



River ecosystem processes: A synthesis of approaches, criteria of use and sensitivity to environmental stressors



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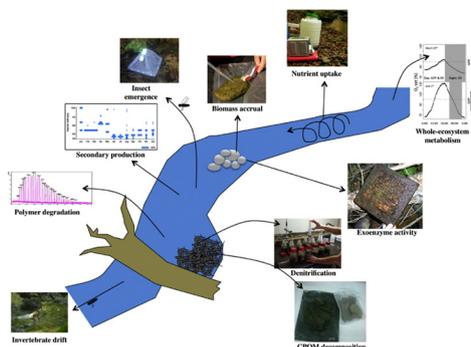
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HIGHLIGHTS

- River ecosystems should be assessed by their structure and functioning.
- Ecosystem functioning is rarely taken into account.
- A synthesis of river ecosystem processes is proposed.
- Approaches, criteria of use and sensitivity to stressors are described.
- Our synthesis contributes to a more functional view in river research and management.

GRAPHICAL ABSTRACT



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ABSTRACT

River ecosystems are subject to multiple stressors that affect their structure and functioning. Ecosystem structure refers to characteristics such as channel form, water quality or the composition of biological communities, whereas ecosystem functioning refers to processes such as metabolism, organic matter decomposition or secondary production. Structure and functioning respond in contrasting and complementary ways to environmental stressors. Moreover, assessing the response of ecosystem functioning to stressors is critical to understand the effects on the ecosystem services that produce direct benefits to humans. Yet, there is more information on structural than on functional parameters, and despite the many approaches available to measure river ecosystem processes, structural approaches are more widely used, especially in management. One reason for this

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discrepancy is the lack of synthetic studies analyzing river ecosystem functioning in a way that is useful for both scientists and managers. Here, we present a synthesis of key river ecosystem processes, which provides a description of the main characteristics of each process, including criteria guiding their measurement as well as their respective sensitivity to stressors. We also discuss the current limitations, potential improvements and future steps that the use of functional measures in rivers needs to face.

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1. Introduction

Ecosystem structure refers to the physical features of the ecosystem and the organisms (*i.e.* microbes, plants and animals) that inhabit it. Ecosystem functioning, on the other hand, refers to the set of processes that regulate the fluxes of energy and matter in ecosystems as a consequence of the joint activity of these organisms (Tilman et al., 2014). Thus, ecosystem structure and functioning can be viewed as the two sides of a same coin. In the case of rivers, structure encompasses variables such as channel form, water characteristics, or composition of the biological communities, whereas functioning refers to processes such as metabolism, organic matter decomposition and secondary production (Sandin and Solimini, 2009). Although structure and functioning influence each other, their relationship is not straightforward, and often one cannot be automatically inferred from the other (Cardinale et al., 2012). Furthermore, environmental stressors can affect structure and functioning in contrasting ways (Fig. 1) (Sandin and Solimini, 2009).

The concept of ecosystem functioning is gaining popularity among environmental scientists and managers alike (Jax, 2010). This interest is based on a number of reasons, among which two stand out. Firstly, one can be directly interested in ecosystem functioning, as it is the backbone of ecosystem services (Millennium Ecosystem Assessment, 2005), some of which can be translated into monetary benefits (Quintessence Consortium, 2016). For instance, the capacity of rivers to retain nutrients contributes to water purification, a relevant regulating service (Loomis et al., 2000). Likewise, fish production can be a key provisioning service for the local communities as well as a source of income derived from recreational angling (Hernández-Morcillo et al., 2013). In this sense, management actions may be fully or partially focused on fish production, metabolism and nutrient cycling (Bunn et al., 2010; Kupilas et al., 2016; Lepori et al., 2005). Secondly, ecosystem functioning can be viewed as an integral component of ecological status. This is, for instance, the case of the EU Water Framework Directive (EC, 2000), which defines ecological status as “an expression of the quality of the structure and functioning of aquatic ecosystems associated with surface waters”.

Traditionally, many methods have been developed to characterize ecosystem structure, and incorporated into environmental assessment protocols. Despite the growing demand, however, much less progress has been made to develop and standardize methods to measure ecosystem functioning, or to incorporate them into the assessment of river ecological status (Palmer and Febria, 2012). Functional indicators of ecological status are still in their infancy (Bunn et al., 2010; Young et al., 2008) and are not the focus of this paper; still, we hold that promoting the measurement of ecosystem functioning will favor their development and implementation.

While researchers and managers recognize the importance of ecosystem functioning, water authorities remain in general reluctant to measure river ecosystem processes. The main reasons for reluctance are the widespread consideration of being too expensive, difficult to perform or interpret, or simply that these measurements yield results not directly applicable to management. We oppose to these objections, and hold that there is sufficient scientific knowledge to provide suitable and efficient functional measures, that may be tailored to the needs of the water authorities. Although some processes are complex to measure or require very specific equipment, others are not, and measurements could be performed straightforward in combination with the structural variables commonly assessed.

The aim of this paper is to present a synthesis of key river ecosystem processes. We provide a description of the main characteristics of each process, including criteria guiding their measurement as well as their respective sensitivity to stressors. We also discuss the current limitations, potential improvements and future steps that the use of functional measures in rivers needs to face. Our ultimate purpose is to contribute to the adoption of a more functional perspective in river research and management.

