathways, food web size structure and composition and ecosystem multifunctionality through the alteration of the basal food resources that constitute the sustenance of stream food webs and physical and chemical fluvial components. The results obtained from this multi-level approach will be highly valuable to design management solutions for mitigating the effects of land cover change and conserve headwater stream ecosystem functioning and service provision.

The specific objectives of this thesis are focused on the following aspects:

- Address the effects of historic and current land cover on DOM quantity and composition and how these effects are influenced by the distance of a certain land cover patch to the stream and catchment topography.
- Investigate the role of catchment land cover in determining food resource type (allochthonous vs autochthonous) and quantity and determine how changes in food resources affect macroinvertebrate food assimilation, energy flow pathways and food web structure.
- Assess the response of macroinvertebrate community size structure to variations in food resource type and quantity to test whether the efficiency in energy transfer among trophic levels and community carrying capacity are altered by land cover changes.
- Examine the local scale abiotic and biotic pathways through which land cover controls the rates of fluvial ecosystem functions related to ecosystem energetics and address land cover change effects on ecosystem multifunctionality.

1.5 Layout of the thesis

The structure of the thesis is organized as follows:

In Chapter I, a general overview and the background to the research objectives are presented first. At the end of this chapter the general and specific objectives of the thesis are outlined.

In Chapter II, a detailed description of the study area, the methodology followed to characterize the land cover and the stream selection is provided.

The chapters III to VI address the specific research objectives of the thesis. Each of the chapters includes an abstract, introduction, methods, results, discussion, conclusion, references and supplementary material section.

A brief description of the investigations conducted in each chapter is described below.

Chapter III. No land cover legacy in fluvial dissolved organic matter.

Chapter III explored the effects of current and historic land cover on DOM quantity and composition to test for legacy effects of historic land cover. DOM molecular composition was characterized by ultrahigh-resolution mass spectrometry and the relative abundance and average mass of 8 molecular groups and total compound richness was calculated. We described how DOM molecular composition varied along a land cover gradient and how it was modulated by catchment area, altitude, hill slope and the distance of a certain land cover patch to the stream. Moreover, we related the main compositional gradients of DOM in the study streams to compound richness, relative abundance and average mass of each molecular group, and to DOM properties characterized by size-exclusion chromatography and spectroscopy.

Chapter IV. Catchment land cover influences macroinvertebrate food web structure and energy flow pathways.

In chapter IV, we analyzed how land cover changes altered food resource type and quantity and how changes in food resources affected macroinvertebrate food assimilation, energy flow pathways and food web structure. We measured the variation of food resource type and quantity along a land cover gradient. To estimate food resource importance to food webs, we determined how the variation of food resources effected the assimilation of allochthonous vs autochthonous food resources and the percentage of biomass sustained by each of these resources through the combination of stable isotopes (δ^2H and δ^{15}N) and macroinvertebrate biomass measures. Moreover, to account for modifications in food web structure with food resource variation, we assed biomass distribution among the different macroinvertebrates groups that conform each community.

Chapter V. The effect of forest cover loss on stream macroinvertebrate community size structure.

Chapter V addressed the response of macroinvertebrate size structure to the variations in food resource type and quantity with land cover change and tested whether the ecosystem carrying capacity and the efficiency in energy transfer among trophic levels were altered. Size spectra were constructed for each stream macroinvertebrate community based on the relationship of organism biomass and density. We analyzed how size spectrum intercept and slope changed in relation to the variations in food resource type identified in chapter IV and total food resource quantity. To identify how community size structure was modified, we also characterized the variations in taxa richness, density, biomass and average body size of the entire community of each feeding group with food resource type and quantity.

Chapter VI. Influence of forest cover on stream ecosystem multifunctionality.

Chapter VI investigated the effects of land cover change on fluvial ecosystem multifunctionality from the ecosystem energetics perspective. Ecosystem multifunctionality was assessed considering five ecosystem functions: wood decomposition rate, biofilm growth rate as the accumulation rate of chlorophyll *a* and ephilitic biomass, and stream metabolism including GPP and ER. A path analysis was performed to identify the local scale abiotic and biotic pathways by which land cover controls each ecosystem function. Local scale abiotic factors included non-living physical and chemical components such as leaf litter, river channel area, canopy cover, light availability, water temperature, nutrient concentration, electric conductivity and DOM while local scale biotic factors comprised the living components including biofilms, macroinvertebrates and fish. Moreover, the interactions between land cover and other catchment scale factors such as catchment area and geology were also addressed.

Finally, general conclusions and future research lines are described in Chapter VII.

1.6 References

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Chapter II

Study area description, land cover characterization and stream reach selection

Chapter II. Study area description, land cover characterization and stream reach selection

2.1 Study area

The study area is located in the central section of the Cantabrian Mountains, a mountain range which reaches up to 2600 m a.s.l. and spans more than 300 km across northern Spain, parallel to the Cantabrian Sea (Atlantic Ocean; Fig. 2.1). The Cantabrian Mountains constitute a prolongation of the Pyrenees towards the west, from the Aralar mountain range on the east, to the Galician Massif on the west (Alonso, Pulgar & Pedreira, 2007).

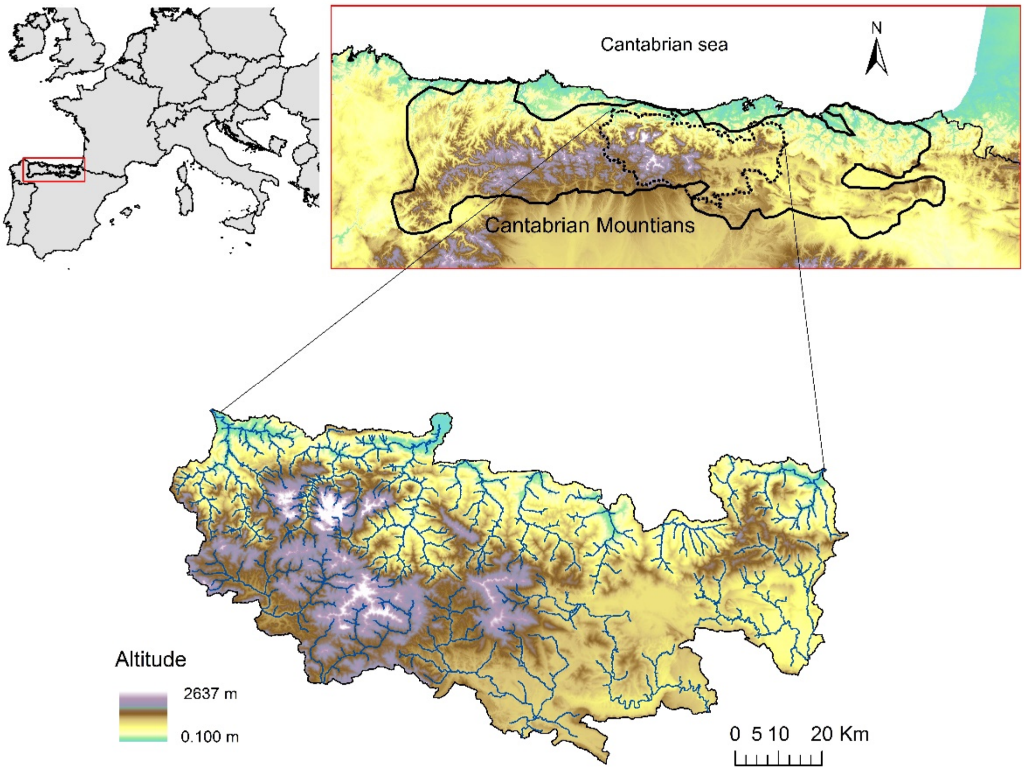

Figure 2.1 - Map of the study are located in the central section of the Cantabrian Mountains, northern Spain.

2.1.1 Geomorphology and geology

The Cantabrian Mountains were mainly formed by two large orogenies: the Hercynian orogeny, in the Late Carboniferous, and the Alpine orogeny, in the Late Cretaceous. In the Hercynian orogeny, the two large continental plates, Gondwana and Laurentia, collided to form the Pangea continent. This collision generated a strong compression that created folds and fractures and lifted sediments giving rise to the primitive Cantabrian Mountains, which were later heavily eroded. Over time, the continents separated and the Iberian plate, where part of the Iberian Peninsula and the Cantabrian Cordillera are located, drifted as an isolated piece of land. During the Mesozoic, the area that the Cantabrian Cordillera currently occupies was a marine catchment and large deposits of sediment accumulated. During the Triassic period, clays and evaporites were deposited in the coastal areas, while during the Lower and Middle Jurassic, marine carbonate rocks formed as a result of sea level rise. The Upper Jurassic and the Lower Cretaceous resulted in detrital deposits in deltaic or fluvial areas, and during the Aptian, a new rise in sea level formed limestone reef rocks. At the end of the Upper Cretaceous, a deepening of the marine basin occurred and as consequence, turbidites were accumulated.

During the Alpine orogeny, the Iberian plate moved towards the north joining the Indo-European plate and in the collision zone formed the Alps, the Pyrenees and the Cantabrian Mountains, which rose again to acquire an aspect similar to the one we currently observe. The sediments accumulated over time (i.e., quartzites, sandstones, shales, conglomerates, coal layers and limestones) were compressed, folded and fractured to form the current mountains. Hereinafter, the Cantabrian Mountains topography was modeled by exogenous processes (e.g., fluvial erosion and landslides) (Alonso *et al.*, 2007). In particular, a series of glacial periods led to the formation of several glaciers that left a clear imprint on the relief (Jiménez & Farias, 2005). These glaciers lead to multitude of erosive forms, such as "U" shaped valleys, glacial cirques, horns (e.g., Cornón peak) or moraines, as well as the large number of deposits of till in the valley bottoms. The imprint of the glacial periods can also be noticed on the snowfields, glacial ice masses, icy caves and seasonal icy soils (Pisabarro *et al.*, 2017).

Currently, the most resistant sediments (e.g., limestones, calcareous rocks) can still be found in the highest areas (e.g., Ancares, Somiedo, Mampodre, San Isidro, Fuentes Carrionas, Ubiña or Picos de Europa) while the softest sediments (e.g., sandstones, siliceous rocks) were eroded by the action of rivers and glaciers and appear predominantly in valley bottoms (Fig. 2.2).

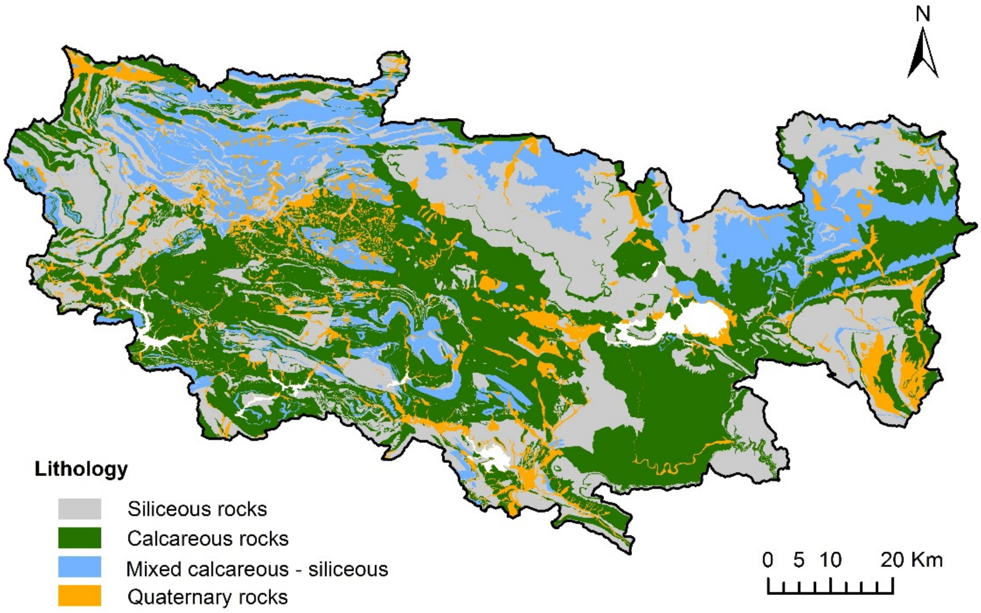

Figure 2.2 – Dominant soil composition in the study area.

Nevertheless, all these processes did not affect the entire area of the Cantabrian Mountains alike and, from a geological and geomorphological point of view, three sectors can be distinguished:

- The Basque-Cantabrian sector, to the east, characterized by moderate elevations that rarely exceed 1500 m in which Mesozoic sediments predominate.
- The Asturian Massif in the center, with the highest elevations (up to 2600 m), has a limited presence of Mesozoic sediments, which were eroded revealing the Paleozoic rocks raised during the Alpine orogeny.
- The western sector, located on the border between Asturias and Galicia, presents again lower elevations. In this sector, there are no Mesozoic sediments and the Alpine orogeny is only visible in some tertiary basins limited by faults.

The study area considered in this thesis comprises an area between the Basque-Cantabrian sector and the Asturian Massif, including the massif of Picos de Europa completely. This massif is mainly composed of limestones formed during the Paleozoic (in the Carboniferous). Limestones are permeable substrates which caused water to circulate underground forming shafts and caves in the interior of the calcareous massif avoiding the erosion of the Picos de Europa and favoring its individualization in the landscape (Fig. 2.3).

Figure 2.3 – Limestone peaks characteristic of the most elevated areas of the Picos de Europa massif (Andara massif, most eastern part of Picos de Europa).

2.1.2 Climate

The Cantabrian Mountains, despite being considered the only oceanic mountain in Spain with a mean annual temperature of 9.5 ± 2.2°C and mean annual precipitation of 1150 ± 210 mm (Ninyerola, Pons & Roure, 2007b a), are not climatically homogeneous. They have a unique climate for the southern latitude in which they are located with two climatically differentiated zones - the Atlantic and the Mediterranean slopes - due to the dominance of oceanic winds and their different influence in each zone.

The Atlantic slope or northern slope, is narrow and steep with just a few tens of kilometers (40 - 50 km) between the sea and the water divide at 2600 m a.s.l. This slope is highly influenced by the Atlantic Ocean and, thus, characterized by an Atlantic climate. Oceanic winds collide with the mountains, condensing and leading to abundant precipitations (mean annual precipitation of 1315 ± 200 mm ; Ninyerola *et al.*, 2007a; Fig. 2.4 a) and fresh temperatures (mean annual temperature of 8.8 ± 1.8°C; Ninyerola *et al.*, 2007b ; Fig. 2.4 b). The Atlantic slope is therefore rainy throughout the year with slight differences in temperatures during the day and among seasons.

In contrast, the Mediterranean slope or southern slope, is characterized by a much smaller descent in altitude (1500 m) in a much larger distance (hundreds of kilometers), what generates a broad space of climatic transition characterized by a continental sub-Mediterranean climate. After the oceanic winds collide with the mountains in the Atlantic slope, dry winds descend towards the Mediterranean slope (Foëhn effect) causing a more arid climate. The Mediterranean slope, is thus characterized by higher temperatures and lower precipitations than the Atlantic slope (mean annual temperature is 10.2 ± 2.2°C and mean annual precipitation 990 ± 230 mm; Ninyerola *et al.*, 2007 a, b; Fig. 2.4 a and b) with greater contrast between summer and winter. Summers have warm temperatures and marked droughts while winters are cold and rainy.

In the water divide, as altitude increases the thermal regime becomes stricter. In the most elevated areas, temperatures below 2ºC are common during the winter months and annual precipitation—occurring in the form of snow for much of the year above 1000 m— often exceeds 2000 mm (Aemet, 2011).

Although the north-south climatic gradient is the most dominant in the Cantabrian Mountains, there is also a transition from wetter climatic conditions in western areas to drier conditions on eastern areas.

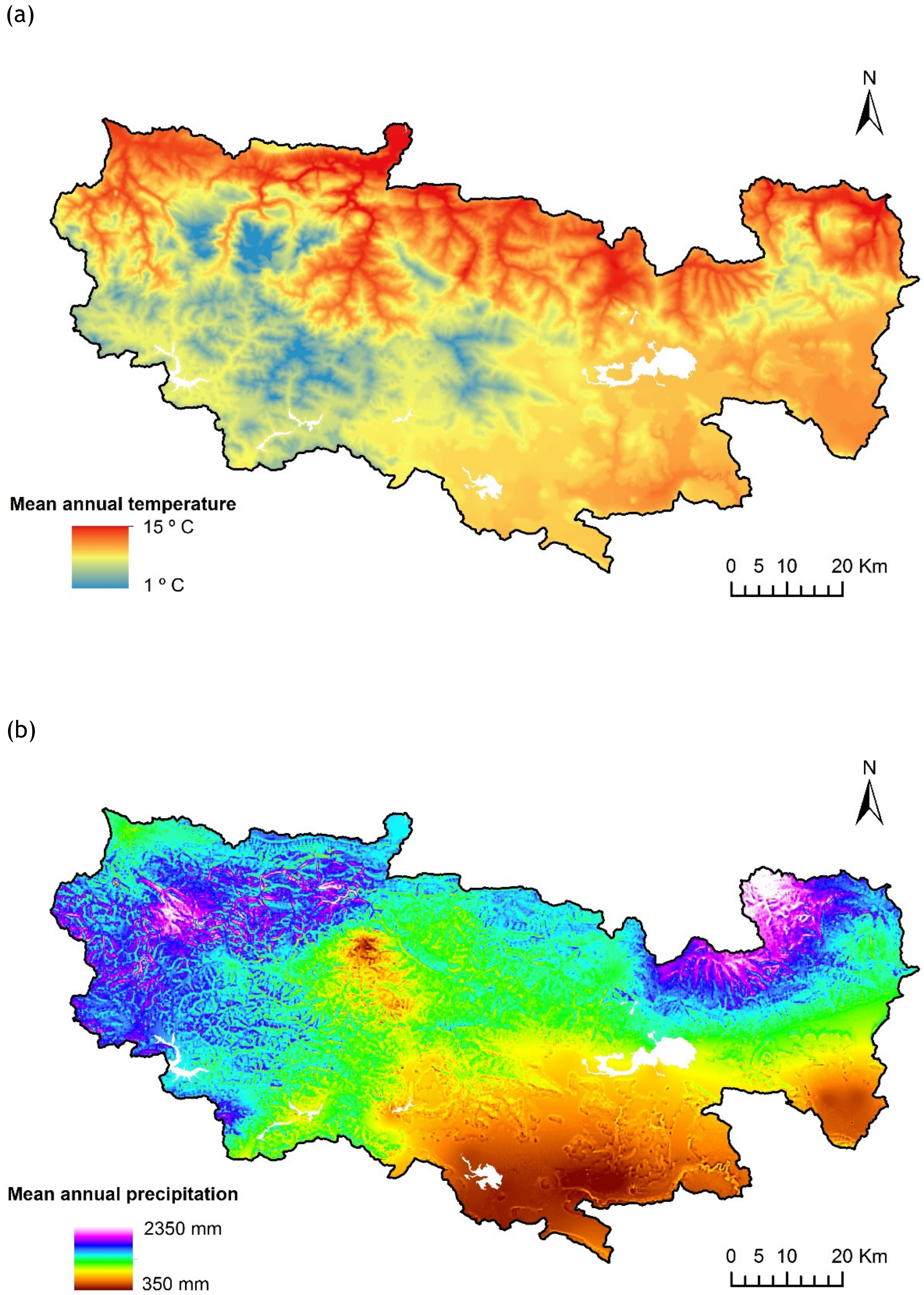

Figure 2.4 – Mean annual precipitation (a) and temperature (b) in the study area according to Ninyerola et al. (2007 a, b).

2.1.3 Socioeconomics

The Cantabrian Mountains have been intimately linked to the human presence since Neolithic times (Fernández Mier, López Gómez & González Álvarez, 2013). For centuries, agriculture, cattle raising and forestry have been the pillars of the economy and consequently, landscape organization has always been linked to these land uses.

Since Middle Ages, agriculture was predominantly characterized by small plots of arable land, next to the villages at the bottom of the valleys, for vegetables, cereal and linen production. Cattle raising was extensive, based on transhumance from higher pastures in summer to valleys bottoms in winter as well as short-term seasonal movements (often less than 100 km), what resulted in a landscape dominated by vast extensions of grasslands. Forestry for wood, firewood and fodder production dominated across the study area while clear-cutting forest harvesting was particularly relevant in the eastern sector of the Cantabrian Mountains. Here, in the mid-eighteenth century, the Royal Artillery Factory opened to supply guns, ammunition and other foundry products to the Royal Spanish Navy ships. The artillery production required large amounts of wood, what resulted in the completely deforestation of many forested areas (e.g., Miera and Pas river catchments). These harvested areas were maintained on an herbaceous state by the use of fire for pasture generation for cattle grazing (González-Pellejero *et al.*, 2014). Moreover, mining had special relevance from the second half of the nineteenth until the mid-nineteenth century. During this period, the Cantabrian Mountains were exploited for the extraction of iron (mines in Picos de Europa), coal (in the mountains of Palencia and León) and zinc (in the Central Massif and Ándara), what led to the installation of small forges next to the rivers.

At present, the landscape of the Cantabrian Mountains reflects the evolution of the economic activity and the population dynamics in the last decades. The Cantabrian Mountains have experienced a strong development of tertiary activities linked to the use of territorial resources for recreational uses, leisure and tourism, the depopulation of rural municipalities towards urban areas (mainly located in coastal municipalities), and the abandonment of the basic productive activities. This abandonment of productive activities has particularly centered in the cease of traditional agricultural activities and retreat of the surface of agrarian use, the decline in extractive mining and forges operation, the disappearance of transhumance, and the reorientation of the farming economy. In this sense, farming has been reconverted to an intensive system specialized in dairy production and industrial activities linked to the production of cheese and butter. The cease of these activities has resulted into the landscape re-naturalization with an important advance of the forest masses in some locations. Nevertheless, cattle raising is still an activity that employs a high percentage of the active population and the intensive system of dairy production coexists with an extensive farming for meat production. Thus, many grasslands are still maintained for cattle grazing, mostly by the use of fire.

Hence, the current landscape of the Cantabrian Mountains is characterized by the alternation of old grasslands maintained over time for cattle grazing, old-growth mature forests, and young secondary forests in abandoned grasslands and agricultural plots (Fig. 2.5), in concert with villages and larger urban areas, predominately along the coastline.

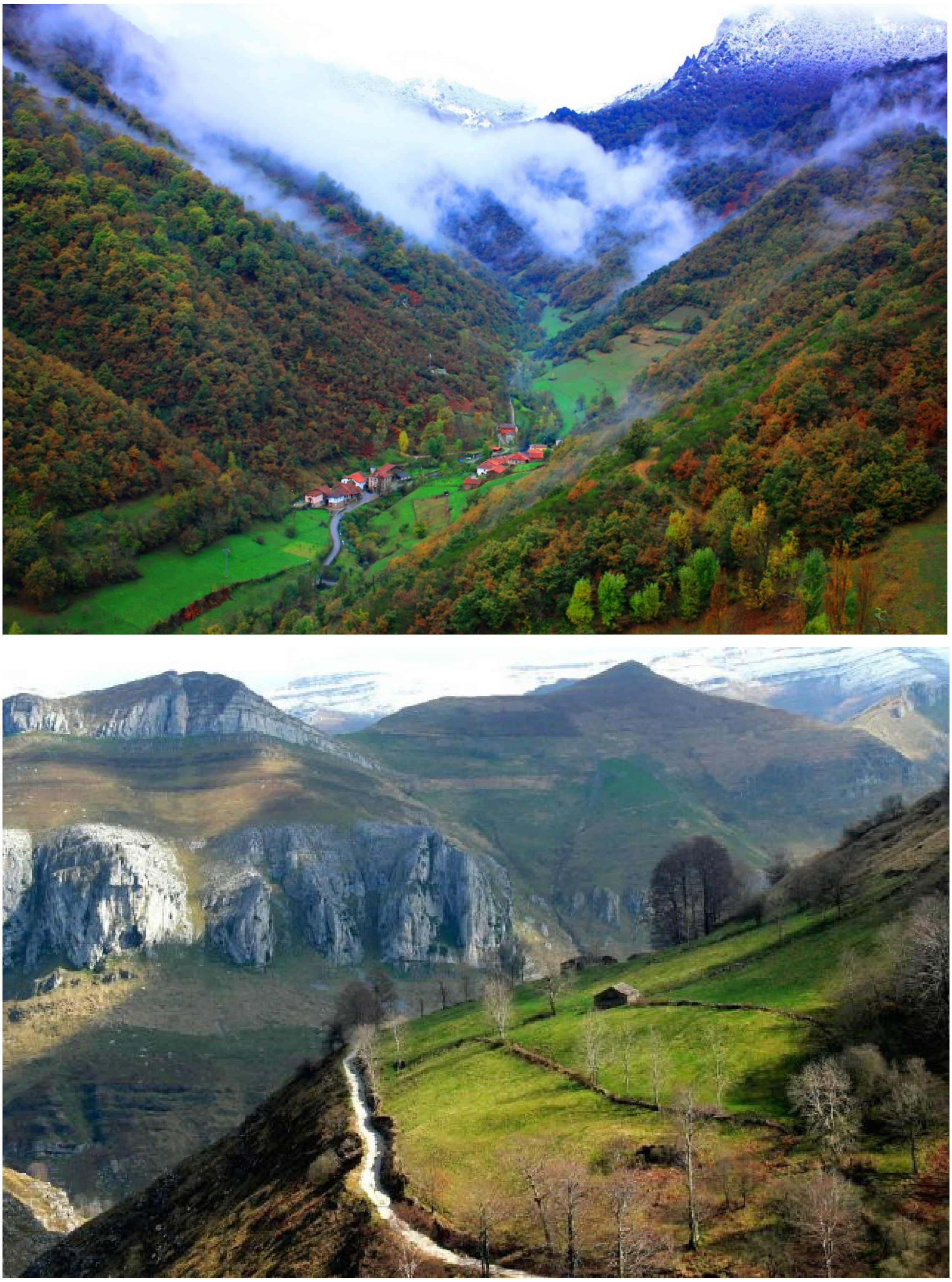

Figure 2.5 – In the study area old-growth forest alternate with vast extensions of grasslands that have been maintained over time for cattle grazing.

2.1.4 Flora and fauna

The Cantabrian Mountains represent the north-south and east-west border in the distribution of many species and communities of flora and fauna (Pleguezuelos, Márquez & Lizana, 2002; Madroño, González & Atienza, 2004; Palomo, Gisbert & Blanco, 2007).

2.1.4.1 Flora

The Cantabrian Mountains house one of the greatest plant richness of the European mountains with a high percentage of endemicity (Jiménez-Alfaro, 2009), favored by the Atlantic and sub-Mediterranean climatic conditions that characterize this mountain region. Vegetation is distributed according to climatic characteristics, the orientation of the slopes and the composition and edaphic structure in four bioclimatic floors: hill (0 - 500 m), montane (500 - 1500 m), sub-alpine (1500 - 2200 m) and alpine (> 2200 m) floors.

The hill floor in the Atlantic slope is characterized by presence of mixed deciduous forest composed of beeches (*Fagus sylvatica* L.), oaks (*Quercus robur* L.), chestnuts (*Castanea sativa* Mill.), elms (*Ulmus* L.), hazels (*Corylus avellana* L.) and ashes (*Fraxinus excelsior* L.). Moreover, there is a significant presence of holm oak forests, perennial forests characteristic of the Mediterranean region that grow in this Atlantic slope because of the high light reflection and permeability of calcareous substrates. Holm oak forests are predominantly composed of holly oaks (*Quercus* ilex L.), laurels (*Laurus nobilis* L.), strawberry trees (*Arbutus unedo* L.), hazels (*Corylus avellana* L.) and hawthorns (*Crataegus monogyna* Jacq.). In the Mediterranean slope, the hill floor is dominated by semi-deciduous forests and sclerophyllous formations (e.g., *Quercus pyrenaica* Willd. and *Quercus ilex* L. *subsp. Rotundifolia*) accompanied by vast extensions of heaths (*Erica australis* L.). In this floor numerous croplands as well as abundant plantations of eucalyptus (*Eucaliptus globulus* Labill.) can be found.

The montane floor in the Atlantic slope is dominated by is indigenous oak (*Quercus robur* L.) on sunny slopes facing south with a rich undergrowth and beech forests (*Fagus sylvatica* L.) on shady slopes forming monospecific forests. In the sub-Mediterranean slope, the montane floor is dominated by sessile oaks (*Quercus petraea* Matt. Liebl.) and birch groves (*Betula pubescens* Ehrh).

The last wooded formations occupying the higher altitudes in the sub-alpine floor are predominantly white birch forest (*Betula alba* Ehrh.). Above 1800 m, almost all the vegetation is composed of small size plants (e.g., bushes and herbaceous plants), being dominant junipers, legumes and grasses, which form mountain grasslands.

Riparian vegetation is predominantly composed of alders (*Alnus glutinosa* (L.) Gaertn.), elms (*Ulmus glabra* Huds.), ashes (*Fraxinus excelsior* L.), hazels (*Coryllus avellanea* L.) and willows (*Salix spp.* L.) and shrub vegetation dominated by dogwoods (*Cornus sanguinea* L.), spindles

(*Euonymus europaeus* L.), various brambles (e.g., *Rubus ulmifolius* Schott. and *Rubus caesius* L.) and lianas (*Tamus communis* (L.) Caddick & Wilkin, *Rubia peregrine* L., *Hedera spp.* L.). The most abundant herbaceous plants are nettles (*Urtica dioica* L.), horsetails (Equisetum spp.), ferns such as the soft shield-fern (*Polystichum setiferum* (Forssk.) Woynar) and sweet grasses (e.g., false brome *Brachypodium sylvaticum* (Huds.) Beauv.).

Riverbed vegetation is mostly composed of bryophytes (e.g., mosses such as *Plagiomnium undulatum (Hedw.) T.J.Kop., Rhynchostegium riparioides* Hedw. and *Thamnobryum alopecurum* Hedw., and hepatics such as *Conocephalum conicum* (L.) Dum.) and macrophytes (e.g., R*anunculus spp* L., *Nasturtium officinale* W.T. Aiton, *Apium nodiflorum* Lag. and *Potamogeton crispus* L.). Diatom communities are dominated by the genera Nitzschia, Navicula and Gomphonema.

2.1.4.2 Fauna

The Cantabrian Mountains also concentrate one of the largest vertebrate richness in the Iberian Peninsula. The Cantabrian Mountains are inhabited by 18 species of amphibians, 22 of reptiles, 190 of breeding birds and 67 of mammals, out of the 22, 35, 263 and 67 species that can be found in the Iberian Peninsula (Pleguezuelos, Márquez & Lizana, 2002; Madroño, González & Atienza, 2004; Palomo *et al.*, 2007).

The largest mammals are bears (*Ursus arctos*), wolfs (*Canis lupus*), foxes (*Vulpes vulpes*), ungulates such as red deers (*Cervus elaphus*), chamoises (*Rupricapra rupricarpa*), roe deers (*Capreolus capreolus*) or wild boars (*Sus scofra*). Rodents (e.g., *Apodemus silvaticus, Chinomys nivalis*), mustelids (e.g., *Martes foina*) and bats (e.g., *Barbastellus barbastellus*) are very common and there is a broad presence of moles (*European Talpa*), rabbits (*Oryctolagus coniculus*) and hares (*Lepus capensus*).

Birds are dominated by goshawks (*Accipiter gentilis*), pikes (*Dryocopus martius*), capercaillies (*Tetrao urogallus*), yellow-billed choughs (*Pyrrhocorax graculus*), wallcreepers (*Tichodroma muraria*), grey partridges (*Perdix perdix*), snowfinchs (*Montifringilla nivalis*) and black woodpeckers (*Dryocopus martius*). It is also possible to find many passerines such as the goldfinch (*Carduelis carduelis*) or the wren (*Troglodytes troglodytes*), as well as birds of prey as the golden eagle (*Aquila chrysaetos*), the Egyptian vulture (*Neophron pernocterus*), the griffon vulture (*Gyps fulvus*) or the buzzard (*Buteo buteo*).

The most characteristic terrestrial invertebrates are large forest beetles (e.g., *Rosalia alpina* or *Lucanus cervus*), mollusks such as the quimper snail (*Elona quimperiana)* and reptiles (e.g., *Anguis fragilis*).

Fluvial ecosystems host numerous native fish species among which outstand the salmon (*Salmo salar*), the brown trout (*Salmo trutta*), the minnow (*Phoxinus vigerri*), the eel (Anguilla

anguilla) and the sea lamprey (Petromyzon marinus) and numerous families of benthic invertebrates. Other non-aquatic species closely linked to fluvial ecosystems are the otter (*Lutra lutra*), the Pyrenean desman (*Galemys pyrenaicus*) or the dipper (*Cinclus cinlcus*).

2.1.5 Aquatic ecosystems

The Cantabrian Mountains comprises a wide network of rivers and numerous lakes and lagoons originated during the glacial periods, among which stand out the Lakes of Covadonga (Enol and La Ercina) or the Somiedo Lakes.

The river network in the study area encompasses a total of nine river catchments. Seven of these catchments (Sella, Deva, Nansa, Saja-Besaya, Miera, Pas and Ason) are situated in the Atlantic slope and drain to the Atlantic Ocean, while two are located in the Mediterranean slope and drain to the Mediterranean Sea (Ebro river) and to the Atlantic Ocean (Douro river; Fig. 2.6 a). The climatic and geomorphologic characteristics that differentiate the Atlantic and Mediterranean slopes, also define the characteristics of the rivers located in each of them.

The rivers in the Atlantic slope are short and steep, especially in their head, due to the proximity of the mountains to the sea, and have small catchments since they run embedded in mountain valleys. The combination of altitude and slope generates high erosive power in the most elevated river reaches, which causes rectilinear channels, often forming gorges, dominated by large size substrates (i.e., large blocks and boulders). In lower areas, when the river still carries a certain amount of sediments, rivers can show a greater sinuosity. The regular and abundant precipitations in the Atlantic slope lead to mighty rivers characterized by a regular flow regime and the absence of severe summer droughts (Fig. 2.6 b).

In contrast, rivers in the Mediterranean slope are long and gently sloping with extensive catchments located between large mountainous systems because of the shallower relief. Compared to rivers in the Atlantic slope, rivers in the Mediterranean slope tend to be more meandering due to their lower erosive power and are composed of smaller size substrates. Moreover, these rivers have highly irregular flows with seasonal floodings and strong summers droughts (Fig. 2.6 b) linked to the differences in pluviometry along the year, this is, rainy winters and dry summers.

Moreover, this river network encompasses many subterranean rivers, favored by the large areas of calcareous sediments in the Cantabrian Mountains (Fig. 2.2). These subterranean rivers are generated by water infiltration through shafts and vertical ducts in calcareous massifs (e.g., Picos de Europa) and emerge to the main rivers through many spring sources and peripheral upwelling.

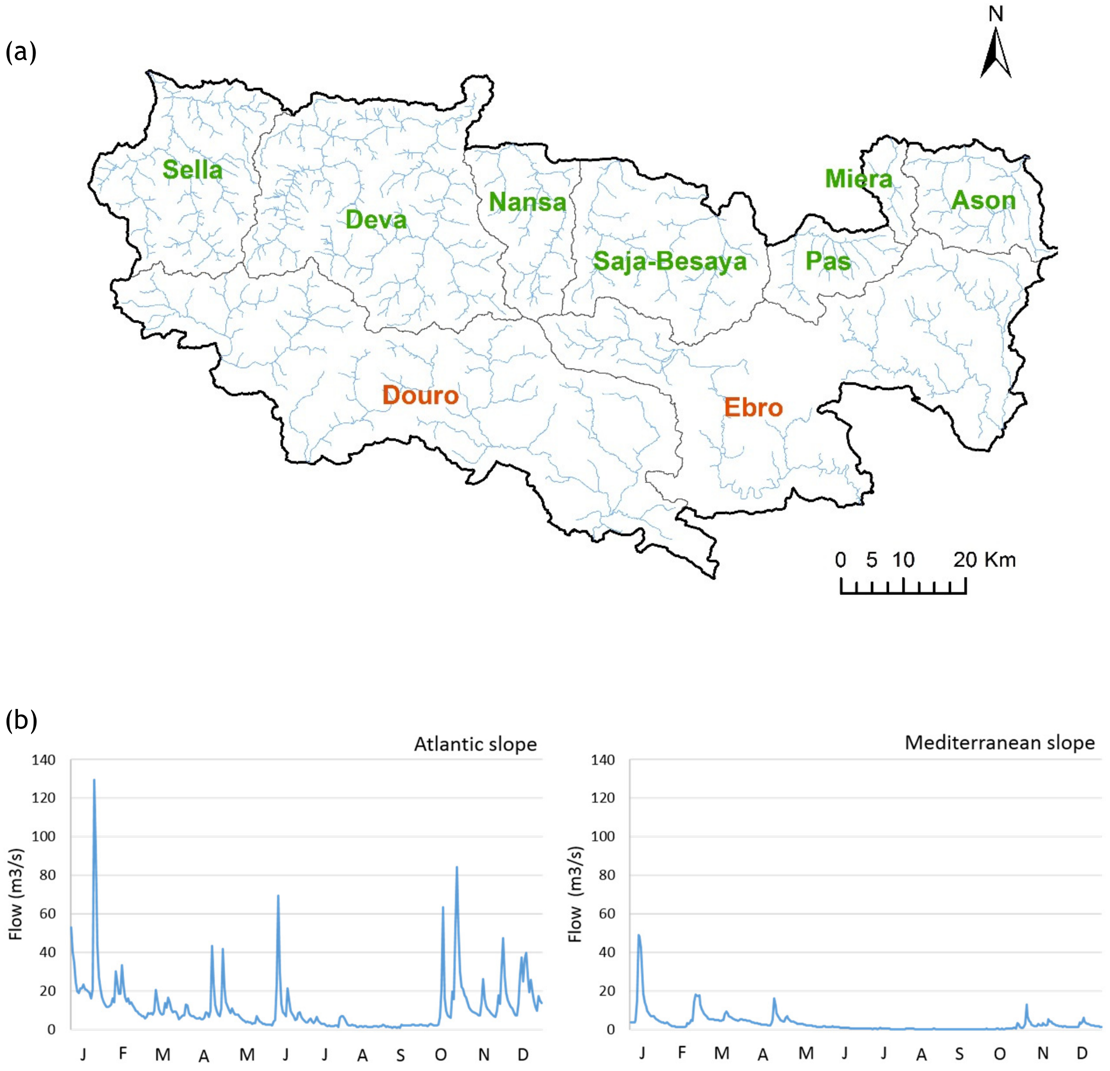

Figure 2.6 – (a) The study area encompasses 9 rivers, 7 in the Atlantic slope (green) and 2 in the Mediterranean slope (orange). (b) Rivers situated in the Atlantic slope are characterized by an elevated flow, a regular flow regime and the absence of summer draughts (on the left) while rivers in the Mediterranean slope show a lower flow and marked summer draughts (on the right).

2.2 Land cover characterization

Since the objective of this thesis is to understand the effects of catchment land cover on mountain stream food web structure, energy flow pathways and ecosystem multifunctionality, current and historic land cover in the study area was characterized. The land cover information was obtained from the land cover classification performed in the RIVERLANDS project (http://riverlands.ihcantabria.es/). The overall aim of RIVERLANDS project was to understand the mechanisms by which current and past land uses affect river processes. Hence, land cover was characterized in the year 2009 and in the year 1984, as a distinctive historic state of land cover, to capture differences on landscape dynamics (Fig. 2.7).

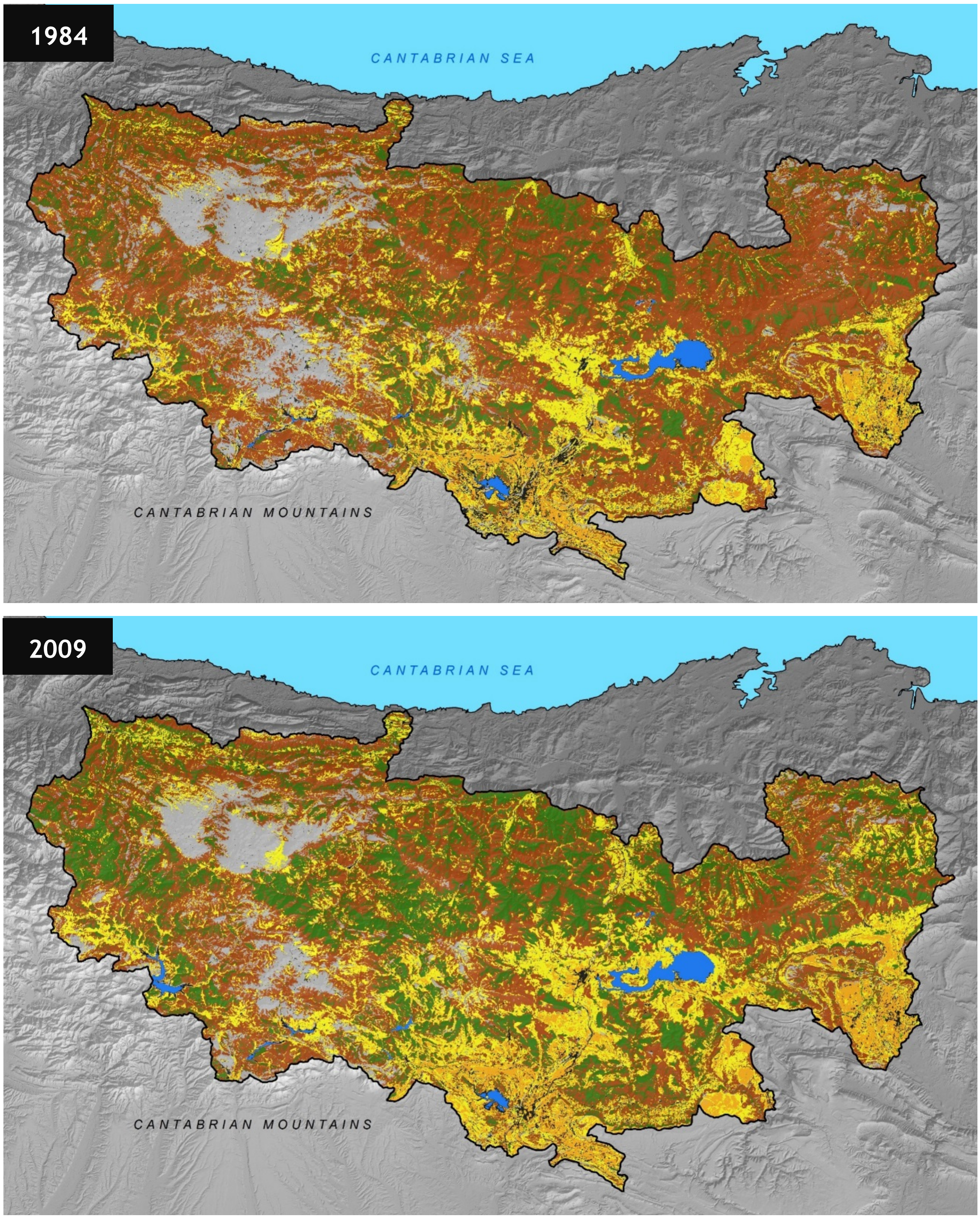

Figure 2.7 - Land cover characterization of the study area in 1984 and 2009. Broad leaf forests are represented in green, shrublands in brown, agriculture land in orange, pasture in yellow, rock outcrops in grey, urban infrastructures in black and water bodies in blue.