2. Classification of processes

The list of processes that can be measured in rivers is very long, and ranges from purely physical processes to others more biologically mediated (Palmer and Febria, 2012). Here, we focus exclusively on

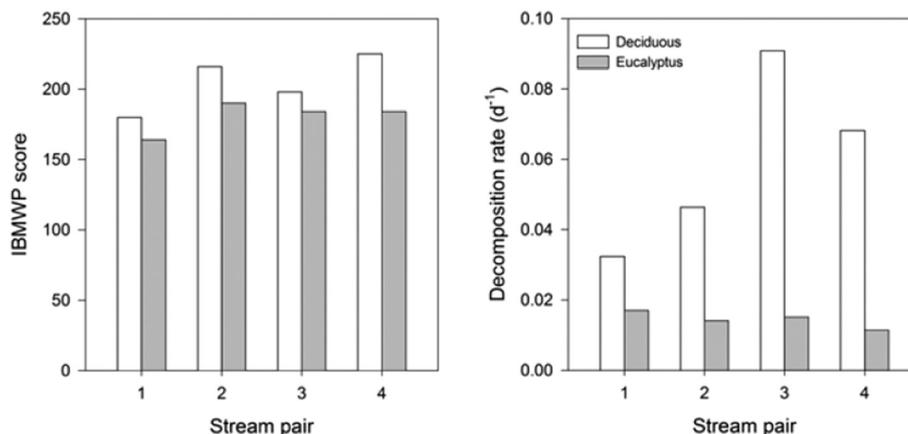


Fig. 1. Differential response of structural and functional metrics to an environmental stressor in 8 paired streams (*i.e.* pairs are very similar streams) in the northern Iberian Peninsula. Half of them were surrounded by native deciduous vegetation, the other half by Eucalyptus plantations. The small differences in IBMWP (Iberian Biological Monitoring Working Party) (left panel), an invertebrate-based biotic index, between deciduous and Eucalyptus streams, contrast with strong differences in decomposition rate of alder leaves measured in coarse mesh bags (right panel). Unpublished data provided by Eloisegi et al.

biologically-mediated processes occurring within the stream channel which cover most ecologically relevant processes in rivers. Therefore, we do not consider riparian or terrestrial processes such as riparian shading, or physical processes such as meander migration or hydraulic retention of organic matter, which certainly can influence fluxes of energy and matter but have no significant direct biotic contribution.

After thorough review of the literature, we compiled a total of nineteen processes. To organize these processes and make them more comparable, we classified them into five categories that cover different aspects of ecosystem functioning: i) organic matter decomposition, ii) nutrient cycling, iii) metabolism, iv) pollutant dynamics, and v) community dynamics. For each process, we briefly explain its general

Table 1

Key river ecosystem processes with their definition, most commonly used methods and relevant conceptual and methodological references.

Process	Definition	Conceptual references	Most commonly used methods	Methodological references
Organic matter decomposition				
Coarse particulate organic matter decomposition	Decomposition of coarse (>1 mm) organic matter particles (e.g. leaves, wood, fruits) driven by microbial decomposers and animal detritivores	(Gessner et al., 1999; Tank et al., 2010; Young et al., 2008)	Field assay (litter-bag method, cotton-strips, wooden sticks)	(Benfield, 2006; Graça et al., 2005)
Fine particulate organic matter decomposition	Decomposition of fine (<1 mm) particles (e.g. leaf pieces, feces) driven by microbial decomposers and animal detritivores	(Bundschuh and McKie, 2015; Tank et al., 2010)	Field assay with very fine mesh bags or laboratory bioassay	(Bonin et al., 2000; Mattingly, 1986; Yoshimura et al., 2008)
Dissolved organic matter uptake and degradation	Uptake and degradation of dissolved (<0.45 µm) organic matter (e.g. humic substances, proteins, sugars) driven by microbial heterotrophs	(Findlay and Sinsabaugh, 2003; Mineau et al., 2016; Prairie, 2008)	Field DOM additions or laboratory bioassay	(Fellman et al., 2009; Kaplan and Newbold, 1995; Servais et al., 1987)
Exoenzymatic activities	Expression of microbial enzymes related to the acquisition of carbon and nutrients from organic matter	(Arnosti et al., 2014; Romani et al., 2012; Sinsabaugh and Follstad Shah, 2012)	Laboratory assay with fluorescence-linked artificial substrates	(Graça et al., 2005; Kemp et al., 1993; Romani et al., 2009)
Nutrient cycling				
Whole-ecosystem nutrient uptake	Uptake of nutrients, primarily by microbes and plants, at the reach-segment scale	(Mulholland and Webster, 2010; Newbold, 1996; Schlesinger and Bernhardt, 2013)	Field nutrient addition	(Covino et al., 2010; Martí and Sabater, 2009; Webster and Valett, 2006)
Compartment-specific nutrient uptake	Uptake of nutrients, primarily by microbes and plants, at the organism or community scale	(Dodds et al., 2004; Mulholland and Webster, 2010)	Field incubation in enclosures	(Hoellein et al., 2009; Reisinger et al., 2015; von Schiller et al., 2009)
Individual nutrient cycling processes	Individual processes within the cycle of a particular nutrient (e.g. nitrification, denitrification, N fixation)	(Mulholland and Webster, 2010; Schlesinger and Bernhardt, 2013)	Laboratory assay (nitrapyrin-inhibition method, DEA, acetylene-reduction method)	(Groffman et al., 2006; Kemp and Dodds, 2001; Marcarelli and Wurtsbaugh, 2006)
Metabolism				
Whole-ecosystem metabolism	The balance of energy created (primary production) and used (respiration) within a river reach	(Tank et al., 2010; Young et al., 2008)	Field diel oxygen method	(Bott, 2006; Demars et al., 2015)
Compartment-specific metabolism	The balance of energy at the organism or community scale	(Tank et al., 2010)	Field incubation in enclosures	(Bott et al., 1997, 1978)
Biomass accrual	The gain in biomass of primary producers over time	(Biggs, 1996)	Field incubation on substrate	(Bowden et al., 2006; Lowe and Laliberte, 2006; Steinman et al., 1996)
Pollutant dynamics				
Whole-ecosystem dissolved pollutant attenuation	The capacity of the river to attenuate dissolved pollutants (e.g. pharmaceuticals, metals) in transport	(Rivera-Utrilla et al., 2013)	Field mass balance or addition	(Writer et al., 2011)
Compartment-specific dissolved pollutant uptake	The capacity of an organism or community to take up and bioaccumulate dissolved pollutants	(Peters et al., 2013; Zenker et al., 2014)	Field or laboratory incubation in enclosures	(Arnot and Gobas, 2006; Van Geest et al., 2010)
Solid and adsorbed pollutant degradation	Degradation of solid pollutants (e.g. microplastics) and pollutants adsorbed to solids (e.g. POPs adsorbed to sediments)	(Eerkes-Medrano et al., 2015; Gross and Kalra, 2002)	MALDI-TOF MS and MALDI-TOF Imaging	(Rivas et al., 2016a; Daniel Rivas et al., 2016b)
Community dynamics				
Invertebrate drift	Voluntary or accidental movement of invertebrates downstream with the current	(Brittain and Eikeland, 1988; Waters, 1972)	Field sampling with drift net	(Elliott, 1970; Smock, 2006)
Secondary production	Increase of invertebrate biomass through time	(Dolbeth et al., 2012; Huryn and Wallace, 2000)	Field sampling and application of instantaneous growth or size-frequency method	(Benke and Huryn, 2006)
Fish migration	Movement of fish from one part of a water body to another on a regular basis, usually to feed or reproduce	(Binder et al., 2011; Lucas et al., 2001)	Field sampling with capture-dependent or capture-independent methods	(Zale et al., 2012)
Recolonization	Reestablishment of a invertebrate or fish community in an area that was previously perturbed	(Detenbeck et al., 1992; Mackay, 1992)	Field substrate incubations (invertebrates) or monitoring (fish)	(Smock, 2006; Zale et al., 2012)
Insect emergence	Life cycle process by which flying insects leave the aquatic environment to search for a mate in the terrestrial environment	(Ballinger and Lake, 2006; Gratton and Zanden, 2009)	Field traps	(Smock, 2006)
Consumption and related physiological processes	The ingestion of food by an animal in a given time, and other related processes such as egestion, excretion, respiration and growth	(Benke and Huryn, 2006; Cummins and Klug, 1979)	Laboratory bioassay	(Canhoto et al., 2005; Lamberti et al., 2006; Peckarsky, 2006)

Table 2
Classification of river ecosystem processes according the spatial (from patch/habitat to river segment; based on Frissell et al. (1986)) and temporal (from hours to months/years) scales that they integrate. Complexity (level of expertise required) and cost (personnel, equipment and other expenses) of their implementation and the possibility of using automatic devices and historic data are also indicated. Bullet's size indicates importance at each particular scale, complexity and cost and possibility of being automated or of using historic data. No bullets are included when a given process is not relevant at the selected scale or the information indicated does not apply to the case at hand.

Process	Spatial scale			Temporal scale				Other			
	Patch/habitat	Reach	Segment	Hours	Days	Weeks	Months/years	Complexity	Cost	Automated?	Historic data?
Organic matter decomposition											
Coarse particulate organic matter decomposition	●	•				●	•	•	•		
Fine particulate organic matter decomposition	●				●	•		•	•		
Dissolved organic matter uptake and degradation	●	•			●	•		•	•		
Exoenzymatic activities	●			●				•	•		
Nutrient cycling											
Whole-ecosystem nutrient uptake		●	•	●	•			•	•	•	•
Compartment-specific nutrient uptake	●			●	•			•	•		
Individual nutrient cycling processes	●			●	•			•	•		
Metabolism											

characteristics and significance, and how it can be approached, providing the most relevant conceptual and methodological references (summarized in Table 1). To avoid overwhelming the reader with a long list of methods, we also provide a set of five criteria to help scientists and managers selecting the process that best suits their specific needs (summarized in Table 2).

The first criterion is the spatial (from patch/habitat to river segment; based on Frissell et al. (1986)) and the temporal (from hours to years) scales integrated by each process. The second criterion is complexity, which refers to the level of expertise required for measuring a certain process. Thus, low complexity processes can be measured by personnel with none or minimum expertise. Intermediate complexity implies the participation of personnel with some degree of training (*i.e.* basic taxonomic and/or analytical skills). High complexity corresponds to processes that can only be measured by expert personnel (*i.e.* high

taxonomic and/or chemical analysis skills). The third criterion is cost, and depends on the equipment, number of personnel as well as sampling and laboratory expenses required for obtaining a measure of a certain process at one site. Here, we consider cost to be low when the measurement does not require (i) specialized equipment (*e.g.* specific sensors, traps, electrofishing gear), (ii) a high number of personnel (*i.e.* more than two people for field and/or laboratory work), and (iii) intensive field and/or laboratory work (*i.e.* more than two field sampling campaigns and/or more than one full day of laboratory work). Intermediate or high cost implies, respectively, that the measurement requires at least one or two of the above conditions (*i.e.* (i), (ii) or (iii)). The complexity and cost of measuring a certain process vary depending on the specific method used. Here, we show the complexity and cost associated with the easiest and cheapest method that can be used to measure a particular process. We have chosen not to assign a monetary price to