Land cover information was obtained from a multitemporal classification of remote sensing imagery (see Álvarez-Martínez *et al.*, 2010). Initially, two Landsat 5 TM images of the years 2009 and 1984 (Path 202, Row 30) with a 30 meter spatial resolution were downloaded from the Earth Explorer catalog of the United States Geological Survey as Level 1 Product Generation System standard products. These images corresponded to the first and last available TM images covering the whole study area with a minimum cloud cover and a relatively high sun elevation angle. We avoided using different satellite sensors (such as Landsat 7ETM+ or Landsat 8OLI) to avoid bias in the following analyses of land cover change (Lillesand, Kiefer & Chipman, 2008). The images were radiometrically, atmospherically and topographically corrected using the freely available algorithms (GRASS, 2013) proposed in Álvarez-Martínez et al. (2018). The topographical correction is crucial for avoiding misclassifications in land cover change analyses as it reduces the variance of spectral responses for each land cover type by flat-normalizing reflectance values at each pixel. The topographical correction was optimized by the use of a high resolution digital elevation model (DEM), which was originally obtained at 5-meters from interpolated LiDAR data (CNIG, 2014) but resampled to 30 meters to match the spatial resolution of the Landsat imagery.

Land cover was classified into eight major land cover classes with enhanced spectral differences using Landsat imagery in the study area: (i) Broadleaf forests dominated by various species of oaks (*Quercus pyrenaica* Willd., *Quercus robur* L. and *Quercus petraea* Matt. Lieb.), birches (*Betula spp*. L.), beeches (*Fagus spp*. L.) and riparian formations; (ii) conifer afforestations at different growing states; (iii) shrublands (*Erica spp* L., *Ulex spp* L., *Cytisus spp*. L. and *Genista spp*. L., among others) ranging from forest ecotones to fringe communities in steeper areas; (iv) agricultural land; (v) grasslands and pastures in valley bottoms; (vi) rocky surfaces, including a gradient from sparse grass vegetation to bare land; (vii) anthropic areas including human settlements, mines, quarries and other infrastructures; and (viii) large water patches. For each class, we collected a complete dataset of reference points across the study area; a total of approx. 3000 points allowed assessment of variability of spectral responses of each class. We accounted for spatial autocorrelation using Global Moran's I statistic measured with ArcGIS 10.2 (ESRI, 2016) and current aerial photographs (CNIG, 2014), Google Street View and field visits for ensuring a correct interpretation of ground data. The 2009 reference dataset was refined for the 1984 reference dataset by using orthorectified aerial photographs from 1980-1986 (CNIG, 2014). 10% of all reference points were randomly selected for algorithm training while validation was performed with the remaining 90%.

Image classification was performed over: (i) six non-thermal bands of 2009 and 1984 TM images, (ii) the Normalized Difference Vegetation Index and Tasselled Cap Transformation components (Brightness, Greenness and Wetness) for each year, as a measure of total photosynthesis and the productivity of vegetation, and (ii) topographical constraints represented by slope and altitude, derived from the 30-m DEM, that may enhance differentiation among land-cover classes that have similar spectral responses but occur at different topographies (e.g., mountain summits, steep slopes). We applied a per-pixel classification approach using a Maximum Likelihood algorithm (Strahler, 1980; Conese & Maselli, 1992) over the multiband dataset. The Maximum Likelihood algorithm assigned each image pixel to a particular land cover class on the base of a maximum membership probability (Hagner & Reese, 2007) generating a hard classification.

2.3 Stream selection

The forest cover per pixel information was integrated in a Synthetic River Network (SNR) delineated for the study area. SRNs are computer-based geospatial simulations of riverine landscapes that include digital elevation models, synthetic hydrography, and their coupling (for more information, see Barquin et al. 2015 and Benda et al. 2016) and provide the proper spatial framework and hierarchical organization to sort out environmental information. The SRN of the study area was delineated from flow directions inferred from a 25 m DEM using the Buildgrids and Netrace software packages, which are included in the ‘NetMap’ platform (www.terrainworks.org; Benda *et al.*, 2016), and the algorithms described by Clarke et al. (2008). The obtained SRN was divided by river confluences into reaches of 100 to 500 m length.

The SRN allowed establishing the percentage of each land cover class upstream of each stream reach, at catchment level and in a 200 m buffer along the entire river network upstream each river reach to further test the influence of the land cover spatial scale in chapter III. To account for land cover history, land cover change was calculated as the difference in forest cover between the years 2009 and 1984. (Fig. 2.8).

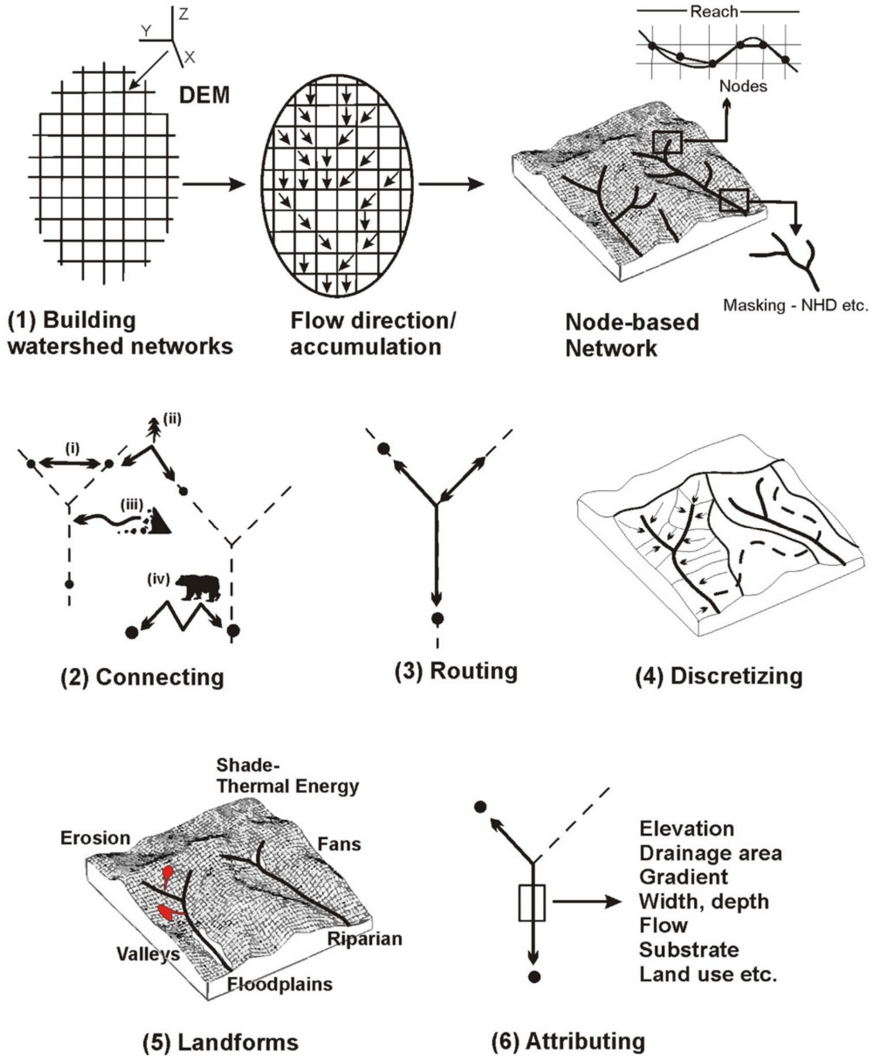

Figure 2.8 - Schematic representation of how Synthetic River Networks were extracted from Digital Elevation Models. Figure extracted from (Benda et al., 2016).

We selected 31 headwater streams along a land cover gradient based on the current forest cover in their upstream catchment (Fig. 2.9 a). In the selected streams, current forest cover ranged from 0% to 79.5% and showed an increase in the past 25 years (from 1984 to 2009; Fig. 2.9 b). However, while in some catchments this increase was minimal (lower than 2%) in others reached up to 24%.

The selected streams were located at different altitudes ranging from 200 m a.s.l. to 1640 m a.s.l. Catchment area ranged from 4.94 to 86.85 km^2, mean hill slope from 26.84% to 90.41% and geology from mostly calcareous (91.97%) to completely siliceous (0% of calcareous rock in the catchment). All streams lacked sewage effluents or pollution sources and agricultural and urban areas were scarce (< 1% of the catchment area).

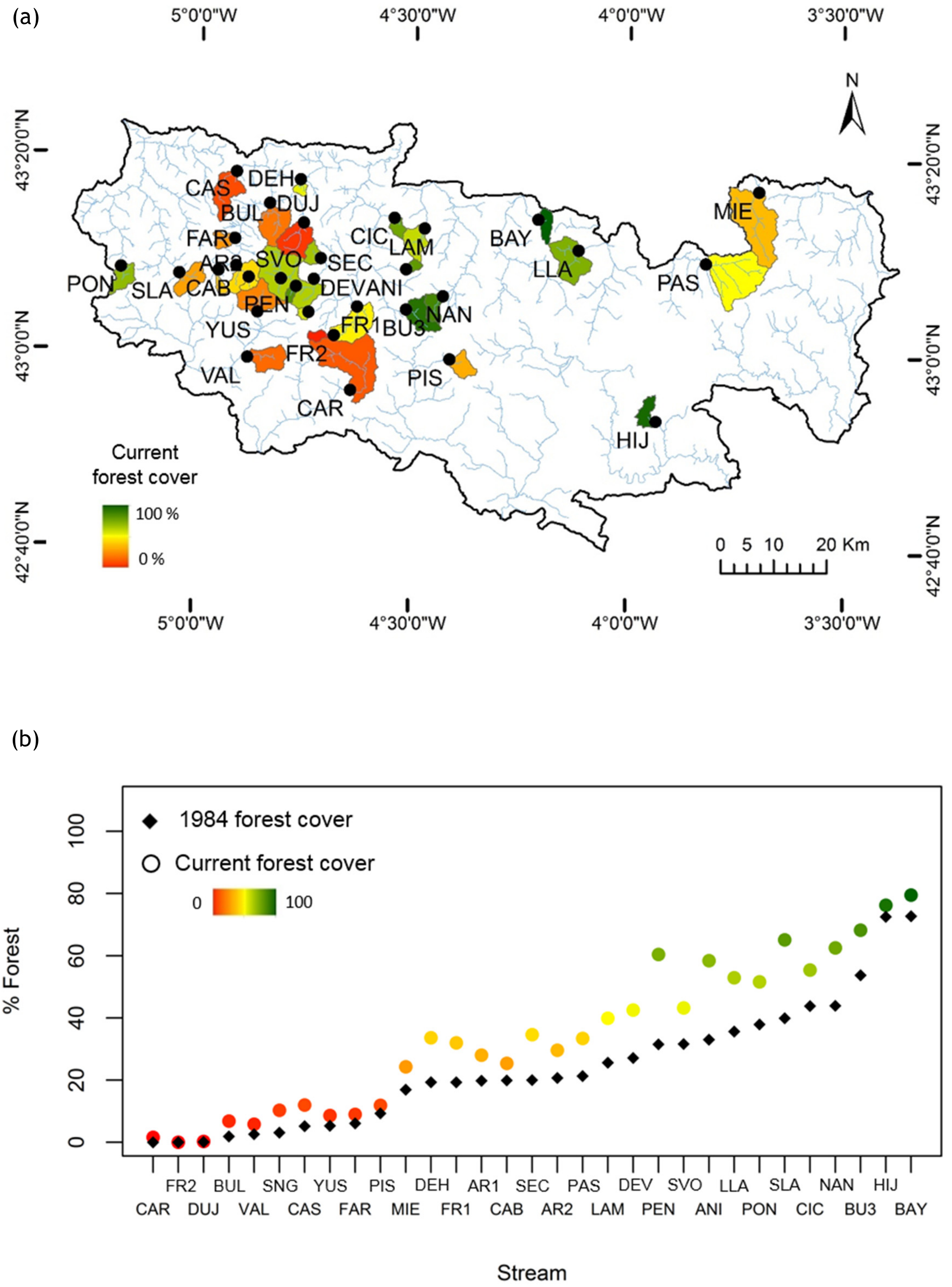

Figure 2.9 - (a) 31 streams (stream codes are shown; SM 2.1) were selected based on the current forest cover in the upstream catchment. Catchments are colored according to current forest cover. (b) Variation in forest cover between 1984 (black diamonds) and 2009 (circles colored according to current forest cover in the catchment) in each of the study streams (stream codes are shown; SM 2.1).

In chapter III, only 24 streams of the 31 streams were considered (Fig. 2.10 a) as 5 streams showed sample contamination and in 2, which are extremely closed to a spring, water DOM might not be significantly affected by land cover. For chapters IV and V, 10 streams (Fig. 2.10 b) were selected due to the methodological constrains of macroinvertebrate biomass determination. In chapter VI all the study streams were considered.

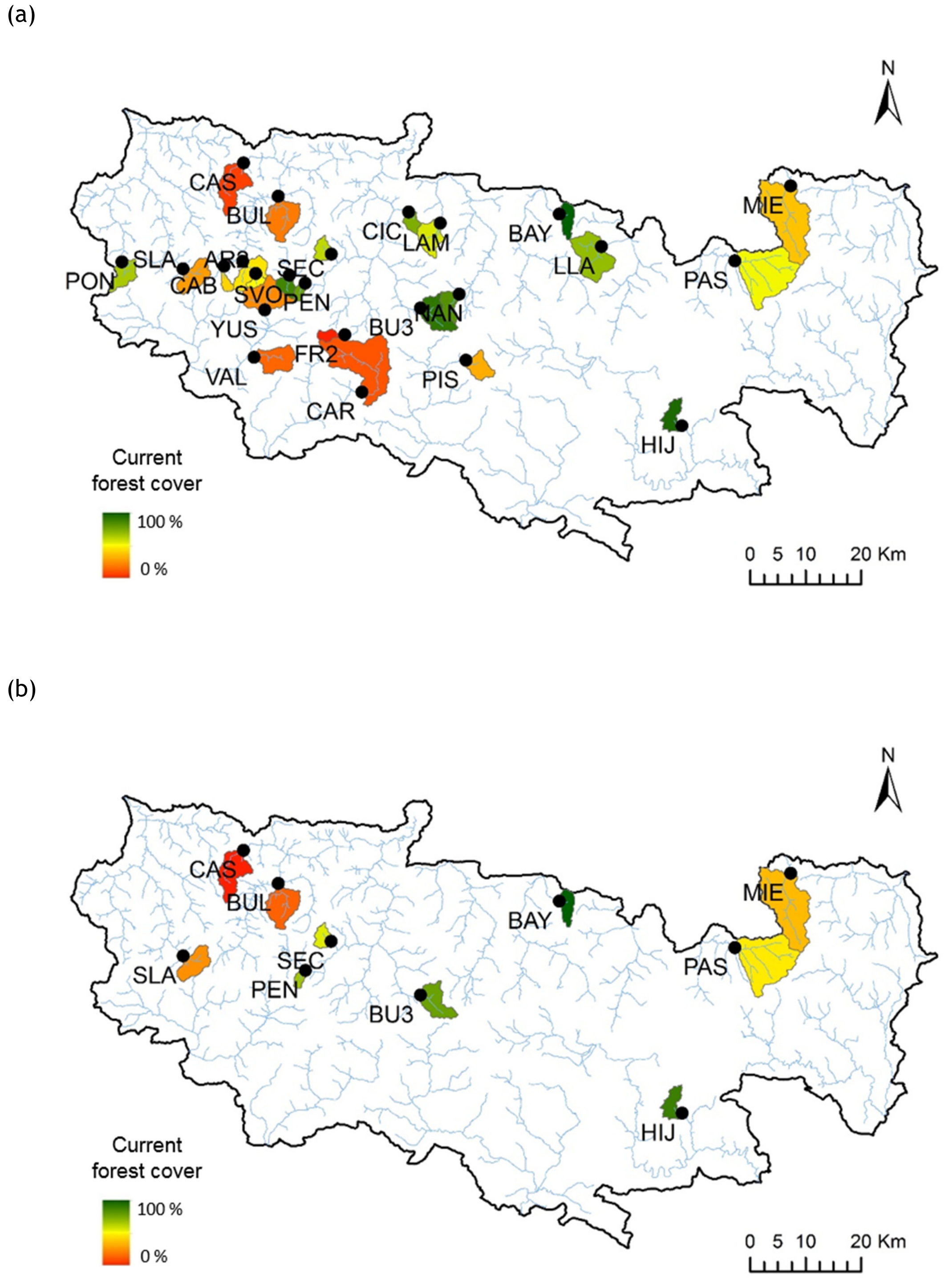

Figure 2.10 – Out of the 31 streams, only 24 streams (stream codes are shown; SM 2.1) were considered in chapter III (a) and 10 streams (stream codes are shown; SM 2.1) in chapters IV and V (b). Catchments are colored according to current forest cover in the upstream catchment.

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2.5 Supplementary material 2

Supplementary material 2.1 – Environmental characteristics of the study streams and correspondence between stream name and the stream code that is used chapters III to VI.

Stream name	Stream code	Catchment area (km^2)	Altitude (m a.s.l.)	Forest cover (%)	Calcareous rock (%)	Hill slope (%)
Aniezo	ANI	5.30	769	58.4	0.00	57.41
Arenal 1	AR1	27.57	963	28.0	15.45	49.49
Arenal 2	AR2	6.89	1210	29.6	0.00	46.64
Bayones	BAY	16.15	267	79.5	0.00	39.24
Bullon 3	BU3	24.54	630	68.2	2.04	48.83
Bulnes	BUL	31.90	228	6.8	91.00	81.59
Cable	CAB	5.34	1051	25.4	30.32	48.78
Carrion	CAR	86.85	1340	1.6	14.80	40.02
Casaño	CAS	34.03	306	1.02	78.41	58.04
Cicera	CIC	8.39	521	55.4	10.59	40.89
Dehesa	DEH	5.02	133	33.6	76.52	68.50
Deva	DEV	81.45	619	42.5	21.56	60.33
Duje	DUJ	34.09	910	0.3	68.78	70.50
Farfada	FAR	10.81	590	9.0	91.97	90.41
Frio 1	FR1	43.66	547	32.0	7.42	55.49
Frio 2	FR2	7.17	1640	0.0	1.37	49.50
Hijedo	HIJ	14.56	767	76.2	0.00	26.84
Lamason	LAM	25.67	527	39.9	34.37	42.87
Llares	LLA	43.75	263	52.9	0.00	45.29
Miera	MIE	82.23	188	24.3	4.48	50.58
Nansa	NAN	15.11	878	62.5	0.00	42.07
Pas	PAS	76.95	288	33.4	0.00	54.99
Penalba	PEN	4.94	817	60.4	0.00	68.62
Pisuerga	PIS	20.32	1259	11.9	0.00	39.73
Ponga	PON	17.21	868	51.6	28.35	59.65
Salvoron	SVO	9.81	1010	43.2	0.00	57.99
Sanglorio	SNG	5.51	1408	10.3	0.00	46.18
Seco	SEC	8.73	629	34.6	47.33	83.88
Sella	SLA	23.01	586	65.1	3.66	56.46
Valponguero	VAL	26.52	1162	5.8	15.10	53.36
Yuso	YUS	32.51	1296	8.6	0.00	48.99

Chapter III

No land cover legacy in fluvial dissolved organic matter

Chapter III. No land cover legacy in fluvial dissolved organic matter

This study, performed by Edurne Estévez, José M. Álvarez-Martínez, Thorsten Dittmar, José Barquín & Gabriel A. Singer, is under preparation to be submitted for publication in a SCI journal.

Abstract

Dissolved organic matter (DOM) represents the largest source of organic carbon in fluvial ecosystems. The majority of DOM in rivers has shown to have terrestrial origin, what indicates that most of the compounds in DOM leach directly from vegetation or from degraded organic matter in catchment soils. This makes DOM composition highly dependent on vegetation cover and extremely sensitive to changes in land cover. Although both vegetation and soil properties are strongly affected by land cover alterations, the transformation of vegetation occurs on a shorter time scale than the consequent transformation of soil organic matter characteristics. This suggests that past land cover might have a strong effect on DOM properties. However, how historic land cover affects DOM properties is still poorly understood as it has only been described for clear-fell forest harvesting in experimental facilities. The primary objective of this study is to address the effect of land cover history (grasslands for extensive cattle raising) on DOM quantity and composition in headwater mountain streams, to determine whether there is a legacy effect after land abandonment. Moreover, we aim to address how the spatial scale of land cover (riparian area vs entire catchment) and catchment topography influence the effects of land cover on DOM composition. Our results show that DOM properties reflected gradients of forest cover and hill slope in the riparian area. More oxygenated and slightly more aromatic terrestrial DOM reached the streams in more forested catchments, what was associated to the increase in tannins and polycyclic aromatic compounds and the decline of lignin and younger soil and fresh vegetation materials as oxygen-poor aliphatic compounds. Gentle hill slopes were associated to a greater DOM quantity, characterized by the dominance of aromatic and humic compounds (i.e., phenolic and polycyclic aromatic compounds) but less lignin and in-stream produced or fresh vegetation derived compounds (carbohydrates, fatty acids and proteins). Nevertheless, no effect of historic land cover on DOM composition was detected, possibly because of the rapid vegetation recovery after land abandonment and the dominance of vegetation composition, in opposition to soil OM, in defining DOM properties in low flow conditions.

3.1 Introduction

Freshwater ecosystems, despite covering a relatively small percentage of Earth's surface, have an important role in the global carbon cycle as they transport, mineralize, and bury large amounts of organic carbon (Cole *et al.*, 2007; Battin *et al.*, 2008). Dissolved organic matter (DOM) represents the largest pool of organic carbon in most freshwater ecosystems (Wetzel, 2001) and its majority has shown to be of terrestrial origin (Raymond & Bauer, 2001; Jaffé *et al.*, 2013). Indeed, most of the compounds in freshwater DOM leach directly from terrestrial vegetation (e.g., leaves, twig, fruits; Kaplan & Newbold, 1993) in or close to the aquatic environment, or originate from degraded terrestrial vegetation accumulated in the catchment soils and reach the stream via subsurface soil flow paths (Fiebig, Lock & Neal, 1990; Mei *et al.*, 2012). Thus, in freshwater ecosystems, DOM properties (i.e., composition and concentration) are strongly defined by the catchment vegetation composition (e.g., vegetation types such as herbaceous, shrub or arboreal vegetation) and the soil environment. This fact makes DOM properties extremely sensitive to changes in land cover.

Transformations of land cover, in concert with climate change, are major environmental threats to the stability and conservation of freshwater ecosystems (Millennium Ecosystem Assessment, 2005) and predicting effects of land cover change on carbon cycling is a primary objective of current research (Schindler & Hilborn, 2015). Our ability to observe the process of land cover transformation itself has greatly benefitted from increasing space-borne remote sensing efforts. However, it has remained a challenge to study the effects of land cover transformation on ecosystems on the ground in space and time, constraining our capacity to understand ecosystem responses over large areas or extended time periods. Space-for-time substitution, which assumes that spatial and temporal variation are equivalent, is an essential approach to overcome the time scale limitation while avoiding long term studies (Pickett, 1989). For space-for-time substitution to be a valid and applicable approach to study and predict effects of land cover transformation, we need to ascertain that responses of interest trace the pressure (i.e., land cover transformation) at a reasonably short time scale if not immediately. In other words, in hindsight, we must be able to show that historic land cover – if different from current land cover – does not have an effect on the response of interest. Nevertheless, the absence of a legacy of historic land cover has often been directly assumed and several studies have used observable patterns of spatial variation to make predictions into the future.

These studies relating DOM properties to present day catchment land cover have consistently reported variations in DOM quantity, composition, origin, and reactivity with land cover. DOM quantity (measured as dissolved organic carbon or DOC concentration) is shown to increase proportionally to wetland, peatland and pasture coverage (Laudon, Köhler & Buffam, 2004; Giling *et al.*, 2014; Masese *et al.*, 2017). Regarding DOM composition, the contribution of aromatic DOM of high molecular weight is reduced in agricultural, pasture and urban areas in comparison to forested areas, while the in-stream produced protein-like DOM of low molecular weight (Frost *et al.*, 2006; Lu *et al.*, 2014; Masese *et al.*, 2017) with high bioavailability increases (Seitzinger, Sanders & Styles, 2002; Chantigny, 2003). Moreover, DOM originating from peatland- and wetland-dominated catchments is reported to be highly colored and aromatic with a greater proportion of polyphenolic aromatic compounds and characterized by low bioavailability (Ågren *et al.*, 2008; Asmala *et al.*, 2013).

However, when land cover changes, the transformation of soil organic matter characteristics occurs over longer time scales than the transformation of vegetation composition and coverage (Trumbore, 2009). Vegetation bulk properties can recover in short time periods as a similar leaf area index can be achieved in 8 years for some vegetation types and climatic conditions (Elliott, Boring & Swank, 2002). In contrast, soil organic matter recovery requires at least 40 years as suggested by the meta-analysis conducted by Guo & Gifford (2002); although some studies consider this recovery time to be longer than 80 years (e.g., Marin-Spiotta *et al.*, 2009). This indicates that soil v remembers past land cover, what confers a buffering capacity that might prevent new vegetation cover to immediately influence stream DOM properties. Hence, historic land cover could contribute to shaping fluvial DOM properties long after the land cover change happened.

Despite the recognition of the potential legacy effects of past land cover (Bürgi, Östlund & Mladenoff, 2017), only few studies have directly tested the effect of land cover *change* (as opposed to current land cover itself) on DOM properties. In fact, all available studies addressed the same land cover change, i.e., clear-fell forest harvesting and the immediately following natural recovery (Hobbie & Likens, 1973; Meyer & Tate, 1983; Yamashita *et al.*, 2011; Burrows *et al.*, 2013; Cawley *et al.*, 2014; Lee & Lajtha, 2016). All these studies were performed in forest experimental facilities such as Coweeta Hydrologic Laboratory (e.g., Meyer & Tate, 1983; Yamashita *et al.*, 2011), J. Andrews Experimental Forest (Lee & Lajtha, 2016) and Hubbard Brook Experimental Forest (Hobbie & Likens, 1973; Cawley *et al.*, 2014) and considered a similar time period since the harvesting (i.e., between 20 and 50 years). The only exception is the investigation by Burrows *et al.* (2013), who analyzed the effects of recent (1-19 years old) clear-fell harvesting of natural forests. Such studies on effects of clear-fell forest harvesting showed an increase in DOC (Hobbie & Likens, 1973; Meyer & Tate, 1983; Tate & Meyer, 1983). Also, compared to old-growth forest streams, DOM in streams draining catchments with harvested forests had a greater contribution of proteinacous and microbially derived compounds but a reduced contribution of aromatic, humic and fulvic-like compounds (Yamashita *et al.*, 2011; Burrows *et al.*, 2013; Cawley *et al.*, 2014; Lee & Lajtha, 2016).

Land cover changes occurring worldwide are more diverse than clear-fell forest harvesting and, by consequence, more generalized effects of land cover history on DOM properties remain largely unknown to date. In reality, these recent land cover changes belong to two predominant and juxtaposed transformation processes (Lambin *et al.*, 2001; Millennium Ecosystem Assessment, 2005; Rudel *et al.*, 2005). In some areas, forest removal has been followed by an intensive use of the territory often for industrialized agricultural activities (crop-farming as well as livestock keeping) and settlement construction (urbanization) that last to present days (Geist & Lambin, 2002; Plieninger *et al.*, 2016). In other areas, the historic use of the territory for extensive cattle raising (i.e., grasslands) and agriculture for local population subsistence, has been progressively abandoned since the middle of the 20th century (Barandiaran & Manterola, 2000), fostering secondary succession processes that mostly result in forest recovery (Pugnaire et al., 2006).

The effects of land cover *change* on fluvial DOM properties might be quite distinct depending on the direction of the land cover change. An intensive use after an abrupt forest removal might have more immediate effects on DOM properties than secondary succession and forest recovery as this is a highly gradual process. In this specific case, the slow land cover change in combination with soil memory and buffering capacity, and the use of the land for cattle raising and agriculture for during decades or centuries, suggests that historic land cover might still have strong and long-lasting effects on soil organic matter properties and consequently, on the properties of the DOM reaching the fluvial ecosystem. Hence, the time period since the secondary succession was initiated (e.g., 10 vs. 100 years) might be key to determine the actual effects of this type of land cover change on fluvial DOM composition.

How land cover influences fluvial DOM properties also depends on the distance to the stream (Sakamaki & Richardson, 2011). The majority of studies consider land cover at a catchment scale (e.g., Graeber *et al.*, 2012; Heinz *et al.*, 2015; Masese *et al.*, 2017), assuming that streams integrate all land uses of the entire draining catchment. However, land cover in the riparian area is expected to exert a greater influence on DOM properties because it is more directly connected to the fluvial ecosystem. Therefore, significant differences could arise when land cover changes are not homogenous within the catchment. For instance, the effects of land cover changes on fluvial DOM composition might be dampened when changes occur distant from the stream and riparian land cover remains invariant, whereas exacerbated when land cover only varies in the riparian area.

Catchment properties such as catchment area (Mulholland, 1997; Ågren *et al.*, 2007), elevation (Johnson et al., 2000; Hazlett et al., 2008) or hill slope highly influence the properties of the DOM reaching the stream (Hinton, Schiff & English, 1998; Chaplot & Poesen, 2012). Hill slope controls both the organic matter accumulation in soils and the transfer of DOM to river network by modifying water residence times and hydrologic flow paths (Xenopoulos *et al.*, 2003; Frost *et al.*, 2006; Lee & Lajtha, 2016). However, how hill slope affects fluvial DOM properties and modulates the effect of land cover is still unclear. Gentle hill slopes have shown to slow the movement of water through land (Frost *et al.*, 2006). A slower water movement in concert with the greater soil organic matter content associated to a more elevated organic matter accumulation generated by the leaf litter accumulation on catchment soils with decreasing hill slope (Orndorff & Lang, 1981; Selva *et al.*, 2007), could result in increasing quantity, degree of humification and aromaticity of the DOM reaching the stream. Nevertheless, in low flow conditions, gentle hill slopes have also been associated to flows through deeper soil layers (Lee & Lajtha, 2016) rich in microbially processed and protein-like DOM (Lajtha *et al.*, 2005). This contributes to a more proteinaceous and microbially derived DOM reaching the streams.

The primary objective of this study is to address the effect of historic and current land cover on DOM quantity and composition in headwater mountain streams to determine whether there is a land cover legacy effect when land is abandoned after its previous use (i.e., grasslands for extensive cattle raising). We expect an overall dominance of terrestrial DOM with a greater proportion of humic and aromatic DOM in more forested streams with an undisturbed history due to the greater organic matter accumulation in catchment soils. In contrast, streams draining more extensive grasslands are expected to be dominated by more recent and proteinaceous DOM. For catchments with a legacy of grassland vegetation prior to secondary succession we predict stream DOM with features typical of streams draining recent grasslands despite a high forest cover in the catchment associated to a strong soil buffering capacity. Moreover, we aim to investigate how the distance of a certain land cover patch to the stream (i.e., whether land cover is expressed at the scale of the riparian buffer vs the entire catchment) and catchment topography (catchment area, altitude and hill slope) modulate the effects of (historic and current) land cover on DOM quantity and composition.

3.2 Methods

3.2.1 Study area and stream reaches

The study area is located in the central sector of the Cantabrian Cordillera, a mountain range that spans more than 300 km across northern Spain parallel to the coast (Cantabrian Sea; Atlantic Ocean; Fig. 2.1 in chapter II). In this chapter, a total of 24 streams were study in this area (chapter II, Fig. 2.10 a).

3.2.2 Catchment topography

Catchment topography was characterized by the draining catchment area, altitude and hill slope. The draining catchment area and the altitude of the sampling reaches were computed from the 25 m DEM. To measure hill slopes, the slope of each pixel from the 25 m DEM was computed. The per pixel hill slope was integrated in the Synthetic River Network delineated for the study area (chapter II). This allowed establishing the hill slope upstream of each sampled reach, at catchment level and in a 200 m buffer along the entire river network upstream sampled reach to further test the influence of the land cover spatial scale.

3.2.3 Sample collection

In September 2015, water samples were collected from each stream during low flow conditions to minimize DOM variations related to hydrology. Large-volume water samples were on-line filtered through a double layer of pre-combusted (450°C, 4h) Whatman GF/F filters into acid-rinsed Nalgene PETG 2000 ml bottles (Nalgene, Rochester, NY) in the field and kept cooled (approx. 4 ºC) and in darkness until further processing. Additional 40 ml samples were filtered (Whatman GF/F, double layer, pre-combusted) using syringes into acid-rinsed and pre-combusted (450°C, 4h) glass vials with PTFE-lined septa caps for spectroscopic and chromatographic DOM characterization. For the analysis of DOM composition by ultrahigh-resolution mass spectrometry, a volume of 888-5441 ml was acidified to pH 2 with 10-molar ultrapure HCl and DOM was extracted on a solid phase (Agilent Bond Elut 500 mg PPL) and eluted with LC-MS grade methanol (Dittmar *et al.*, 2008). The volume of water to be extracted was computed from DOC data generated during a pilot campaign and aiming at a carbon-to-resin mass ratio of 400-800.

3.2.4 Spectroscopic analysis

We generated absorbance spectra and excitation emission matrices (EEMs) simultaneously on a combined fluorescence/UV–Vis absorbance spectrofluorometer (Aqualog, Horiba Scientific, Edison NJ, USA). Fluorescence intensities were measured at excitation wavelengths from 250 to 450 nm (5-nm increments) and emission wavelengths from 210 to 600 nm (1.7-nm increments). Absorbance units were converted to Napierian absorption coefficients following $a[\lambda] = 2.303\ A[\lambda] / l$, where $a[\lambda]$ is a wavelength-specific absorption coefficient (m^{-1}), $A[\lambda]$ is the measured absorbance value and l is the path length of the cuvette (in m). The water Raman peak of Milli-Q water served as reference for fluorescence measurements. EEMs were corrected for blanks (MilliQ) and absorbance (inner filter effect) following McKnight et al. (2001) using in-house algorithms coded in R (Fuß *et al.*, 2017). Data from the Rayleigh scatter regions were deleted and a triangle of zeros was set below the Rayleigh band.

From absorbance data, we computed the specific UV absorption at 254 nm ($SUVA_{254}$) as an indicator of aromaticity (Weishaar et al., 2003) by dividing the decadal absorption coefficient $A[\lambda] / l$ by DOC (in ppm). The absorption coefficient ratio E_2:E_3 ($a[\lambda = 250]$: $a[\lambda = 365]$) gave further information about aromaticity and molecular weight of DOM (De Haan & De Boer, 1987, Helms et al., 2008, Peuravuori & Pihlaja, 1997). Absorption slope was computed for the wavelength range 250-500 nm by using a non-linear estimation method based on the Levenberg-Marquardt algorithm for fitting the standard equation ($a[\lambda] = a_0 \exp(S[\lambda_0 - \lambda])$) with λ_0 = 250 nm; Helms et al., 2008, Loiselle et al., 2009). Absorption slope semi-quantitatively describes the ratio of fulvic to humic acids and was shown to correlate with molecular weight of fulvic acids (Helms et al., 2008, Twardowski et al., 2004). Furthermore, we computed spectral slope, which is the ratio of spectral slopes for a short wavelength region (275-295 nm) and a long wavelength region (350-400 nm). Spectral slope is inversely correlated with molecular weight of DOM and has been identified as an indicator of photodegradation (Helms et al., 2008).

From EEMs we computed the fluorescence index (FI) as the ratio of emission intensity at 450 to that at 500 nm for an excitation wavelength of 370 nm (McKnight et al., 2001). The FI is used to distinguish terrestrially derived DOM (FI~1.2) from microbially derived DOM (FI~1.8). Lastly, the humification index (HIX) defined as the area under the emission spectrum from 435-480 nm divided by the sum of the peak areas from 435-480 nm and 300-345 nm, all at an excitation wavelength of 254 nm, was computed following Ohno (2002). HIX represents the relative humic substance content or extent of humification (Ohno & Bro, 2006). EEMs were also used for parallel factor analysis (PARAFAC), a multivariate three-way modeling technique that decomposes the fluorescence signal into individual components and provides estimates of the relative contribution of each component to the additively formed total signal (Bro, 1997, Stedmon & Bro, 2008). Prior to PARAFAC, Rayleigh scatter regions with missing data were interpolated to speed up the modeling process (Bro et al., 2006). PARAFAC was performed using the DOMFluor toolbox for MATLAB 2007 following the manual by Stedmon and Bro (2008). We allowed a maximum of 10 components, rigorously assessed residual EEM plots and validated final models by split-half validation and random initialization as recommended (Stedmon & Bro, 2008). The results of the PARAFAC model were queried (Tucker Congruence Coefficient = 95%) in the OpenChrom/OpenFluor database (http://www.openfluor.org) in order to search for quantitative matches with previously published and validated PARAFAC models (Murphy *et al.*, 2014). Three PARAFAC components (Table 3.1) were found to provide a robust description of DOM fluorescence of our dataset. For further analysis we used relative fluorescence of these three PARAFAC components and the ratio between the first two components (C1:C2).

Table 3.1 – Fluorescent components of DOM identified by parallel factor analysis (PARAFAC). Given are PARAFAC components, observed excitation and emission wavelengths for maximum fluorescence, alignment with fluorescence peaks identified in previous studies, and a literature-based component description.

PARAFAC component	Excitation and emission maxima (nm)	PARAFAC components and peaks in previous studies	Description
C1	Ex 360 (< 250) Em 478	C3[1], C2[2], mixture of A and C peaks[3], C2[4]	UV humic - like material, humic acid - type
C2	Ex 315 (< 250) Em 412	C4[1], C1[2], C peak[3], C1[4], C1[5]	Humic - like, terrestrial, possibly fulvic acid - type
C3	Ex < 250 Em 328	C5[6], C4[7]	Protein - like, possibly tryptophan - like

[1] Stedmon, Markager & Bro, 2003

[2] Yamashita *et al.*, 2011

[3] Coble, 2007

[4] Santín *et al.*, 2009

[5] Graeber *et al.*, 2012

[6] Yamashita *et al.*, 2010

[7] Shutova *et al.*, 2014

3.2.5 Chromatographic analysis

Size-exclusion chromatography (SEC), in combination with UV- and IR-organic carbon detection and UV-organic nitrogen detection (Huber *et al.*, 2011), was used to measure DOM quantity, as the DOC concentration, and three chromatographically separated DOM size fractions: (i) non-humic high molecular weight substances of hydrophilic character such as polysaccharides, proteins and amino sugars (HMWS), (ii) humic or humic-like substances with high aromaticity including humic buildings blocks (HS), and (iii) low molecular weight substances (LMWS) encompassing neutral, hydrophilic and amphiphilic substances (alcoholes, aldehydes, ketones, sugars, amino acids). We expressed each fraction as a fraction of total C (% LMWS, % HMWS and % HS). DOC concentration (mg C/L) was measured using the column-bypass. Moreover, we determined the hydrophobic organic carbon fraction (HOC; µg/L), which does not pass through the chromatographic column, by subtracting the sum of the 3 fractions from the DOC concentration.

3.2.6 Ultrahigh-resolution mass spectrometric analysis

For an in-depth characterization of DOM, we used ultrahigh-resolution Fourier-Transform Ion Cyclotron Mass Spectrometry (FT-ICR-MS). Leachates were adjusted to 15 ppm C in 1:1 ultrapure water/methanol before broadband mass spectrometry on a 15 Tesla Solarix FT-ICR-MS (Bruker Daltonics, Bremen, Germany) in electrospray ionization (ESI) negative mode (300 accumulated scans, 0.1 sec ion accumulation time, 240 µL/h flow rate) searching for masses from 150 to 1000 Da. After internal mass calibration, raw mass lists were exported for further data processing using in-house code in R (see del Campo, Gómez & Singer; SM 3.1). Briefly, data below a method detection limit were deleted before alignment of peaks across samples. Molecular formulae were assigned to m/z values assuming single-charged deprotonated molecular ions and Cl-adducts for a maximum elemental combination of $C_{100}H_{250}O_{80}N_4P_2S_2$. To exclude unlikely formula assignments, we employed (i) a rigorous assessment of mass error and its partitioning into random and systematic components, (ii) a search for stable isotope confirmation by daughter peaks, and (iii) homologous series assessment. The final dataset consisted of approx. 3900 sum formulae covering on average 63% of total spectrum intensity. FT-ICR-MS data are 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; 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 8 molecular groups, 7 of which are non-overlapping (SM 3.2), based on elemental composition and derived structural information such as double bond equivalents and a computed aromaticity index (Koch & Dittmar, 2006). We computed the total number of differing formulae (molecular richness), the total intensity and the average molecular mass for all 8 molecular groups.

3.2.7 Data analysis

First, a canonical analysis of principal coordinates (CAP; Anderson & Willis, 2003) based on Bray-Curtis dissimilarity was used to build a model explaining variation of DOM molecular composition with current forest cover (including the forest cover in the catchment and forest cover in the buffer) and topographical variables (catchment area, altitude, catchment level hill slope and buffer hill slope). Here, Bray-Curtis dissimilarity was computed from relative peak intensities of the FT-ICR-MS spectra, that is, absolute peak intensities normalized by the total sum of peak intensities of a given spectrum. The constraining variables were subjected to a step-wise forward selection procedure using adjusted r^2 values (Blanchet, Legendre & Borcard, 2008) to develop a parsimonious model explaining DOM molecular composition (tests were done using 9999 permutations). Once the most explanatory variables (current forest cover in the buffer and buffer hill slope) were identified, these were used as initial conditions for a conditioned CAP to test for an additional legacy effect of historic land cover, i.e. an effect of land cover *change* itself (here being increased forestation), on DOM molecular composition. For this, past forest cover was used as a single constraining variable while the previously identified explanatory variables were used as conditions.

To further describe the main compositional gradients of DOM identified by the unconditioned CAP, we computed correlations (function ‘envfit’ with 999 permutations) between the significant CAP axes and total compound richness, number of compounds and average mass in each molecular group, and the spectroscopic and chromatographic variables. Significant variables ($p < 0.05$) are shown as post-hoc fitted arrows in the CAP ordination space. All statistical analyses were performed in R (version 3.3.0, R Project for Statistical Computing, Vienna, Austria) using the packages *vegan* (Oksanen *et al.*, 2017) and *packfor* (Dray, Legendre & Blanchet, 2011).

3.3 Results

The total DOM pool was composed of a large number of compounds. In total mass spectrometry detected 46087 distinguishable peaks in our samples. In individual streams peak richness ranged from 10297 to 18232 and only 10.3% (4747) of the peaks were ubiquitous. These numbers include multiple peaks generated as part of isotope patterns from identical sum formulae yet the applied method cannot distinguish among the many possible structural isomers and actual compound richness must therefore be notably higher. A total of 3906 individually detected peaks (and their eventually existing isotope patterns) were assigned to molecular formulas, i.e. 8.5% of all peaks detected, yet these covered on average 63% of a spectrum summed peak intensities. The majority of assigned compounds were phenolic (74.9%), among which 52.2% were depleted in O (O/C < 0.5) and 22.7% rich in O (O/C > 0.5), followed by aliphatic compounds (8.9%) and polyphenols (8%). Proteins (3.43%), fatty acids (2.61%), combustion-derived polycyclic aromates (1.54%) and carbohydrates (0.13%) were the least numerous. These molecular groups follow a non-overlapping definition. Occurrence of heteroatoms or CRAMS, which have potentially overlapping definitions, comprised on average 37% and 53.2% of a spectrum compound richness, respectively. Patterns based on abundance of all these molecular groups (i.e., relative intensity) resembled those based on their counts: phenolic compounds were the most abundant (86.7 ± 2%), especially phenols depleted in O (66.5 ± 3.9%), followed by polyphenols (6.3 ± 1.1%), aliphatic compounds (4.4 ± 1.7%), fatty acids (1.0 ± 0.21%), combustion-derived polycyclic aromatics (0.91 ± 0.25%), proteins (0.28 ± 0.1%) and carbohydrates (0.04 ± 0.02%). On average 69 ± 2% of a spectrum assigned intensity could be attributed to CRAMS.

CAP results showed that current forest cover in the buffer and buffer hill slope (p = 0.003 and p = 0.032 respectively) explained 24.6% of the variability in DOM composition among streams (Fig. 3.1 a).

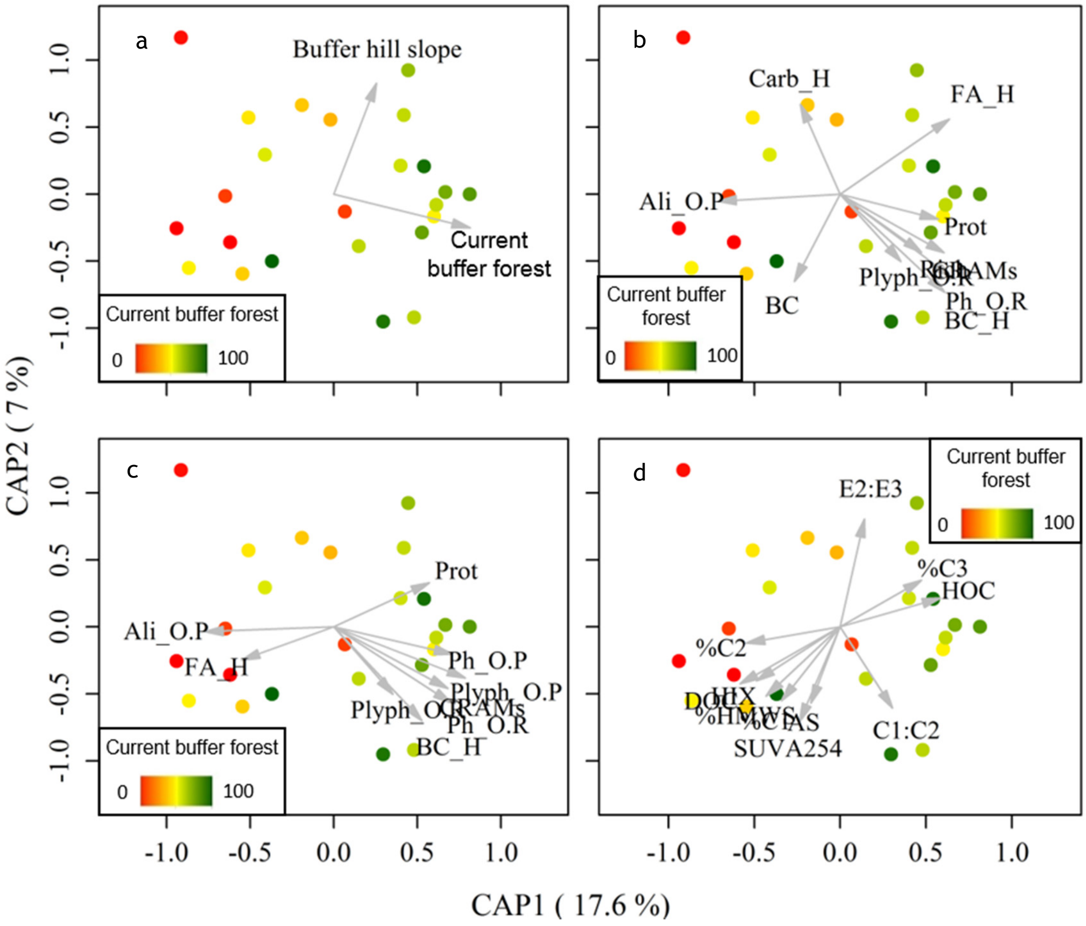

Figure 3.1 – Canonical analysis of principal coordinates (CAP) based on Bray-Curtis dissimilarity of DOM molecular composition. (a) Changes of DOM composition are driven by the major constraints: current forest cover in 200 m buffer (Current buffer forest), which mainly defines canonical axis (CAP 1) and hill slope in 200 m buffer (Buffer hill slope), which mainly defines canonical axis (CAP 2). The changes in DOM composition are strongly correlated to (b) the number of compounds in each molecular group, (c) their average mass (Supplementary Material 3.5) and (d) the spectroscopic and chromatographic variables (Supplementary Material 3.5). Only significantly correlated variables ($p < 0.05$) are shown. The sites are colored according to current forest cover in the 200 m buffer (Figure 3.2).

While CAP axis 1 (CAP 1) explained 17.6% (p = 0.001) and was strongly positively correlated with current forest cover in the buffer, CAP axis 2 (CAP 2) explained an additional 7% of the variance (p = 0.036) and was positively related to buffer hill slope (Fig. 3.1 a). Spearman correlations of DOM compound relative intensities and CAP axes (Fig. 3. 1 a) showed that CAP 1 was negatively related to the abundance of O-poor aliphatics, phenolic compounds and carbohydrates. In contrast, CAP 1 was positively associated with highly unsaturated O-rich phenolics, fatty acids (especially those rich in oxygen), proteins, combustion-derived polycyclic aromatics with heteroatoms, O-rich polyphenols and CRAMS (Fig. 3.3 a). CAP 2 was positively related to the relative intensity of P-containing carbohydrates, O-poor phenols, fatty acids and O-rich aliphatics and CRAMS and negatively to polyphenols (O-rich and O-poor), combustion-derived polycyclic aromatics, O-poor aliphatics and to a lesser extent O-rich phenolic compounds (Fig. 3.3 b). Chemical diversity (i.e., molecular richness) increased with CAP 1 and decreased with CAP 2 (Fig. 3.1 b; SM 3.3). In general, the number of compounds in each of the defined molecular groups followed the same pattern as their relative intensity, except for CRAMS, which increased in number with decreasing CAP 2, and for O-poor polyphenols and O-rich aliphatics, which did not show a significant relationship with any CAP axes (Fig. 3.1 b; SM 3.3 and 3.5). Moreover, CAP 1 was positively related to the average mass of combustion-derived polycyclic aromatics with heteroatoms, polyphenols, phenols, proteins and CRAMS, while it was negatively related to fatty acids and O-poor aliphatic compounds. CAP 2 was accompanied by higher protein average mass but lower average mass of combustion-derived polycyclic aromatics with heteroatoms, polyphenols, O-rich phenols and CRAMS, fatty acids and O-poor unsaturated phenols. Neither combustion-derived polycyclic aromatics without heteroatoms, O-rich aliphatics, nor carbohydrates showed a significant variation of average molecule mass with any CAP axes (Fig. 3.1 c, SM 3.3 and 3.5).

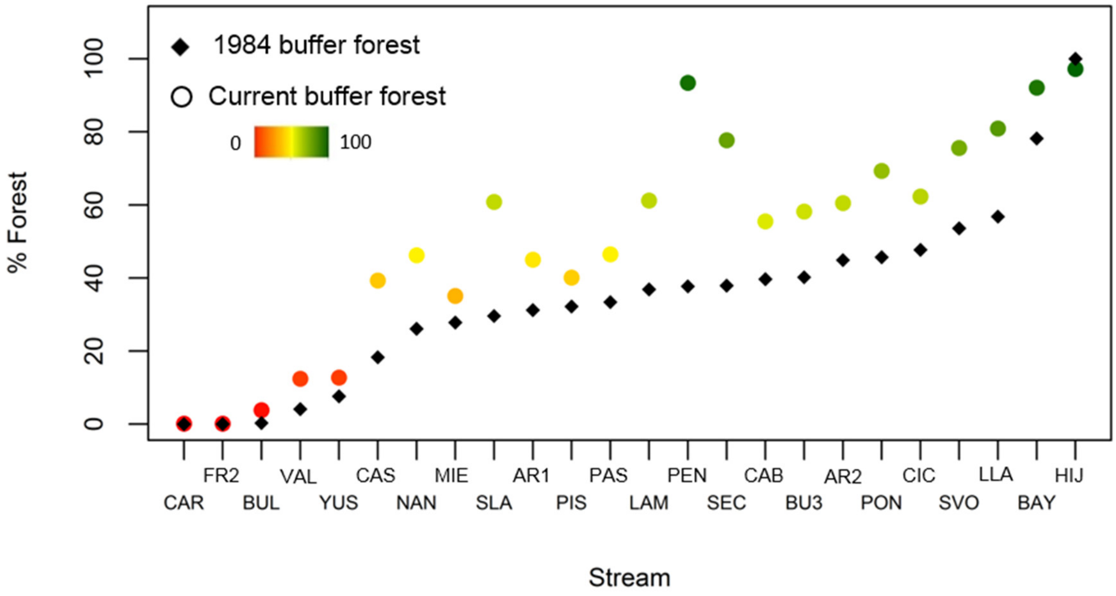

Figure 3.2 – Variation forest cover in 200 m buffer between 1984 (black diamonds) and 2009 (circles colored according to current forest cover in 200 m buffer) in each of the study streams.

DOC concentration was relatively low across all streams (from 0.3 to 2.7 mg C/L) but higher at lower values of both CAP axes (Fig. 3.1 d, SM 3.4 and 3.5). Similarly, the absorption slope and HIX were higher at lower values of the CAP axes. The absorption ratio E_2:E_3 increased with CAP 2 while $SUVA_{254}$ declined. The spectral slope and FI did not show any significant variation with CAP axes, probably because of the merely slight variation in these parameters among streams (Fig. 3.1 d, SM 3.4 and 5). Indeed, FI ranged from 1 to 1.3, suggesting that DOM was mostly of a terrestrial origin throughout all streams. The PARAFAC modeling of EEM spectra revealed 3 independent components (C1 – C3; Table 3.1). Components C1 and C2 corresponded to humic-like materials. C1 could be characterized as similar to humic acid-like fluorophores, while C2 with shorter emission and excitation wavelengths compared to C1, was identified as a fulvic acid-like component. Fluorescence features of C3 suggest a protein-like component, possibly tryptophan-like. The relative fluorescence of these components varied highly among streams (% C1 from 28.4% – 45%, % C2 from 27.13% - 47.10%, and % C3 from 9.85% to 44.49%). % C1 and % C2 decreased with both CAP axes although % C1 was more strongly associated to CAP 2, % C2 was more strongly associated to CAP 1. On the contrary, % C3 increased with both with both CAP axes (Fig. 3.1 d, SM 3.4 and 3.5). C1:C2 ratio increased with CAP 1 and declined with CAP 2 (Fig. 3.1 d, SM 3.4 and 5).

The results from size-exclusion chromatography showed that HS and LMWS were most abundant (% HS ranged from 33.1% to 87% and % LMWS from 10.8% to 65.5%), but their variability was not associated to any of the CAP axes (Fig. 3.1 d, SM 3.4 and 5). However, HMWS, which represented less than 1% of total DOM (% HMWS ranged from 0.01% to 0.08%), declined with CAP 1 and increased with CAP 2. HOC also increased with both CAP axes (Fig. 3.1 d, SM 3.4 and 5).

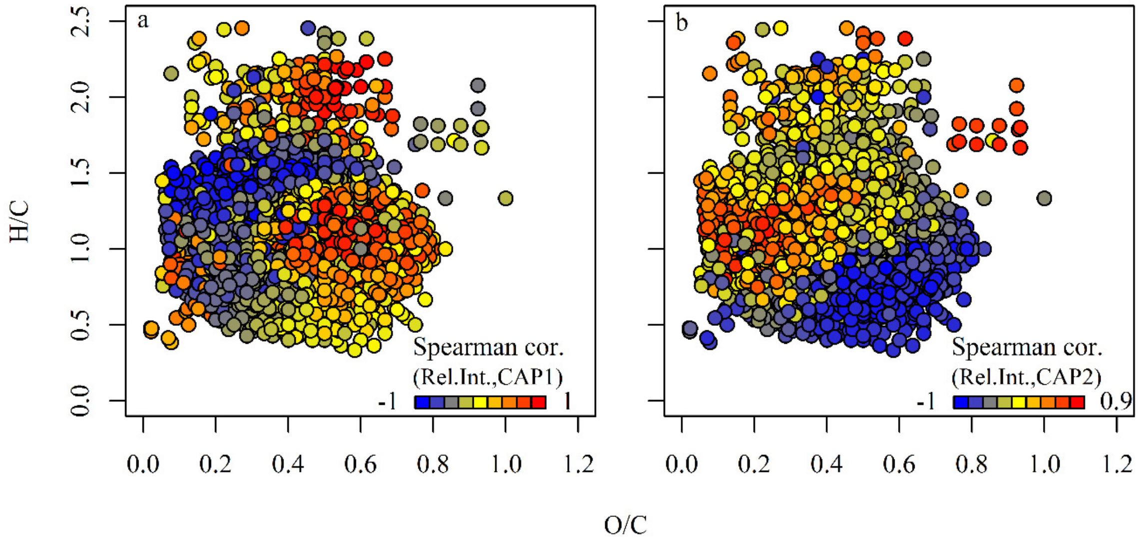

Figure 3.3 – Van Krevelen diagrams show the major changes in DOM composition associated with the two significant canonical axes defined by (a) current forest cover in 200 m buffer and (b) hill slope in 200 m buffer. Point color indicates correlation of relative intensities of individual molecules with the canonical axis across all sampled streams. Red/blue molecules increase/decrease in relative intensity with forest cover and hill slope.

The change in forest cover in the buffer from 1984 to 2009 (Fig. 3.2) showed no significant effect on DOM composition as it only explained an additional 3.6% (p = 0.405) of compositional variation of DOM besides the conditions current forest cover in the buffer and buffer hill slope. In this case, neither CAP 1 nor change in forest cover in the buffer were significant (p = 0.407 and p = 0.399, respectively).

3.4 Discussion

Results showed the dominance of terrestrial DOM in all the study streams and the variation in DOM properties with current land cover and catchment topography of the riparian area, pointing to a strong influence of land cover and topographical features in the immediate vicinity of the stream rather than at a catchment-wide scale. However, the studied land cover *change* had no effect on DOM properties, possibly because of the rapid vegetation recovery after land abandonment, suggesting an absence of a land cover legacy on DOM properties, at least at low flow conditions.

3.4.1 DOM composition across streams

The total compound richness in DOM was large and only few compounds were ubiquitous. The majority of the detected compounds, which were also the most abundant in terms of intensity, were phenolic compounds and polyphenols with formulae commonly associated to lignins and tannins (Kim, Kramer & Hatcher, 2003; Hertkorn *et al.*, 2006; Sleighter & Hatcher, 2007). These compound categories are likely derived from complex biopolymers in plant tissues, what clearly points to a terrestrial origin of DOM, primarily from the leaching and decomposition of plant litter and soils. Carbohydrates, proteins and lipids, labile compounds most commonly produced in-stream or from fresh leaf litter (Marschner & Kalbitz, 2003; Koch & Dittmar, 2006; Sleighter & Hatcher, 2007, 2008), were scarce, both in number and abundance. This high dominance of terrestrially derived compounds and the limited availability of carbohydrates, proteins and lipids could be explained by the environmental factors that characterize mountain streams and the sampling period. Direct vegetation inputs might have been at their minimum (Pozo et al., 1997) because the study was conducted right before the leaf litter fall in autumn. Moreover, steep slopes and fast flowing waters cause short transport times even during the low flow summer season limiting the stream capacity to process and degrade terrestrial DOM and the accumulation of the terrestrial vegetation components in the stream (Goñi, Teixeira & Perkeya, 2003; Roach, 2013). At the same time, low nutrient concentration, low water temperature and turbulent hydraulic conditions reduce algae growth and development, limiting in-stream DOM production (Goldman & Carpenter, 1974; Horner & Welch, 1981; Hill & Knight, 1988).

3.4.2 Variation of DOM properties with land cover

Current land cover was the strongest determinant of DOM composition in the study streams. More oxygenated and slightly more aromatic terrestrial DOM reached the streams in the most forested catchments. The greater DOM oxygenation was associated to a higher proportion of O-rich phenols with formulas previously associated to tannins and lower proportion of O-poor phenolics with formulas previously associated to lignin (Kim *et al.*, 2003; Sleighter & Hatcher, 2007). Both lignin and tannins are compounds mostly present in fresh vegetation and leaf litter, rather than degraded organic matter accumulated in soils (Raymond & Spencer, 2014), which vary substantially with vegetation composition (i.e., vegetation types and plant species; Osono & Takeda, 2004; Yano *et al.*, 2005; Coq *et al.*, 2010). Current land cover in the study catchments, although expressed as the percentage of forest cover, follows a gradient of vegetation composition, which ranges from grasslands, dominated by herbaceous vegetation in the least forested catchments, to arboreal vegetation in the most forested streams, with a dominance of bush vegetation at intermediate levels of forest cover. The observed differences in lignin and tannins with land cover suggest more herbaceous vegetation to be related to a greater relative abundance of lignin and arboreal vegetation to be related to a relative higher content of tannins. Given that phenols and polyphenols show the greatest relative abundances and variation among streams, the main mechanism explaining the differences in fluvial DOM composition with land cover might be strongly related to the characteristics of the dominant vegetation in the catchment.

Moreover, the greater abundance of combustion-derived polycyclic aromatics and lower abundance of O-poor unsaturated aliphatics in forested streams resulted in more humic and aromatic DOM. This DOM humification and aromaticity in forested streams was also shown by the low absorbance slope and the high C1:C2, two indices describing the relative content of humic and fulvic acids. C1, which is defined as a humic component, was strongly associated with polyphenols and polycyclic aromatics, compounds that are understood as being derived from decomposing vascular plants and combustion processes, respectively (Wickland, Neff & Aiken, 2007; Yamashita *et al.*, 2011). In contrast, fluorophores similar to C2, which is defined as a fulvic component, have been identified as derived from O-poor phenols and highly unsaturated compounds (Lu *et al.*, 2013). Humic acids are considered older and more diagenetically altered products than fulvic acids (Aiken *et al.*, 1985). Indeed, the humic acid-like C1 may be derived from older soil organic matter than the fulvic acid-like C2, suggesting that DOM reaching the stream in forested catchments may originate from older organic matter accumulated in catchment soils, probably enhanced by the greater organic matter production (i.e., litter input to top soil) of deciduous forests in comparison to grasslands (Kögel-Knabner, 2002). Thus, besides vegetation composition, the organic matter accumulation and degradation in soils is identified as a strong determinant of fluvial DOM composition. This agrees with previous studies that showed a greater influence from young soils in streams draining grasslands and a higher influence from aromatic and decomposed plant-derived materials in highly forested streams (Lu *et al.*, 2015; Seifert *et al.*, 2016). Hence, differences in fluvial DOM composition with land cover might also be explained by the characteristics of soil OM.

Contrary to initial expectations, forest cover was also associated with a greater relative abundance of proteins and fatty acids, what was also supported by the behavior of protein-like fluorophore C3. Mass spectrometry is semi-quantitative and can just detect the relative changes among compounds and not the absolute abundances because of the lack of calibration standards and the differences in ionization efficiency. As these compounds showed a relative low abundance and limited variation among streams, we could initially think that the relative increase of proteins and fatty acids might have been the result of the decline in the relative abundance of dominant compounds as lignin-like O-poor phenolics. Nevertheless, proteins and fatty acids were also associated with HOC in the CAP ordination. Anthropogenic wastewaters have shown to be enriched in aliphatic compounds with high H/C ratios containing S (Gonsior *et al.*, 2011; Wagner *et al.*, 2015) and HOC (Imai *et al.*, 2002). Thus, the actual greater abundance of these compounds might be identifying a set of streams (CAS and PAS) slightly affected by wastewaters, probably from small settlements.

This fluvial DOM composition reflected the land cover in the riparian area (200 m buffer from the stream), agreeing with previous studies where riparian land use explained more of the DOM properties than land uses in the entire catchment (Gergel, Turner & Kratz, 1999; Hedges *et al.*, 2000; Molinero & Burke, 2009). This evidences that land cover in a narrow area from the stream exerts most influence on DOM properties, particularly in low flow conditions, even though the land cover in the entire catchment is still often considered. The fact that DOM properties in headwater streams with small catchment area reflect the land cover in the riparian area, suggests a stronger influence of riparian land cover in large rivers, in which many catchment areas are less closely connected to the fluvial ecosystem than the most distant areas in small catchments. This highlights the need to further test how the effects of spatial scale of land cover on DOM properties vary with catchment size and whether these are influenced by other land uses (e.g., agriculture).

3.4.3 Variation of DOM properties with hill slope

Hill slope also showed a strong influence on fluvial DOM properties. Streams draining catchments with gentle hill slopes received a greater quantity of DOM of terrestrial origin, characterized by the dominance of more aromatic and humic compounds (i.e., phenolic and polycyclic aromatic compounds) but less non-decomposed organic matter products such as lignin (i.e., O-poor phenolics) and in-stream produced or fresh vegetation derived compounds (carbohydrates, fatty acids and proteins). The more aromatic and terrestrial DOM was associated to greater values of SUVA and HIX indices and C1 PARAFAC component, indicating the presence of older and more diagenetically altered organic matter in less steep catchment soils. Moreover, the increasing proportion of HMWS and the concomitant decrease in E_2:E_3 in streams with gentle hill slopes, suggests an increase in the average molecular weight of DOM. Although this increase in the proportion of HMWS has often been related to the increase in the relative amount of carbohydrates (Marschner & Kalbitz, 2003), in our study, these two properties of DOM were not associated in the CAP ordination. It is important to note that SEC provides an actual quantification of abundance of very large carbohydrate and proteinaceous molecules (biopolymers with molecule masses > 10 kDa), while our mass-spectrometric analysis was limited to molecules < 1 kDa and provides only a semi-quantitative estimate relative to other compounds. Moreover, polar carbohydrates also experience poor retention on the used PPL resin and comparably low ionization efficiency in the FTMS. The SEC-based finding of high carbohydrates at gentle hill slopes is surprising as it suggests very fresh material to co-occur with degraded humic and aromatic compounds. Carbohydrates are often obtained from either in-stream production (Koch & Dittmar, 2006; Sleighter & Hatcher, 2007) or if terrestrially derived, from recent plant organic matter in surface soils in conjunction with lignin (Ohno *et al.*, 2010). However, lignin showed an opposite pattern and agreed with the relative abundance of carbohydrate compounds obtained by mass spectrometry.

These results show that topography plays a significant role in determining what ultimately reaches the stream, although, as previously reported by Li et al. (2015), catchment topography was less important than land cover in determining DOM properties. We suggest that organic matter accumulation and degradation in catchment soils and water residence time are the main mechanisms explaining the differences in DOM composition with hill slope. Steep slopes likely lead to a low accumulation of organic matter in catchment soils while they increase direct OM-input by leaching from living vegetation, fresh leaf litter and young soil materials. This is consistent with the increase in the proportion of lignin and carbohydrates. On the contrary, gentle hill slopes likely increase plant organic matter accumulation in soils, which – in concert with longer water residence times – increases the importance of highly decomposed and diagenetically older DOM sourced from soils and composed of highly unsaturated and aromatic compounds classically associated with humified OM. These findings support recent studies that demonstrated that gentle hill slopes (i) limit leaf litter transport from valley sides into streams enhancing the leaf litter accumulation in soils (Hart, Hibbs & Perakis, 2013; Smits *et al.*, 2015, chapter IV) and (ii) increase the contact time between water and soil organic matter due to a slower water movement and a longer residence time (Boyer *et al.*, 1997; Mulholland, 1997; Hinton *et al.*, 1998). Moreover, our results agree with water flow through deeper soil layers, yet they disagree with water flow through soil layers rich in microbially processed and protein-like DOM (e.g., Yano *et al.*, 2004; Rumpel & Kögel-Knabner, 2011; Huang *et al.*, 2015).

3.4.4 Land cover legacy effect on DOM properties

Despite forest cover being responsible for the largest variation in DOM composition among streams, no land cover change legacy effect was detected. This absence of a historic land cover effect opposes our initial expectations and the results obtained by the few studies that analysed land cover legacy effects on fluvial DOM properties. The previous studies (Hobbie & Likens, 1973; Meyer & Tate, 1983; Yamashita *et al.*, 2011; Burrows *et al.*, 2013; Cawley *et al.*, 2014; Lee & Lajtha, 2016) considered a different trajectory of land cover change, i.e. the succession following timber harvest, but considered a similar period since land cover changed as our study (20-50 years) and observed an increase in protein and microbial-like DOM during forest recovery.

In headwater streams, the properties of DOM reaching the fluvial ecosystem are mostly determined by both catchment vegetation composition and soil OM. In our conceptual framework and the context of this study, soil organic matter would be mainly responsible for a legacy effect of land cover change. This is because the time period needed to achieve a similar soil organic matter composition in catchments previously used as grassland for cattle raising or for cultivation (i.e., agriculture) compared to old growth-forested catchments is most often longer than the time scale considered in our study (i.e., 25 years; Poeplau *et al.*, 2011). Our approach was to not directly measure soil organic matter composition but to exploit the strongly similar current vegetation composition in catchments with different historic land cover and thus, likely different soil properties. In our opinion, there are three major explanations for the absence of land cover legacy effects on fluvial DOM.

First, the most plausible explanation would be that vegetation composition is more dominant in determining DOM properties than soil OM. This explanation is supported by the results obtained for the variations in DOM composition with current land cover, which were mostly explained by vegetation content in lignin and tannins. Further, it would indicate that, once the vegetation composition is recovered (i.e., similar current forest cover), even if soil organic matter composition differs, no effect of historic land cover could be detected in fluvial DOM.

Second, we note that the effects of secondary succession processes (i.e., secondary forest development) on soil organic matter remain poorly understood and recovery time scales are still largely unknown, mainly because several factors including soil type and clay mineralogy, soil texture, topography or climate interact to determine soil organic matter storage and composition (Jobbágy & Jackson, 2000; de Koning, Veldkamp & López-Ulloa, 2003; López-Ulloa, Veldkamp & de Koning, 2005; Heim *et al.*, 2009). Thus, it is also possible that secondary succession processes in soil organic matter operated at shorter time scales (< few decades) than often considered, and that the absence of land cover legacy is simply due to a lack of differentiation in soil organic matter and vegetation composition despite opposing land cover history at decadal time scales. This explanation agrees with studies reporting soil DOC content to reach pre-alteration levels in tropical climates in less than 20 years (e.g., Guggenberger & Zech, 1999).

Third, it needs to be considered that different flow paths, and consequently, soil organic matter pools, are activated with varying hydrology. During high flows and heavy precipitation events, water flows through superficial and shallow soil horizons whereas during low flow, water flows through deeper ones (Fellman *et al.*, 2009; Sanderman *et al.*, 2009). As our study was performed during low flow conditions, a similar DOM composition in deeper soil horizons would explain the absence of land cover legacy. However, differences in shallower soil horizons organic matter composition among catchments with different land cover history, would still be possible. Shallower flow paths have been associated to the export of large amounts of younger, plant-derived DOM (Kaiser *et al.*, 1997; Butman *et al.*, 2012) as organic matter from fresh plant materials are lost with soil depth (Fröberg, Berggren Kleja & Hagedorn, 2007; Malik & Gleixner, 2013). Thus, high flows would enhance the fraction of more recent soil organic matter in fluvial

DOM with potentially higher discriminating power among current and historic land cover. This suggests that the absence or presence of a land cover legacy effect on fluvial DOM properties could be tied to phases of the hydrological cycle. For the moment, we can only conclude that without understanding how secondary succession processes affect soil organic matter properties, it is not possible to ascertain the absence of a land cover legacy effect.

3.5 Conclusions

Our study presents a comprehensive analysis of the detailed molecular composition of fluvial DOM in a gradient of forest cover. Moreover, to our knowledge, it represents the first study attempting an analysis of land use legacy effects related to secondary succession processes following the abandonment of grasslands historically used for extensive cattle raising. The results demonstrate the relevance of current forest cover and, to a lesser extent, hill slope in the stream riparian area in determining the quantity and composition of the DOM reaching the fluvial ecosystems. They point out vegetation composition (grasslands – shrublands – forests), organic matter accumulation and degradation and water residence time in catchments soils as the main factors defining the DOM properties. The absence of land cover legacy suggests that – for catchment-wide landscape ecology encompassing streams as drainage systems – in low flows, the vegetation composition, might be more important for defining fluvial DOM properties than soil OM. Given that land abandonment is expected to increase in the near future and low order streams represent the first link in the DOM processing and transport from catchment soil sources, it is critical to gain a deeper understanding of how historic land cover and secondary succession processes affect soil organic matter, and thus, fluvial DOM properties.

3.6 References

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3.7 Supplementary material 3

Supplementary material 3.1 - Details on FT-ICR-MS data processing.

Following internal calibration, peaks with S/N > 1 were exported from Bruker-DataAnalysis software. 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 organic matter (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 in 5% steps) 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 approx. 170 k compounds with an improved alignment of small peaks. After removal of approximately 67% singlets, 67 contaminants with known mass and 258 peaks showing up dominantly (S/N < 20) in 2 process blanks (extracts from ultrapure water acidified to pH 2), 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 0.5 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], $H \leq 2C+2+N$, 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). A high 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 spectra. This m/z error tolerance is formula-specific as it depends on N. 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 to lower the systematic error by m/z recalibrations done similarly to Koch et al.,(2007). In contrast to these authors, we used general additive models (Hastie & Tibshirani, 1990) of error dependent on m/z rather than segment-wise polynomial fits of m/z on ion cyclotron frequency.

Supplementary material 3.2 - Description of the molecular groups defined based on the elemental composition and structural information obtained from FT-ICR-MS. Only the first seven molecular groups have non-overlapping definitions (all except carboxylic-rich alicyclic molecules). * DBE = double bond equivalents

Molecular group	Molecular sub-group	Description	Definition
Black carbon	without heterotatoms	Combustion-derived polycyclic condensed aromates (PCAs), no heteroatoms	Aromaticity index > 0.66 & S = 0 & Se = 0 & N = 0 & P = 0
	with heterotatoms	Combustion-derived PCAs, no heteroatoms	Aromaticity index > 0.66 & (S > 0 or Se > 0 or N > 0 or P > 0)
Polyphenols	O-rich	PCAs with aliphatic chains, typically soil-derived, rich in oxygen	Aromaticity index ≥ 0.5 and ≤ 0.66 & O:C ≥ 0.5
	O-poor	PCAs with aliphatic chains, typically soil-derived, poor in oxygen	Aromaticity index ≥ 0.5 and ≤ 0.66 & O:C < 0.5
Highly unsaturated phenols	O-rich	Phenols, typical for soil-derived humic material, rich in oxygen	Aromaticity index<0.5 & H:C < 1.5 & O:C > 0.5 and <0.9
	O-poor	Phenols, typical for soil-derived humic material, poor in oxygen	Aromaticity index<0.5 & H:C < 1.5 & O:C≤0.5
Aliphatics	O-rich	Unsaturated aliphatic compounds, rich in oxygen	H:C ≥1.5 and ≤2 & O:C > 0.5 and ≤0.9 & N = 0
	O-poor	Unsaturated aliphatic compounds, poor in oxygen	H:C ≥1.5 and ≤ 2 & O:C ≤ 0.5 & N = 0
Fatty acids	without heterotatoms**	Saturated fatty acids without heterotatoms	H:C > 2 & O:C < 0.9 & S = 0 & Se = 0 & N = 0 & P = 0
	with heterotatoms	Saturated fatty acids with heterotatoms	H:C > 2 & O:C < 0.9 & (S > 0 or Se > 0 or N > 0 or P > 0)
Carbohydrates	without heteroatoms**		O:C ≥ 0.9& S = 0 & Se = 0 & N = 0 & P = 0
	with heteroatoms		O:C ≥ 0.9& (S > 0 or Se > 0 or N > 0 or P > 0)
Proteins		Unsaturated aliphatic and with at least 1 N	H:C ≥ 1.5 and ≤ 2, O:C ≤ 0.9 & N > 0
Carboxylic-rich alicyclic molecules			DBE/C ≥ 0.3 & DBE/C ≤ 0.68 & DBE/H > 0.2 & DBE/H ≤ 0.95 & DBE/O ≥ 0.77 & DBE/O ≤ 1.75

***Not present in the study streams*

Supplementary material 3.3 - Correlation of the total compound richness and the number and average mass of the compounds in each molecular group with the resulted canonical analysis of principal coordinates ordination of DOM molecular composition (relative intensities) based on the environmental drivers (current forest in the buffer and buffer hill slope). Significant correlations (*p* < 0.05) are highlighted in bold.

Molecular group	Number of compounds			Average mass		
	CAP 1	CAP 2	r^2	CAP 1	CAP 2	r^2
Black carbon without heterotatoms	-0.38	-0.93	**0.41**	-0.37	-0.93	0.22
Black carbon with heterotatoms	0.64	-0.77	**0.79**	0.60	-0.80	**0.67**
O-rich polyphenols	0.58	-0.82	**0.31**	0.58	-0.82	**0.31**
O-poor polyphenols	0.93	0.37	0.04	0.90	-0.44	**0.67**
O-rich highly unsaturated phenols	0.72	-0.69	**0.71**	0.78	-0.63	**0.72**
O-poor highly unsaturated phenols	1.00	0.02	0.12	0.96	-0.28	**0.47**
O-rich aliphatics	-0.99	0.11	0.06	0.98	0.19	0.21
O-poor aliphatics	-1.00	-0.07	**0.44**	-1.00	-0.05	**0.51**
Fatty acids with heterotatoms	0.75	0.66	**0.62**	-0.91	-0.41	**0.30**
Carbohydrates with heteroatoms	-0.33	0.95	**0.41**	0.98	-0.18	0.08
Proteins	0.95	-0.31	**0.30**	0.87	0.50	**0.37**
Carboxylic-rich alicyclic molecules	0.81	-0.59	**0.48**	0.85	-0.53	**0.73**
Molecular richness	0.74	-0.68	**0.34**	**-**	**-**	**-**

Supplementary material 3.4 - Correlation of the spectroscopic and chromatographic variables with the resulted canonical analysis of principal coordinates ordination of DOM molecular composition (relative intensities) based on the environmental drivers (current forest in the buffer and buffer hill slope). Significant correlations (p < 0.05) are highlighted in bold.

Spectroscopic and chromatographic variables	**CAP 1**	**CAP 2**	**r^2**
Dissolved organic carbon	-0.81	-0.59	**0.44**
$SUVA_{254}$	-0.32	-0.95	**0.46**
Absorbance slope	-0.29	-0.96	**0.28**
Slope ratio	0.96	-0.27	0.02
E_2:E_3	0.17	0.99	**0.56**
Fluorescence index	-0.12	0.99	0.04
Humification index	-0.77	-0.64	**0.33**
% C1	-0.53	-0.85	**0.35**
% C2	-0.98	-0.22	**0.26**
% C3	0.81	0.59	**0.28**
C1:C2	0.44	-0.89	**0.38**
Hydrophobic organic carbon fraction	0.94	0.34	**0.32**
% Low molecular weight substances	0.73	0.69	0.15
% High molecular weight substances	-0.64	-0.77	**0.38**
% Humic substances	-0.76	-0.65	0.08

Supplementary material 3.5 - Correspondence between the spectroscopic and chromatographic variables and code used in Figure 3.1.

Spectroscopic and chromatographic variables	Code
Black carbon without heterotatoms	BC
Black carbon with heterotatoms	BC_H
O-rich polyphenols	Polyph_O.R
O-poor polyphenols	Polyph_O.P
O-rich highly unsaturated phenols	Phen_O.R
O-poor highly unsaturated phenols	Phen _O.P
O-rich aliphatics	Ali_O.R
O-poor aliphatics	Ali_O.P
Fatty acids with heterotatoms	FA_H
Carbohydrates with heteroatoms	Carb_H
Proteins	Prot
Carboxylic-rich alicyclic molecules	CRAMs
Dissolved Organic Carbon	DOC
$SUVA_{254}$	$SUVA_{254}$
Absorbance Slope	AS
Slope Ratio	SR
E_2:E_3	E_2:E_3
Fluorescence Index	FI
Humification index	HIX
% Component 1	% C1
% Component 2	% C2
% Component 3	% C3
Ratio Component 1 to Component 2	C1:C2
Hydrophobic organic carbon fraction	HOC
% Low molecular weight substances	% LMWS
% High molecular weight substances	% HMWS
% Humic substances	% HS

Chapter IV

Catchment land cover influences macroinvertebrate food web structure and energy flow pathways

Chapter IV. Catchment land cover influences macroinvertebrate food web structure and energy flow pathways

This chapter has led to the article entitled: "Catchment land cover influences macroinvertebrate food web structure and energy flow pathways in mountain streams" by Edurne Estévez, José Manuel Álvarez-Martínez, Mario Álvarez-Cabria, Christopher T. Robinson, Tom J. Battin & José Barquín. It has been submitted for publication in the journal Freshwater Biology.

Abstract

Understanding how different food resources sustain stream food webs is fundamental towards increasing our knowledge on trophic structure and energy flow pathways in fluvial ecosystems. In small mountain streams, food webs can be sustained by autochthonous (instream primary production) and allochthonous (inputs from the terrestrial ecosystem) organic resources, with their relative importance highly dependent on catchment land cover. The aim of this study was to understand the role of catchment land cover in determining food resource type (autochthonous and allochthonous) and quantity, and how this affects macroinvertebrate food assimilation, food web structure and energy flow pathways in mountain streams through the combination of stable isotopes (δ^2H and δ^{15}N) and macroinvertebrate biomass measures. Results showed that stream food webs were highly dependent on the type and quantity of food resources available. Forested streams were sustained mainly by allochthonous resources, while streams flowing through grassland/shrub landscapes were sustained mostly by autochthonous resources. The response differed between feeding groups, pointing towards feeding mode as a key trait in determining organism adaptation capacity to variations in food resources. Detritivores showed a fixed assimilation of allochthonous resources independent of resource quantity, while omnivore assimilation was determined by the dominant food resource. This differential behavior led to an increase in detritivore biomass and a conservation of omnivore biomass with increasing forest cover, which had a bottom-up effect on carnivores, who also increased allochthonous assimilation. This was reflected in food web structure as community biomass was more equally distributed among the organisms composing the food webs when forest cover increased. The differences between mean assimilation and actual biomass sustained by a resource (i.e., energy flow pathway) reached 20%, highlighting the importance of combining food resource assimilation and organism biomass estimates to understand trophic relations and energy flow pathways in stream food webs.