Process	Spatial scale			Temporal scale				Other			
	Patch/habitat	Reach	Segment	Hours	Days	Weeks	Months/years	Complexity	Cost	Automated?	Historic data?
Whole-ecosystem metabolism		●	●	●	●	●	●	●	●	●	●
Compartment-specific metabolism	●			●	●			●	●		
Biomass accrual	●	●		●	●	●	●	●	●		
Pollutant dynamics											
Whole-ecosystem dissolved pollutant attenuation		●	●	●	●			●	●		
Compartment-specific dissolved pollutant uptake	●			●	●	●		●	●		
Solid and adsorbed pollutant degradation	●				●	●	●	●	●		
Community dynamics											
Invertebrate drift	●	●		●	●			●	●		
Secondary production		●				●	●	●	●		
Fish migration			●			●	●	●	●	●	●
Recolonization	●	●				●	●	●	●		
Insect emergence	●	●		●	●	●		●	●		
Consumption and related physiological processes	●	●		●	●	●		●	●		

each process measurement because the monetary cost of measuring a process is site-specific. The fourth criterion is the possibility to generate automatic measurements, which depends mainly on the potential implementation of automatic sensors to measure a certain process. Closely related to this is the fifth criterion, the possibility to use historic data, which refers to the potential of using past, often automated, measurements of a certain processes to analyze mid- to long-term temporal changes in that process.

Finally, we also included case studies from the scientific literature providing evidence of the sensitivity of each process to various environmental stressors (summarized in Table A.1). The list of stressors is not exhaustive and reflects proximate factors that can directly affect ecosystem processes and are strongly related to human activities. For some processes (e.g. coarse particulate organic matter decomposition, whole ecosystem metabolism) there is ample evidence of their sensitivity to most stressors. In contrast, for other processes (e.g. fine particulate organic matter decomposition, whole-ecosystem dissolved pollutant attenuation), we found no evidence of stressor effects in the literature, either because they are not sensitive or because more studies are still required. Therefore, the interest of this classification is twofold; on the one hand, it allows selecting the most appropriate processes likely to be affected by the stressors present in the area of study; on the other hand, it illustrates the areas where more research is needed.

2.1. Organic matter decomposition

2.1.1. Coarse particulate organic matter decomposition

The decomposition of coarse particulate organic matter (CPOM; organic particles > 1 mm) constitutes a major pathway of energy transfer and nutrient recycling in rivers (Tank et al., 2010). This process involves leaching of soluble compounds, microbial conditioning by fungi and bacteria, and fragmentation by invertebrate consumers or by physical abrasion. It eventually leads to conversion of CPOM into smaller particles, incorporation of organic carbon into secondary production, and mineralization (Gessner et al., 1999). Although decomposition of large wood can also be an important ecosystem process, here we focus on decomposition of CPOM of small size (e.g. leaf litter) which is the most commonly measured process. Litter decomposition has a high potential as an indicator of impaired river ecosystem functioning because it is an integrative process, it is affected by a wide range of environmental factors and it is relatively easy to measure (Chauvet et al., 2016). In fact, it has been used to evaluate the impact of stressors or the success of restoration efforts (Young et al., 2008), although not for routine monitoring of rivers, with few exceptions such as the Waikato Regional Council in New Zealand (Collier and Hamer, 2014).

CPOM decomposition measurements generally involve placing pre-weighed organic substrates in the stream and estimating the mass lost over time (Benfield, 2006; Graça et al., 2005). The substrates are retrieved either regularly over the course of the study or in a single retrieval. Generally, decomposition rates are determined by fitting mass-loss data to an exponential model, assuming a first-order irreversible reaction rate. The rate of mass loss is usually expressed as percent of the initial mass lost per day or per accumulated degree-day to compensate for temperature effects.

The “litter bag method”, the most classic approach that uses leaf litter as substrate, allows measuring the natural decomposition process and estimating the contribution of microorganism vs. invertebrates by combining bags of different mesh sizes (Benfield, 2006). As CPOM decomposition is sensitive to leaf quality, the intra- and inter-specific variability in chemical composition of leaf litter is a factor to take into account. Approaches with artificial substrates, such as wooden sticks (Arroita et al., 2012), cotton strips (Tiegs et al., 2013) or DECOTAB (Kampfraath et al., 2012), allow minimizing the possible confounding effects of the variability in the chemical composition of leaves, especially if the substrate is nutrient-free. In addition, they are less susceptible to artificial fragmentation and generally easier to transport. Nevertheless,

these approaches mostly measure microbial decomposition, and the degradation of some of these materials cannot be easily translated into the natural functioning of rivers.

Measurements of CPOM decomposition generally integrate the patch/habitat scale and a time frame from weeks to months, depending on the substrate type used (Table 2). The complexity of these measurements is low, requiring basic field and laboratory skills. The cost associated with this measurement is also low because it requires only basic equipment, minimum personnel and two field campaigns if a single retrieval is used. In general, small streams present fewer practical problems than do large ones. Complexity and costs can increase with some methods (e.g. DECOTAB). CPOM decomposition is highly sensitive to many environmental stressors, especially those that affect the performance of decomposers (Table A.1).

2.1.2. Fine particulate organic matter decomposition

Fine particulate organic matter (FPOM; organic particles from 0.45 µm–1 mm) in running waters consists of a mixture of particles, from feces to other organic fragments generated both in streams and in adjacent soils (Bundschuh and McKie, 2015). FPOM contributes substantially to the total pool of carbon in running waters, and it is an important carrier of nutrients, metals and other chemicals (Yoshimura et al., 2008). Moreover, it is a key food source for heterotrophic microbes and invertebrate collectors (Tank et al., 2010). However, despite the recognition of its importance in stream ecosystems, the dynamics of FPOM in running waters have been investigated less frequently than the dynamics of CPOM.