4.1 Introduction

Identifying the mechanistic role of food resources that sustain riverine food webs is a central target of current ecological research (Dodds *et al.*, 2014) and is crucial for testing theories of ecosystem structure and function (Minshall *et al.*, 1985). Stream food webs rely on two types of food resources depending on their origin: in-stream produced autochthonous organic matter such as algae and allochthonous organic matter imported from the adjacent terrestrial ecosystem in the form of wood, leaf litter and terrestrial invertebrates. The relative availability of allochthonous versus autochthonous food resources has been shown to depend on ecosystem size and catchment land cover (Allan, 2004). Thus, a large number of studies over the last decades have evaluated the effect of ecosystem size and land cover on stream food webs (e.g., Vannote *et al.*, 1980; Bruns & Minshall, 1985; Grubaugh, Wallace & Houston, 1997; Hall, Wallace & Eggert, 2000; Whiting, Whiles & Stone, 2011).

Food resource contribution to stream food webs has historically been addressed from the organism consumption point of view, by analyzing gut contents (Rosi-Marshall & Wallace, 2002; Wellard Kelly *et al.*, 2013). This technique is highly valuable for examining predator-prey relationships and food web interactions; however, it is strongly laborious and only represents a snap-shot of ingestion. More recently, the use of stable isotopes has become widespread as it enables the characterization of assimilated organic matter, rather than just the recently ingested food resources (Rounick & Winterbourn, 1986; Layman *et al.*, 2012), allowing a more accurate estimation of the importance of food resources. Therefore, several studies such as Collins *et al.* (2016a); Hayden, McWilliam-Hughes & Cunjak (2016) and Rosi-Marshall *et al.* (2016) are now focusing on understanding the assimilation of food resources with increasing ecosystem size (e.g., revisiting the River Continuum Concept; Vannote *et al.*, 1980). Nevertheless, much less attention is being paid to catchment land cover, which despite diminishing its capacity of determining food resource availability with increasing river size, can be highly relevant for small streams, as these are tightly coupled to their catchments (Conners & Naiman, 1984).

One of the main reasons for this lack of attention is derived from the use of stable carbon isotopes ($\delta^{13}C$) to characterize assimilated diets. Although $\delta^{13}C$ in organism tissues is the most commonly used stable isotope because carbon directly reflects the dominant food resource consumed, the $\delta^{13}C$ signal needs to differ sufficiently between food resources to define the contribution of allochthonous versus autochthonous resources. However, these differences often are not achieved in low order or mountain streams because algae growing in high water velocities are depleted in $\delta^{13}C$ (Trudeau & Rasmussen, 2003), resulting in overlapping signals of $\delta^{13}C$ among food resources (especially with leaf litter).

In this regard, stable hydrogen isotopes (δ^2H) have recently shown potential to differentiate between allochthonous and autochthonous food resources in streams because terrestrial plants are strongly enriched in δ^2H compared to algae (~ 100‰ difference; e.g., Sabo *et al.*, 2010; Dekar *et al.*, 2012), even in forested headwater streams (Doucett *et al.*, 2007). Thus, the use of δ^2H has opened the door to more specifically addressing food resource contribution to low order or mountain stream food webs and evaluating the effects of land cover alteration. Nevertheless, to estimate the contribution of different food resources to food webs, a measure of organism biomass sustained by each food resource is required. Although organism biomass is always considered when the importance of resources are addressed from gut content analyses, it still is lacking in many studies based on assimilation estimates from stable isotopes (e.g., Zah *et al.*, 2001; Ishikawa *et al.*, 2014; Fellman *et al.*, 2015; González-Bergonzoni *et al.*, 2017) and consequently, the importance of different food resources to food webs in these studies might have been misestimated.

Food resource processing and incorporation into food webs likely depends not only on quantity but also on quality (Marcarelli *et al.*, 2011). Resource quality is defined by chemical composition (i.e., carbon to nutrient ratio, fatty acid content, structure of carbon molecules) and determines its palatability and lability (Lau, Leung & Dudgeon, 2008; Twining *et al.*, 2016a b). Autochthonous food resources have higher quality and greater lability than leaf litter (Thorp & Delong, 2002) and even though many aquatic invertebrates show trophic plasticity (i.e., capacity to feed on diverse food resources; Mihuc, 1997; Simpson *et al.*, 2004; McNeely, Clinton & Erbe, 2006), several studies have reported a preference for high-quality food resources (e.g., Leberfinger *et al.*, 2011). However, recent studies have documented an opposite relationship, showing some macroinvertebrate feeding groups (e.g., shredders, macroinvertebrate predators) preferentially feeding on allochthonous food resources, even when these are less abundant than autochthonous resources (e.g., McCutchan & Lewis, 2002; Lau *et al.*, 2009a). This contrast demonstrates that the response of different macroinvertebrate taxa to the interaction of food resource quality with variation in food resource quantity, and its effects on food web structure and composition, remain poorly understood.

The aim of this study was to understand the role of catchment land cover in determining food resource type (allochthonous vs autochthonous) and quantity, and how changes in food resource type and quantity affect macroinvertebrate food assimilation, food web structure and energy flow pathways (biomass they sustained by autochthonous vs allochthonous food resources) in mountain streams. We hypothesized that food resource type and quantity will reflect catchment land cover, with greater importance of allochthonous relative to autochthonous food resources as forest cover increases. We predicted that changes in food resources would lead to shifts in macroinvertebrate assimilation of allochthonous and autochthonous food resources and consequently in dominant energy flow pathways (autochthonous vs allochthonous resources sustained). Furthermore, we anticipated that changes in food resources will affect stream food web structure. We expected macroinvertebrate community biomass to be more evenly distributed among all taxa composing food webs when autochthonous and allochthonous food resources are equally available and to be unevenly distributed when one resource dominates because a smaller number of macroinvertebrate taxa would dominate the community. The aim of this study was to understand the role of catchment land cover in determining food resource type (allochthonous vs autochthonous) and quantity, and how changes in food resource type and quantity affect macroinvertebrate food assimilation, food web structure and energy flow pathways (biomass they sustained by autochthonous vs allochthonous food resources) in mountain streams. We hypothesized that food resource type and quantity will reflect catchment land cover, with greater importance of allochthonous relative to autochthonous food resources as forest cover increases. We predicted that changes in food resources would lead to shifts in macroinvertebrate assimilation of allochthonous and autochthonous food resources and consequently in dominant energy flow pathways (autochthonous vs allochthonous resources sustained). Furthermore, we anticipated that changes in food resources will affect stream food web structure. We expected macroinvertebrate community biomass to be more evenly distributed among all taxa composing food webs when autochthonous and allochthonous food resources are equally available and to be unevenly distributed when one resource dominates because a smaller number of macroinvertebrate taxa would dominate the community.

4.2 Methods

4.2.1 Study area and stream reaches

The study area is located in the central sector of the Cantabrian Cordillera, a mountain range that spans more than 300 km across northern Spain parallel to the coast (Cantabrian Sea; Atlantic Ocean; Fig. 2.1 in chapter II). In this chapter, a total of 10 streams were studied in this area (chapter II, Fig. 2.10 b).

4.2.2 Macroinvertebrate biomass

We quantified the macroinvertebrate biomass in each stream at the end of the low flow season 2014 (end of the summer) as it represents the moment when many macroinvertebrates achieve the highest biomass and, consequently, the greatest demand for food resources. In each stream, we collected one benthic Surber sample (mesh 500 μm, 0.09 m^2) each in 3 pools and 3 runs randomly selected along a 100 m reach. In each reach, the total number of pools was counted, a unique number was assigned to each pool and 3 random numbers were drawn to select the sampled pools. The same procedure was repeated for the selection of the 3 runs. Surber samples were composited, preserved in 90% ethanol and returned to the laboratory. In the lab, samples were divided into < 1 mm and > 1 mm size fractions. Macroinvertebrates retained in the > 1mm sieve were identified to family level (except Oligochaeta, which were identified to subclass and Chironomidae, which were identified to subfamily and grouped in Chironomidae Tanypodinae and Chironomidae non-Tanypodinae), and then measured using ImageJ software (version 1.47; National Institutes of Health, Bethesda, Maryland). Body mass was calculated using published body length-dry mass equations (Smock, 1980; Burgherr & Meyer, 1997; Benke *et al.*, 1999; Rosati, Barbone & Basset, 2012). Body length was measured as the distance from the anterior part of the head to the last abdominal segment, excluding gills and antennae. Macroinvertebrate biomass was corrected for sample area (0.54 m^2) and expressed in g/m^2.

4.2.3 Food resource characterization

In each stream, we determined the quantity of food resources that can sustain stream food webs: wood, leaf litter, fine particulate organic matter < 1mm (FPOM), macro-algae, biofilms, and dissolved organic carbon (DOC) as a quantitative measure of dissolved organic matter. DOC (mg C/L) was measured from a water sample collected at each study stream. Water samples were filtered in the laboratory within 24 h after collection (pre-combusted Whatman GF/F) and DOC analyzed by high-temperature catalytic oxidation on a Shimadzu TOC-V CSH analyzer (Shimadzu Corporation, Kyoto, Japan). From the composite Surber samples, we separated wood, leaf litter, FPOM and macro-algae. Samples were dried to constant mass at 70ºC, weighed, ashed at 500°C for 4 h, and reweighed to yield ash free dry mass (g/m^2).

One cobble was collected from the same pools and runs as the Surber samples (n = 6) to estimate chlorophyll *a* concentration and epilithic biomass of benthic biofilms. All cobbles from each stream were brushed individually into a tray and from the composite slurry, three aliquots of the same volume were filtered through three separate pre-ashed 45-µm glass-fiber filters. Filters were transported cold (4°C) to the laboratory and preserved at -20°C until analysis. Chlorophyll *a* was extracted from one filter in 90% acetone at 4ºC for 24 h in the dark. Absorbance was read on a Hach-Lange DR-5000 UV/visible spectrophotometer and converted to pigment concentration. Epilithic biomass content was determined from a second filter using a modified version of the technique described by Sinsabaugh et al. (1991). Filters were dried to constant mass at 95°C, weighed, ashed at 550°C for 2 h, and reweighed to yield ash free dry mass (g). Chlorophyll *a* concentration (mg/m^2) and epilithic biomass (g/m^2) were corrected for cobble surface area. Surface area was calculated measuring the three longest axes of each cobble and using these measures in the equation developed by Graham, McCaughan & McKee (1988).

4.2.4 Stable isotope analysis

We measured δ^2H and δ^{15}N isotopic signals of macroinvertebrates by collecting 10 macroinvertebrate kick samples with a 500-µm mesh net from all existing habitats (pools, runs, riffles, cascades) in each stream along the same 100 meter reach used above. Individuals were sorted to family level in the field and each family was kept in an individual vial with deionized water for 8 h to allow gut clearance and then frozen at -20°C until analysis. Macroinvertebrate δ^2H values were corrected for background water values since there is a water contribution to the δ^2H isotopic composition (Solomon *et al.*, 2009). Each water sample collected from each stream was filtered through a Filter-Lab polypropylene filter (0.22 µm) and conserved frozen in vials without air headspace until analysis. For autochthonous and allochthonous resources, we determined isotopic signals of macro-algae (when present), leaf litter and biofilm. The biofilm sample was obtained from the remaining filter of the epilithic biofilm slurry. Non-quantitative samples of macro-algae and leaf litter were collected from all existing habitats (pools, runs, riffles, cascades) along each 100 m study reach and composited into single macro-algae and leaf litter samples. All the samples were kept frozen at -20°C until analysis.

In the laboratory, food resources and macroinvertebrates samples were dried at 55°C and then ground using a mortar and pestle. Ancylids and hydrobiids were removed from their shells for isotope analysis. A subsample from each preparation was retained for δ^2H analysis while the remaining sample was acidified (1 ml, 1M HCl) to remove carbonates and again dried at 55°C until a constant weight was reached (we performed a δ^{13}C analysis that did not separate allochthonous and autochthonous food resources, but not used in the present study). Prior to the laboratory analysis, macroinvertebrate families were assigned to a feeding group following Tachet et al. (2002; SM 4.1). Four macroinvertebrate feeding groups were defined based on the type of food ingested: herbivores that mainly forage on living microphytes and macrophytes; detritivores that feed on plant detritus; carnivores that prey on living invertebrates; and omnivores that did not show a clear preference in food type ingested (mostly gatherers, gatherer-scrapers and filter-feeders). Specifically, hydraenids and elmids were considered detritivores based on recent studies (see Elliott, 2008). Once each family was assigned to a feeding group, macroinvertebrates were grouped by taxonomic order (except gastropods which were grouped to class level and chironomids which were grouped in Chironomidae Tanypodinae and Chironomidae non-Tanypodinae) and feeding group. Only those groups (feeding groups by order) that represented > 1% of the community biomass were considered in this chapter.

For δ^2H analysis, ground samples were sent to the Colorado Plateau Stable Isotope Laboratory (Northern Arizona University, Flagstaff, Arizona) where they were weighed, encapsulated in Ag capsules and measured in an isotope-ratio mass spectrometer (Thermo- Finnigan TC/EA and Delta PLUS -XL; Thermo Electron Corporation, Bremen, Germany). The methods of Doucett et al. (2007) were followed to report the value of non-exchangeable hydrogen. Water samples were analyzed for δ^2H by headspace equilibration with H_2 gas and a Pt catalyst using a Thermo-Finnigan Gas-Bench II. For δ^{15}N analysis, samples were weighed into 5 x 9 mm tin capsules and analyzed at the UC Davis Stable Isotope Facility in a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK).

4.2.5 Calculation of allochthonous assimilation and biomass sustained by allochthonous resources

We calculated the percentage of allochthonous assimilation (% AA) for each of the defined macroinvertebrate groups (taxonomic order and feeding type) in each stream as:

$$\%\text{AA} = \frac{\delta\ {}^{2}\text{H}_{Consumer-corrected} - \delta\ {}^{2}\text{H}_{Autoc}}{\delta\ {}^{2}\text{H}_{Alloc} - \delta\ {}^{2}\text{H}_{Autoc}} \times 100$$

where $\delta^2\text{H}_{Consumer\text{-}corrected}$ is the consumer δ^2H signal corrected for water incorporation, $\delta^2\text{H}_{\text{Autoc}}$ is the δ^2H signal of the autochthonous end member, and $\delta^2\text{H}_{\text{Alloc}}$ is the δ^2H signal of the allochthonous end member. The % AA of the entire community was the mean % AA value of all the macroinvertebrate groups present in each stream.

The δ^2H signal for the different defined consumer groups was corrected ($\delta^2\text{H}_{Consumer\text{-}corrected}$) because there is a water contribution to isotopic composition of the non-exchangeable fraction of H in consumer tissues. The $\delta^2\text{H}_{Consumer\text{-}corrected}$ was calculated following Vander Zanden *et al.* (2016) using the expression:

$$\delta\ {}^{2}\text{H}_{Consumer-corrected} = \frac{\delta\ {}^{2}\text{H}_{Consumer} - \left(\omega_{Compound} \times \delta\ {}^{2}\text{H}_{Water}\right)}{\omega_{Compound}}$$

where $\delta^2\text{H}_{\text{Consumer}}$ is the directly measured consumer signal, $\delta^2\text{H}_{Water}$ is the δ^2H value of the stream water for each site, and $\omega_{Compound}$ is the total contribution of environmental water in tissue H. The value of $\omega_{Compound}$ was calculated as:

$$\omega_{Compound} = 1 - (1 - \omega)^{\text{tp}_{Consumer}}$$

where $\text{tp}_{Consumer}$ is the trophic position (tp) of the consumer quantified as the number of trophic levels above primary producers, and ω is the proportion of tissue H derived from ambient water, which was assumed to be on average 17% for all consumers based on published values (Solomon *et al.*, 2009).

The tp was calculated as$\text{tp}_{Consumer} = 2 + \frac{\delta^{15}\text{N}_{Consumer} - \delta^{15}\text{N}_{Base}}{3.4}$where $\delta^{15}\text{N}_{Consumer}$ is measured directly, $\delta^{15}\text{N}_{Base}$ is the minimum δ^{15}N value of a primary consumer in each stream (mainly Ephemeroptera and Gastropoda, although in some streams were Coleoptera, Diptera or Crustacea) and 3.4‰ is the assumed enrichment in δ^{15}N per trophic level (Post, 2002).

The δ^2H_{Alloc} was considered being equal to the leaf litter δ^2H signal (δ^2H_{Leaves}). In many streams (6 out of 10), a few macroinvertebrate groups had a less negative value than the δ^2H_{Leaves}. We believe that this was related to the composite character of the δ^2H_{Leaves} signal (i.e., some individual leaves might have had less negative values than the overall sample). In these cases, the difference between δ^2H_{Leaves} and these macroinvertebrate groups was low (10.71‰ ± 8.73). Thus, we assigned the macroinvertebrate group with the least negative value as the allochthonous end member.

To estimate δ^2H_{Autoc}, we used the δ^2H signal from macro-algae (δ^2H_{Algae}) for those streams where we could obtain an algae signal due to the presence of clear patches of macro-algae material and where δ^2H_{Algae} was the most negative value (3 streams out of 10 sampled). For the remaining streams, the biofilm δ^2H signal was not a valid autochthonous end member. Biofilms consist of multiple components (algae, microbial heterotrophs and allochthonous organic matter such as fungi, bacteria, and fine detritus; Stoodley et al. 2002) and this mixture of autochthonous and allochthonous components results in an intermediate deuterium signal between δ^2H_{Algae} and δ^2H_{Leaves}. Therefore, we estimated the δ^2H_{Autoc} signal following a similar approach to Collins et al. (2016a). In each stream, we estimated δ^2H_{Autoc} from the $\delta^2H_{Biofilm}$ and a correction factor. This correction factor was based on the ratio of δ^2H_{Algae} to $\delta^2H_{Biofilm}$ weighted by the biofilm ratio of chlorophyll *a* to epilithic biomass (determined in the streams where δ^2H_{Algae} was a valid end member) because the amount of chlorophyll *a* relative to total organic matter in biofilms drives the difference between δ^2H_{Algae} and $\delta^2H_{Biofilm}$.

The % AA was expressed as an average ± 1 standard error (± SE) of all the defined macroinvertebrate groups for the entire community as well as for herbivores, detritivores, carnivores and omnivores. Moreover, we calculated the percentage of biomass that was sustained by allochthonous resources (% BSAR) at the community level as well as for herbivores, detritivores, carnivores and omnivores separately. The % BSAR is an indicator of the energy pathways through which the food webs are sustained. The % BSAR defines the % biomass that is fueled through the allochthonous energy pathway while its opposite, 100 - % BSAR, through the autochthonous energy pathway. The % BSAR was calculated as the weighted mean of % AA and the biomass (B) of each macroinvertebrate group (i) for all the macroinvertebrate groups (n) that comprise herbivores, detritivores, carnivores and omnivores, respectively, and all the macroinvertebrate groups at the community level:

$$\%\ \mathrm{BSAR} = \frac{\sum_{i=1}^{n} \%\ \mathrm{AA}_i \times \mathrm{B}_i}{\sum_{i=1}^{n} \mathrm{B}_i} \times 100$$

4.2.6 Data analysis

We used a principal component analysis as an unconstrained ordination technique to summarize variation in food resource availability in the selected streams. The principal component analysis was performed based on the quantity of each food resource in each stream. To test if the environmental variables explained food resource availability, we correlated the environmental variables (% of forest cover and hill slope) with the components of the principal component analysis. As principal component analysis axis 1 (PC1) defined a gradient changing from low to high input of allochthonous food resources (i.e., degree of allochthony), the score of each stream in PC1 was used to perform linear regression analyses to assess its effect in % AA assimilation and % BSAR for the entire community and for the different feeding groups. Given that only one group of herbivores (Gastropoda) was present in half of our study streams, we did not perform individual statistical analysis for this group. Nevertheless, herbivores were considered in all analyses concerning the entire community.

To test the effects of food resource type and quantity on food web structure, linear regression analyses were performed between the potential food resources and macroinvertebrate biomass. Specifically, detritivore biomass was regressed against leaf litter biomass. We did not include wood biomass in calculations because this resource has a high biomass contribution but it is rarely consumed by macroinvertebrates (Richardson, Zhang & Marczak, 2010). Omnivore biomass was regressed against total basal food resource biomass, which was determined as the sum of FPOM, leaf litter, biofilm and macro-algae biomass. Carnivores were regressed against their potential prey biomass (sum of herbivore, detritivore and omnivore biomass). In addition, linear regression analyses were performed between PC1 and the % biomass of detritivores, carnivores and omnivores. To assess how the total macroinvertebrate community biomass was distributed among the macroinvertebrate groups composing the community with the degree of allochthony, a linear regression analysis was performed between PC1 and the coefficient of variation (CV) of biomass in each stream. The biomass CV was calculated as the mean biomass of all defined macroinvertebrate groups in each stream divided by their standard deviation. Non-normally distributed variables were ln-transformed prior to analysis. All statistical analyses were performed in R software (version 3.3.0, R Project for Statistical Computing, Vienna, Austria).

4.3 Results

4.3.1 Catchment land cover versus stream food resources

Food resources varied widely among streams (Table 4.1). Wood was present in all streams except for MIE and wood biomass ranged from 0.2 to 32.1 g/m^2. Leaf litter biomass ranged from 1.6 to 100.1 g/m^2, FPOM from 0.8 to 15.1 g/m^2, chlorophyll *a* from 15 to 56.1 mg/m^2, epilithic biomass from 4.5 to 12.8 g/m^2, and DOC concentration from 0.2 to 3.1 mgC/L. Macro-algae were only present in CAS (0.6 g/m^2).

Table 4.1 - Food resource biomass: wood (g/m^2), leaf litter (g/m^2), fine particulate organic matter (FPOM, g/m2), macro-algae (algae, g/m2), chlorophyll a (mg/m^2), epilithic biomass (g/m^2) and dissolved organic carbon (DOC, mgC/L) in the study streams. These variables were used to perform the PCA analysis and construct the degree of allochthony gradient.

Stream Code	Wood (g/m^2)	Leaf litter (g/m^2)	FPOM (g/m^2)	Algae (g/m^2)	Chlorophyll *a* (mg/m^2)	Epilithic biomass (g/m^2)	DOC (mgC/L)
BAY	14.6	3.8	1.5	0.0	36.4	5.6	3.1
BU3	32.1	100.1	6.6	0.0	19.5	4.7	0.9
BUL	2.8	4.4	1.3	0.0	45.6	12.8	0.2
CAS	0.2	6.0	1.0	0.6	56.1	12.1	0.5
HIJ	25.3	6.9	1.1	0.0	15.0	4.9	2.6
MIE	0.0	1.6	0.1	0.0	38.2	9.5	0.8
PAS	0.48	5.0	0.8	0.0	52.4	11.5	1.3
PEN	12.2	32.6	15.1	0.0	23.1	4.7	0.2
SEC	10.1	14.3	3.8	0.0	35.4	5.9	0.6
SLA	9.6	22.1	3.2	0.0	24.6	4.5	0.9

The first two principal components (PC1 and PC2) explained 75.5% of the variation in food resource type and quantity, with PC1 explaining 53.1% of the variation (Fig. 4.1). PC1 was positively associated with streams characterized by high wood, leaf litter and FPOM biomass and negatively with chlorophyll *a*, and macro-algae and epilithic biomass. PC2 explained 22.4% of the variation and was negatively related to DOC and wood biomass and positively with the remaining food resources. PC2 differentiated two streams (BAY and HIJ) that were characterized by a low availability of food resources, except for high DOC concentration and wood biomass. Forest cover in the catchment was positively and significantly correlated with PC1 (0.81; $r^2 = 0.86$, $p < 0.001$) and negatively with PC2 (-0.58; $r^2 = 0.86$, $p < 0.001$), while hill slope was positively correlated with PC2 (0.95; $r^2 = 0.51$, $p = 0.07$). Therefore, we considered PC1 as a proxy of the degree of allochthony of the streams (Fig. 4.1).

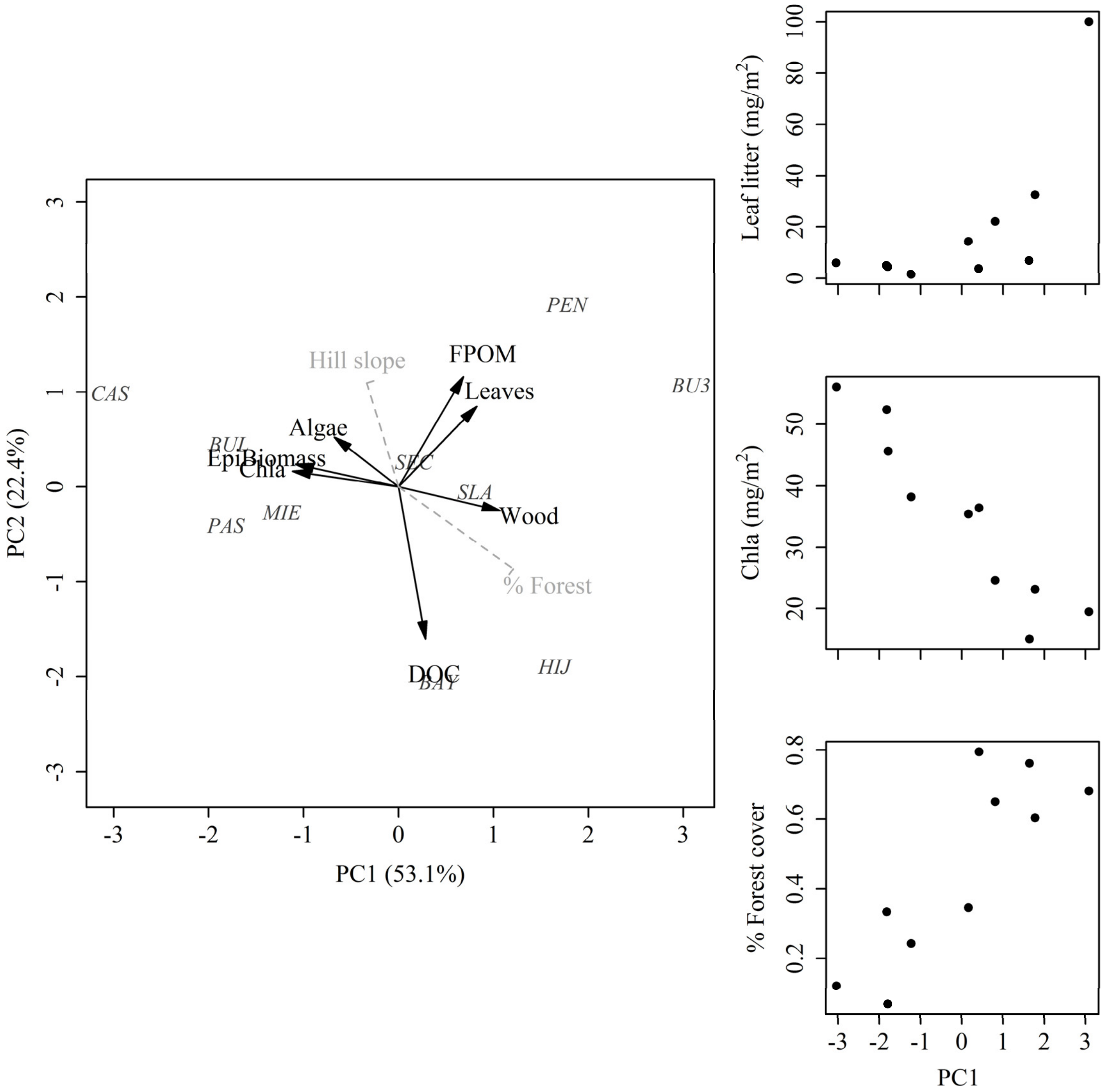

Figure 4.1 - Principal component analysis of the study streams based on food resource availability. The first two principal components (PC1 and PC2) explained 75.5% of the data variation. In the main graph, study streams are shown in italics, black arrows indicate the food resource relationship with the principal components and study streams and grey T- ended arrows show the correlation of principal components and environmental variables (% of forest cover in the catchment and hill slope, in grey). Smaller graphs show the individual relationship between PC1, the axis defining the relative degree of allochthony among streams, and the quantity of chlorophyll a and leaf litter, and the percentage of forest cover.

4.3.2 Assimilation and biomass sustained by food resources

The % AA varied widely within macroinvertebrate communities from 35.8 ± 7.03% to 83.2 ± 7.08% and increased significantly with the degree of allochthony ($r^2 = 0.57$, $p = 0.01$; Fig. 4.2). The % AA was > 50% in all the streams except for CAS, the stream most dominated by autochthonous food resources, and SLA. The % BSAR was 29% in the most autochthonous stream (CAS) and strongly increased to 85% in the most allochthonous stream (BU3; Fig. 4.2). The % BSAR was correlated with the degree of allochthony ($r^2 = 0.78$, $p < 0.001$). When we compare the % BSAR and % AA for the entire community, the % BSAR was lower than % AA in the most autochthonous streams. Differences between % BSAR and % AA decreased with increasing degree of allochthony ($r^2 = 0.58$, $p = 0.010$; SM 4.2), becoming % BSAR even higher than % AA in the two most allochthonous streams (PEN and BU3; Fig. 4.2; SM 4.2).

Herbivore invertebrates were present only in half of the study streams (BUL, MIE, PAS, PEN and SLA; Table 4.2) and their % AA was strongly autochthonous (% AA ranged from 0 ± 0% to 45.1 ± 0%). As only one herbivore group was present in the study streams (Gastropoda), the % AA and % BSAR were equal.

Table 4.2 - Biomass (g/m^2) and relative percentage (%) of herbivores, detritivores, omnivores and carnivores in each study stream.

Stream Code	Herbivores (g/m^2)/(%)	Detritivores (g/m^2)/(%)	Omnivores (g/m^2)/(%)	Carnivores (g/m^2)/(%)
BAY	0.0 (0.0)	35.2 (21.1)	66.5 (39.8)	65.4 (39.1)
BU3	0.0 (0.0)	400.2 (24.4)	1063.7 (64.8)	176.3 (10.8)
BUL	451.4 (31.1)	69.9 (4.8)	774.9 (53.4)	155.3 (10.7)
CAS	0.0 (0.0)	372.3 (13.6)	1109.0 (40.5)	1258.6 (45.9)
HIJ	0.0 (0.0)	96.3 (34.8)	72.6 (26.3)	107.4 (38.9)
MIE	5.4 (1.7)	43.6 (13.8)	204.3 (64.8)	62.0 (19.7)
PAS	126.9 (16.0)	68.0 (8.6)	404.5 (51.1)	192.3 (24.3)
PEN	12.4(1.3)	450.4 (51.7)	142.6 (16.4)	266.5 (30.6)
SEC	0.0 (0.0)	207.0 (32.6)	68.2 (10.7)	360.1 (56.7)
SLA	56.4 (7.6)	221.8 (29.8)	281.5 (37.8)	184.8 (24.8)

The detritivore % AA was strongly allochthonous in all streams (range from 65.7 ± 24.1% to 91.0 ± 12.7%). Given the low variability among streams, no relationship was obtained between the degree of allochthony and % AA ($r^2 = 0.24$, $p = 0.16$). This result suggests that the % AA is independent of allochthonous resource quantity for detritivores (Fig. 4.2). Similarly, the % BSAR for detritivores was strongly allochthonous (58.3% - 98.5%), with no relationship with the degree of allochthony (r^2 = 0.27, *p* = 0.12; Fig. 4.2). Omnivores showed a much more variable % AA (range from 29.2 ± 11.2% to 82.9 ± 27.8%) that significantly increased with the degree of allochthony (r^2 = 0.5, *p* = 0.021; Fig. 4.2). The % BSAR showed the same pattern, ranging from 21.3 to 79.4% and significantly increased with the degree of allochthony (r^2 = 0.53, *p* = 0.02). Carnivores showed a similar pattern as omnivores. Carnivore % AA ranged from 27.4 ± 3.2% to 75.8 ± 20.3%, while % BSAR ranged from 27.1% to 88.8%; both significantly increased with the degree of allochthony (r^2 = 0.46, *p* = 0.03 and r^2 = 0.52, *p* = 0.02, respectively; Fig. 4.2). Therefore, the difference between the % BSAR and % AA only varied with the degree of allochthony in omnivores (r^2 = 0.51, *p* = 0.029; SM 4.2) except in CAS, despite being the most autochthonous stream, the difference between % BSAR and % AA was relatively low (7.9%).

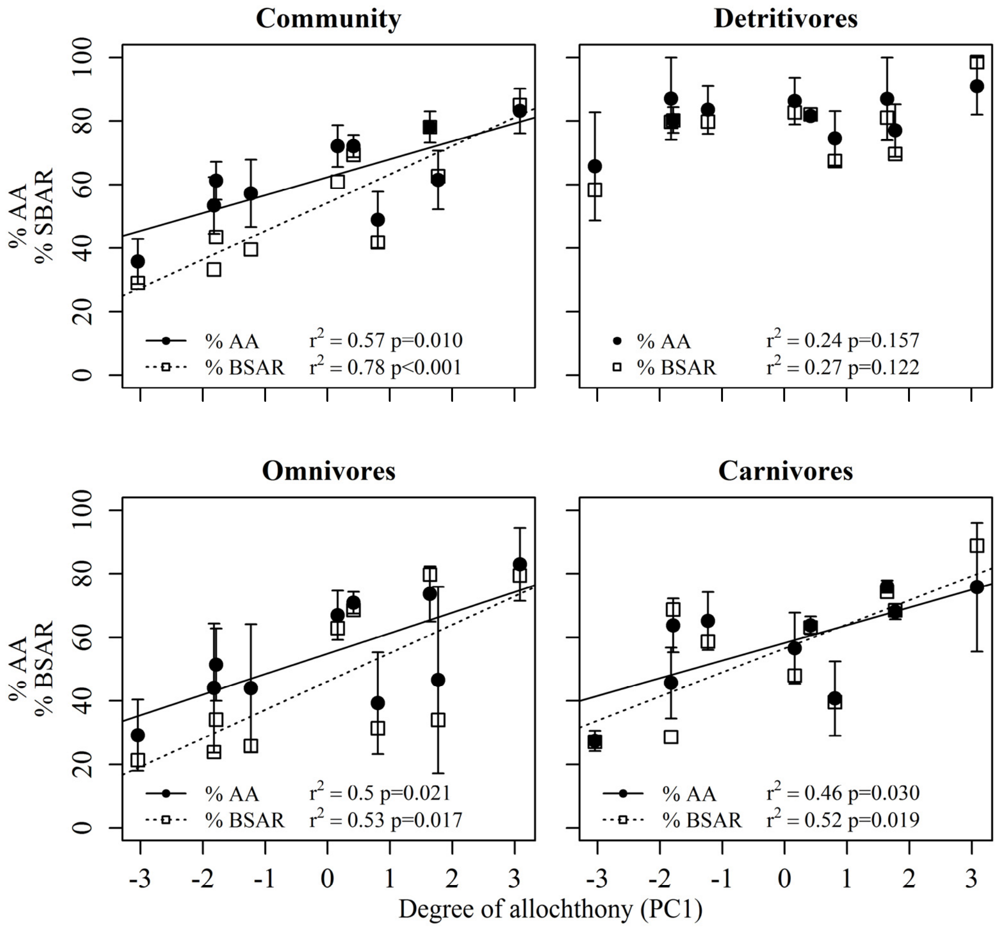

Figure 4.2 - Mean percentage (± SE) of allochthonous assimilation (●, top equation) and percentage of biomass sustained by allochthonous resources (□; bottom equation) in relation to the degree of allochthony (PC1) for the entire macroinvertebrate community, detritivores, omnivores and carnivores. Regression lines are shown for significant (*p* < 0.05) linear regressions.

4.3.3 Food web structure

Herbivore biomass represented a low percentage of the macroinvertebrate community, ranging from 0% to 31.1% and being > 2% in 3 streams (BUL, PAS and SLA). Detritivore biomass was related to leaf litter biomass (r^2 = 0.66, p = 0.004) and their percentage in the macroinvertebrate community ranged from 4.8 to 34.8% strongly increasing with the degree of allochthony (r^2 = 0.53, p = 0.02; Table 4.2, Fig. 4.3).

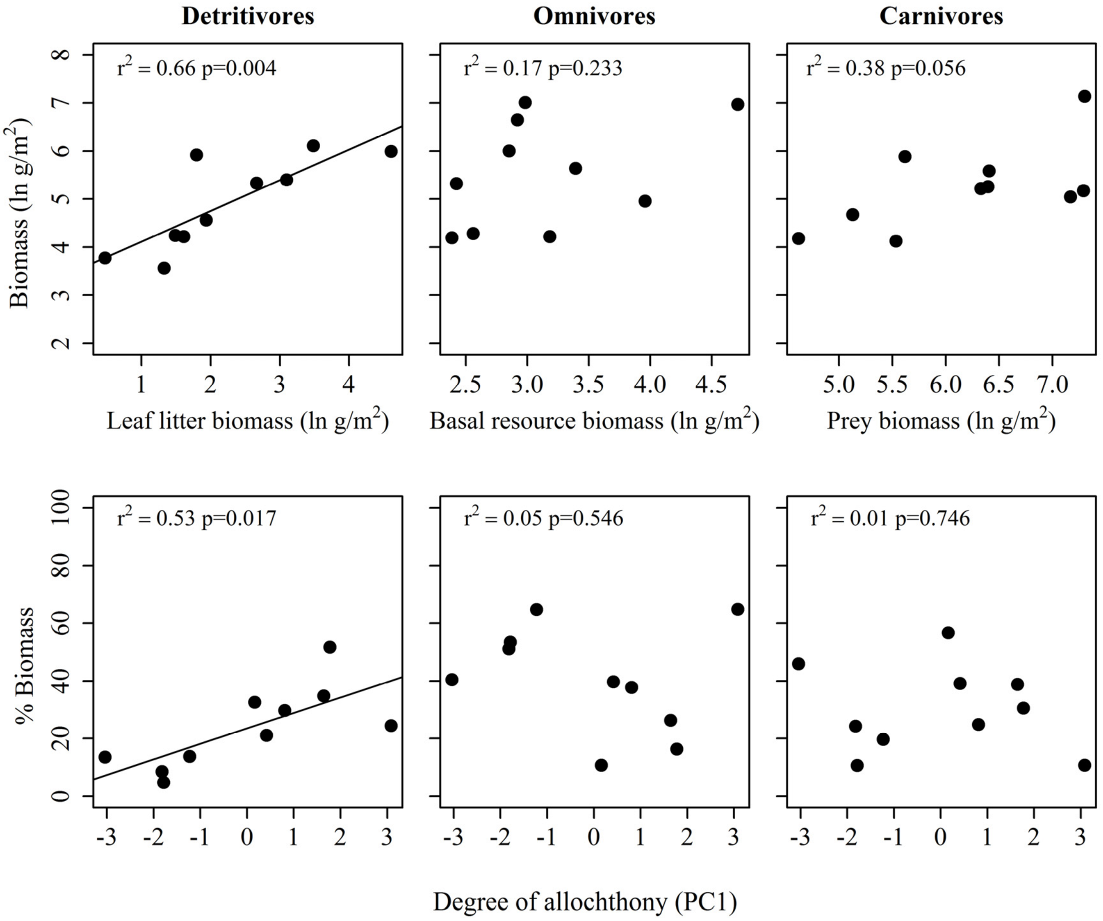

Figure 4.3 - Relationships of detritivore, omnivore and carnivore invertebrate biomass to the leaf litter, basal resources and prey biomass, respectively (top row), and their relative biomass (%) with the degree of allochthony (PC1; bottom row). Regression lines are shown for significant ($p<0.05$) linear regressions.

Food resource biomass was not related to omnivore biomass (r^2 = 0.17, p = 0.23; i.e., omnivore biomass was similar in streams with different food resource biomass; Table 4.2, Fig. 4.3). The percentage of omnivore biomass in the macroinvertebrate community was not related to the degree of allochthony (r^2 = 0.05, p = 0.55; Table 4.2, Fig. 4.3). Carnivore biomass ranged from

10.3 to 56.7% but was not related to the degree of allochthony ($r^2 = 0.01$, $p = 0.746$; Table 4.2, Fig. 4.3) and, although carnivore biomass increased with its potential prey biomass, this relationship was marginally non-significant ($r^2 = 0.38$, $p = 0.056$). Macroinvertebrate biomass CV showed a strong decrease with the degree of allochthony ($r^2 = 0.38$, $p = 0.056$; Fig. 4.4), indicating a more equal biomass distribution within the food web when the degree of allochthony increased.

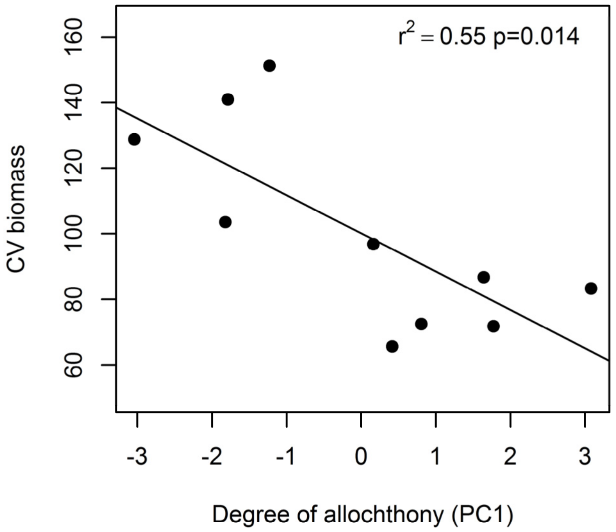

Figure 4.4 - Relationship between the macroinvertebrate biomass coefficient of variation (CV) and the degree of allochthony (PC1).

4.4 Discussion

In this study, we addressed an important question discussed for decades: what is the relative contribution of allochthonous versus autochthonous food resources in supporting stream food webs? By combining stable isotope analysis (δ^2H and $\delta^{15}N$) and macroinvertebrate biomass estimates, we produced a quantitative answer for this question. This approach allowed determining not only macroinvertebrate assimilation of food resources but also reflected how energy is transferred through the food webs, providing better understanding on how catchment land cover shapes food web structure in mountain streams.