FPOM decomposition can be estimated from changes in mass through time following exposure to organisms. A known quantity of material is enclosed in very fine mesh bags, which are incubated in the field (Mattingly, 1986; Yoshimura et al., 2008). However, this technique is only appropriate to estimate microbial decomposition. Furthermore, very fine mesh restricts water exchange between the bag and the surrounding environment, thus FPOM decomposition rates may be underestimated. An alternative approach consists in offering a known quantity of FPOM to invertebrate communities in laboratory mesocosms. This approach allows examining the role of invertebrates; however, the stream community and environmental factors will not be representative of natural conditions. Microbial FPOM decomposition may also be indirectly estimated by measuring microbial respiration (Bonin et al., 2000; Yoshimura et al., 2008). A quantity of FPOM slurry is collected in the field and the respiration rate of microbes on the FPOM is measured as the dissolved oxygen decline over time.

Measurements of FPOM decomposition generally integrate the patch/habitat scale and a time frame from days to weeks, depending on the approach used (Table 2). The complexity of these measurements is intermediate due to the difficulties inherent to working with small particles (e.g. lost mass may lead to overestimation of decomposition rates). As in the case of CPOM, small streams present fewer practical problems than do large ones, especially for *in situ* measurements. The cost associated with the simplest approach (i.e. fine mesh bag experiments) is low because it requires only basic equipment, minimum personnel and two field campaigns if a single retrieval is used. A number of studies show how the quantity and quality of FPOM can be altered by environmental stressors, and we may expect similar effects of environmental stressors as for CPOM decomposition (Bundschuh and McKie, 2015). However, we have found no evidence about the direct effects of diverse environmental stressors on FPOM decomposition (Table A.1).

2.1.3. Dissolved organic matter uptake and degradation

Dissolved organic matter (DOM) is a complex mixture of organic compounds, which represents the largest pool of transported organic matter in running waters and plays an essential role in river ecosystem functioning (Prairie, 2008). Among several key functions, DOM supplies carbon and nitrogen for heterotrophic production, thereby affecting the transfer of energy to higher trophic levels. Measurements of DOM

uptake and degradation thus inform about the potential of DOM to be degraded or to be passively transported downstream (Findlay and Sinsabaugh, 2003). The study of DOM uptake and degradation has traditionally focused on the factors affecting the degradability of different quality materials. However, as for CPOM or FPOM decomposition, DOM decomposition measurements may be used to compare the carbon degradation capacity among communities and ecosystems.

DOM uptake and degradation is commonly estimated from changes in dissolved organic carbon (DOC) concentration following the exposure of water samples to microorganisms. The most common approach is to use bioassays, where DOM is offered to a microbial inoculum (Kaplan and Newbold, 1995; Servais et al., 1987). Thereby, DOC uptake and degradation is estimated from the difference between DOC concentration before and after incubation. In addition to DOC concentration, changes in other properties of DOM during the incubations may be analyzed, such as molecular size fractions (Fischer et al., 2002), optical properties (Catalán et al., 2013) and stable isotopes (Geeraert et al., 2016). Microbial biomass and composition may be also followed during the incubations.

Although less commonly done, DOM uptake and degradation may be also measured with whole-ecosystem approaches by using DOM additions similar to those carried out to determine whole-ecosystem nutrient uptake (see Section 2.2.1) (Mineau et al., 2016). These additions typically consist in elevating DOM concentrations (monomers or leachates) over background values using slug or constant-rate additions (Fellman et al., 2009) or by using additions of ^{13}C -labeled DOM (Kaplan et al., 2008). Some studies have also determined DOM uptake and degradation at the reach or segment scale through mass balance and modelling approaches (Wollheim et al., 2015).

Measurements of DOM decomposition integrate from patch/habitat to reach scale and a time frame from days to weeks, depending on the approach used (Table 2). The complexity and cost associated with the simplest approach (*i.e.* bioassays) are intermediate, requiring analytical skills and a DOC analyzer. Complexity and costs increase with other approaches and with the size of the stream, especially for *in situ* additions. The measurement of DOM decomposition is currently not being automated and historic data are not being used (Table 2); however, the use of automatic DOM sensors has high potential for *in situ* measurements of DOM uptake and degradation (Spencer et al., 2007). DOM uptake and degradation may be altered by shifts in the metabolism of microorganisms, which is affected by multiple environmental stressors (Table A.1).

2.1.4. Exoenzymatic activities

Exoenzymes are catalyst substances produced by prokaryotic and eukaryotic cells, including bacteria and fungi but also algae and protozoa (Arnosti et al., 2014; Sinsabaugh and Follstad Shah, 2012). Most exoenzymes are involved in the breakdown of large macromolecules into soluble monomers that can be taken up and metabolized. In running waters, exoenzymatic activities are strongly involved in the decomposition of allochthonous organic matter, the main source of energy and nutrients for heterotrophs (Romaní et al., 2012). Exoenzymatic activities have been incorporated as indicators into some river assessment protocols, for instance, to determine which nutrients are the limiting factors in river sediments (USEPA, 2007).

Exoenzymes are commonly measured in river biofilms growing on different substrates (*e.g.* fine sediments, rocks, wood) (Graça et al., 2005; Romaní et al., 2012, 2009). Some of the most frequently measured exoenzymes include lipase, leucine-aminopeptidase, β -glucosidase, β -xylosidase and alkaline phosphatase, all related to the acquisition of carbon or nutrients through the breakdown of organic molecules. Most exoenzymes can be determined spectrofluorometrically by using fluorescence-linked artificial substrates. For sampling, artificial colonizing surfaces are commonly preferred over natural surfaces. In any case, it is important to measure the area of the sampled surface and/or

microbial biomass to standardize exoenzymatic activity values and make them more comparable across sites and sampling times.