4.4.1 Land cover versus food resources

Our results emphasized the importance of land cover in determining food resource type and quantity in streams. Grassland/shrub dominated streams (CAS, BUL, MIE, PAS) were characterized by a higher proportion of biofilm biomass (with higher chlorophyll *a* content) and macro-algae, and a lower proportion of allochthonous food resources. Nevertheless, due to the mountainous character of these streams, autochthonous resource biomass was, in general, low compared to lower order streams or non-mountainous streams (e.g., Sabater *et al.,* 2000). In the study streams, algae were mostly found in biofilms and macro-algal presence was limited regardless of catchment land cover, likely because algae growth and development is restricted by environmental factors characteristic of mountain streams (e.g., low nutrient concentration, low water temperature, turbulent hydraulic conditions; Goldman & Carpenter, 1974; Horner & Welch, 1981; Hill & Knight, 1988).

In contrast, streams with a greater percentage of forest in their catchment showed a higher proportion of allochthonous food resources and lower amounts of macro-algae and chlorophyll *a* in biofilms. Contrary to expectations, leaf litter biomass in the two most forested streams (BAY, HIJ) was as low as in the least forested streams (Table 4.1). Differences in reach-scale environmental variables that control leaf litter inputs (e.g., canopy cover; Swanson *et al.*, 1982) or reach-scale organic matter retention (e.g., channel hydraulics; Johnson & Covich, 1997) may explain the low leaf litter biomass in these two streams. However, canopy cover was high (68% in BAY, 75% in HIJ) and mean water velocity low (0.05 m/s in BAY, 0.04 m/s in HIJ) relative to the other streams, with no large differences in hydraulic retentive structures in the river reaches.

Since sampling occurred in late summer and deciduous trees generate most of the leaf litter in autumn, low leaf litter availability due to invertebrate and microbial processing over several months after leaf fall may have been possible. Because this pattern was not consistent among streams (it only occurred in BAY and HIJ, and leaf litter availability differed highly from other forested streams), it cannot be assumed as a plausible explanation for the low leaf litter availability in the two highly forested streams. On the contrary, DOC from terrestrial origin (e.g., humification index was 0.81 and 0.92, and $SUVA_{254}$ was 4.47 and 5.38 in BAY and HIJ, respectively, chapter III) was highest in BAY and HIJ streams, which could be related to gentle hill slopes in these catchments relative to the other streams (39% in BAY and 27% in HIJ, > 49% in others). Lower slopes may limit leaf litter transport from valley side-slopes into streams as found in a recent study (Smits *et al.*, 2015). Here, leaf litter would accumulate mostly in forest soils and decomposed, thereby increasing dissolved organic matter concentrations to adjacent streams. The low amount of leaf litter but high dissolved organic matter and wood input might have major implications for stream ecosystems because these resources are mainly processed and assimilated by microbial and fungal assemblages with little carbon passing to macroinvertebrates (Eggert & Wallace, 2007; Karlsson, 2007), but this was not addressed in our study.

4.4.2 Food resource assimilation and food web structure

Similarly to previous studies (Hondula *et al.*, 2014; Collins *et al.*, 2016b), δ^2H isotopes effectively differentiated between autochthonous and allochthonous food resources, which allowed determining how macroinvertebrate assimilation varied among feeding groups and with land cover. Food resource assimilation differed among feeding groups with changes in food resource type availability. As suggested in previous studies based on stable isotope analysis (Collins *et al.*, 2016a; Hayden *et al.*, 2016), detritivores showed a fixed assimilation of mainly allochthonous resources along the examined land cover gradient, even when these resources were scarce. In contrast, omnivores showed variable assimilation, which ranged from highly autochthonous to mostly allochthonous food resources. This variable assimilation might be related to a more plastic resource ingestion than for detritivores, as omnivore macroinvertebrates encompassed various feeding modes that included filter feeding, gathering and gatherer-scraping. These feeding modes allow them to forage on food resources that can differ in the proportion of autochthonous versus allochthonous organic matter content (e.g., biofilms, FPOM) and increases the allochthonous proportion with increasing forest cover. This capacity to ingest a wider variety of food resource needs to be supported by effective assimilation, which might be enhanced by a more diverse gut microbiota as suggested by Harris (1993), although is still poorly understood.

The differential behavior between detritivores and omnivores had major implications for food web structure. The fixed assimilation of detritivores made them highly susceptible to the quantity of allochthonous resources in a stream, so that when these were scarce, detritivore biomass was low and increased when allochthonous resources incremented. Omnivore biomass, however, was similar across streams and did not respond to variation in food resource type. The stable omnivore biomass, together with the variable assimilation of the most dominant food resource, contradicts previous studies (e.g., Marcarelli *et al.*, 2011) that related omnivore biomass maintenance in streams dominated by allochthonous resources to their selection capacity for high-quality food (autochthonous resources in our study). These findings suggest that the plasticity in omnivore assimilation is important towards explaining how they maintain biomass across the land cover gradient and points to feeding mode as a key trait in determining organism adaptation capacity to variations in food resources.

The greater proportion of detritivore biomass, together with the higher allochthonous assimilation by omnivores, had a bottom-up effect that was propagated to carnivores. Carnivores not only exhibited a biomass gain but also an increase in allochthonous resource assimilation with the degree of allochthony. Moreover, the increase in detritivore and carnivore biomass, and omnivore biomass maintenance, led to fewer differences in biomass among macroinvertebrate groups (i.e., the absence of dominant feeding groups) with an increase in forest land cover. Thus, contrary to the initial expectations of a more even macroinvertebrate community biomass distribution when autochthonous and allochthonous food resources were equally available, a more balanced biomass distribution within the macroinvertebrate community was reached in forested streams when allochthonous food resources dominated. How this biomass redistribution was achieved, through variations in the number of organisms or in the body size, and whether this is reflected in ecosystem functioning, deserves further research.

4.4.3 Energy flow pathways: estimating food resource allocation to food webs

To date, specific resource importance to food webs has been quantified either from ingestion measures from gut content analyses and assimilation efficiencies derived from literature (e.g., Benkf & Bruce Wallace, 1997; Hall, Likens & Malcom, 2001; Cross *et al.*, 2013) or by measuring macroinvertebrate resource assimilation (mainly δ^{13}C or δ^{2}H stable isotopes) without incorporating biomass values (e.g., Cole *et al.,* 2011; Collins *et al.,* 2016a; but see Junker & Cross, 2014). Both methods might not represent actual assimilated energy and might lead to erroneous estimations of food resource importance to food webs. For example, mean allochthonous assimilation increased with forest cover but was never strongly autochthonous (always > 30% and < 50% only in 2 streams) because of the presence of detritivores that fed on allochthonous resources in all streams. However, significant differences appeared in food webs when comparing mean allochthonous assimilation with the actual biomass sustained by allochthonous resources. Specifically, the actual biomass sustained by allochthonous resources was slightly higher than the mean allochthonous assimilation in streams dominated by allochthonous resources (PEN, BU3), but considerably lower in streams having greater autochthonous resources with differences reaching up to a 20%. These differences appear to be driven by greater macroinvertebrate biomass, especially of omnivores, supported by autochthonous resources compared to allochthonous resources, which becomes more noticeable when autochthonous resources are more abundant (i.e., in grassland/shrub dominated streams). This demonstrates that organism assimilation data on its own only provide a measure of resource assimilation preference, and not accounting for organism biomass would lead to an over-estimate in the importance of allochthonous resources as subsidies to food webs. Consequently, information relative to food web structure (i.e., organism biomass) that supplements organism assimilation is needed to link the relative importance of food resources to food webs and identify dominant energy flow pathways.

This study was based on a unique food resource and macroinvertebrate standing stock biomass measure at the end of the low-flow season, just before leaf litter fall in autumn, when allochthonous food resources are minimum and autochthonous resources peak due to flow stability, high water temperature and light availability (Rolls, Leigh & Sheldon, 2012). Hence, the importance of food resources to stream food webs found in our study is only representative of the most autochthonous moment of the year. Our findings show that at this time of year stream food webs at the community level were sustained by the most abundant food resource; i.e., food webs in grassland/shrub dominated streams were more sustained by autochthonous sources and forested streams were sustained by allochthonous resources. These results agree with previous studies (e.g., Rosi-Marshall & Wallace, 2002; Lancaster *et al.*, 2005) on the relevance of the allochthonous energy pathway in supporting mountain stream food webs, even when allochthonous food resources are at their minimum. In contrast to conceptual frameworks such as the River Continuum Concept (Vannote *et al.*, 1980) or River Production Model (Thorp & Delong, 1994), we also show the capacity of the autochthonous energy pathway in supporting food webs during low-flow summer season, in low order stream ecosystems, even if they are metabolically net heterotrophic. The fact that even in the most autochthonous period of the year, allochthonous energy pathways dominated and few streams were strongly supported by autochthonous food resources, strengthens the idea that low order mountain streams food webs might be heavily sustained by allochthonous food resources annually.

To fully understand the importance that food resources to food webs, a seasonal or annual measure of the autochthonous and allochthonous energy flow would be necessary because food resources and macroinvertebrate standing stock biomass vary seasonally (Torres-Ruiz, Wehr & Perrone, 2007; Díaz Villanueva, Buria & Albariño, 2010). This cannot be achieved from our sampling scheme because estimates of energy flow based on standing stock biomass might substantially differ when using macroinvertebrate secondary production (e.g., high variability of production to biomass ratios when comparing large and small macroinvertebrates; Benke, 1993). Moreover, secondary production should not be inferred from published production to biomass ratios when strong differences in food resources among study streams are present (e.g., large land cover or river size differences) because secondary production highly depends on food resource quality (Huryn & Wallace, 2000) and, consequently, substantial differences in secondary production among organisms within the same taxa may exist among streams. Hence, the quantification of how much energy flow depends on autochthonous or allochthonous resources over different seasons or annually would be more accurate when combining measures of food resource availability and organism food resource assimilation, in concert with macroinvertebrate secondary production estimates.

4.5 Conclusions

In conclusion, the presence of low order mountain streams dominated by different vegetation land cover, together with the dependence of stream food webs on dominant food resource types, highlight the importance of catchment land cover in determining energy flow pathways in streams. Moreover, food web structure alteration, with less dominant taxa in concert with variations in dominant taxa, might compromise certain ecosystem functions. Therefore, a better understanding of how ecosystem functioning (e.g., stream metabolism or organic matter decomposition) might respond to changes in food web structure and energy pathways will improve our ability to predict how vegetation land cover change affects mountain stream ecosystems. Lastly, differences between the assimilation data and the actual biomass sustained by a food resource emphasizes the need to simultaneously measure the percentage of assimilated resources and organism biomass to accurately determine the dominant energy flow pathways and ultimately understand the role of food resources in defining stream food web structure.

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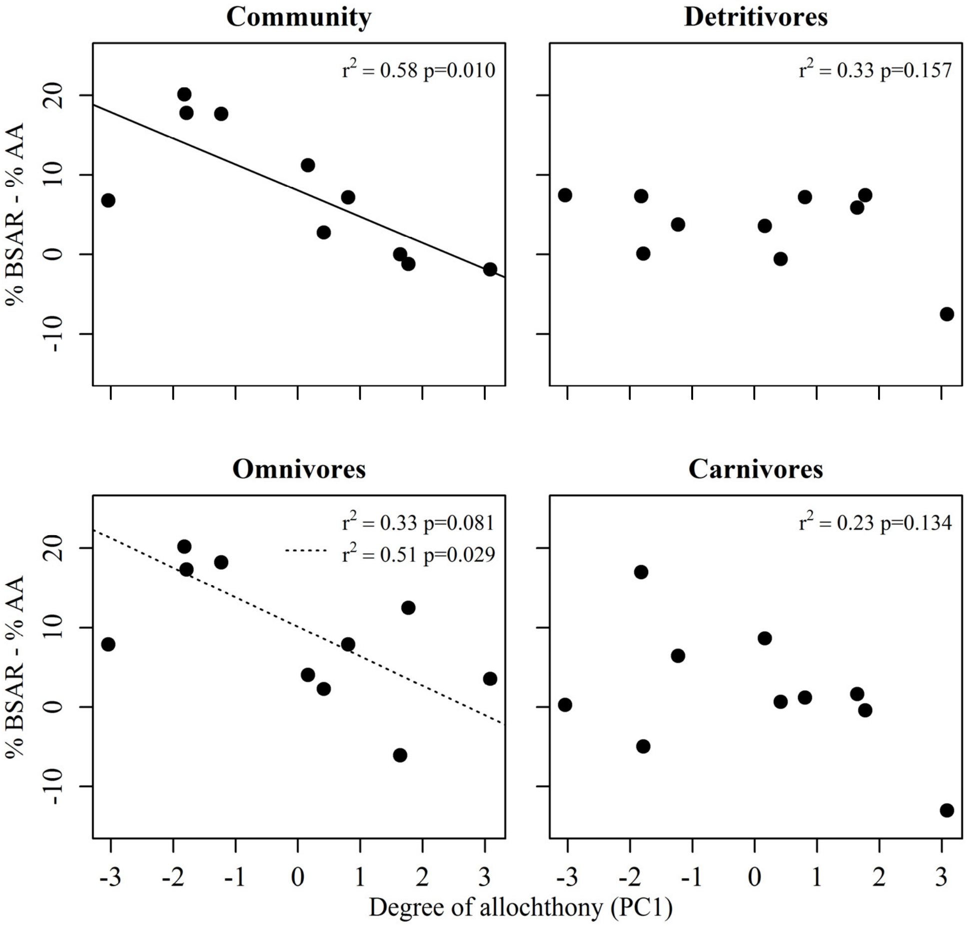
4.7 Supplementary material 4

Supplementary material 4.1 - Macroinvertebrate taxa that represented more than 1% of biomass in the study streams and assigned feeding group (H = Herbivore, D = Detritivore, O = Omnivore, C= Carnivore).

Taxa	Feeding group	Taxa	Feeding group
Aeshnidae	C	Hydraenidae	D
Ancylidae	H	Hydrobiidae	H
Athericidae	C	Hydropsychidae	O
Baetidae	O	Lepidostomatidae	D
Beraeidae	D	Leptophlebiidae	O
Brachycentridae	O	Leuctridae	D
Caenidae	O	Limnephilidae	D
Ceratopogonidae	C	Limoniidae	C
Chironomidae non-Tanypodinae	O	Nemouridae	D
Coenagrionidae	C	Odontoceridae	D
Cordulegasteridae	C	Perlidae	C
Dixidae	O	Philopotamidae	O
Dolichopodidae	C	Polycentropodidae	C
Dryopidae	D	Psychodidae	D
Dytiscidae	C	Psychomyiidae	O
Elmidae	D	Rhyacophilidae	C
Empididae	C	Sericostomatidae	D
Ephemerellidae	O	Simuliidae	O
Ephemeridae	O	Chiromomidae Tanypodinae	C
Gammaridae	O	Tipulidae	D
Heptageniidae	O		

Supplementary material 4.2 - Relationship of the difference between % BSAR and % AA and the degree of allochthony (PC1) for the entire macroinvertebrate community, detritivores, omnivores and carnivores. Regression lines are shown for significant (*p* < 0.05) linear regressions. The dotted line describes the relationship between the difference between % BSAR and % AA and the degree of allochthony when CAS is not considered.



Chapter V

The effect of forest cover loss on stream macroinvertebrate community size structure

Chapter V. The effect of forest cover loss on stream macroinvertebrate community size structure

This chapter has led to the article entitled: "Effects of variations in forest cover on stream macroinvertebrate community size structure" by Edurne Estévez, Aitor Larrañaga, Carlos Alonso & José Barquín. It has been submitted for publication in the journal Functional Ecology.

Abstract

Forest cover loss is one of the most extensive and influential human impacts on fluvial ecosystems as it highly influences hydrology, water temperature or the food resources available to stream communities. The effects of changes in food resource quantity and type have long been studied on the structure and composition of stream macroinvertebrate communities; however, little attention has been paid to address how these changes affect the community size structure. In the case of forest cover loss, predicting the response of community size structure to changes in food resources is strongly hampered by how resource quality and lability and organism life strategy affect body size. The objective of this study is to assess the effect of changes in food resource quantity and type due to forest cover loss on river macroinvertebrate size structure to test whether the ecosystem carrying capacity (size spectrum intercept) and the energy transfer efficiency among trophic levels (size spectrum slope) are altered by food resource changes. Results showed that community size spectra slopes remained invariant to the alteration of food resource type by forest cover loss. This indicated an internal compensatory regulation of the community size structure for the maintenance of the trophic transfer efficiency (size spectrum slope) only adjusting its carrying capacity (size spectrum intercept) to total food resource quantity through variations in macroinvertebrate abundance. The compensatory mechanisms by which macroinvertebrate communities adjusted to forest cover loss occurred by means of detritivore-omnivore substitution, although the shifts in size structure differed between these feeding groups. Omnivore response was numerical (i.e., increase in density and consequently in biomass) not related to body size or taxonomic composition and driven by changes in large organisms. In contrast, detritivores showed a reduction in body size, not paralleled by a reduction of total biomass or density, that was related to a taxonomic replacement of larger case-building trichopterans (i.e., Odontoceridae, Limnephilidae and Beraeidae) by smaller detritivores (e.g., Elmidae). These findings evidenced that while omnivore size structure was modified only by body size distribution, detritivore size structure was modified via shifts in both body size distribution and taxonomic composition.

5.1 Introduction

One of the major components of global change is the transformation of land uses in the territory (Millennium Ecosystem Assessment, 2005), which is leading to the loss of landscape natural vegetation cover, especially forest, due to an intensification of extensive cattle raising, agriculture, forestry and urbanization (J. Brandt, 1999; Geist & Lambin, 2002; Bürgi, Hersperger & Schneeberger, 2004).

Changes in landscape natural vegetation have strong effects on river ecosystems as these are tightly linked to the terrestrial environment in their draining catchment (Hynes, 1975). For instance, catchment forest cover highly influences hydrology, sediment yield or water temperature in the receiving streams (Schoonover, Lockaby & Helms, 2006; Burcher, Valett & Benfield, 2007; Hannah *et al.*, 2008). Moreover, vegetation cover determines the origin (i.e., type) of food resources available for stream macroinvertebrate communities (Allan, 2004): in-stream produced autochthonous organic matter such as algae vs allochthonous organic matter imported from adjacent terrestrial ecosystems (e.g., wood and leaf litter). Particularly, forest cover loss is associate to the decline of leaf litter biomass and the increase in algae biomass, and therefore, to the alteration in opposed directions of allochthonous and autochthonous food resource quantity (Gregory *et al.*, 1991; Sweeney, 1993). This alteration of the food resource type implies an increase in quality of the overall food resources available for stream macroinvertebrate communities because allochthonous organic matter has lower quality and greater lability than autochthonous organic matter (Thorp et al. 2002). Most studies analyzing the effects of changes in food resources on stream macroinvertebrate communities have focused on differences in resource quantity (e.g., Hawkins and Sedell 1981, Delong and Brusven 1998, Rosi-Marshall and Wallace 2002, Collins et al. 2016) and the few studies analyzing variations in food resource quality, have only considered a single resource, essentially leaf litter (e.g., LeRoy and Marks 2006, Larrañaga et al. 2009). Moreover, the effects of changes in food resources have traditionally been assessed on community structure and composition (Minshall, 1967; Cummins, 1974; Doisy & Rabeni, 2001; Baumgartner & Robinson, 2017), but no attention has been given to the effects that these changes produce on community size structure (e.g., Dell et al. 2015).

Size spectrum, which represents the distribution of the abundance of organisms in relation to their body size, is a descriptor of community size structure (Sheldon, Prakash & Sutcliffe, 1972; Kerr & Dickie, 2001). The size spectrum intercept informs of the ecosystem carrying capacity, this is, the total organismal abundance that can be supported in the community (Gaedke, 1993; Daan *et al.*, 2005; Sweeting *et al.*, 2009), while the size spectrum slope describes the rate at which abundance changes with increasing body size. In most freshwater ecosystems, the abundance of organisms declines with body size (i.e., negative size spectra slope). If all the organisms in the community belong to the same trophic level, this decline follows the allometric scaling laws of the metabolic rate and size spectrum slopes of - 0.75 are expected (Damuth 1981, Peters 1983, Brown et al. 2004). In contrast, multitrophic communities are size structured by predation and, since the transfer of energy between prey and predator is inefficient (10%-13% of prey biomass is converted into predator production; Pauly and Christensen 1995, Jennings 2007), the size spectrum slope is theorized to be close to - 1 (Jennings & Mackinson, 2003; Blanchard *et al.*, 2009). Thus, in multitrophic communities, the slope of the size spectrum is indicative of the efficiency in energy transference among trophic levels (Woodward et al. 2005, Andersen et al. 2009).

Community size structure research has shown that, in absence of disturbances, the size spectrum is relatively regular and predictable (e.g., Sheldon et al. 1972) because communities tend to be trophically efficient (Kerr, 1974; Jennings & Mackinson, 2003). In contrast, how size spectrum responds to natural or human disturbances is far from clear. Numerous studies have demonstrated that ecosystem carrying capacity, and thus size spectrum intercept, varies according to the nature of disturbance that is acting (e.g., increase with nutrient enrichment or decline after floods; Robinson and Uehlinger 2008, Davis et al. 2010). Although size spectrum slope has been considered to be relatively stable, even in communities subjected to disturbances because of compensatory regulation mechanisms of the community size structure (Klug *et al.*, 2000; Downing *et al.*, 2008), recent investigations suggest that it may be altered by disturbances. Some studies (e.g., Petchey and Belgrano 2010) have theorized that in disturbed ecosystems the size spectra slope increases because of a decline in the community trophic transfer efficiency, whereas shallower size spectra slopes (even greater than - 0.75) have been predicted in ecosystems subsidized by allochthonous food resources (Trebilco *et al.*, 2013) due to a lower energy limitation (in these ecosystems the rate of transfer and renewal of allochthonous resources is extrinsic to the community; Polis and Strong 1996). This contrasting size spectrum response to disturbances highlights the need to understand whether disturbances modify size spectrum through the alteration of organism size structure or, on the contrary, the organism size structure is altered and adjusted to maintain the community size spectrum and the efficiency in energy transference.

Variations in size spectrum in response to a disturbance result from the joint change in organism body size and abundance of organisms in a particular size. However, predicting the size response of stream communities to forest cover loss is strongly hampered by discrepancies on how resource quality and lability and organism life strategy affect body size. The two types of resources (allochthonous and autochthonous food resources) define two different energy channels which not only differ in the properties of the food resources themselves (quality and lability), but are hypothesized to be structurally and functionally different, providing the basis for the life history characteristics (i.e., life strategy) of the organisms that conform each of these energy channels (Rooney & McCann, 2012). This suggests that, as allochthonous food resources have lower quality than the autochthonous ones (Thorp & Delong, 2002), smaller organisms might be expected in ecosystems dominated by allochthonous resources because nutritional constraints to secondary production might limit organism growth (McNeely, Finlay & Power, 2007). However, as allochthonous energy channel is less competitive than the autochthonous one because it is donor-controlled (Polis and Strong 1996), the dominant life strategy in this energy channel is characterized by a slow growth and long reproduction cycles (k strategy taxa; MacArthur and Wilson 1967), what might result in larger body sizes. On the contrary, the autochthonous energy channel is hypothesized to be a more competitive environment as food resource abundance is consumer density – dependent (Hill & Knight, 1987), what might favor a life strategy of growing and reproducing fast and lead to smaller body sizes. Size responses in predators, which couple these two energy channels (Rooney *et al.*, 2006), are even more difficult to predict as they depend on the effect that the food resource change has on primary consumers. Macroinvertebrate predators primarily consume small prey (prey with smaller body size than their predator; Brose et al. 2006, Woodward and Warren 2007) and for this reason, predators may benefit more from an increase in abundance and biomass of small prey than from similar increases in large prey or in small prey body size.

The aim of this study is to assess the response of macroinvertebrate community size structure to variations in total food resource quantity and food resource type (i.e., autochthonous vs allochthonous resources) driven by forest cover loss in mountain streams. We are also interested on testing whether the ecosystem carrying capacity (size spectrum intercept) and the efficiency in energy transfer among trophic levels (size spectrum slope) are altered by food resource changes. Moreover, we aim to understand how community size structure is modified: via shifts in the taxonomic composition (i.e., taxa richness or taxa replacement), in body size distribution or via a combination of both. We expect an increase in community carrying capacity (size spectrum intercept) due to greater macroinvertebrate biomass with increasing food resource quantity and quality (i.e., increasing dominance of autochthonous food resources). Moreover, we anticipate opposed responses in biomass among macroinvertebrates based on the type of food they ingest due to the variation in opposed directions of food resource types with forest cover loss (i.e., a decline in leaf litter relative quantity while increase in biofilm quantity and quality). Nevertheless, we cannot predict whether this variation in biomass results from a change in density or in body size and is accompanied by a change in taxonomic composition and if it leads to the maintenance or alteration of the efficiency in energy transfer among trophic levels (size spectrum slope).

5.2 Methods

5.2.1 Study area and stream reaches

The study area is located in the central sector of the Cantabrian Cordillera, a mountain range that spans more than 300 km across northern Spain parallel to the coast (Cantabrian Sea; Atlantic Ocean; Fig. 2.1 in chapter II). In this chapter, a total of 10 streams were studied in this area (chapter II, Fig. 2.10 b).

5.2.2 Macroinvertebrate sampling

We collected Surber samples (mesh 500 µm, 0.09 m^2) in 3 runs and 3 pools randomly chosen at each sampling reach (Chapter IV). Samples samples were divided into < 1 mm and > 1 mm size fractions. Macroinvertebrates retained in the > 1mm sieve were identified to family level (except for Hydrachnidiae, Oligochaeta which were identified to subclass and Planipenne which were identified to order), measured using ImageJ software (version 1.47; National Institutes of Health, Bethesda, Maryland). Individual body size was calculated using published body length - dry mass equations as described in Chapter IV (Smock, 1980; Burgherr & Meyer, 1997; Benke *et al.*, 1999; Rosati, Barbone & Basset, 2012). Within this chapter, only organisms with a mass greater than 0.125 mg were considered. Organisms smaller than this mass are undersampled as most are washed out when using a 1 mm sieve (Gruenert et al. 2007).

Macroinvertebrates were assigned to a feeding group based on the type of food ingested following Tachet et al. (2002; SM 5.3) as performed in chapter IV: herbivores that mainly forage on living microphytes and macrophytes; detritivores that feed on plant detritus; carnivores that prey on living invertebrates; and omnivores that did not show a clear preference in food type ingested (mostly gatherers, gatherer-scrapers and filter-feeders). Specifically, the Hydraenidae and Elmidae were considered detritivores based on recent studies (see Elliott 2008).

5.2.3 Food resource characterization

In each stream, we measured the quantity of food resources that can sustain stream food webs: wood, leaf litter, benthic fine particulate organic matter (FPOM; < 1mm), macro-algae, biofilms (chlorophyll *a* and ephilitic biomass) and dissolved organic carbon (DOC) as a quantitative measure of dissolved organic matter as described in chapter IV. DOC (mg C/L) was measured from a water sample by high-temperature catalytic oxidation on a Shimadzu TOC-V CSH analyzer (Shimadzu Corporation, Kyoto, Japan). Wood, leaf litter, FPOM and macro-algae biomass was obtained from the composite Surber samples. These food resources were dried to constant mass at 70ºC, weighed, ashed at 500°C for 4 h, and reweighed to yield ash free dry mass (g/m^2). Chlorophyll *a* concentration and epilithic biomass of benthic biofilms were measured from cobbles collected from the same pools and runs as the Surber samples. Chlorophyll *a* was extracted 90% acetone at 4°C for 24 h in the dark and absorbance was read on a Hach-Lange DR-5000 UV/visible spectrophotometer and converted to pigment concentration. Epilithic biomass content was obtained using a modified version of the technique described by Sinsabaugh et al. (1991).

5.2.4 Data analysis

5.2.4.1 Variation in food resource type and quantity

We summarized the variation in resource type (Table 4.1) using a Principal Component Analysis (PCA) and tested the correlation of the PCA axes with the percentage of forest cover in the catchment. As the PCA axis 1 (PC1) defined a gradient changing from high to low quantity of autochthonous food resources as from low to high quantity of allochthonous resources linked to the percentage of forest cover in the catchment (chapter IV, Fig. 4.1), the scores of the streams in the PC1 were used as a measure of the degree of allochthony. Moreover, we calculated the total food resource quantity in each stream as the sum of all the food resource standardized biomass to ensure an equal contribution of the different food resources. We did not include DOC in the calculation as this resource is not directly consumed by macroinvertebrates (Richardson, Zhang & Marczak, 2010). This way, we ranked the streams according to the quantity of all food resources (SM 5.1). The total food resource quantity was unrelated to the degree of allochthony (PC1; Spearman correlation $r = -0.18$, $p = 0.632$).

5.2.4.2 Trophic transfer efficiency and carrying capacity

To characterize the size - abundance relationships and identify differences in the efficiency in trophic energy transfer and the ecosystem carrying capacity with changes in food resource quantity and type, we performed ordinary least square regression between the standardized total food resource quantity and the degree of allochthony, and the slope and intercept of the macroinvertebrate community size spectra. We constructed one size spectrum for each stream macroinvertebrate community. Regularly distributed size classes were created within the $\log_{10}$ body size range for each site. Six size classes were used as it maximized the number of size classes, while avoiding having empty size classes. Size spectra intercepts and slopes were calculated from regressions between the center of the size class and $\log_{10}$ of the number of organism per size class (White, Enquist & Green, 2008).

5.2.4.3 Taxonomic composition and biomass distribution

The taxonomic composition was characterized by measuring the taxa richness of the entire community and of each feeding group, and examining differences in taxonomic composition of the entire community and of each feeding group using non-metric multidimensional scaling

(NMDS) based on pairwise Jaccard dissimilarities. Similarly, in order to evaluate differences in biomass distribution in the entire community and in each feeding group, we performed NMDS based on Bray–Curtis dissimilarities of the 5 most abundant taxa biomass. We selected 5 taxa as it was the minimum number of taxa needed to represent at least a 50% of community biomass in all the study streams. To identify the influence of changes in total food resource quantity and type on taxonomic composition and biomass distribution, we performed a vector fitting analysis (*envfit* function) and fitted averaged total resource quantity and the degree of allochthony to the NMDS ordinations. Significance values were generated with 9999 random permutations. As variations in total resource quantity comprise the change in both autochthonous and allochthonous food resource quantity in the same direction and, thus, affect all feeding groups alike, for total food resource quantity, these analyses were only performed at community level.

5.2.4.4 Size structure

Finally, in order to identify the size structure response to changes in food resource quantity and type, we first calculated total macroinvertebrate density (ind/m^2), biomass (mg/m^2) and average body size (mg) for the entire macroinvertebrate community and each feeding group. We performed ordinary least square regression analyses between total resource quantity and the degree of allochthony and macroinvertebrate density, biomass and average body size. For total resource quantity, these analyses were only performed at the community level, while for the degree of allochthony, these analyses were performed for entire macroinvertebrate community and for each feeding group. When significant differences were obtained for a feeding group, we repeated the above described ordinary least square regression analyses for large and small organisms independently. The threshold value between large and small was considered the average body size of all the organisms belonging to each feeding group. Similarly, when significant differences were obtained for a specific size category (e.g., large and/or small organisms), the ordinary least square regression analyses were repeated for each taxon within that specific size category when present in more than 3 streams. Normality was checked from residuals and $\log_{10}$ – transformation was used when needed to remove heteroscedasticity.

As herbivores were not present in all the streams and more than 5 organisms were only collected in 4 of them, we did not carry out any individual statistical analyses for this group. Nevertheless, herbivores were considered in all the analyses concerning the entire community. All statistical analyses were performed in R software (version 3.3.0, R Project for Statistical Computing, Vienna, Austria).

5.3 Results

5.3.1 Trophic transfer efficiency and carrying capacity

Size spectra showed a significant decline in macroinvertebrate abundance with body size in all the streams (SM 5.2). Size spectra slopes ranged from - 0.64 to - 1.3 and confidence intervals showed that they did not differed from - 0.75 and - 1 in any stream except for SLA where it was lower than - 1 (Table 5.1).

Table 5.1 – Macroinvertebrate size spectra slopes and intercepts in the study streams. 5% - 95% confidence intervals (CI) are shown.

Stream Code	**Slope**			**Intercept**		
	Estimate	**5% CI**	**95% CI**	**Estimate**	**5% CI**	**95% CI**
BAY	-0.64	-1.26	-0.02	1.38	1.04	1.73
BU3	-0.93	-1.35	-0.50	2.42	2.01	2.83
BUL	-1.30	-1.95	-0.66	2.37	1.96	2.77
CAS	-0.85	-1.09	-0.61	2.57	2.35	2.78
HIJ	-0.88	-1.11	-0.65	1.38	1.25	1.52
MIE	-0.69	-1.15	-0.23	1.62	1.32	1.91
PAS	-0.68	-1.05	-0.31	1.88	1.61	2.15
PEN	-0.71	-1.02	-0.40	2.07	1.84	2.31
SEC	-0.90	-1.11	-0.69	1.93	1.72	2.14
SLA	-0.73	-0.94	-0.53	1.98	1.85	2.11

Size spectra slopes did not vary neither with the degree of allochthony nor with the total food resource quantity (Fig. 5.1 a and b, respectively). On the contrary, size spectra intercept increased with total food resource quantity (Fig. 5.1 c) but did not respond to the degree of allochthony (Fig. 5.1 d).

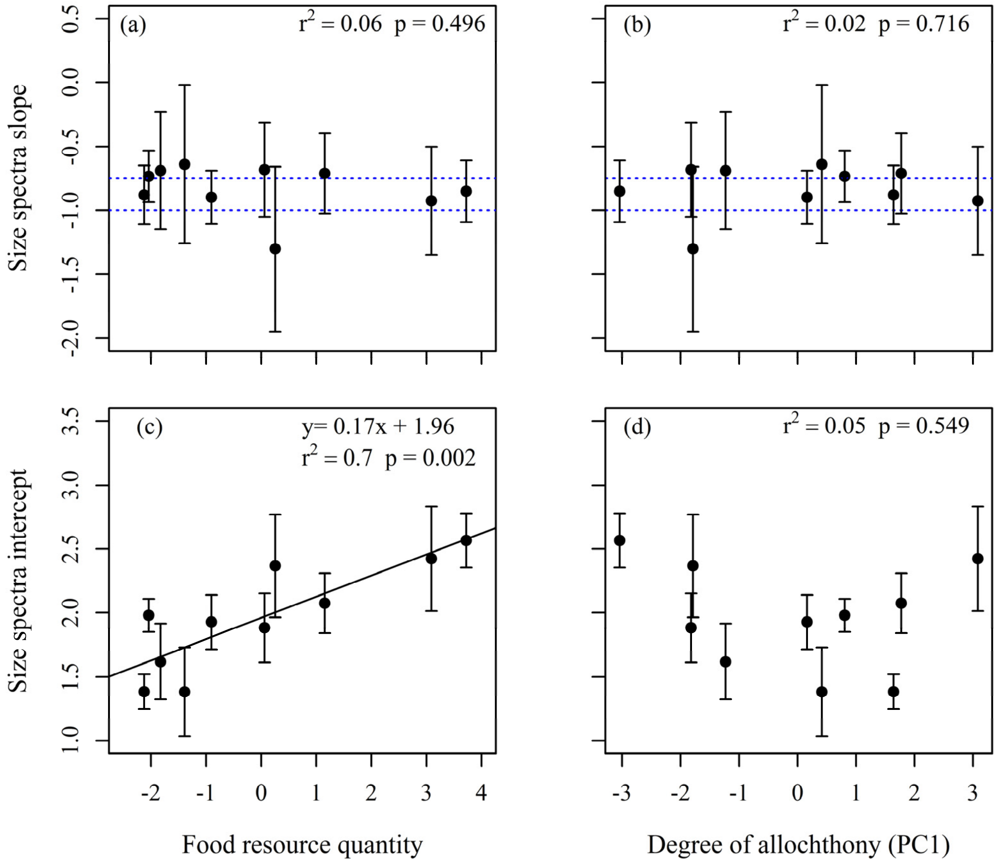


Figure 5.1 - Linear regressions between total food resource quantity (a, c) and the degree of allochthony (b, d) and macroinvertebrate size spectra slope (a, b) and intercept (c, d). Confidence intervals for size spectra slopes and intercepts (5% - 95%) and regression lines and regression equations for significant ($p < 0.05$) linear regressions are shown. Blue dotted lines denote - 0.75 and - 1 size spectra slopes.

5.3.2 Taxonomic composition and biomass distribution

Macroinvertebrate community taxa richness ranged from 17 to 34 in the study streams and only 5 taxa belonging to the Baetidae, Elmidae, Heptageniidae, Hydraenidae and Leuctridae families were common across streams. Omnivore taxa richness ranged from 6 to 10, while detritivore and carnivore taxa richness from 4 to 10. Despite these differences among streams, no variation in taxa richness (0.93 ± 0.8; $r^2 = 0.14$, $p = 0.281$), community composition ($r^2 = 0.12$, $p = 0.638$) or biomass distribution among the most dominant taxa occurred with total resource quantity ($r^2 = 0.06$, $p = 0. 812$).

Taxa richness did not respond to the degree of allochthony, neither the entire community nor the feeding groups (Table 5.2).

Table 5.2 - Results from linear regression analyses between the degree of allochthony and the taxa richness, macroinvertebrate density, biomass and average body size for the entire community and for each feeding group. Estimates and standard errors are shown. Significant p values (*p* < 0.05) are highlighted in bold.

	Richness			Density (ind/m^2)			Biomass (mg/m^2)			Average body size (mg)		
	Estimate	**SE**	**r^2**	**Estimate**	**SE**	**r^2**	**Estimate**	**SE**	**r^2**	**Estimate**	**SE**	**r^2**
Community	0.40	0.93	0.02	-0.05	0.07	0.07	-0.05	0.07	0.06	0.00	0.02	0.00
Detritivores	0.36	0.32	0.14	-0.11	0.07	0.22	0.00	0.07	0.00	**0.11**	**0.03**	**0.65**
Omnivores	0.00	0.29	0.00	-0.06	0.08	0.07	-0.07	0.08	0.10	-0.02	0.03	0.03
Omnivores*	0.04	0.37	0.00	**-0.16**	**0.07**	**0.45**	**-0.20**	**0.06**	**0.62**	-0.04	0.04	0.13
Carnivores	0.16	0.49	0.01	0.04	0.07	0.03	0.00	0.09	0.00	-0.04	0.04	0.09

* *Except for BU3*

The degree of allochthony was only related to a change in detritivore taxonomic composition (Fig. 5.2, SM 5.3), which was mostly driven by the disappearance of case-building caddis trichopterans when decreasing the degree of allochthony. Specifically, Odontoceridae were present in medium and highly allochthonous streams (BAY, BU3, HIJ, PEN, SEC and SLA), Limnephilidae in the 3 most allochthonous streams (BU3, PEN and SLA) and Beraeidae only in one of the most allochthonous streams (PEN).

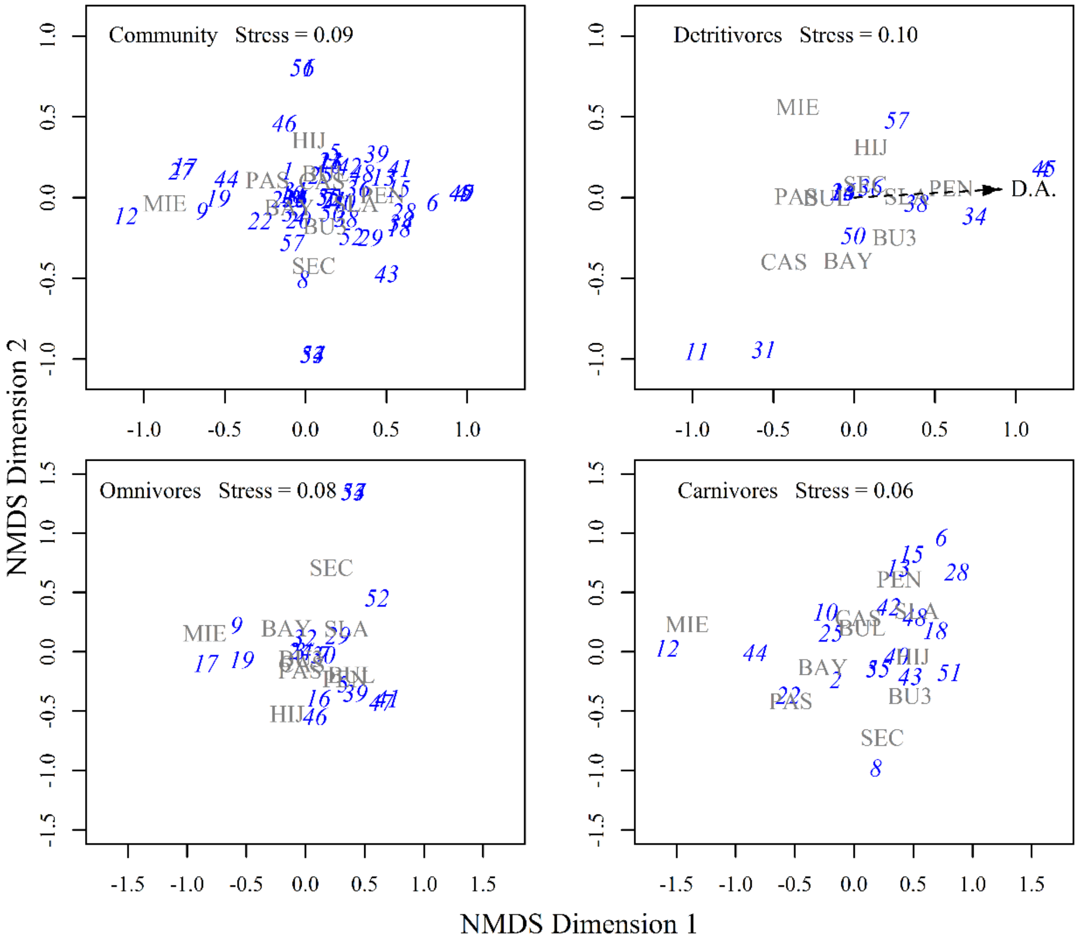


Figure 5.2 - Non-metric multidimensional scaling (NMDS) ordinations based on Jaccard dissimilarity performed to assess differences in taxonomic composition in the entire community and in each feeding group. Stream codes are shown in grey. Blue numbers denote the macroinvertebrate taxa (SM 5.3). The black arrow shows the significant correlation ($p < 0.05$) between ordination axes and the degree of allochtohony (D.A.).

Despite the absence of differences in taxa composition (except in detritivores), biomass distribution among the most dominant taxa varied with the degree of allochthony in the entire community and in omnivores and carnivores (Fig. 5.3, SM 5.3). At the community level, herbivores (Ancylidae) and omnivores as Baetidae, Heptageniidae and Hydropsychidae dominated in biomass in the most autochthonous streams (BUL, CAS, MIE and PAS). Detritivores as Odontoceridae and Sericostomatidae, carnivores as Cordulegasteridae and omnivores as Leptophlebidae and Gammaridae (only in BU3) appeared among the most dominant taxa when the degree of allochthony increased (Fig. 5.3). Among omnivores, Hydrophilidae dominated in the most autochthonous streams (BUL, CAS, MIE and PAS) and when the degree of allochthony increased, non-predator Chironomidae (e.g., Orthocladinae and Chironominae subfamilies; BAY, BU3 and HIJ) and Gammaridae biomass incremented. Ephemeropterans were also highly dominant in all the streams but the most dominant ephemeropteran taxa varied with the degree of allochthony. Baetidae, Heptageniidae and Ephemerellidae, dominated in the most autochthonous streams (BUL, CAS, MIE and PAS; although Ephemerellidae only in the 2 most autochthonous streams BUL and CAS) while Leptophlebidae, in the most allochthonous streams (SEC and BU3). Among carnivores, Dolichopodidae and Polycentropodidae dominated in the most autochthonous streams while Limoniidae, Cordulegasteridae, Tanipodinae (Chironomidae) and Perlidae increased in biomass with the degree of allochthony (Fig. 5.3).

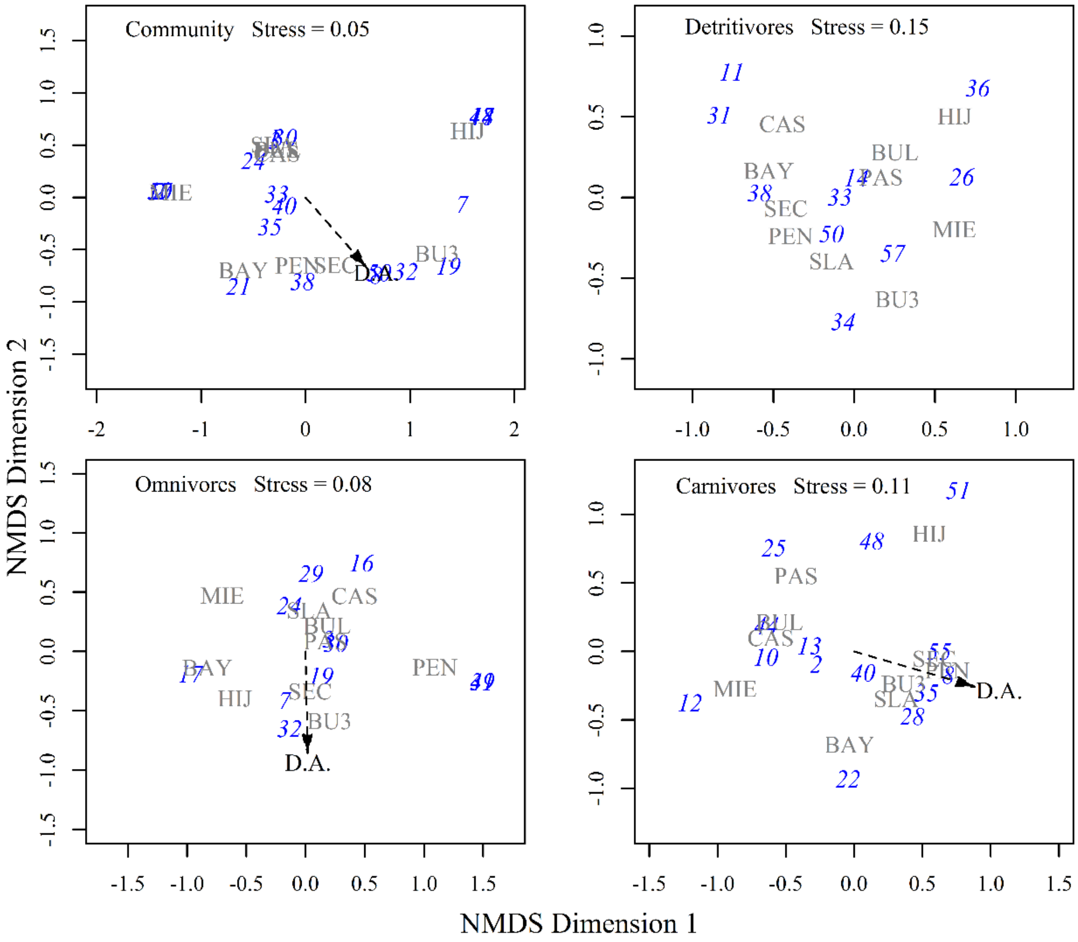


Figure 5.3 - Non-metric multidimensional scaling (NMDS) ordinations based on Bray-Curtis dissimilarity performed to evaluate differences in biomass distribution among the most dominant taxa in the entire community and in each feeding group. Stream codes are shown in grey. Blue numbers denote the macroinvertebrate taxa (SM 5.3). The black arrow shows the significant correlations (p < 0.05) between ordination axes and the degree of allochtohony (D.A.).

5.3.3 Size structure

The community size structure showed an increase in macroinvertebrate density and biomass with total food resource quantity (0.15 ± 0.04; $r^2 = 0.6$, $p = 0.008$ and 0.16 ± 0.03; $r^2 = 0.73$, $p = 0.001$, respectively) without variation in community average body size (0.02 ± 0.02; $r^2 = 0.11$, $p = 0.344$).

In contrast, at the community level the size structure did not respond to the degree of allochthony (Table 5.2) as neither macroinvertebrate density nor biomass, nor average body size varied with the degree of allochthony.

Table 5.3 - Results from linear regression analyses between the degree of allochthony and the density, biomass and average body size of large and small detritivores and omnivores. Estimates and standard errors are shown. Significant p-values (*p* < 0.05) are highlighted in bold.

Taxa			**Density (ind/m^2)**			**Biomass (mg/m^2)**			**Average body size (mg)**		
			Estimate	**SE**	**r^2**	**Estimate**	**SE**	**r^2**	**Estimate**	**SE**	**r^2**
Detritivores	Large		-0.03	0.08	0.02	0.04	0.07	0.03	**0.07**	**0.02**	**0.52**
	Small		**-0.19**	**0.07**	**0.47**	**-0.19**	**0.07**	**0.47**	0.00	0.00	0.00
		Elmidae	**-0.19**	**0.07**	**0.46**	-0.14	0.07	0.31	-	-	-
		Hydraenidae	-0.05	0.06	0.06	-0.06	0.06	0.13	-	-	-
		Leuctridae	-0.19	0.10	0.31	-0.17	0.11	0.25	-	-	-
		Nemouridae	0.15	0.13	0.43	0.08	0.18	0.10	-	-	-
		Sericostomatidae	0.05	0.07	0.10	0.06	0.11	0.07	-	-	-
Omnivores	Large		-0.05	0.09	0.04	-0.07	0.09	0.08	-	-	-
	Large*		**-0.19**	**0.07**	**0.52**	**-0.21**	**0.06**	**0.60**	-	-	-
		Baetidae	-0.09	0.11	0.08	-0.08	0.11	0.06	-	-	-
		Chironomidae Non-Tanypodinae	0.17	0.04	0.89	0.16	0.06	0.79	-	-	-
		Ephemerellidae	-0.11	0.11	0.21	-0.13	0.11	0.28	-	-	-
		Ephemeridae	0.00	0.00	1.00	0.23	0.14	0.74	-	-	-
		Heptagenidae	**-0.20**	**0.07**	**0.54**	**-0.14**	**0.05**	**0.57**	-	-	-
		Hydropsychidae	-0.1	0.06	0.30	-0.14	0.08	0.33	-	-	-
	Small		-0.06	0.07	0.09	-0.07	0.07	0.11	-	-	-
	Small*		-0.14	0.07	0.35	-0.16	0.07	0.41	-	-	-

** Except for BU3*

However, detritivore and omnivore size structure was altered by the degree of allochthony. Detritivores average body size, but not density and biomass, declined with the decrease on allochthony (Table 5.2). In this sense, case-building trichopterans that disappeared when the degree of allochthony declined (i.e., Odontoceridae, Limnephilidae and Beraeidae), showed a greater average body size than the overall mean detritivore body size (Fig. 5.4). Additionally, small detritivores (and not large ones) increased in both density and biomass and not in body size when the degree of allochthony declined (Table 5.3). Among the small detritivores (e.g., Elmidae, Hydraenidae and Leuctridae), only Elmidae increased significantly in both density and biomass when allochthony decreased (Table 5.3). On the contrary, omnivore density and biomass increased, without an alteration of average body size, when decreasing allochthony (except in the most allochthonous stream BU3; Table 5.2). This was mainly caused by the increase in both density and biomass of large omnivores but not small ones (Table 5.3). Regarding the response of specific taxa, Baetidae, Ephemerellidae, Heptageniidae and Hydropsychidae increased in both density and biomass when decreasing allochthony, but only Heptageniidae experienced a significant increment (Table 5.3).

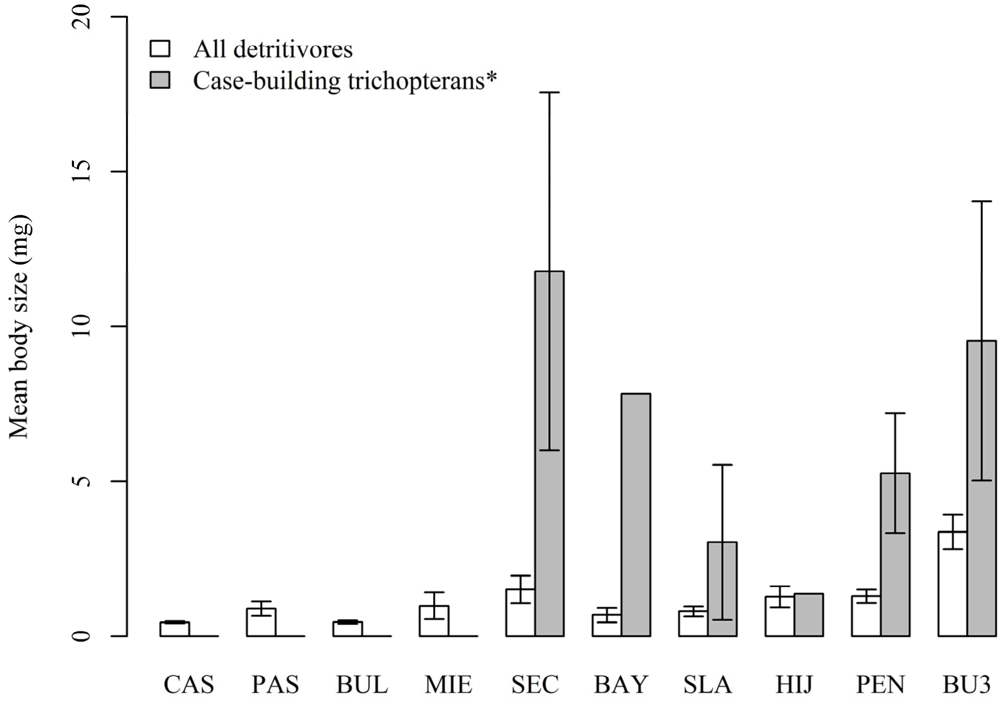


Figure 5.4 - Detritivore mean (± standard error) body size. Case-building trichopterans that appeared above a given degree of allochthony (Odontoceridae, Beraeidae and Limnephilidae; grey bars) show a larger mean body size than the remaining detritivore taxa (white bars). Streams are ordered following the allochthony gradient from left to right.

5.4 Discussion

This study addresses the effects that changes on food resources because of forest cover loss have on stream macroinvertebrate size spectrum. The combination of the community composition and size structure analysis, in concert with the community disaggregation by feeding modes, has shown how the community size structure adapts to maintain the trophic transfer efficiency when food resource type varies, only adjusting its carrying capacity to total food resource quantity.

5.4.1 Community size spectrum

Size spectrum is a synthetic indicator of the function and structure of biotic communities. Hence, understanding how size spectra respond to environmental alterations may provide information on how these affect community abundance, trophic interactions and patterns of energy transfer among trophic levels or ecosystem productivity and stability (Brown *et al.*, 2004; Woodward *et al.*, 2005a; Brose *et al.*, 2006; Hatton *et al.*, 2015).

In this study, macroinvertebrate communities were strongly size structured as shown by the significant decline in macroinvertebrate density with body size in all the streams. The average size spectra slope (- 0.83) was closer to the slope proposed for single trophic level communities (- 0.75; Damuth 1981, Peters 1983, Brown et al. 2004) than for multitrophic communities (- 1; Jennings and Mackinson 2003, Blanchard et al. 2009). However, none of the size spectra slopes (except for SLA) was significantly different from - 1, what indicates that, despite the dominance of primary consumers (SM 5.4), macroinvertebrate communities might be trophically size structured due to predation from both carnivore macroinvertebrates and fish community. Hence, the size spectra slopes may reflect the gradual loss of energy transfer from smaller to larger organisms through predation as shown by Kerr and Dickie (2001).

The response of macroinvertebrate community size spectra to food resource alteration by forest loss differed between intercept and slope. Size spectra intercept (the ecosystem carrying capacity) increased with increasing food resource quantity, as initially expected and reported in previous studies (e.g., Kobayashi and Kagaya 2004). In contrast, it was not affected by changes in food resource type. It needs to be considered that the alteration of food resource type by forest cover loss is not paralleled to a variation in total food resource quantity because the largest quantity of food resources occurs at minimum and maximum levels of forest cover (SM 5.1), while the minimum quantity, at intermediate levels of forest cover (SM 5.1). Thus, the fact that carrying capacity was adjusted to total resource quantity, independently of the dominant resource type, suggests that ecosystem carrying capacity might be more sensitive to changes in resource quantity than in the overall quality. This carrying capacity adjustment to resource quantity was achieved by variations in the number of organisms, and consequently in the community biomass, without changes in taxonomic composition, possibly because the variation in all available food resources had a similar effect in all macroinvertebrate taxa, independently of their feeding mode.

Size spectrum slope remained invariant despite the great variations in food resource quantity and type among streams. Although size spectrum is free of biogeographical constraints and the organism taxonomic identity is relegated to a second place enabling a large comparability among communities and ecosystems (Friberg *et al.*, 2011), how size spectrum slope responds to disturbances is, up to date, largely contradictory. Both the alteration (including the decline and the increase) and the stability of size spectra slopes in response to disturbances have been theorized (Petchey & Belgrano, 2010; Trebilco *et al.*, 2013) and several empirical studies support these theoretical predictions. For instance, just considering aquatic ecosystems, size spectra slope decreased in fluvial macroinvertebrate communities affected by non-native forest plantations (Martínez *et al.*, 2016) or in fish communities affected by urbanization (Benejam *et al.*, 2016) while it increased in marine fish communities impacted by fisheries (Shin *et al.*, 2005) and remained invariant in fluvial fish communities subjected to an increasing resource availability and predation intensity (Murry and Farrell; 2013).

The size spectra slope stability found in our study shows that stream macroinvertebrate communities maintained the trophic transfer efficiency despite the strong variations in food resources when forest cover is lost, suggesting that, as observed in previous investigations (e.g., Murry and Farrell 2013), communities tend to be trophically efficient even when they are affected by disturbances. This disagrees with the general prediction that disturbances alter the community spectrum and, particularly, with the size spectra slopes predicted for ecosystems subsidized by allochthonous food resources. Hereof, size spectra slopes greater than - 0.75 have been theorized in these ecosystems because inputs of external resources would reduce the energy limitation allowing the energetic transfer adjustment through body sizes without a decrease on organism abundance (Trebilco *et al.*, 2013). According to this, we should have observed an increase in slope with forest loss and the shallowest size spectra slopes in the most forested streams. However, size spectra slopes did not vary along the forest cover gradient and the shallowest slopes did not correspond with any of the most forested streams, contradicting not only this theory, but the shallow size spectra slopes observed in river fish communities (Benejam *et al.*, 2018), lake plankton communities (del Giorgio & Gasol, 1995) or marine benthic macroinvertebrate communities (Dinmore & Jennings, 2004) subsidized by allochthonous food resources.

Our findings are in line with recent theories that suggest that biological communities stabilize and adapt to the environmental changes and disturbances to be trophically efficient, only adjusting their carrying capacity to their nature (Alonso-González, In Preparation). This suggests that the time period after the disturbance may be crucial to identify whether the size spectra slope is altered or has recovered reaching pre-disturbance values. Moreover, size spectra are the result of the combination of bottom-up and top-down controls (Finlay *et al.*, 2007; Greenwood *et al.*, 2007; Davis *et al.*, 2010). Some disturbances preferentially affect the abundance of large body size organism, this is, the top of the food webs (e.g., fisheries exploit the largest organisms; (Jennings, Reynolds & Mills, 1998; Shin *et al.*, 2005), while others the abundance of small size organisms, this is the bottom of the food web (e.g., nutrient supply; Cyr and Downing 1997). Consequently, depending on the disturbance nature and if not sufficient time for the community adaptation is allowed, an increase or a decline in size spectrum slope could be reported. Although the time after the disturbance in combination with the disturbance nature could constitute a plausible explanation for the differences in the response of size spectra slope to disturbances observed among studies, it still remains challenging given the wide combination of communities, ecosystems and disturbances studied. Indeed, this contrasting response among studies evidences, that to be able to predict values of trophic efficiency and identify similar response patterns to disturbances, a stronger theoretical framework, which recognizes how size spectra of different communities behave in natural environmental gradients and respond to different types of disturbances needs to be developed.

5.4.2 Community size structure compensatory regulation

In presence of a disturbance, an internal compensatory regulation of the community size structure is required to maintain the trophic transfer efficiency, what implies that the abundance of one group (e.g., taxon or size class) needs to be balanced by the opposite change in one or more groups (Downing *et al.*, 2008). In the studied macroinvertebrate communities, this was achieved by biomass redistribution among feeding groups without changes in overall community composition and richness. Detritivores and carnivores dominated in highly allochthonous streams while herbivores and omnivores, in the most autochthonous streams (SM 5.4). These changes on feeding groups with forest cover loss traced the variation in food resources, what resembles the changes predicted by the River Continuum Concept (Vannote *et al.*, 1980), as increases in both forest cover loss and stream order result in the increase in autochthonous food resources and the simultaneous decline in allochthonous food resources. Size spectrum stability particularly reflected the antagonistic response in size structure between omnivores and detritivores because these feeding groups were the most dominant in the study streams. Although the simultaneous decline in leaf litter quantity and the increase in biofilm biomass with forest cover loss represented an opposed impact for these feeding groups (detritivores fed mostly on leaf litter, omnivores strongly depended on biofilm biomass; chapter IV), they did not experience the same response in opposed directions (e.g., a change in density).

Detritivores declined in body size because of a taxa replacement for smaller size organisms whereas omnivores increased in density and biomass through a biomass redistribution among dominant taxa, showing that while detritivore size structure was modified via shifts in both body size distribution and taxonomic composition, omnivore size structure was modified only by body size distribution. Further, the disappearance of large detritivores (case-building trichopterans) in concert with the increase in large omnivore density seems to counterbalance and had no effect on carnivores, which did not vary their size structure with forest cover loss. This suggests that carnivores might be more affected by changes in small organism density than in large organism density or body size. The only exception was BU3, which despite being the most allochthonous stream, it showed a great density and biomass of omnivores, especially of

Gammaridae. In this stream, gammarids were the most dominant taxa and represented 23% of the entire community biomass and 40% of omnivore biomass. Why this taxon represents such a high biomass and does not adjust to the observed pattern cannot be explained in the present study. Nevertheless, we believe that it might be benefiting from the low biomass of larger predators such as fish (117 g/m^2; SM 5.1) compared to other streams and the vast amount of allochthonous resources in this stream (i.e., leaf litter biomass was threefold larger than in any other stream; Table 4.1), as previous stable isotopes analyses revealed that in this stream gammarids fed mostly on allochthonous resources (99.9% of allochthonous food resource assimilation; chapter IV).

These findings evidenced the mechanisms that govern the response of macroinvertebrate size structure to alterations in food resource type, providing insight into how stream macroinvertebrate communities might respond to catchment disturbances. These mechanisms are related to the properties that distinguish the autochthonous and allochthonous energy channels, essentially dominant life strategy (r vs k strategy taxa) and feeding mode. Life strategy defines whether changes in size structure occur through variations in organism abundance or body size. The increase in resource quantity in the allochthonous energy channel, in concert with a slow growth and long life cycles (k strategy), might result in the increase in body size rather than in changes in total density. On the contrary, the increase in resource quantity in the autochthonous energy channel, combined with a fast reproduction (r strategy), might lead to an increase in the abundance of organisms, without affecting body size. Feeding mode defines organism capacity to adapt to available food resources (Collins *et al.*, 2016a; Hayden *et al.*, 2016, chapter IV) and can dictate variations in taxonomic composition when food resources change. Detritivores show a strict feeding behavior that makes them highly sensitive to alterations in their food resources, what can result in changes on taxonomic composition. In contrast, omnivores, which encompass taxa with various feeding modes that include filter feeding, gathering and gatherer-scraping, have a high trophic plasticity that confers them the capacity to feed on available food resources and, when food resources vary, persist only varying in density.

5.5 Conclusions

In conclusion, our study shows how stream macroinvertebrate communities regulate their size structure to maintain the energy transfer efficiency to higher trophic levels (e.g., fish) adjusting its carrying capacity to total food resource quantity when forest cover is lost. This internal regulation seems to be related to the interplay between the organism life strategy (k vs r strategy), which determine whether the response affects the number of individuals or their body size, and feeding mode (capacity to adapt to available food resources), which stablishes if these changes are accompanied by variations in taxonomic composition. Since community structure and ecosystem processes (e.g., metabolism, feeding rate, reproduction) are essentially linked to body size, understanding how community size structure responds to forest loss will improve our predictions of forest cover loss effects on ecosystem functioning.

5.6 References

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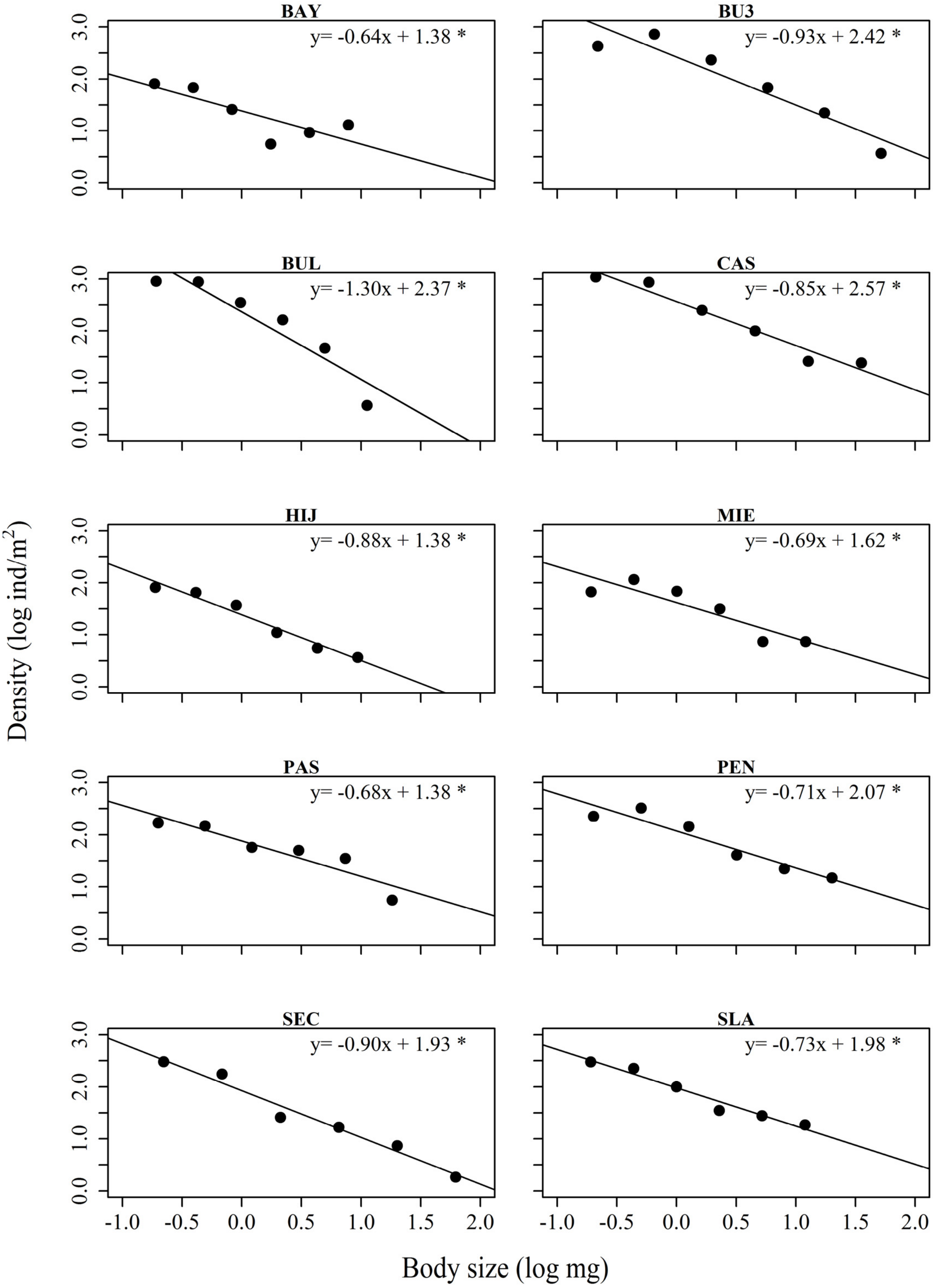
5.7 Supplementary material 5

Supplementary material 5.1 - Standardized total food resource quantity and fish biomass in the study streams.

Stream Code	Resource quantity (stand.)	Fish biomass (g/m^2)
BAY	0.42	748.3
BU3	3.08	117.0
BUL	-1.78	2315.3
CAS	-3.04	2318.7
HIJ	1.64	591.1
MIE	-1.23	1775.5
PAS	-1.82	9520.6
PEN	1.77	745.1
SEC	0.16	1033.5
SLA	0.81	266.5

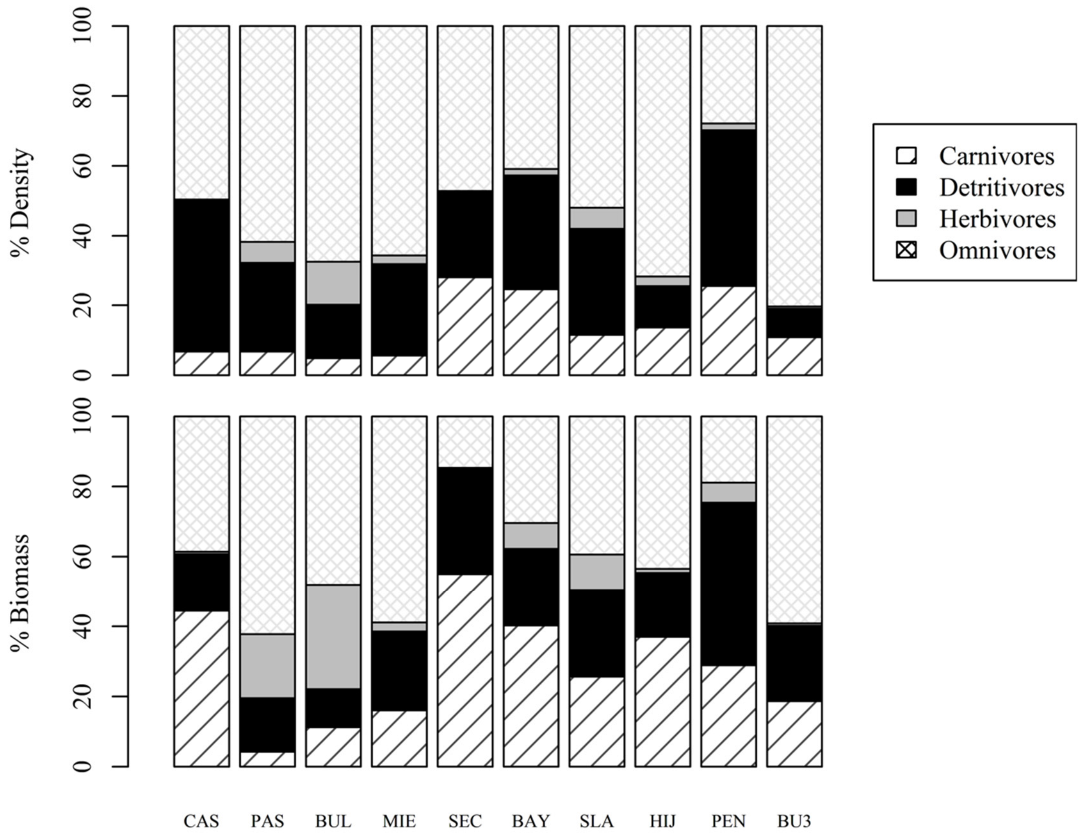
Supplementary material 5.2 - Community size spectrum of each study stream. Regression lines and their equation are shown. * Significant linear regressions ($p < 0.05$).



Supplementary material 5.3 - Macroinvertebrate taxa present in the study streams, numeric code used in the non-metric multidimensional scaling (NMDS) ordination representations in Figures 5.2 and 5.3 and feeding group (H = Herbivore, D = Detritivore, O = Omnivore, C = Carnivore).

Taxa	Numeric code	Feeding group	Taxa	Numeric code	Feeding group
Ancylidae	1	H	Hydrophilidae	29	O
Athericidae	2	C	Hydropsychidae	30	O
Baetidae	3	O	Lepidostomatidae	31	D
Beraeidae	4	D	Leptophlebiidae	32	O
Brachycentridae	5	O	Leuctridae	33	D
Ceratopogonidae	6	C	Limnephilidae	34	D
Chironomidae Non-Tanypodinae	7	O	Limoniidae	35	C
Cordulegasteridae	8	C	Nemouridae	36	D
Dixidae	9	O	Niphargidae	37	O
Dolichopodidae	10	C	Odontoceridae	38	D
Dryopidae	11	D	Oligochaeta	39	O
Dugesiidae	12	C	Perlidae	40	C
Dytiscidae	13	C	Philopotamidae	41	O
Elmidae	14	D	Planariidae	42	C
Empididae	15	C	Planipenne	43	C
Ephemerellidae	16	O	Polycentropodidae	44	C
Ephemeridae	17	O	Psychodidae	45	D
Erpobdellidae	18	C	Psychomyiidae	46	O
Gammaridae	19	O	Ptychopteridae	47	O
Glossosomatidae	20	H	Rhyacophilidae	48	C
Goeridae	21	H	Scirtidae	49	H
Gerridae	22	C	Sericostomatidae	50	D
Haliplidae	23	H	Sialidae	51	C
Heptageniidae	24	O	Simuliidae	52	O
Hydrachnidiae	25	C	Sphaeriidae	53	O
Hydraenidae	26	D	Stratiomyidae	54	O
Hydrobiidae	27	H	Chiromomidae Tanypodinae	55	C
Hydrometridae	28	C	Thaumaleidae	56	H
			Tipulidae	57	D

Supplementary material 5.4 - Relative density and biomass of each macroinvertebrate feeding group (carnivores, detritivores, herbivores and omnivores). Streams are ordered following the allochthony gradient from left to right.



Chapter VI

Influence of forest cover on stream ecosystem multifunctionality

Chapter VI. Influence of forest cover on stream ecosystem multifunctionality

This study, performed by Edurne Estévez, Tamara Rodríguez - Castillo & José Barquín, is under preparation to be submitted for publication in a SCI journal.

Abstract

Land use-land cover changes, in concert with climate change, are the most extensive and influential human impacts on fluvial ecosystems. Changes in land cover, through the alteration of hydrology, catchment vegetation, soil erosion, sediment yield or canopy cover, can have strong effects on numerous environmental factors and ecosystem components including water temperature, light intensity, nutrient concentration, organic matter or macroinvertebrate community composition. All these, control multiple ecosystem functions that occur simultaneously in fluvial ecosystems (ecosystem multifunctionality). However, how these factors, which act at a local scale, interact to determine stream ecosystem functioning rates and how these interactions are influenced by other distal factors (e.g., climate, altitude, geology), remains poorly understood. The main objective of this study was to examine local scale abiotic and biotic pathways by which catchment land cover determines the rates of ecosystem functions related to ecosystem energetics (e.g., wood decomposition, biofilm growth, GPP and ER) in mountain headwater streams. This study also aims at addressing land cover change effects on fluvial ecosystem multifunctionality and investigate the direct and indirect interactions between land cover and catchment scale factors (i.e., geology and catchment area). Results showed that forest cover controlled ecosystem functions predominantly through two abiotic factors: minimum water temperature in the case of wood decomposition and light availability in the case of biofilm growth, GPP and ER. These abiotic factors completely outweighed the influence of organism density or standing stock biomass on ecosystem functions. Moreover, results revealed an interaction between forest cover and catchment area, which defined the level of stream canopy cover and thus, controlled ecosystem functions mediated by autotrophic organisms (biofilm growth and primary production) through light availability. This interaction was reflected in the ecosystem multifunctionality, which varied more than 50% among streams and was the result of the increase in wood decomposition and the decline in primary production with forest cover and the increase in biofilm growth and primary production with catchment area.

6.1 Introduction

Land cover change is one of the most extensive and influential components of global change (Vitousek *et al.*, 1997) and has led to the transformation of more than 43% of the Earth's land surface (Daily, 1995). Land cover changes highly influence fluvial ecosystems due to the tight connection between rivers and their surrounding terrestrial ecosystem. This sets land cover alteration, in concert with climate change, as one of the major environmental threats to biodiversity and ecosystem functioning in fluvial ecosystems and, thus, to the services they provide to human societies (Millennium Ecosystem Assessment, 2005).

Land cover, which acts at a large geographical scale (i.e., distal factor; Dodds *et al.*, 2015), controls environmental factors and river ecosystem components acting at more local scales (i.e., reach scale). For instance, land cover, through the alteration of catchment vegetation and canopy cover, can modify soil erosion and sediment yield (Yang *et al.*, 2003; Burcher, Valett & Benfield, 2007), hydrology (e.g., change in intensity or timing of flow; Schoonover, Lockaby & Helms, 2006; Belmar *et al.*, 2018) and affect stream water temperature, light intensity, nutrient concentration and water velocity (Ahearn *et al.*, 2005; Foley *et al.*, 2005; Julian, Stanley & Doyle, 2008). Moreover, previous chapters have demonstrated the strong influence of forest cover on dissolved organic matter composition (chapter III) and on the size structure and composition of macroinvertebrate communities (chapters IV and V). All these local biotic and abiotic factors control the rates of a large number of ecosystem functions that simultaneously occur in fluvial ecosystems, which define the ecosystem multifunctionality. Ecosystem multifunctionality is as an integrative descriptor of ecosystem functioning which can be highly informative of the ecosystem health (Costanza, 1992) and the provisioning of key ecosystem services (Millennium Ecosystem Assessment, 2005). Therefore, over the last years, there has been an increasing interest to characterize and quantify ecosystem multifunctionality in river ecosystems (e.g., Wagg *et al.*, 2014; Allan *et al.*, 2015; Antiqueira, Petchey & Romero, 2018).

The numerous ecosystem functions that define the ecosystem multifunctionality, describe diverse processes, from purely physical processes such as meander migration or hydraulic retention of organic matter, to biologically mediated processes that regulate the fluxes of energy and matter in the ecosystem as a consequence of organism activity (Tilman, Isbell & Cowles, 2014). Biologically mediated ecosystem functions encompass those functions related to nutrient cycling, pollutant dynamics, organic matter decomposition (e.g., particulate organic matter decomposition or dissolved organic matter uptake and degradation), metabolism (e.g., ecosystem metabolism and primary producer biomass accrual) and community dynamics (e.g., invertebrate drift, secondary production, fish migration or insect emergence; von Schiller *et al.*, 2017). In this chapter, with the aim of understanding the effects of land cover changes on organic matter dynamics and ecosystem energetics, we will focus on relevant functions that describe the autotrophic production and the processing of allochthonous organic matter, this is, the two energy pathways (autochthonous and allochthonous) that sustain river food webs. These functions include the growth of primary producers (i.e., biofilm growth), the decomposition of allochthonous organic matter and the ecosystem metabolism: GPP and ER. Biofilm growth represents the gain of primary producers biomass over time (Tank *et al.*, 2010) and highly defines the organic matter available for a food web within the autotrophic energy channel, while the allochthonous organic matter decomposition shows the organic matter consumption in the allochthonous energy channel (Gessner, Chauvet & Dobson, 1999). Ecosystem metabolism, as it encompasses all the interrelated processes that fix (GPP) and mineralize organic matter of all autotrophic and heterotrophic organisms in the ecosystem (ER; Hall *et al.*, 2016), is an integrative indicator of nutrient and organic matter cycling in the ecosystem (Williamson *et al.*, 2008) and thus, of the energy sources for food webs.

Numerous empirical studies have investigated the rates and local factors that control functions related to ecosystem energetics in fluvial ecosystems. This research has demonstrated that biofilm growth is mainly affected by water velocity, light intensity, temperature, nutrient concentration and macroinvertebrate grazers (Horner & Welch, 1981; Bothwell, 1988; Feminella, Power & Resh, 1989; Arnon *et al.*, 2007). Organic matter decomposition rates, however, strongly depend on water temperature, nutrient concentration, water velocity and residence time or macroinvertebrate (i.e., shredders), microbial and fungal communities (Gulis & Suberkropp, 2003; Mille-Lindblom & Tranvik, 2003; Dang *et al.*, 2009; Abril *et.*, 2015; Graça, 2001). Regarding the ecosystem metabolism, dominant controls of GPP include water temperature, light and nutrient availability and photosynthetic organisms (e.g., benthic algae, phytoplankton; Guasch, Martí & Sabater, 1995; Hill, Ryon & Schilling, 1995; Mulholland *et al.*, 2001; Pastor *et al.*, 2017), while ER highly depends on organic matter quantity and composition and water temperature, velocity and residence time (Young & Huryn, 1999; Acuña *et al.*, 2004; Houser, Mulholland & Maloney, 2005; Roberts, Mulholland & Hill, 2007). Besides, as ER incorporates the respiration of all the organisms in the community (autotrophic and heterotrophic), it is highly controlled by the composition and biomass of organisms in the ecosystem, but particularly by photosynthetic algae and heterotrophic bacteria and fungi (Hall, 2016). All these local factors are not only affected by changes in land cover, but simultaneously controlled by multiple distal factors such as climate, geology, altitude or catchment topography (e.g., Gibbs, 1970; Hinton, Schiff & English, 1998; Meyer *et al.*, 1999; Johnson *et al.*, 2000). However, how land cover interacts with other distal factors that simultaneously influence local factors and how these local factors interact among them to determine the rates of multiple ecosystem functions, remains still poorly understood. Further, the effect of land cover alteration has been, so far, assessed directly on individual ecosystem functions or on a few functions in isolation (e.g., Encalada, CAlles, Ferreira, Canhoto, & Graça, 2010; Houser, Mulholland, & Maloney, 2005; Von Schiller et al., 2008; Roger G. Young & Huryn, 1999). Hence, how the river ecosystem multifunctionality is affected by the changes in land cover has not yet been described.

The main objectives of this study were: (1) to examine the local scale abiotic and biotic pathways by which forest cover controls ecosystem functions related to ecosystem energetics in mountain headwater streams, (2) address the effects of catchment forest cover change on fluvial ecosystem multifunctionality, and (3) investigate the direct and indirect interactions between land cover and catchment scale factors affecting ecosystem functioning simultaneously.

6.2 Methods

6.2.1 Study area and stream reaches

The study area is located in the central sector of the Cantabrian Cordillera, a mountain range that spans more than 300 km across northern Spain parallel to the coast (Cantabrian Sea; Atlantic Ocean; Fig. 2.1 in chapter II). In this chapter, a total of 31 streams were studied in this area (chapter II, Fig. 2.9). However, while GPP and ER were estimated in all the study streams, wood decomposition and biofilm growth were only measured in 22 and 25 streams respectively due to the sample loss in the remaining streams (SM 6.1).

6.2.2 Ecosystem multifunctionality

Ecosystem multifunctionality was assessed considering 5 biologically mediated ecosystem functions related to ecosystem energetics that describe autotrophic production and processing of allochthonous organic matter: wood decomposition, biofilm growth as the growth of autotrophic components (chlorophyll *a* accrual rate) and the growth of both autotrophic and heterotrophic components (epilithic biomass accrual rate), and GPP and ER. Ecosystem multifunctionality was quantified in a single multifunctionality index calculated for each stream as the average of the 5 functions previously normalized scaling the mean value of each function in the range [0,1].

6.2.2.1 Wood decomposition

Wood decomposition rates were measured on untreated wooden sticks of Canadian poplar wood (*Populus nigra x canadensis*; Betik, 15 x 1.8 x 0.22 cm). The sticks were arranged in groups of five. Three groups were placed in a randomly selected run in the stream reach and retrieved after approximately 80 days (the number of days ranged from 72 to 86 as the number of streams and the distance among them disabled accounting for the exact same number of days). Sticks were only recovered from 22 of the 31 streams as they were lost in the remaining streams (SM 6.1). Upon removal, the sticks were stored in individual zip-lock bags, transported cold (4 ^{0}C) to the laboratory and frozen at -20 ^{0}C until analysis. Then, the sticks were washed with tap water and brushed to remove invertebrates and other attached material, oven-dried (70 ^{0}C, 72 h) and ashed (500 ^{0}C, 5 h) to determine AFDM (Aristi *et al.*, 2012). Wood decomposition rates were estimated according to a negative exponential model (Petersen & Cummins, 1974):

$$\mathrm{M_t} = \mathrm{M_0} \times e^{-(kt)}$$

where M_0 (g) is the initial organic matter obtained from the mean organic matter of 50 sticks that had been incubated during 24 hours in tap water, Mt (g) is the mean remaining organic matter at time t (d), and k is the decomposition rate (d^{-1}). For each stream, the average decomposition rate was calculated.