Measurements of exoenzymatic activities are done at the patch scale and integrate a time frame of hours or less (Table 2). The complexity and cost of these measurements are intermediate, requiring analytical skills and a spectrofluorometer (Table 2). Because they depend on both microbial metabolism and resource availability (Sinsabaugh and Shah, 2011), exoenzymatic activities can be affected by several environmental stressors (Table A.1).

2.2. Nutrient cycling

2.2.1. Whole-ecosystem nutrient uptake

Whole-ecosystem nutrient uptake describes the reach-scale process by which dissolved nutrients, principally the limiting nutrients nitrogen and phosphorus, are removed from the water column and immobilized in particulate form or transformed into gaseous forms that leave the system permanently (Newbold, 1996). It is strongly related to the self-purification capacity of running waters and constitutes an ecosystem service *per se* by reducing nutrient loads downstream (Schlesinger and Bernhardt, 2013). The mechanisms for nutrient uptake can be physical (*i.e.* residence time in benthic and hyporheic zones controlled by hydrodynamic and geomorphological characteristics), chemical (*i.e.* sorption) and biological (*i.e.* microbial immobilization, uptake by primary producers) (Mulholland and Webster, 2010). Whole-ecosystem nutrient uptake has been used to evaluate the effects of river restoration (Newcomer Johnson et al., 2016), but despite its potential use as an indicator of ecological status, it has not yet been implemented in management schemes.

Whole-ecosystem nutrient uptake is commonly measured using nutrient enrichments (Martí and Sabater, 2009; Webster and Valett, 2006). This approach basically consists in increasing the concentration of nutrients in the water column and measuring to what extent these nutrients are taken up along the study reach. The nutrients can be injected using constant rate or pulse additions. In constant rate additions, high nutrient concentrations resulting from the addition experiments may underestimate uptake rates at ambient nutrient levels (Mulholland et al., 2002). This can be avoided by using multiple enrichments (Payn et al., 2005). In contrast to the constant rate addition, the pulse addition does not create homogenous conditions along the reach and the contact time between nutrients and sediments is shorter, which may cause lower uptake. However, the pulse addition method is easier to implement, because it requires less equipment and sampling only at one downstream site. In addition, the pulse addition method can be used in larger streams where the constant rate additions are impracticable (Tank et al., 2008). Some methodological advances have occurred recently. Runkel (2007) suggested a transport-based approach for the analysis of time-series and steady-state data during tracer addition experiments. This approach involves fitting a transient storage model that includes uptake terms to identify uptake rate coefficients for both the main channel and storage zones. On the other hand, Covino et al. (2010) proposed a novel approach to quantify nutrient uptake kinetics from ambient to saturation. Heffernan and Cohen (2010) suggested an approach with great potential for continuous monitoring of whole-ecosystem assimilatory nutrient uptake using automated nutrient sensors.

Whole-ecosystem nutrient uptake can also be measured using stable isotope injections (Hall et al., 2009). This approach basically consists in enriching the stream with a stable isotope without altering significantly the background nutrient concentration. The approach is restricted to nitrogen because phosphorus has no stable isotope. Usually ^{15}N is used and it is injected together with a conservative tracer in a constant-rate injection. Because the background concentration is not altered, this approach allows measuring uptake rates at ambient conditions. Another great advantage is that it allows disentangling the contribution of the different assimilatory (*e.g.* algal and microbial uptake) and dissimilatory

(e.g. nitrification, denitrification) uptake pathways to nitrogen uptake, as well as describing how nitrogen is incorporated into the stream food web and mineralized back to the water column. The main disadvantage of this approach is its cost, especially of the stable isotope analyses.

The approaches explained above provide measures of the gross uptake of nutrients, but some researchers advocate for measuring net uptake instead or additionally to gross uptake (von Schiller et al., 2015). Net uptake represents the balance between gross uptake and release processes, and may thus be more representative of nutrient balances in river networks. It is measured by performing a mass balance of background nutrient concentrations along a reach or segment. By comparing net uptake and gross uptake measures it is possible to estimate release rates. Whole-ecosystem net uptake can also be approached using larger-scale models validated with nutrient data (Mineau et al., 2015).

Whole ecosystem nutrient uptake integrates from reach- to segment-scale and a time frame from hours to days; however, the introduction of automatic nutrient sensors may allow integrating wider time frames (Rode et al., 2016) (Table 2). The complexity and cost using the simplest approach (i.e. pulse additions) are intermediate, requiring personnel with field work and laboratory skills as well as equipment for the determination of solute concentrations (Table 2). The complexity and cost become highest if the stable isotope approach is used. Measurements in large river reaches may be unfeasible due to high costs and technical complexity. Automatization of net uptake measures and use of historic data is becoming a reality (Rode et al., 2016). Whole-ecosystem nutrient uptake is mainly controlled by the biomass and activity of primary producers and microbes; therefore, nutrient uptake is strongly affected by multiple environmental stressors (Table A.1).

2.2.2. Compartment-specific nutrient uptake

Compartment-specific nutrient uptake describes the uptake of nutrients by a compartment of the ecosystem (e.g. biofilm on rocks, biofilm on leaf litter, macrophytes) (Mulholland and Webster, 2010). If measured in several compartments it may allow disentangling the specific contribution of a particular compartment to whole-ecosystem nutrient uptake (Dodds et al., 2004; Hoellein et al., 2009). Although the focus is on assimilatory uptake, other dissimilatory uptake processes (e.g. nitrification, denitrification) or abiotic uptake processes (e.g. sorption) can be integrated in the measurements. The response of compartment-specific uptake to several environmental stressors (Table A.1) makes it a potentially interesting functional indicator; however, this application has been poorly explored (Proia et al., 2017).

Compartment-specific nutrient uptake is commonly measured with microcosm incubations of benthic (Hoellein et al., 2009) or planktonic (Reisinger et al., 2015) compartments. This approach consists in enclosing the compartment in chambers or bottles, and measuring nutrient concentrations at the beginning and end of the incubation. These incubations are commonly used to determine the uptake of different forms of nitrogen or phosphorus. A control treatment (e.g. water without benthic compartment) may also be used. The incubations can be performed *in situ* or in the laboratory. The characteristics of the chamber (size, shape, material) depend on the compartment to be studied. It is recommended to keep water circulation, with aquarium pumps when chambers are big, or with magnetic stir bars when they are smaller. Either natural or artificial substrata may be used for the incubations, depending on the objectives. Nutrient uptake measurements may be coupled to metabolic measurements if dissolved oxygen dynamics are followed in parallel (see Section 2.3.2) (Hoellein et al., 2009). An alternative but more costly way to determine compartment-specific nutrient uptake is by determining the isotopic content of particular compartments during stable isotope additions (von Schiller et al., 2009).

Compartment-specific uptake integrates the patch/habitat scale, and a time frame from hours to days (Table 2). The complexity and cost of measurements using non-isotopic approaches is intermediate, requiring personnel with analytical skills as well as equipment for the

determination of nutrient concentrations (Table 2). There are fewer studies on the effect of environmental stressors on compartment-specific than on whole-ecosystem nutrient uptake (Table A.1). In addition, the sensitivity of compartment-specific uptake to environmental stressors is highly dependent on the studied compartment (e.g. photoautotrophs vs. heterotrophs).

2.2.3. Individual nutrient cycling processes

Nutrient cycling at the whole-ecosystem scale comprises a wide range of processes (Mulholland and Webster, 2010; Schlesinger and Bernhardt, 2013). Within the term individual nutrient cycling processes, we include here nutrient cycling processes other than assimilatory uptake, which is the focus of Section 2.2.2. As a relevant example, we include dissimilatory uptake processes related to nitrogen cycling (i.e. nitrification, denitrification, fixation). Some of these measures have been used in the assessment of river restoration; for instance, to examine the improvement of in-stream nitrogen removal (Klocker et al., 2009). In addition, these measures show high potential as indicators of ecological status (Udy et al., 2006).

Nitrification (i.e. the transformation of ammonium to nitrate performed by some bacteria and archaea) is typically measured in the laboratory with the nitrapyrin-inhibition method (Kemp and Dodds, 2001). Briefly, a certain amount of sample is placed in “inhibited” and “reference” bottles along with stream water. In the inhibited bottle, nitrapyrin is added to block the conversion of ammonium to nitrate, thereby inhibiting nitrification. In the reference bottle nitrification can occur. The bottles are then incubated. After incubation, the slurry is sampled and extracted ammonium concentration is analyzed. To determine the nitrification rate, the difference in ammonium concentration between the inhibited and reference bottles is calculated and scaled by assay duration and the rates are expressed per unit sample area and/or per unit organic matter content. The incubation samples may be amended with additional ammonium if the aim is to quantify maximum potential rate.

Denitrification (i.e. the transformation of nitrate to gaseous nitrogen by some bacteria) is typically measured in the laboratory with denitrification enzyme activity (DEA) assays (Richardson et al., 2004). In performing a DEA assay, all factors that may limit denitrification are removed so the functioning enzymes can be fully expressed. Stream sediments and oxygen-free stream water are incubated under anaerobic conditions with pure acetylene to prevent the reduction of N_2O to N_2 . Non-limiting quantities of nitrate and available carbon are added and the slurry is continuously shaken to eliminate diffusion constraints. Finally, chloramphenicol is added to inhibit the synthesis of new enzymes, ensuring the observed N_2O production is exclusively a result of pre-existing enzymes. Chloramphenicol can also inhibit the expression of existing enzymes, especially when sampling occurs over several hours. For this reason, the assay is limited to few hours, during which at least two gas samples (initial and final) should be analyzed.

Nitrogen fixation (i.e. the conversion of atmospheric nitrogen into ammonia performed by N-fixing bacteria) can be measured by use of an acetylene-reduction assay (Marcarelli and Wurtsbaugh, 2006). Samples (usually biofilm slurries *in situ*) are placed into sealed bottles, injected with acetylene gas to achieve a headspace, and shaken. Standards that contain a known concentration of ethylene and blanks to control for non-biological production of ethylene are also run. After a few hours, gas samples are collected in serum vials, and ethylene and acetylene are measured on a gas chromatograph. Concentrations of ethylene in the samples are compared with the concentrations in the standards and the amount of N_2 fixed is calculated by using an ethylene: N_2 conversion ratio.

Measurements of individual nutrient cycling processes integrate the patch/habitat scale and last from hours to days (Table 2). Overall, their complexity and costs are intermediate, requiring personnel with analytical skills as well as specialized equipment. Individual N cycling

processes can be affected by all environmental stressors considered here (Table A.1).

2.3. Metabolism

2.3.1. Whole-ecosystem metabolism

Whole-ecosystem metabolism, *i.e.* the collective metabolic activity of organisms in the ecosystem, has key implications for nutrient, carbon and energy cycling and fluxes in river ecosystems, and determines their life-supporting capacity (Tank et al., 2010). Metabolism includes gross primary production (GPP), *i.e.* the synthesis of new organic matter from solar energy and inorganic carbon, and ecosystem respiration (ER), *i.e.* the oxidation of this organic matter to use its energy. Due to its integrative character and easiness to measure, also automatically, whole-ecosystem metabolism has a high potential as an indicator of ecological status (Young et al., 2008). However, with few exceptions (Collier and Hamer, 2014), metabolism is currently only measured in scientific-oriented studies to assess river restoration efforts or the effect of particular stressors (Kupilas et al., 2016).