6.2.2.2 Biofilm growth

Biofilm growth rates were measured using unglazed clay tiles (area: 240 cm^2). Six tiles were distributed in the same run as wooden sticks and concurrently retrieved, although they were only recovered from 25 of the 31 streams (SM 6.1). Upon retrieval, tiles were immediately transported cold to the laboratory and frozen at -20 ^{0}C until analysis. Each tile was submerged in 90% acetone at 4 ^{0}C for 24 hours in the dark to extract the chlorophyll *a*. Absorbance was read on a Hach-Lange DR-5000 UV/visible spectrophotometer and converted to pigment concentration (Steinman *et al.*, 2017). Afterwards, epilithic biomass content was calculated using a modified version of the technique of Sinsabaugh et al. (1991). Each tile, after chlorophyll *a* was measured, was brushed into a tray and filtered through a pre-ashed 70-µm glassfiber filter, dried to constant weight at 95^{0}C, weighed, ashed at 500 ^{0}C for 2 h, and reweighed to yield ash free dry mass (AFDM; Barquín & Death, 2006). We assumed that only the upper surface area (120 cm^2) of each tile was exposed to light and consequently, accessible for colonization by biofilm. The biofilm growth rates (mg $Chla.m^{-2}.d^{-1}$ and mg $AFDM.m^{-2}.d^{-1}$) were estimated by dividing the chlorophyll *a* concentration and epilithic biomass in the unglazed clay tiles by the tile surface area and the incubation days. For each stream, the average biofilm growth rate was calculated.

6.2.2.3 GPP and ER

GPP and ER were estimated using the single-station open channel method based on dissolved oxygen diel changes. Dissolved oxygen concentration and water temperature were measured with oxygen loggers (HOBO® model U26-001 dissolved oxygen data logger, Onset Computer Corporation, Cape Cod, MA, USA) calibrated in water-saturated air before deployment. Loggers were deployed in the stream bottom in well-mixed stream areas recording information at 5 minute intervals for a minimum of 72 hours. Net ecosystem production (NEP; $gO_2.m^{-2}.d^{-1}$) was estimated as the sum for a 24 hours period of the instant NEP. Instant NEP at time t was calculated as:

$$\mathrm{NEP(t) = z \times (dC/dt - k \times [Cs - C])}$$

where C is the dissolved oxygen concentration measured, Cs the oxygen concentration at saturation, k is the reaeration coefficient and z is water depth. The reaeration coefficient for each stream was estimated using an empirical equation developed for mountain streams characterized by pool and riffle sequences as the ones considered in this study (Melching, et al. 1999). Average night-time respiration (ANR; $gO_2.m^{-2}.h^{-1}$) was calculated as the average NEP during the night hours, while ER ($gO_2.m^{-2}.d^{-1}$) was estimated as the ANR extrapolated to 24 hours. GPP ($gO_2.m^{-2}.d^{-1}$) was calculated as $\mathrm{GPP = NEP - ER}$. GPP and ER were reported as the mean value of the days when they were measured.

6.2.3 Environmental factors

We selected environmental factors that have been previously reported to highly influence the selected ecosystem functions (conceptual diagram in Fig. 6.1). The selected environmental factors were divided into two groups according to the scale at which they operate: catchment scale factors and local scale factors (i.e., reach scale). Local scale factors were, in turn, divided by their nature: biotic factors that comprise densities and standing stock biomass of living components and abiotic factors that include non-living chemical and physical components of the ecosystem.

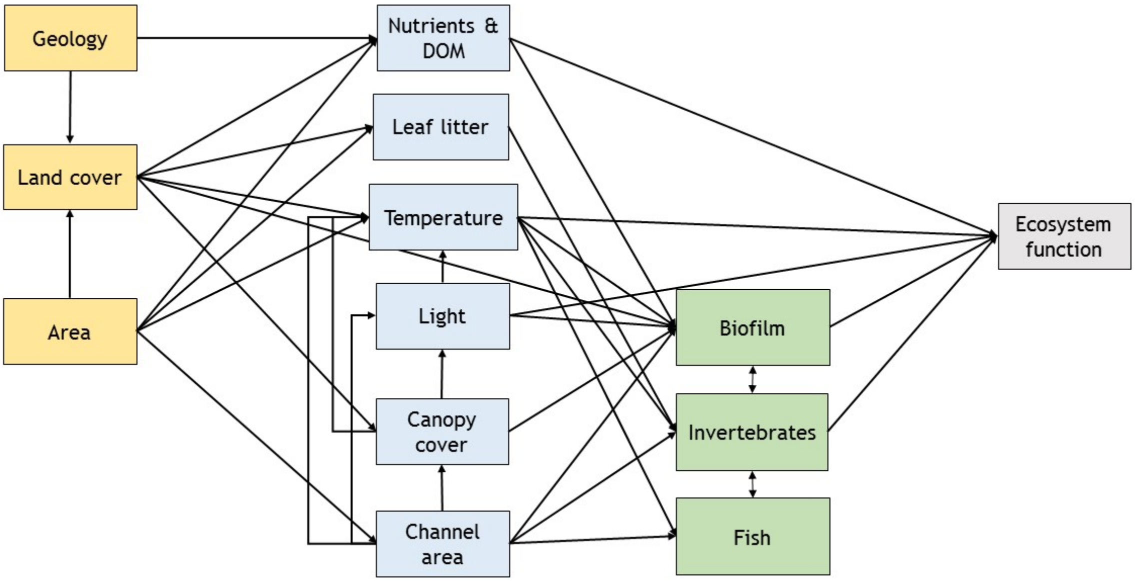


Figure 6.1 - Conceptual diagram describing the catchment (dark yellow) and local scale abiotic (blue) and biotic (green) factors hypothesized to influence the 5 stream ecosystem functions considered in this study.

6.2.3.1 *Catchment scale factors*

We considered three catchment scale factors: land cover, geology and catchment area. Land cover was characterized by the percentage of forest cover in each of the stream catchments. The percentage of forest cover was obtained from the classification of remote sensing imagery (Landsat 5 TM images of the year 2009) as described in chapter II.

Catchment area was derived from a digital elevation model, originally obtained at 5 meters from interpolated LiDAR data (CNIG, 2014), but resampled to 30 meters to match the spatial resolution of the Landsat imagery used to characterize catchment land cover. Geology was characterized by nine geological classes: calcareous, siliceous, sedimentary and volcanic rocks, conglomerates, sands, shales, slates and clays. The geological classes were obtained after an expert-knowledge reclassification of the lithological classes in the Geological Map of Spain (MAGNA), developed by the Spanish Geologic Institute (IGME) at a scale 1:200000. The geological information was integrated in a Synthetic River Network already delineated for the study area (Álvarez-Cabria *et al.*, 2017) using flow directions inferred from a 25 meter digital elevation model with the NetStream software (chapter II; Fig. 2.8) and the average percentage of each geologic class was obtained for each of the selected stream catchments. As the classes are obtained as percentage of the total catchment area, they are correlated among them (SM

6.2). Thus, for simplicity, only the calcareous class was selected. This geological class, together with shales and slates, dominates in the selected streams and can have a high influence on the solutes dissolved in stream water (Gibbs, 1970).

6.2.3.2 Local scale biotic factors

To characterize local scale biotic factors, we selected organisms from all the trophic levels: primary producers characterized by biofilms, primary consumers characterized by the macroinvertebrate community, and secondary consumers characterized by the fish community.

Fish communities were surveyed by electrofishing using a backpack electroshocking device (Honda ELT 60 I G) on an area representative of each river reach (aprox. 100 m). Captured individuals were weighed and the fork length measured, and from these measurement, fish density (ind.m^{-2}) and biomass (gr.m^{-2}) were estimated.

Macroinvertebrate density (ind.m^{-2}) and biomass (mg.m^{-2}) were calculated from a composite sample of 6 benthic Surber samples (mesh 500 µm, 0.09 m^{2}), each collected from 3 pools and 3 runs randomly selected in the 100 m river reach. Macroinvertebrates in < 1 mm fraction were identified and counted while only the macroinvertebrates in the > 1mm fraction were measured to calculate individual body size using published body length–dry mass equations (see chapters IV and V). Macroinvertebrate density was measured in the 31 study streams while macroinvertebrate biomass only in the 10 streams in chapters IV and V. Moreover, macroinvertebrate density and biomass were independently calculated for four different macroinvertebrate feeding groups: detritivores, herbivores, omnivores and carnivores (although carnivore density and biomass is not considered in this chapter), as described in chapters IV. The new macroinvertebrate families which were not present in the 10 streams considered in chapters IV and V (SM 6.3) were assigned to a feeding group following Tachet et al. (2002).

Biofilm standing stock biomass was characterized by the biomass of autotrophic components, defined by chlorophyll *a* concentration, and the biomass of both autotrophic and heterotrophic components, defined by the biofilm epilithic biomass. Chlorophyll *a* concentration (mg Chla.m^{-2}) and epilithic biomass (mg AFDM.m^{-2}) were measured in 6 cobbles collected from the same runs and pools as Surber samples for macroinvertebrate characterization. Cobbles were brushed and from the slurry, chlorophyll *a* was measured by spectrophotometry after extraction in acetone, while epilithic biomass was measured using a modified version of Sinsabaugh et al. (1991), as described in chapter IV.

6.2.3.3 Local scale abiotic factors

We selected eight environmental abiotic factors at the local scale: nutrient concentration, DOM quantity and composition, leaf litter, light availability, water temperature, canopy cover and ecosystem size. These factors were selected because of their potential to influence the ecosystems functions considered in this study (see references from previous studies in the introduction).

To characterize nutrient concentration, we measured the concentration of nitrate (µgN/L) and nitrite (µgN/L) and the electric conductivity (µS/m). Nitrate and nitrite concentration was measured by continuous spectrometry (AA3 Autoanalyzer, SEAL Analytical GmbH, Norderstadt, Germany) from one water sample collected from the water column in each stream. Electric conductivity was in situ measured using an YSI 556 Multi-Parameter Handheld Meter (YSI Inc., Yellow Springs, OH, USA). DOM quantity and composition was measured from water sample collected from each stream. DOM quantity was measured as dissolved organic carbon (DOC) concentration (mg C/L) and calculated by size-exclusion chromatography while DOM composition by ultrahigh-resolution mass spectrometry (FT-ICR-MS) as described in chapter III. Based on the results obtained in chapter III (Fig. 3.1), which described the variation in DOM composition with forest cover, we selected three main compounds to characterize the DOM: the relative abundance of O-poor aliphatic compounds, as these strongly declined with catchment forest cover, and phenols and polyphenols rich in oxygen, as they increase with forest cover.

Leaf litter (g/m^2) was quantified from the composite Surber samples collected for macroinvertebrate characterization. Leaf litter was separated, dried to constant mass at 70°C, weighed, ashed at 500°C for 4 h, and reweighed to yield ash free dry mass (see chapter IV for further details).

Light (lux) was recorded at 5 min. intervals for a minimum of 72 hours with HOBO Pendant® Temperature/Light 64K (Onset Computer Corporation, Cape Cod, MA, USA) loggers placed in the center of each stream reach facing up. PAR (µmols.m^{-2}.s^{-1}) was inferred from the light measurements in luxes according to the relationship obtained from simultaneous measurements of light as PAR with a PAR Quantum sensor (Skye Instruments, Liandrindod Wells, Powys, England) and lux with HOBO Pendant® Temperature/Light 64K (Onset Computer Corporation, Cape Cod, MA, USA) in the study area (PAR = 5.2+ 0.01*Lux; $r^2 = 0.96$, $p < 0.001$). Simultaneously to light measures, water temperature (^{0}C) was recorded with oxygen loggers (HOBO® model U26-001 dissolved oxygen data logger, Onset Computer Corporation, Cape Cod, MA, USA) also used to measure oxygen concentration for stream GPP and ER estimates. For each stream, the mean PAR and the water minimum and maximum temperature were calculated.

The percentage of canopy cover of the selected stream reaches was estimated from hemispheric photography. One hemispheric image was collected with a Nikon Coolpix P510 with a fish eye lens in the center of each stream reach. The camera was leveled, horizontally positioned and oriented to magnetic north on a tripod. Canopy cover was determined with the Gap Light Analyzer from the obtained hemispherical images (Frazer, Canham & Lertzman, 1999).

Ecosystem size was defined by the stream cross –sectional channel area (m^2). Channel area was computed from channel depth and width measures obtained from 5 cross-section profiles performed in each river reach with a portable Acoustic Doppler Velocimeter (FlowTracker Handheld Acoustic Doppler Velocimeter, SonTek/YSI Inc., United States).

6.2.4 Data analysis

To identify the relationship between each individual function and catchment scale factors (percentage of calcareous rocks, percentage of forest and catchment area), ordinary least square regression analyses were performed between each catchment scale factor and the ecosystem functions. Moreover, a path analysis was performed to relate catchment scale factors to ecosystem functions via local scale abiotic and biotic intermediaries (Fig. 6.1). The path analysis was based on Spearman correlation analyses as these are less sensitive to outliers than linear regressions and can be used with non-normal distributed data (van Sickle, 2003). Firstly, simple Spearman correlations were performed among catchment scale factors, local scale factors and ecosystem functions as described in the conceptual diagram (Fig. 6.1). Then, partial Spearman correlations were performed; initially between each ecosystem function and the significant local scale factors and continued following the described paths to catchment scale factors (Fig. 6.1). The partial Spearman correlations enabled to identify independent correlations by factoring out the variation explained by confounding factors and, thus, the significant and dominating paths. Although this approach is conservative, it can provide the sufficient evidence of causal pathways between catchment scale factors and ecosystem functions (King *et al.*, 2004).

Finally, to assess the effect of catchment scale factors on ecosystem multifunctionality, a multiple linear regression analysis was performed between the catchment scale factors and the multifunctionality index. The most parsimonious model was selected by stepwise forward selection based on their p-values and Akaike information criteria. Normality was checked from residuals and log-transformation was used when needed to remove heteroscedasticity. Statistical analyses were performed in R software (version 3.3.3 R Project for Statistical Computing, Vienna, Austria) using the packages *vegan* and *ppcor* for Spearman correlations.

6.3 Results

The ecosystem functioning rates varied widely across streams. For example, wood decomposition rate ranged from just 0.004 to a maximum of 0.041 d^{-1}, while chlorophyll *a* accrual rate ranged from 0.07 to 0.57 mg $Chla.m^{-2}.d^{-1}$, and epilithic biomass accrual rate from 0.23 to 0.43 mg $AFDM.m^{-2}.d^{-1}$. GPP ranged from just 0.12 to a maximum of 3.26 $gO_2.m^{-2}.d^{-1}$ and ER from 0.09 to 7.93 $gO_2.m^{-2}.d^{-1}$ (SM 6.1). Consequently, the multifunctionality index ranged from 0.11 to 0.61 (SM 6.1).

6.3.1 Linear relationship between catchment scale factors and ecosystem functions

Direct relationships between catchment scale factors and the ecosystem functions (Fig. 6.2) showed that wood decomposition rate increased with forest cover (Fig. 6.2 b), while epilithic biomass accrual rate increased with catchment area (Fig. 6.2 i). GPP declined with forest cover while increased with catchment area (Fig. 6.2 k and l). On the contrary, neither chlorophyll *a* accrual rate nor ER varied with any of the analysed catchment scale factors (Fig. 6.2 d, e and f and 4 m, n and o, respectively). The percentage of calcareous rocks in the catchment was not significantly related to any of the ecosystem functions (Fig. 6.2 a, d, g, j and m).

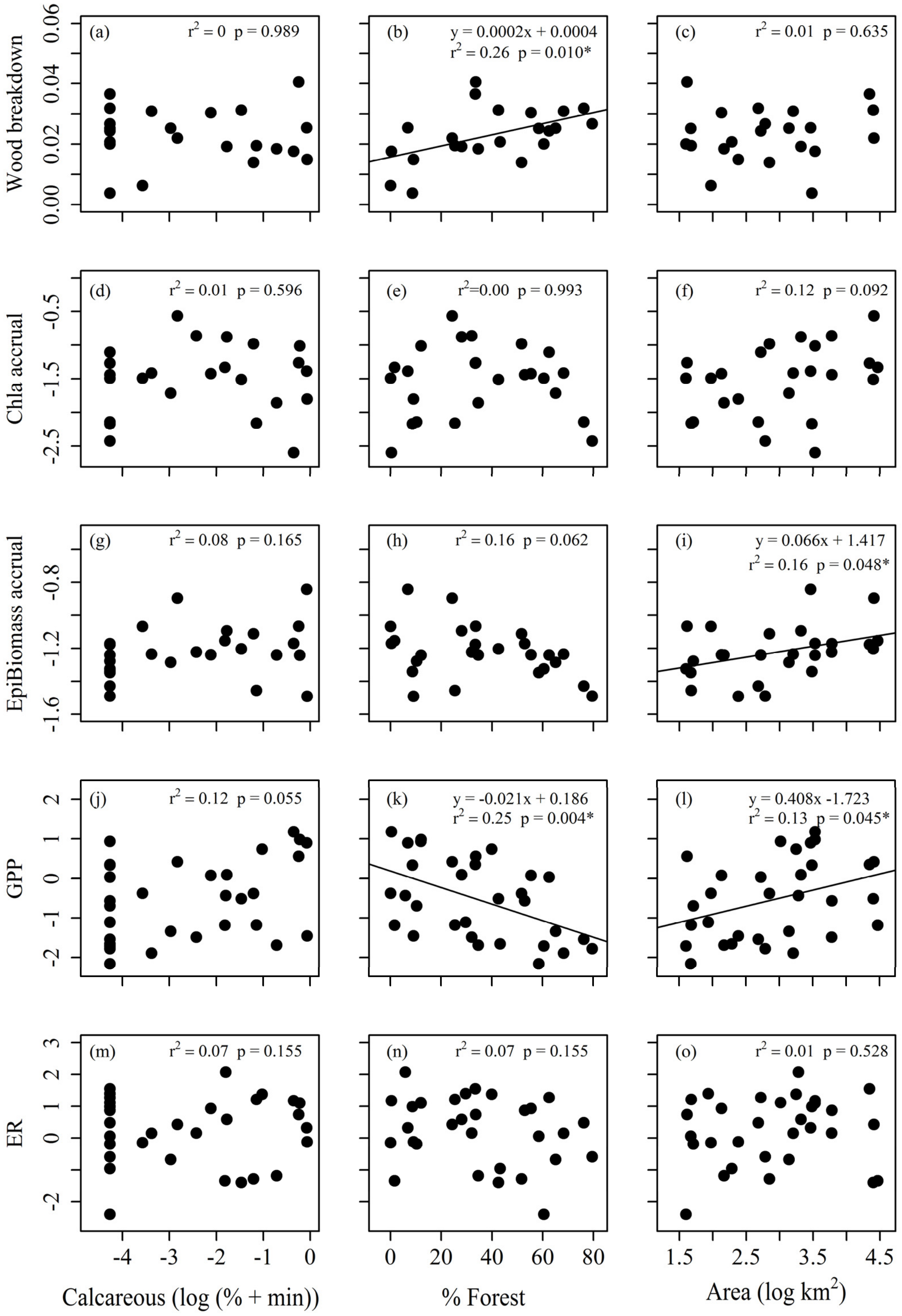


Figure 6.2 - Ordinary least square regression analyses between the percentage of calcareous rocks (a, d, g, l, m), the percentage of forest (b, e, h, k, n) and the catchment area (e, f, i, l, o) and the ecosystem functions: wood decomposition rate (d^{-1}; a, b, c), biofilm chlorophyll a accrual rate (Chla accrual, mg $Chla.m^{-2}.d^{-1}$; d, e, f), biofilm epilithic biomass accrual rate (EpiBiomass accrual; mg $AFDM.m^{-2}.d^{-1}$; g, h,

i), gross primary production (GPP, g $O_2.m^{-2}.d^{-1}$; j, k, l) and ecosystem respiration (ER, g $O_2.m^{-2}.d^{-1}$; m, n, o). * Significant linear regressions ($p < 0.05$). Regression lines and equations are shown for significant ($p < 0.05$) linear regressions.

6.3.2 Path analysis: Catchment and local scale factors

While catchment scale factors were not correlated among them, path analyses identified numerous correlations between catchment scale factors and local abiotic factors. The percentage of forest was positively correlated to canopy cover, water minimum temperature and O-rich polyphenols while negatively to O-poor aliphatics. Catchment area was positively correlated to nitrate and nitrite concentration, maximum water temperature and channel area, and the percentage of calcareous rocks was only positively correlated to nitrate concentration and electric conductivity (Fig. 6.3 a and 6.4 a).

Similarly, several correlations were identified among local scale factors, especially among abiotic factors and between abiotic and biotic factors. Channel area was negatively correlated to canopy cover but positively to mean PAR, maximum water temperature, chlorophyll *a*, epilithic biomass and fish density and biomass. Canopy cover was negatively correlated to both maximum water temperature and mean PAR, which was in turn positively correlated to maximum water temperature, chlorophyll a and epilithic biomass. O-rich polyphenols were negatively correlated to epilithic biomass, while water temperature was positively correlated to biofilm biomass. Maximum water temperature was correlated to epilithic biomass, while minimum water temperature was correlated to chlorophyll *a*. On the contrary, no correlation was detected among local biotic factors (fish, macroinvertebrates and primary producers; Fig. 6.3 a and 6.4 a).

6.3.3 Path analysis: Wood decomposition

Wood decomposition rate was negatively correlated to the density of detritivores while positively with water minimum temperature and nitrite concentration (Fig. 6.3 a). However, only water minimum temperature remained significantly correlated to wood decomposition rates after partialling out the influence of nitrite concentration and detritivore density (neither detritivore density nor nitrite concentration were significantly correlated to wood decomposition rate when the influence of water minimum temperature was considered; Table 6.1), what indicated that wood decomposition rate was indirectly correlated to the percentage of forest cover through water minimum temperature (Fig. 6.3 b).

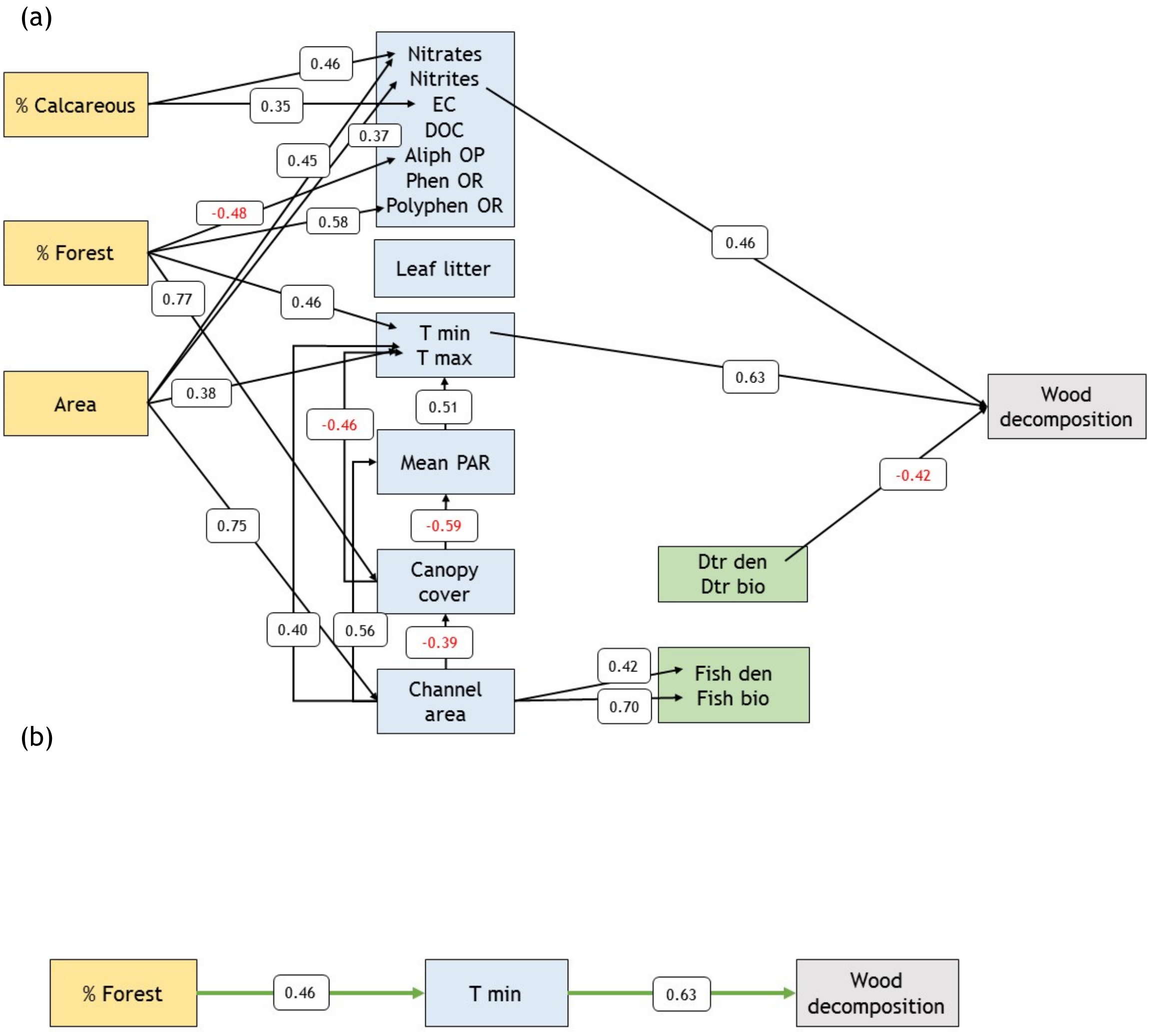


Figure 6.3 - Path diagram exploring the effects of catchment scale factors, via abiotic and biotic local scale intermediaries, on wood decomposition rate. Boxes represent measured factors (catchment scale factors are represented in dark yellow, local scale abiotic factors in blue and local scale biotic factors in green). (a) Black arrows represent significant ($p < 0.05$) Spearman correlations among factors. (b) Green arrows represent significant ($p < 0.05$) partial Spearman correlations when the variation explained by confounding factors is factored out. Numbers associated to arrows denote the magnitude of the correlation: black numbers denote positive correlations and red numbers negatives correlations. * EC = Electric conductivity, DOC = Dissolved organic carbon, Aliph OP = O-poor aliphatics, Phen OR = O-rich phenols, Polyphen OR = O-rich polyphenols, T min = water minimum temperature, T max = water maximum temperature, Dtr =detritivores, den = density, bio = biomass

Table 6.1 - Results of partial Spearman correlations performed to explore the effects catchment scale factors, via abiotic and biotic local scale intermediaries on each ecosystem function. Significant correlations ($p < 0.05$) are highlighted in bold. * T min = water minimum temperature, Dtr =detritivores, DOC = Dissolved organic carbon.

Dependent variable	Independent variables	Influential variables	Partial correlation coefficient	p value
Wood decomposition rate	**T min**	**Nitrites + Dtr density**	**0.58**	**0.007**
	Nitrites	T min+ Dtr density	0.37	0.109
	Dtr density	Nitrites + T min	-0.38	0.098
	Nitrites	T min	0.34	0.141
	Dtr density	T min	-0.38	0.101
GPP	**mean PAR**	**DOC + Epilithic biomass**	**0.57**	**< 0.001**
	DOC	mean PAR + Epilithic biomass	0.33	0.068
	Epilithic biomass	DOC + mean PAR	-0.05	0.794
	DOC	**mean PAR**	**0.36**	**0.049**
	Epilithic biomass	mean PAR	-0.03	0.866
Mean PAR	Channel area	Canopy cover	0.26	0.16
	Canopy cover	**Channel area**	**-0.65**	**< 0.001**
Canopy cover	**Channel area**	**% Forest**	**-0.48**	**0.007**
	% Forest	**Channel area**	**0.73**	**< 0.001**

6.3.4 Path analysis: Biofilm growth

Chlorophyll *a* accrual rate was positively correlated to omnivore biomass and nitrite concentration (Fig. 6.4 a) while epilithic biomass accrual rate was positively correlated to mean PAR and omnivore biomass (Fig. 6.5 a). The positive correlation between chlorophyll *a* and epilithic biomass accrual rates and omnivore biomass indicated that biofilm growth might be affecting omnivore biomass and not vice versa. Therefore, omnivore biomass was not considered in the partial correlation analyses. Consequently, chlorophyll *a* accrual rate was only correlated to nitrite concentration, and thus, indirectly to catchment area (Fig. 6.4 b), while epilithic biomass accrual was correlated to mean PAR. Mean PAR was only significantly and negatively correlated to canopy cover, which was in turn significantly positive correlated to both the percentage of forest cover and catchment area through channel area (Table 6.1). This shows that epilithic biomass accrual rate was ultimately negatively correlated to the percentage of forest while positively to catchment area (Fig. 6.5 b). Nevertheless, the percentage of forest exerted a greater influence on epilithic biomass accrual rate than catchment area as its correlation to canopy cover was stronger than the correlation between canopy cover and channel area (|0.73| vs |0.48|, respectively).

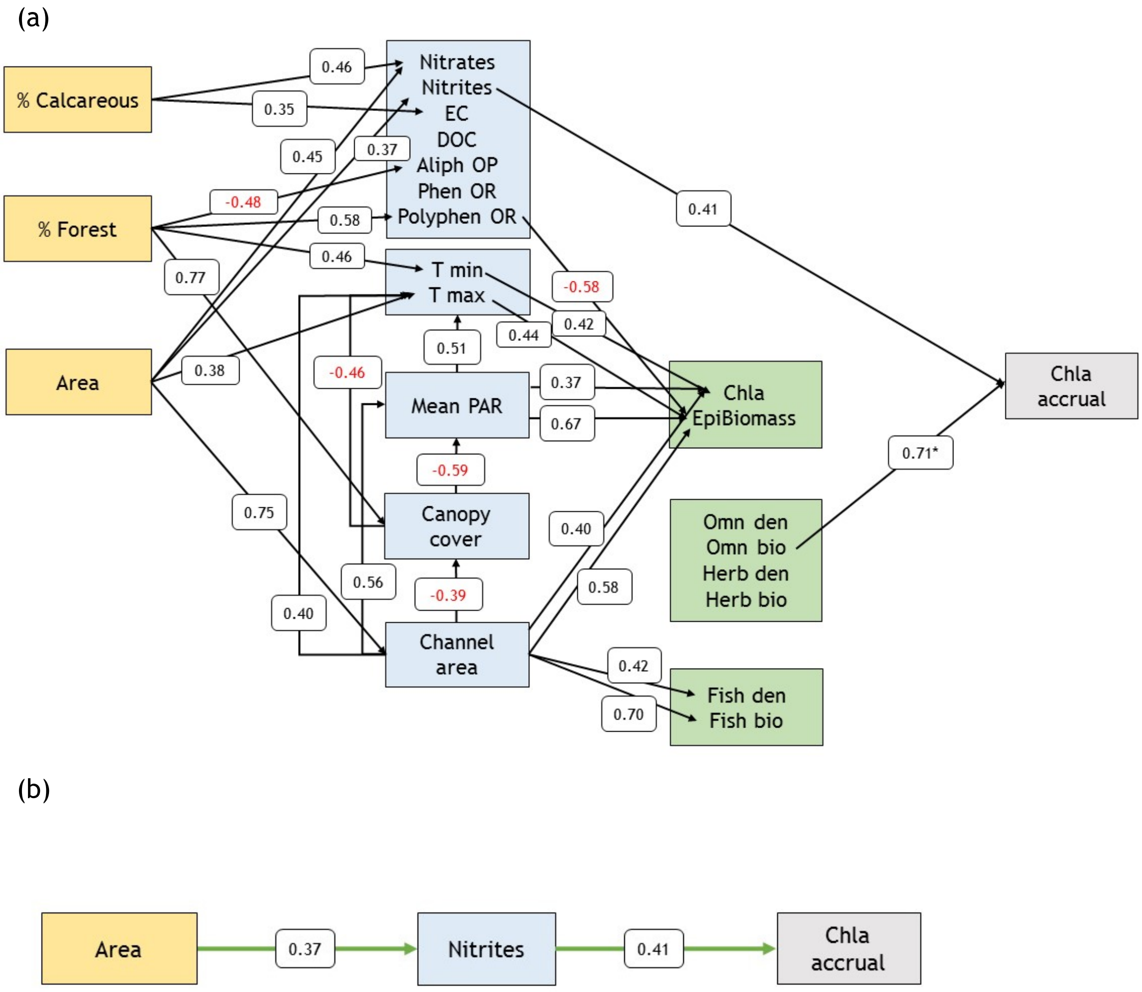


Figure 6.4 - Path diagram exploring the effects of catchment scale factors, via abiotic and biotic local scale intermediaries, on biofilm chlorophyll a accrual rate. Boxes represent measured factors (catchment scale factors are represented in dark yellow, local scale abiotic factors in blue and local scale biotic factors in green). (a) Black arrows represent significant ($p < 0.05$) Spearman correlations among factors. (b) Green arrows represent significant ($p < 0.05$) partial Spearman correlations when the variation explained by confounding factors is factored out. Numbers associated to arrows denote the magnitude of the correlation: black numbers denote positive correlations and red numbers negatives correlations. *EC = Electric conductivity, DO C= Dissolved organic carbon, Aliph OP = O-poor aliphatics, Phen OR = O-rich phenols, Polyphen OR = O-rich polyphenols, T min = water minimum temperature, T max = water maximum temperature, Chla = biofilm chlorophyll a, EpiBiomas s= biofilm epilithic biomass, Omn = omnivores, Herb = herbivores, den = density, bio = biomass

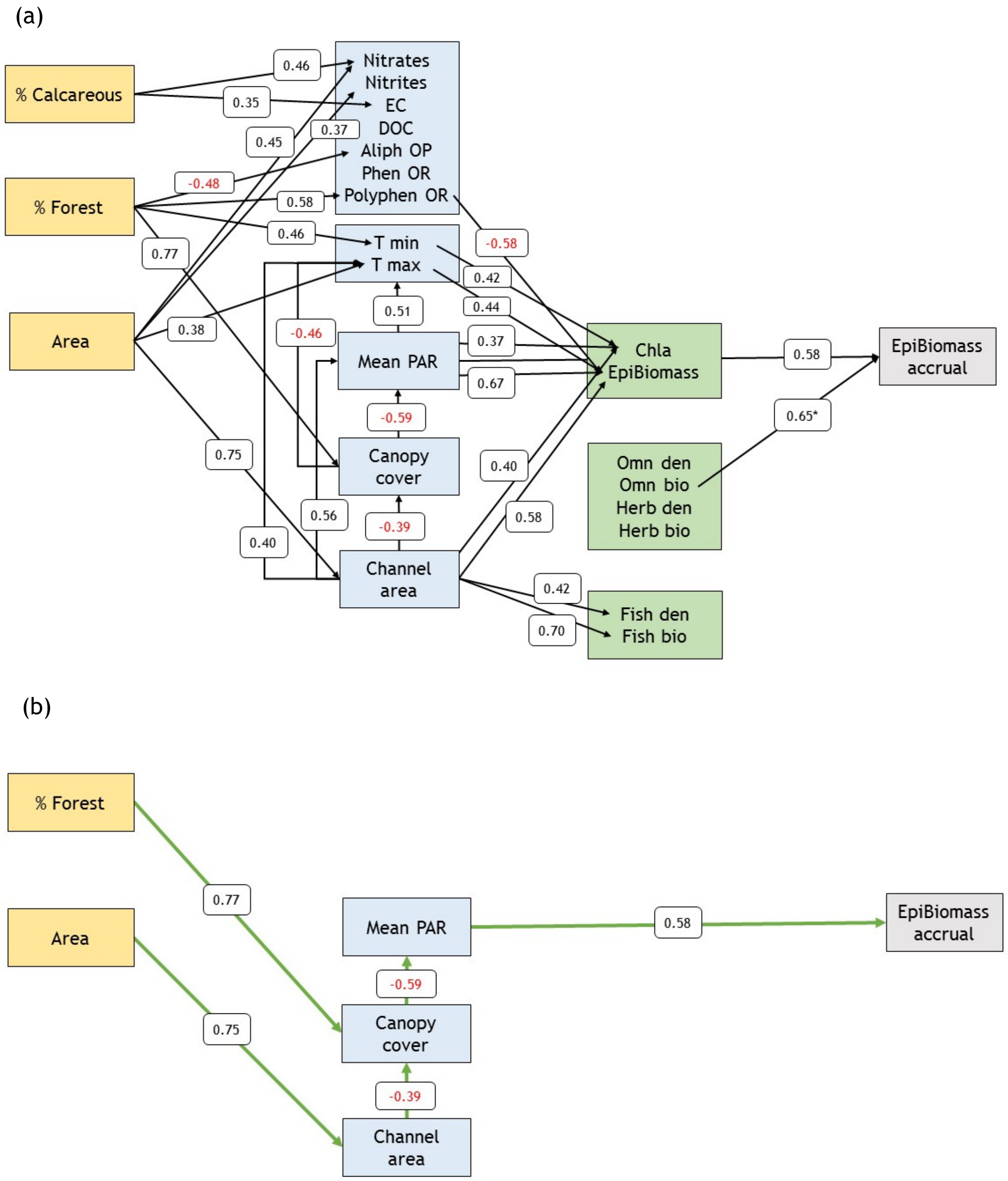


Figure 6.5 - Path diagram exploring the effects of catchment scale factors, via abiotic and biotic local scale intermediaries, on biofilm epilithic biomass accrual rate. Boxes represent measured factors (catchment scale factors are represented in dark yellow, local scale abiotic factors in blue and local scale biotic factors in green). (a) Black arrows represent significant ($p < 0.05$) Spearman correlations among factors. (b) Green arrows represent significant ($p < 0.05$) partial Spearman correlations when the variation explained by confounding factors is factored out. Numbers associated to arrows denote the magnitude of the correlation: black numbers denote positive correlations and red numbers negatives

correlations. * EC = Electric conductivity, DOC = Dissolved organic carbon, Aliph OP = O-poor aliphatics, Phen OR = O-rich phenols, Polyphen OR = O-rich polyphenols, T min = water minimum temperature, T max = water maximum temperature, Chla = biofilm chlorophyll a, EpiBiomass = biofilm epilithic biomass, Herb = herbivores, den = density, bio = biomass

6.3.5 Path analysis: GPP and ER

GPP was positively correlated to mean PAR, DOC and epilithic biomass (Fig. 6.6 a). Partial correlation analyses revealed that only DOC and mean PAR remained significantly correlated to GPP after splitting out the effect of significant factors (Table 6.1). Therefore, considering the above described significant paths between mean PAR and catchment scale factors and the absence of correlation between DOC and any of the catchment or local scale factors, GPP was ultimately negatively correlated to the percentage of forest while positively to catchment area (Fig. 6.6 b).

ER was not directly correlated to any abiotic and biotic local scale factors except for GPP (Fig. 6.6 a), what indicates ER, through GPP, is also correlated to the percentage of forest and catchment area (Fig. 6.6 b).

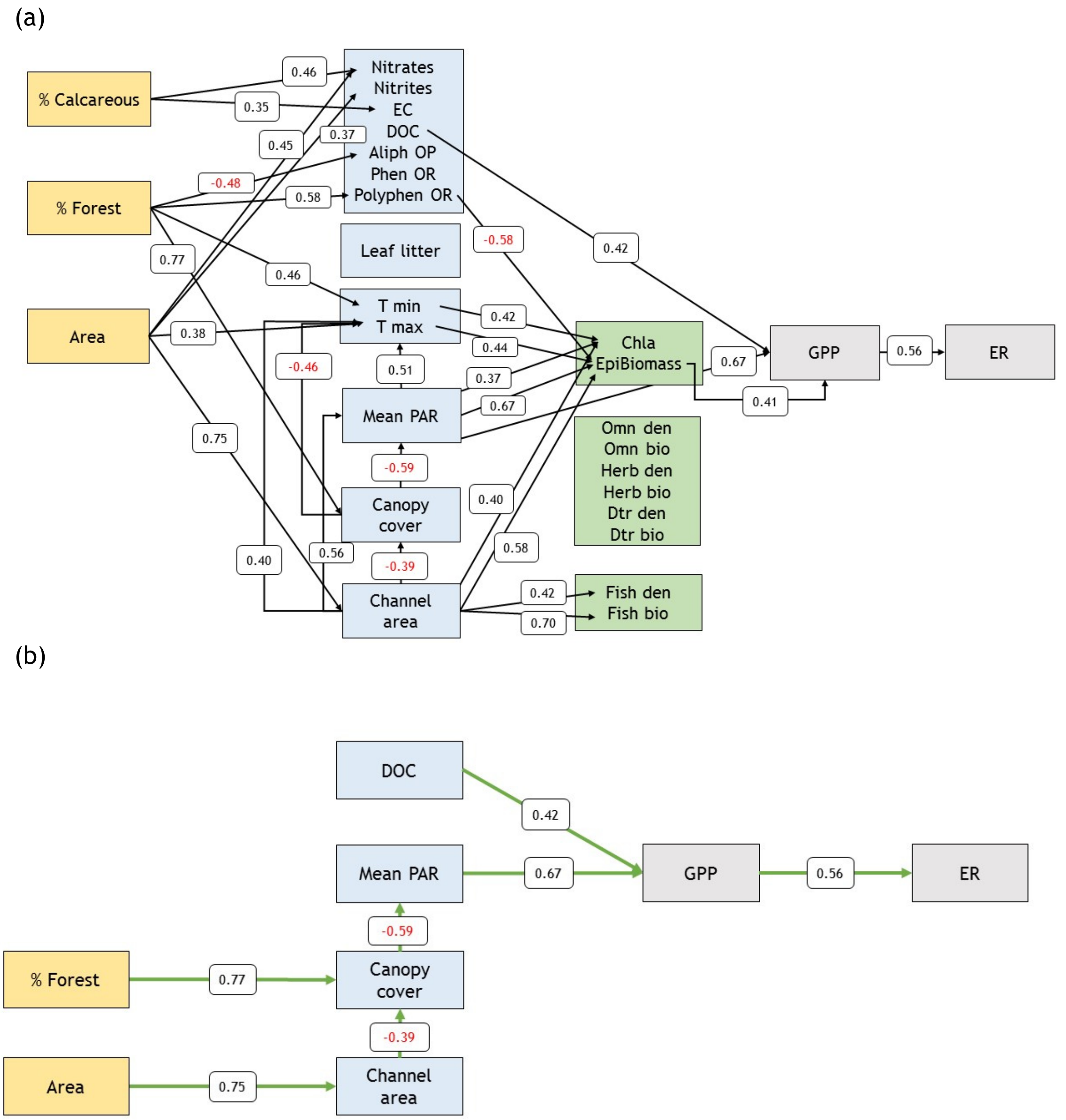


Figure 6.6 - Path diagram exploring the effects of catchment scale factors, via abiotic and biotic local scale intermediaries, on stream gross primary production (GPP) and ecosystem respiration (ER). Boxes represent measured factors (catchment scale factors are represented in dark yellow, local scale abiotic factors in blue and local scale biotic factors in green). (a) Black arrows represent significant ($p < 0.05$) Spearman correlations among factors. (b) Green arrows represent significant ($p < 0.05$) partial Spearman correlations when the variation explained by confounding factors is factored out. Numbers associated to arrows denote the magnitude of the correlation: black numbers denote positive correlations and red numbers negatives correlations. * EC = Electric conductivity, DOC = Dissolved organic carbon, Aliph OP = O-poor aliphatics, Phen OR = O-rich phenols, Polyphen OR = O-rich polyphenols, T min = water minimum temperature, T max = water maximum temperature, Chla = biofilm chlorophyll a, EpiBiomass = biofilm epilithic biomass, Omn = omnivores, Herb = herbivores, den = density, bio = biomass

6.3.6 Ecosystem multifunctionality

Multiple linear regression analyses revealed the percentage of forest and catchment area as the main factors explaining the variation in the multifunctionality index (Table 6.2). The multifunctionality index declined with forest cover while increased with catchment area (Table 6.2, Fig. 6.7 a and b respectively). The percentage of calcareous rocks in the catchment was not related to the multifunctionality index (r^2 = 0.12, *p* = 0.059; data not shown)

Table 6.2 - Results of the most parsimonious model explaining the multifunctionality index.

Dep. variable	Indep. variables	Coefficient	SE	p value	r^2	p value
Multifunctionality Index	Constant	0.294	0.089	0.002	0.38	0.001
	% Forest	-0.003	0.001	0.004		
	Area	0.052	0.025	0.048		

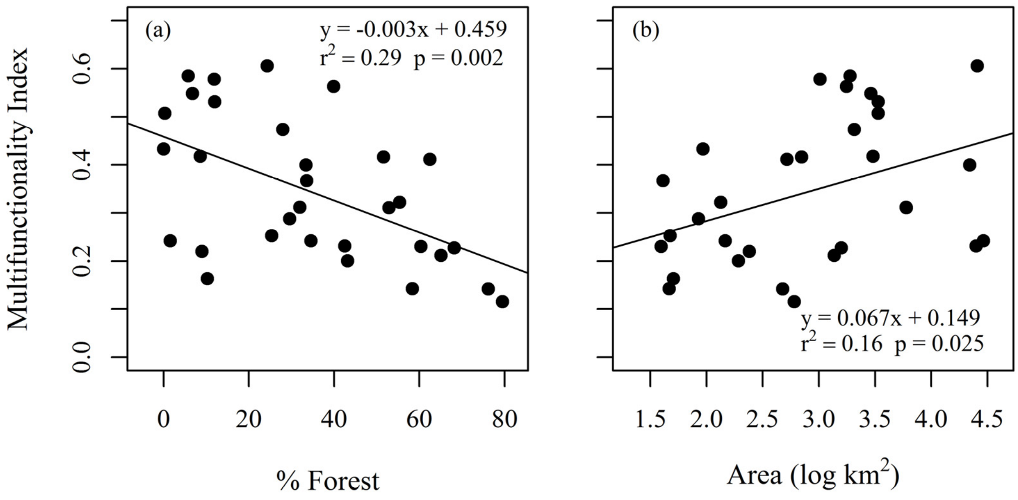


Figure 6.7 - Ordinary least square regression analyses between the multifunctionality index and the % forest cover (a) and catchment area (b). Regression lines and their equations are shown.

6.4 Discussion

This study evidences the forest cover control, by abiotic pathways, of ecosystem functions related to ecosystem energetics: wood decomposition, biofilm growth, GPP and ER and its effects on ecosystem multifunctionality. Furthermore, this study describes the interaction between forest cover and another catchment scale factor, catchment area, and its implications for ecosystem functioning.

6.4.1 Catchment and local factors controlling ecosystem functions

Path analysis revealed multiple interrelations among the factors controlling ecosystem functioning rates, what denoted the complexity of analyzing the abiotic and biotic pathways between land cover and ecosystem functioning while assessing interactions between catchment and local scale factors.

Benthic macroinvertebrate detritivores, bacteria and fungi are considered the main decomposers of organic matter in river ecosystems (Anderson & Sedell, 1979; Gessner, Robinson & Ward, 1998; Webster *et al.*, 1999). Although the relative importance of these organisms still remains unclear (Hieber & Gessner, 2002), macroinvertebrate detritivores are often less dominant as wood decomposers because only a few taxa have a mouthpart morphology that enables a successful processing and ingestion of wood (Eggert & Wallace, 2007). In this study the density of macroinvertebrate detritivores showed a negative correlation with wood decomposition rate, which might indicate an indirect control of wood decomposition rate through a detritivore top-down control. This could be explained by the fact that many detritivores feed on epixylon (biofilm grown on wood debris), rather than on the wood itself, due to epixylon greater quality and lability (Golladay & Sinsabaugh, 1991; Ledger & Winterbourn, 2000). Among other components, epixylon is composed of the bacteria and fungi also responsible for wood decomposition. Therefore, a decline in the density of bacteria and fungi because of a larger density of macroinvertebrate detritivores, might have resulted in the reduction of wood decomposition rate and led to the observed opposed correlation. Nevertheless, minimum water temperature predominantly regulated wood decomposition, showing an overriding effect on macroinvertebrate detritivore density. In the studied mountain streams, water temperature can be low, even during the low flow summer season (minimum temperature recorded was 8ºC). It has been shown that water temperature is a strong determinant of organisms metabolic activity (Brown *et al.*, 2004). Thus, low water temperatures might have limited the microbial and fungal activity reducing wood decomposition rates. Indeed, since the relationship of water temperature and metabolic rates follows an exponential model (e.g., Clarke, 2006), little increments in minimum water temperature, as observed in the study streams (from 8.12 to 15.2 °C), might substantially increase wood decomposition rates (from 0.004 to 0.041 d^{-1}).

Solar radiation has long been considered the principal factor controlling stream water temperature (Caissie, 2006). In our study, this relationship was evident for maximum water temperature. However, minimum water temperature was only correlated to forest cover. Most studies focusing on understanding the controls of stream water temperature usually consider daily mean or maximum temperatures (e.g., Johnson, 2004) because of the strong implications of high temperatures on the viability of disease vectors, the growth, development and distribution of freshwater organisms or the stress of cold water salmonids (Li *et al.*, 1994; Daufresne *et al.*, 2004; Durance & Ormerod, 2007). Hence, the controls of mean and maximum water temperatures have long been recognized, while minimum water temperature dynamics and controls are less well understood. Regarding the effect of forest cover on minimum water temperature, antagonistic responses have been reported. For example, while G. W. Brown & Krygier (1970) and Johnson & Jones (2000) found a decline of minimum water temperature with forest cover, while Malcolm et al.(2008) reported an increment. Our results agree with Malcolm et al. (2008) and suggest that the increase in forest cover and thus, in canopy cover, might lead to the reduction of convective heat losses from both stream water (Monteith & Unsworth, 1990) and catchment soils (Binkley & Fisher, 2013) and consequently, to an increase in minimum water temperature.

Light availability was the major control of ecosystem functions mediated by autotrophic organisms: biofilm growth and GPP, what agrees with previous studies that evidenced the dominance of light as it stimulates algae growth (Steinman, 1992; Hill & Dimick, 2002) and enhances algae primary production (Hill, Mulholland & Marzolf, 2001). The greater biofilm growth under great light conditions might also be related to algal community composition as filamentous algae, which dominate in brighter open streams, have a greater biomass accrual than diatoms, which are more prominent in heavily shaded streams (Denicola, Hoagland & Roemer, 1992). Nevertheless, biofilm growth was only significantly correlated to light when addressed as epilithic biomass accrual and not as chlorophyll *a* accrual. This inconsistency might likely be explained because primary producers in low light environments produce additional chlorophyll *a* to maximize their ability to capture the limited available light (Abal, et al. 1994).

Light availability was directly controlled by canopy cover, which is known to block a substantial proportion of the solar radiation that reaches the stream surface (Dugdale *et al.*, 2018). The degree of canopy cover strongly depended on forest cover. The increase in forest cover in the study streams implies a change in the dominant vegetation in the catchment, which ranges from herbaceous vegetation in grasslands, bushes in shrublands and arboreal vegetation in the most forested catchments. This vegetation change suggests that the increment in shade on the stream might be related to differences in the structure (e.g., shape and size of the canopy and branches; Lim, Treitz, Wulder, St-Onge, & Flood, 2003) and canopy architecture (e.g., leaf area index and leaf inclination; Welles & Norman, 1991) of the vegetation. In addition, the vegetation shading effect was reduced by the stream ecosystem size as streams with a larger channel area showed a greater light penetration. As ecosystem size was largely determined by the area of the draining catchment, these results elucidate an interaction between catchment scale factors: forest cover and catchment area in defining the degree of canopy cover. Thus, since canopy cover controls both light availability and stream maximum water temperature, and organism metabolic activity highly depends on temperature (Brown *et al.*, 2004), the indirect interaction between forest cover and catchment area will have strong effects in all the biologically mediated ecosystem functions. These effects will be particularly relevant for the ecosystems functions driven by autotrophic organisms (e.g., biofilm growth or GPP), as their activity depends on both water temperature and light availability.

GPP, despite being predominantly controlled by light availability, was also related to biofilm biomass (i.e., epilithic biomass). In headwater mountain streams the largest contributor to GPP are biofilm algae attached to cobbles and boulders (phytoplankton and macrophytes are absent or have negligible biomass). However, biofilm effect on GPP might have been outweighed by light availability as, it stimulates both algae growth, and thus biofilm biomass, and algae primary production. GPP was also correlated to DOM quantity. DOM quantity and composition has long been demonstrated to control ER since DOM the main supply of energy and carbon to heterotrophic bacteria (Meyer *et al.*, 1988), but not GPP, as autotrophic organism obtain the carbon form CO_2 fixation, what points to a non causal correlation between DOM quantity and GPP. Regarding ER, DOM of a greater molecular weight, aromaticity and degree of humification has shown to increase bacterial respiration (Asmala *et al.*, 2013; Fasching *et al.*, 2014). In this study, despite the in-depth characterization of DOM composition, which showed an increase in the degree of humification and aromaticity with forest cover mostly driven by the decline of O-poor aliphatics and the increase in O-rich phenols and polyphenols (chapter III; Fig. 3.1), no correlation to ER was obtained. A plausible explanation for the absence of correlation might be that the low relative abundance of these compounds, even in heavily forested streams (polyphenols and aliphatic compounds represents less than 10% while phenols a maximum of 25%) might not be sufficient to detect a correlation to ER. In a correlation analysis, the abundance of these compounds would need to vary over a range relevant for the ecosystem function in question. Nevertheless, the short water residence time in mountain streams associated to the fast water velocity even in low flow conditions, might have limited the bacterial activity (i.e., DOM processing) and thus, the heterotrophic respiration, resulting into the low contribution of DOM to ER. This further suggests that the respiration of autotrophic organisms (autotrophic respiration) might be the most important component of ER, as shown by the strong correlation between GPP and ER, what resembles the results obtained by Beaulieu et al. (2013) and Fuß et al. (2017).

Overall, the results obtained in this study show that biotic factors played weaker roles in defining the rates of ecosystem functioning than abiotic factors, although they were also significant. Macroinvertebrate detritivore density was important in determining wood decomposition rates and biofilm biomass in GPP and ER rates. The only exception was biofilm growth, which was not affected by biotic factors. In fact, the positive correlation between biofilm growth and omnivore biomass might be indicating a bottom up effect of biofilm growth on macroinvertebrate consumers. In this study, only organism densities and biomass (i.e., standing stock) were considered. Accounting for biota growth rates (i.e., secondary production for macroinvertebrates and fish) might have probably rendered stronger correlations to functioning rates. Nevertheless, abiotic factors can simultaneously control organisms growth and their metabolic activity (Gillooly *et al.*, 2001), what might still result in abiotic factors overriding the effects of biota. This is a challenge for future research and applied works as it could substantially help to elucidate the role of main factors controlling these important ecosystem functions and, thus, improve our modelling capacity from local to regional scales.

6.4.2 Ecosystem multifunctionality

Results showed a 50% variation in ecosystem multifunctionality from catchments dominated by forests to areas where forests were less important, evidencing the effect that changes on land cover produce on ecosystem multifunctionality. Although path analyses demonstrated that forest cover can control all the analyzed ecosystem functions (wood decomposition, biofilm growth, GPP and ER), forest cover only exerted a significant control on wood decomposition and GPP. Thus, the variation ecosystem multifunctionality was the result of the increase in wood decomposition and the decline in GPP with forest cover. Nevertheless, ecosystem multifunctionality was also affected by catchment area. As catchment area exerted a strong control on ecosystem functions mediated by autotrophic organisms, ecosystem multifunctionality was also the result of the increase in biofilm growth and GPP with catchment area. Hence, ecosystem multifunctionality reflected the strong interaction between forest cover and catchment area identified in the path analysis, what supports the idea that catchment scale factors can be strong determinants of ecosystem functioning rates (Chadwick *et al.*, 2006; Bernot *et al.*, 2010; Reisinger *et al.*, 2015).

The limited variation in catchment area (from 5.02 to 86.85 km^2; SM 6.4) compared to forest cover (from 0% to 79.5%) suggests that in low order headwater mountain streams, slight increases in catchment area, and consequently in stream ecosystem size, can strongly limit the effect of forest cover on ecosystem functioning, particularly on ecosystem functions mediated by autotrophic organisms. This points out the importance of land cover in controlling ecosystem functioning in small headwater streams, and evidences its decline with stream ecosystem size. Headwater streams held a strategic position in fluvial network as they are longitudinally connected to downstream rivers (Allan & Castillo, 2007) to which they provide water, organic matter, organisms, sediments and nutrients (Alexander *et al.*, 2007; Freeman, Pringle & Jackson, 2007). Therefore, is in the headwater streams, that changes in land cover will not only impact the immediate river reaches, but their effects will be amplified downstream to high order streams. Therefore, strategies for mitigating the effects of the land cover change on fluvial ecosystems should principally focus on the restauration of catchments draining small headwater streams, what will help to maintain a more natural river functioning in the entire fluvial network.

6.5 Conclusions

We underline the strong influence of catchment forest cover on ecosystem functions related to ecosystem energetics (wood decomposition, biofilm growth, GPP and ER), which resulted in the variation of a 50% of ecosystem multifunctionality. Forest cover controlled ecosystem functioning through abiotic pathways, ultimately through minimum water temperature and light availability. Thus, despite biotic factors playing a significant role on ecosystem functioning rates, the dominance of abiotic factors outweighed the influence of biotic factors. Moreover, the interaction between forest cover and catchment area evidenced the dominance of land cover in controlling ecosystem functioning in small headwater streams. Hence, the most effective measures for mitigating land cover change and conserving natural stream ecosystem functioning in fluvial networks should be implemented in catchments draining small headwater streams.

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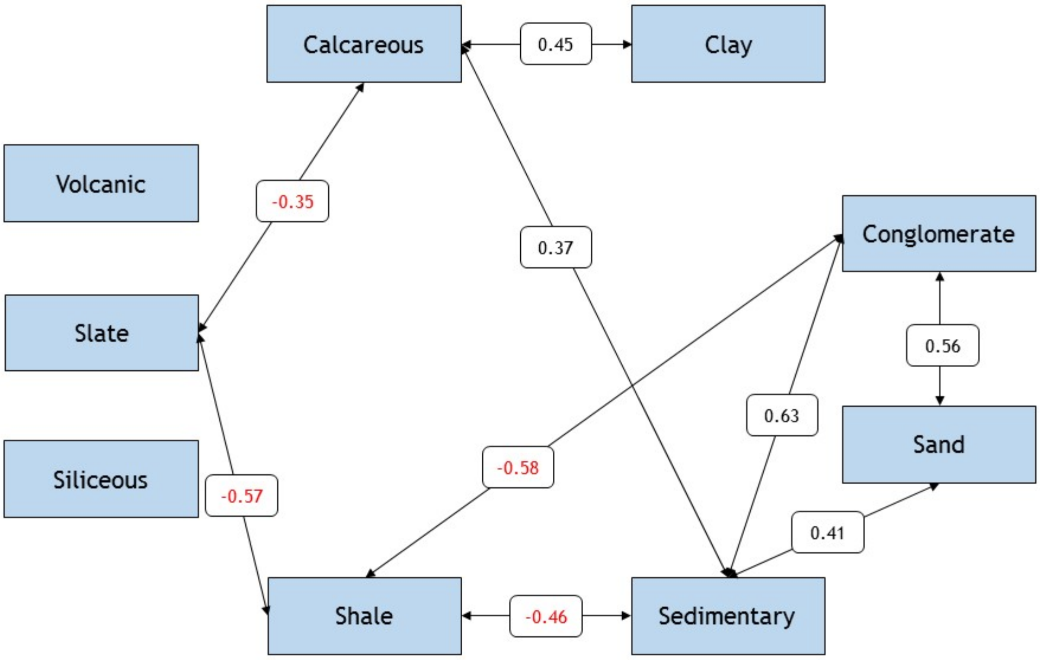
6.7 Supplementary material 6

Supplementary material 6.1 – Ecosystem functions related to ecosystem energetics: wood decomposition, biofilm chlorophyll *a* accrual (Chla accrual), biofilm epilithic biomass accrual, gross primary production (GPP) and ecosystem respiration (ER) and multifunctionality index (MI). While GPP and ER were estimated in the 31 study streams, wood decomposition and biofilm growth were only measured in 22 and 25 streams respectively due to the sample loss (sticks and/or tiles) in the remaining streams.

Stream code	Wood decomposition (d^{-1})	Chla accrual (mgChla. $m^{-2}.d^{-1}$)	Epilithic biomass accrual (mgAFDM.m^{-2}. d^{-1})	GPP (gO_2. $m^{-2}.d^{-1}$)	ER (gO_2. $m^{-2}.d^{-1}$)	MI
ANI	0.025			0.12	1.07	0.14
AR1	0.019	0.42	0.34	1.10	1.81	0.47
AR2				0.33	4.06	0.29
BAY	0.027	0.09	0.23	0.17	0.56	0.11
BU3	0.031	0.24	0.29	0.15	1.17	0.23
BUL	0.025	0.25	0.43	2.46	1.38	0.55
CAB	0.019	0.12	0.23	0.31	3.37	0.25
CAR		0.26	0.32	0.31	0.26	0.24
CAS		0.36	0.29	2.68	3.01	0.53
CIC	0.030	0.24	0.29	1.08	2.54	0.32
DEH	0.041	0.28	0.34	1.75	2.09	0.37
DEV	0.031	0.22	0.30	0.60	0.25	0.23
DUJ	0.018	0.07	0.31	3.26	3.24	0.51
FAR	0.015	0.17	0.23	0.23	0.89	0.22
FR1		0.42	0.29	0.23	1.18	0.31
FR2	0.006	0.22	0.34	0.69	0.87	0.43
HIJ	0.032	0.12	0.24	0.22	1.62	0.14
LAM				2.10	3.98	0.56
LLA		0.24	0.31	0.57	2.40	0.31
MIE	0.022	0.57	0.41	1.53	1.54	0.61
NAN	0.024	0.33	0.29	1.04	3.58	0.41
PAS	0.037	0.28	0.31	1.43	4.73	0.40
PEN	0.020	0.23	0.27	0.18	0.09	0.23
PIS				2.56	3.06	0.58
PON	0.014	0.37	0.33	0.69	0.28	0.42
SVO	0.021			0.19	0.39	0.20
SNG		0.12	0.28	0.50	0.83	0.16
SEC	0.018	0.16	0.29	0.19	0.31	0.24
SLA	0.025	0.18	0.28	0.26	0.51	0.21
VAL				0.65	7.93	0.58
YUS	0.004	0.11	0.26	1.40	2.70	0.42

Supplementary material 6.2 – Spearman correlations among the nine geological classes present in the study streams. Black arrows represent significant ($p < 0.05$) Spearman correlations. Numbers associated to arrows denote the magnitude of the correlation: black numbers denote positive correlations and red numbers denote negative correlations.



Supplementary material 6.3 - Macroinvertebrate taxa present in the study streams that were not present in the 10 streams considered in chapters IV and V and assigned feeding group (H = Herbivore, D = Detritivore, O = Omnivore, C= Carnivore).

Taxa	Feeding group	Taxa	Feeding group
Acari	C	Leptoceridae	O
Calopteridae	C	Lymnaeidae	C
Capniidae	D	Nematoda	C
Chloroperlidae	C	Oligochaeta	O
Copepoda	O	Ostracoda	O
Corixidae	O	Perlodidae	C
Curculionidae	C	Planariidae	C
Dugesiidae	C	Planipenne	C
Erpobdellidae	C	Planorbidae	O
Guerridae	C	Scatophagidae	D
Gyrinidae	D	Sialiadae	C
Hydriidae	C	Tabanidae	C
Hydrometridae	C	Ueonidae	H
Hydroptilidae	H		

Supplementary material 6.4 – Catchment and local scale abiotic factors in the 31 study streams. * EC = Electric conductivity, DOC = Dissolved organic carbon, Aliph OP = O-poor aliphatics, Phen OR = O-rich phenols, Polyphen OR = O-rich polyphenols, T min = water minimum temperature, T max = water maximum temperature.

Stream code	Catchment area (km^2)	Forest cover (%)	Calcareous rocks (%)	Nitrates (μgN/L)	Nitrites (μgN/L)	EC (μS/m)	DOC (mg C/L)	Aliph OP (%)	Phen OR (%)	Polyphen OR (%)	Leaf Litter (g/m^2)	Mean PAR (μmols. m^{-2}.s^{-1})	T min (°C)	T max (°C)	Canopy cover (%)	Channel area (m^2)
ANI	5.30	58.4	0.00	140.91	0.46	177.90	0.45				0.85	9.35	13.64	14.88	75.26	0.26
AR1	27.57	28.0	0.15	348.06	3.53	231.00	0.33	0.59	13.99	5.23	10.11	114.18	12.24	14.74	44.56	1.12
AR2	6.89	29.6	0.00	286.93	0.22	221.00	0.29	0.47	20.52	3.11	2.97	41.30	9.60	11.26	71.28	0.41
BAY	16.15	79.5	0.00	337.72	1.04	51.40	0.70	1.35	23.56	3.60	0.43	21.17	14.86	17.12	67.63	0.77
BU3	24.54	68.2	0.02	294.90	0.64	302.00	0.68	0.62	23.40	3.14	64.46	18.77	13.90	16.20	74.39	0.24
BUL	31.90	6.8	0.91	377.52	0.15	225.00	0.94	0.47	10.84	5.58	1.16	460.90	13.88	16.08	39.84	1.41
CAB	5.34	25.4	0.30	152.37	0.45	136.90	0.33	0.58	14.91	4.76	26.51	19.16	12.98	14.68	69.80	0.75
CAR	86.85	1.6	0.15	54.92	1.07	54.50	1.11	0.80	13.70	7.04	0.52	385.38	10.50	23.36	2.34	3.13
CAS	34.03	1.02	0.78	414.34	0.80	203.00	0.89	0.42	17.47	4.07	1.45	147.87	11.68	16.62	38.72	2.20
CIC	8.39	55.4	0.11	190.98	0.39	307.00	0.53	1.38	25.13	2.31	0.42	38.27	12.94	14.92	56.29	0.55
DEH	5.02	33.6	0.77	343.12	0.68	265.00	0.80				1.70	19.79	13.40	16.42	59.32	0.78
DEV	81.45	42.5	0.22	354.62	3.42	258.00	0.39				0.60	14.64	12.06	14.72	59.89	1.13
DUJ	34.09	0.3	0.69	368.51	0.40	261.00	0.99				0.00	232.82	9.66	13.92	13.57	0.64
FAR	10.81	9.0	0.92	170.98	0.06	202.00	0.14				20.14	16.92	8.12	8.90	60.93	0.22
FR1	43.66	32.0	0.07	358.48	1.69	203.70	0.57				1.44	9.53	11.50	14.08	63.46	0.74
FR2	7.17	0.0	0.01	30.93	0.59	77.40	0.60	0.76	17.28	4.71	0.00	271.87	8.56	17.20	2.51	0.40

Stream code	Catchment area (km^2)	Forest cover (%)	Calcareous rocks (%)	Nitrates (µgN/L)	Nitrites (µgN/L)	EC (µS/m)	DOC (mg C/L)	Aliph OP (%)	Phen OR (%)	Polyphen OR (%)	Leaf Litter (g/m^2)	Mean PAR (µmols. $m^{-2}.s^{-1}$)	T min (°C)	T max (°C)	Canopy cover (%)	Channel area (m^2)
HIJ	14.56	76.2	0.00	151.72	1.13	72.40	2.23	0.72	20.33	4.81	1.61	6.30	9.26	13.20	75.09	0.42
LAM	25.67	39.9	0.34	225.20	0.87	178.50	0.88	0.96	21.99	3.11	8.78	25.43	13.88	14.52	59.83	1.12
LLA	43.75	52.9	0.00	326.67	0.59	135.50	0.47	0.70	25.01	2.10	0.95	89.03	15.20	16.48	51.73	1.39
MIE	82.23	24.3	0.04	362.07	1.49	200.00	0.58	0.62	17.35	3.24	1.18	80.98	14.20	15.80	54.17	2.90
NAN	15.11	62.5	0.00	102.15	1.27	228.00	2.73	0.83	16.70	7.00	2.92	15.84	12.98	15.28	72.22	0.44
PAS	76.95	33.4	0.00	181.24	3.07	106.90	0.61	0.78	25.04	2.06	2.15	112.99	14.32	17.90	36.68	2.38
PEN	4.94	60.4	0.00	45.41	1.02	206.60	0.32	0.74	22.37	2.63	1.37	14.03	11.44	13.00	74.90	0.26
PIS	20.32	11.9	0.00	195.90	2.37	207.00	1.36	0.67	20.04	7.32	2.08	104.08	11.74	16.38	36.17	0.53
PON	17.21	51.6	0.28	118.15	0.09	196.50	0.33	0.41	19.06	2.76	6.10	119.70	8.44	13.58	61.79	0.86
SVO	9.81	43.2	0.00	95.24	0.25	153.90	0.28	0.76	25.26	2.74	2.74	14.63	10.68	12.16	79.86	0.47
SNG	5.51	10.3	0.00	159.62	0.96	246.00	1.14				3.94	16.28	12.56	17.82	39.14	0.28
SEC	8.73	34.6	0.47	262.48	0.53	278.00	0.37	0.82	26.61	4.27	5.00	18.53	11.84	13.20	74.69	0.60
SLA	23.01	65.1	0.04	190.98	0.22	183.40	0.45	0.73	25.12	2.61	11.42	7.60	13.86	14.90	55.25	0.69
VAL	26.52	5.8	0.15	33.14	0.16	65.90	0.50	0.85	15.74	5.48	0.97	37.73	9.88	14.38	21.90	1.25
YUS	32.51	8.6	0.00	0.05	0.12	151.30	0.63	0.58	22.39	3.76	0.99	192.97	9.98	18.36	15.00	0.99

Supplementary material 6.5 – Local scale biotic factors in the 31 study streams. *Chla = biofilm chlorophyll *a*, EpiBiomass = biofilm epilithic biomass, Dtr = detritivores, Herb = herbivores, Omn = omnivores, den = density, bio = biomass.

Stream code	Chla (mg Chla/m^2)	EpiBiomass (mg/m^2)	Dtr den (ind/m^2)	Herb den (ind/m^2)	Omn den (ind/m^2)	Dtr bio (mg/m^2)	Herb bio (mg/m^2)	Omn bio (mg/m^2)	Fish den (ind/m^2)	Fish bio (g/m^2)
ANI	37.33	3289.78	1027.78	0.00	427.78				0.71	3.21
AR1	55.96	10141.36	1435.19	1.85	898.15				0.47	20.01
AR2	25.19	4468.50	1912.96	20.37	1290.74				0.01	0.26
BAY	36.41	5579.17	527.78	3.70	811.11	48.46	15.49	67.19	0.21	2.99
BU3	19.48	4719.92	650.00	27.78	3266.67	405.66	12.96	1123.63	0.02	0.47
BUL	45.64	12820.89	1005.56	400.00	3087.04	170.28	452.74	779.53	0.47	9.26
CAB	21.07	4782.94	2722.22	103.70	1738.89				0.01	0.34
CAR	23.18	12687.66	403.70	0.00	2324.07				0.21	7.06
CAS	56.15	12133.47	1755.56	48.15	2525.93	483.46	20.12	1108.98	0.38	9.27
CIC	33.93	4682.27	1272.22	31.48	3853.70				0.86	13.29
DEH	58.61	3679.48	420.37	48.15	1505.56				0.01	0.02
DEV	18.85	4242.54	935.19	12.96	2870.37				0.20	10.70
DUJ	3.99	2325.76	2411.11	201.85	5318.52				0.23	4.16
FAR	18.73	5937.03	559.26	25.93	1544.44				0.05	0.56
FR1	14.24	3414.24	479.63	20.37	1177.78				0.22	7.09
FR2	58.61	12004.35	850.00	0.00	17940.74				0.41	3.30
HIJ	15.03	4924.28	374.07	12.96	946.30	35.58	1.95	74.96	0.45	2.36
LAM	66.77	10467.49	633.33	22.22	2040.74				0.27	3.88
LLA	40.42	8218.07	125.93	14.81	488.89				0.38	4.32
MIE	38.21	9540.37	312.96	25.93	612.96	78.81	8.74	204.97	0.44	7.21
NAN	77.35	9910.66	1066.67	25.93	1607.41				0.06	1.22
PAS	52.42	11509.57	392.59	96.30	1205.56	107.17	126.87	438.78	1.24	40.66
PEN	23.15	4709.63	2088.89	148.15	2888.89	450.38	54.88	183.75	0.19	2.98
PIS	19.60	7341.05	746.30	11.11	1455.56				0.07	2.62
PON	53.01	9926.81	1024.07	14.81	2968.52				0.09	4.36
SVO	20.39	3897.21	1053.70	24.07	1998.15				0.07	1.80
SNG	12.49	3639.44	1816.67	59.26	3564.81				0.00	0.00
SEC	35.43	5936.23	987.04	29.63	2101.85	207.00	0.00	123.22	0.33	4.13
SLA	24.62	4470.05	2138.89	59.26	1244.44	221.82	71.01	286.11	0.03	1.07
VAL	29.77	7432.68	350.00	11.11	775.93				0.18	8.11
YUS	14.24	8001.11	1174.07	3.70	2592.59				0.37	13.73

Chapter VII

General conclusions and future research

Chapter VII. General conclusions and future research

7.1 General conclusions

Mountain headwater streams are particularly vulnerable to land cover changes due to their close connection to the terrestrial ecosystems, their small size and unidirectional and linear nature and their relative isolation in the river network. Therefore, elucidating land cover change effects on these streams is crucial to unravel how land cover change effects are propagated to downstream ecosystems and extended throughout the fluvial network.

In chapters III, IV, V and VI of this thesis we investigated the effects that land cover change has on mountain stream ecosystems from a multi-level approach, which included physical and chemical fluvial components, organic matter properties, energy flow pathways, food web size structure and composition and rates of ecosystem functioning. Only chapter III specifically assessed the effect of historic land cover, this is, the land cover change per se. In the remaining chapters, since land cover changes occur over long time scales, we applied a space for time substitution approach which assumes that spatial and temporal variation are equivalent. Moreover, this thesis was conducted at the end of the low-flow season to capture the most stable (i.e., flow stability) and autochthonous period of the year (autochthonous resources peak because of stable conditions while allochthonous food resources are minimum right before leaf litter fall in autumn).

Overall, we can conclude that land cover change had a strong impact on the stream energy flow pathways, food web composition and size structure and ecosystem multifunctionality. These impacts were caused through the alteration of the origin, quantity and quality of the organic matter that constitutes the food resource for stream communities and physical and chemical fluvial components.

Following, we present the general conclusions for each chapter of the thesis:

Chapter III. No land cover legacy in fluvial dissolved organic matter

- Land cover in the riparian area determined the quantity and composition of DOM in headwater streams, despite the overall dominance of terrestrial compounds in these streams. In more forested catchments, more oxygenated and slightly more aromatic terrestrial DOM reached the streams, what was associated to the increase in tannins and polycyclic aromatic compounds and the decline of lignin and younger soil and fresh vegetation materials as oxygen-poor aliphatic compounds. Hence, vegetation composition nearby the stream was the main factor defining the fluvial DOM properties, what evidenced a low hydrological connection between the catchment and the stream in low flow conditions.

- Slope in the riparian area strongly influenced the DOM reaching the fluvial ecosystem. A greater DOM quantity, characterized by the dominance of aromatic and humic compounds but less lignin and in-stream produced or fresh vegetation derived compounds was present in streams draining catchments with gentle hill slopes. This effect was associated to a greater organic matter accumulation and degradation and water residence time in catchments soils.
- Historic land cover showed no effect on fluvial DOM properties, what pointed to an absence of land cover legacy in low flow conditions. This absence of land cover legacy effect could be associate to the rapid vegetation recovery after land abandonment and the dominance of vegetation composition, in opposition to soil organic matter, in defining DOM properties in low flows.

Chapter IV. Catchment land cover influences macroinvertebrate food web structure and energy flow pathways

- Land cover determined food resource type available for stream communities, despite the overall dominance of allochthonous food resources in headwater mountainous streams. Grassland dominated streams were characterized by a higher proportion of autochthonous food resources whereas forested streams showed a higher proportion of allochthonous resources. Nevertheless, the quantity of allochthonous food resources also depended on catchment hill slopes, as gentle hill slopes limited leaf litter transport from valley sides into streams.
- The response of macroinvertebrate food resource assimilation to variation in food resources with land cover change differed between feeding groups. Detritivores showed a fixed assimilation of allochthonous resources independent of resource quantity, while omnivore assimilation was determined by the dominant food resource, what demonstrates that feeding mode is a key trait in determining organism adaptation capacity to variations in food resources.
- The differential feeding behavior among feeding groups led to the increased in detritivores and carnivores biomass with forest cover while the omnivore biomass was maintained. This response was reflected in the food web structure. In more forested streams, community biomass was more equally distributed among the organisms composing the food webs, what suggests that community had less dominant taxa.
- Land cover, through the alteration of food type and quantity, determined community resource assimilation and energy flow pathways in streams. Allochthonous food resource assimilation and biomass sustained by allochthonous food resources increased

with forest cover. However, community mean assimilation differed from the actual biomass sustained by a resource (i.e. energy flow pathway), being the biomass sustained by a resource considerably more autochthonous in streams dominated by autochthonous food resources. This demonstrates that organism assimilation data only provides a measure of resource assimilation preference and that a combination of food resource assimilation and organism biomass estimates is necessary to determine energy flow pathways.

Chapter V. The effect of forest cover loss on stream macroinvertebrate community size structure

- Community size spectra slopes remained invariant to the land cover change alteration of food resource type and quantity whereas size spectrum intercept increased with food resource quantity but did not respond to changes in food resource type. This evidenced that stream macroinvertebrate communities regulate their size structure to maintain the energy transfer efficiency to higher trophic levels adjusting its carrying capacity to total food resource quantity.
- Community size structure regulation was achieved by means of detritivore-omnivore substitution. While detritivores dominated in forested streams, omnivores dominated in streams draining grassland.
- The mechanisms that govern the response of macroinvertebrate size structure to alterations in food resource type differed between feeding groups. Omnivore size structure was modified only by body size distribution (numerical response: increase in density and consequently in biomass with forest loss), detritivore size structure was modified via shifts in both body size distribution (reduction of body size with forest loss) and taxonomic composition (taxonomic replacement of larger case-building trichopterans by smaller detritivores with forest loss). This indicates that the internal regulation of the community size structure is an interplay between the organism life strategy (k vs r strategy) and feeding mode (capacity to adapt to available food resources). Specifically, life strategy determines whether the response affects the number of individuals or their body size while feeding mode stablishes if these changes are accompanied by variations in taxonomic composition.

Chapter VI. Influence of forest cover on stream ecosystem multifunctionality

- Land cover controlled ecosystem functions related to ecosystem energetics through abiotic pathways, especially through minimum water temperature in the case of wood decomposition and light availability in the case of biofilm growth, primary production and ecosystem respiration. This dominance of abiotic factors completely outweighed the influence of biotic factors on ecosystem functions.

- Land cover strongly interacted with catchment area to define the level of stream canopy cover and thus, light availability. Specifically, light availability declined with higher canopy cover, which in turn increased with forest cover and declined with catchment area.
- Changes in land cover and catchment area led to the variation of a 50% of the ecosystem multifunctionality. This variation was the result of the increase in wood decomposition and the decline in primary production with forest cover and the increase in biofilm growth and primary production with catchment area.
- The interaction between land cover and catchment area evidenced the dominance of land cover in controlling ecosystem functioning in small headwater mountainous streams.

7.2 Future research

According to the objectives established in this thesis, we identified important consequences of land cover change to headwater mountainous streams, which provide new evidence-based knowledge necessary to design more effective management solutions for mitigating land cover change and conserve headwater stream ecosystem functioning and service provision. In addition, this thesis has also revealed the existence of knowledge gaps in which future research should focus. Below we describe some of these research lines.

- The absence of legacy effects of historic land cover on fluvial DOM might be related to similarities in soil organic matter composition, which was not analysed in this thesis. Hence, future research should focus on understanding how secondary succession processes affect soil organic matter properties across soil horizons and the most recent organic matter layer above the soil. Moreover, high and low flows activate different water flow paths through different soil horizons and only some horizons might differ in organic matter composition with land cover change. Thus, accounting for variation in DOM properties throughout the year would also be necessary to unravel whether there is a legacy effect of historic land cover and to understand the mechanism through which land cover change determines fluvial DOM.
- The variation in DOM composition with current land cover observed in this thesis implies a change in DOM quality and lability (the compounds have a different molecular structure and can have a different resistance to biological degradation). Since DOM is the most important energy and carbon source for stream heterotrophic bacteria, it is necessary to elucidate how the DOM utilization by microorganisms varies with DOM composition (e.g., DOM bioavailability) and the consequences for ecosystem functioning (e.g., microbial respiration).
- Stable isotope results evidenced that omnivore organisms assimilated a wide variety of food resources. Omnivores encompass various feeding modes (i.e., filter feeding, gathering and gatherer- scraping) that enable them to ingest diverse food resources. However, to achieve an effective assimilation of these food resources, omnivores might have a more diverse gut microbiota than other macroinvertebrate feeding groups with a more strict feeding behavior (e.g., detritivores). Future research should focus on characterizing macroinvertebrate gut microbiota and investigating how this varies among organisms with a strict or flexible feeding behavior. Moreover, the effective assimilation of resources with different nutritional quality can affect organism growth. Thus, future investigation should also investigate how macroinvertebrate secondary production varies with the assimilation of different food resources (from autochthonous to allochthonous).

- This thesis only describes the importance of food resources to food webs in the most autochthonous moment of the year. However, food resources and macroinvertebrate standing stock biomass vary throughout the year. Thus, is still necessary to investigate how the energy flow pathways vary with land cover changes on an annual scale to fully understand the importance of the different food resources. This could be achieved by combining food resource availability and organism food resource assimilation measures throughout the year, in concert with macroinvertebrate secondary production estimates.
- The results obtained in this thesis evidenced a community size structure adjustment to maintain the trophic efficiency in response to food resource alteration; however, size structure responses to disturbances are still poorly understood and remain largely contradictory. Thus, understanding how the size spectra of different communities behave in natural environmental gradients and respond to different types of disturbances is necessary to develop a stronger theoretical framework to be able to predict values of trophic efficiency and identify response patterns to disturbances. This would also allow testing whether the results obtained in this thesis are observed in other types of disturbances.
- The dominant energy channel in stream food webs varied with land cover change (i.e., autochthonous energy channel dominates in grassland streams while allochtonous energy channel in forested streams). Since these energy channels have different properties (e.g., organism life strategy; k vs r strategy taxa), food webs might respond differently to disturbances in terms of resilience, resistance or recovery times. Thus, future research should focus on identifying how food webs dominated by either autochthonous or allochthonous energy channels respond to common disturbances (i.e., flooding).
- The effects of land cover change on ecosystem multifunctionality have been characterized by functions that describe organic matter dynamics and ecosystem energetics, essentially, the two energy pathways that sustain river food webs. Hence, investigating how other ecosystem functions such as exoenzymatic activities, nutrient uptake, secondary production or insect emergence are affected by changes in land cover would provide a wider perspective of the effects of land cover change on ecosystem functioning.