Although there are diverse pathways to synthesize and oxidize organic matter, photosynthesis and aerobic respiration prevail in most running waters. Because both processes affect oxygen concentration, which is relatively easy to measure, most techniques to estimate whole-ecosystem metabolism are based on oxygen changes (Bott, 2006; Demars et al., 2015). However, reaeration, *i.e.* the exchange of oxygen with the atmosphere, affects oxygen concentration too, pushing it towards equilibrium with the atmosphere. Therefore, it is necessary to discern this physical force to estimate metabolism. Reaeration increases with the level of departure from oxygen saturation and with the permeability of the ecosystem, linked to its contact surface with the atmosphere and to turbulence. The permeability is defined by the gas exchange coefficient (k) and can be directly measured with additions of a gas (*e.g.* propane, SF₆) together with a conservative tracer (*e.g.* Cl⁻, Br⁻) (Jin et al., 2012), indirectly calculated by the night-time regression method (Hornberger and Kelly, 1975), or estimated using empirical formulae (Raymond et al., 2012). Alternatively, k can be estimated by modelling oxygen curves (see below).

Whole-ecosystem metabolism can be estimated following either the one-station or the two-station method. The former requires a homogeneous river reach upstream from the measurement site over a distance of at least $3v/k$ (where v is flow velocity), and enables to estimate metabolism with data collected by water quality monitoring stations. The latter requires a homogeneous river reach over a shorter distance (at least v/k), and allows estimating metabolism of a delimited stream reach (Reichert et al., 2009). However, the two-station method also requires measuring the variables at two sites (*i.e.* the beginning and the end of the reach) and the water travel time between the two sites. Using either method, GPP and ER can be directly calculated following the mass balance approach, for which several spreadsheets are available (*e.g.* Izagirre et al., 2007), or can be estimated by modelling oxygen curves.

Models are diverse, ranging from simple models that include only two (GPP and ER) or three (GPP, ER and k) parameters and a few input variables, to complex models that include more parameters and input variables (Demars et al., 2015). One must be aware of over parameterization, which can lead to equifinality problems (*i.e.* the same fitting can be reached by many potential parameter value combinations). Once the model is defined, parameters may be inferred by a number of techniques, from standard optimisation (*e.g.* minimisation of the sum of squares or other indicators of optimality) to Bayesian inference. Although computationally more intensive, Bayesian inference (Holtgrieve et al., 2010) provides a framework to make efficient use of information contained into observational variables to reduce parametric uncertainty associated to the prior information. Aquatic metabolism models may also include the possibility to include oxygen stable isotope ratios (Holtgrieve et al., 2010).

Whole-ecosystem metabolism integrates from the reach to the segment scale, and a time frame from hours to years (Table 2). Metabolism can be measured in reaches/segments with homogenous conditions. Stream reaches/segments with significant inflows, high turbulence, low diel oxygen variability or strongly anoxic conditions should be avoided. Rainy periods that may alter the gas exchange between the river and the atmosphere should also be avoided. The complexity of the most basic approach (*i.e.* the one-station method) is intermediate (Table 2). While it is relatively easy to obtain oxygen, temperature and hydraulic data, gas additions to measure k require operators with specific expertise. Parameter estimation can also range from simple (mass balance) to more sophisticated (Bayesian). The cost associated with the measurement of whole-ecosystem metabolism is also intermediate because it requires specialized equipment (*e.g.* dissolved oxygen sensor). One of the most interesting points with metabolism is that measurements of whole-ecosystem metabolism can be automated and historic data can be used to reconstruct past metabolic fluxes (Table 2). Both GPP and ER can be affected by multiple factors; thus, there is ample evidence on the sensitivity of whole-ecosystem metabolism to environmental stressors (Table A.1).

2.3.2. Compartment-specific metabolism

Compartment-specific metabolism describes the carbon balance of a particular compartment of the ecosystem (*e.g.* biofilm on rocks, fine sediment) (Tank et al., 2010). If measured in several compartments it may allow disentangling the specific contribution of a particular compartment to whole-ecosystem metabolism (Hoellein et al., 2009). Compartment-specific metabolism has been proposed as an indicator of ecological status (Bunn et al., 2010), and it has been incorporated into some monitoring schemes (Lazorchak et al., 1998).

Compartment-specific metabolism can be measured in benthic and planktonic compartments by following changes in dissolved oxygen concentration over time using closed systems (Bott et al., 1997). This approach consists in enclosing the compartment in chambers (or bottles), completely filling the chambers with water and closing them hermetically, and measuring oxygen concentration at the beginning and at the end of the incubation (or constantly over incubation time). Chamber characteristics (*i.e.* size, shape, material) depend on the compartment to be studied. It is recommended to keep controlled temperature and water re-circulating, with aquarium pumps when chambers are large, or with magnetic stir bars when they are small. Chambers filled only with water can be used as controls to correct the results with oxygen depletion caused by other mechanisms. Compartment gross primary production and respiration can be disentangled by combining incubations in light with incubations in dark: net compartment metabolism is obtained from incubations in light, compartment respiration from incubations in dark, and gross primary production from the difference between both.

Another interesting approach for obtaining measures of compartment-specific metabolism is the saturation pulse quenching analysis (Schreiber, 2004). This technique is based on the principle that light absorbed by photosynthetic pigments can be measured by pulse amplitude modulated (PAM) fluorometers. This energy can be driven to the photochemical energy conversion in photosynthetic reaction centers, be emitted as basal fluorescence, or be dissipated into heat. Some PAM instruments can assess the photosynthetic performance in biofilms by measuring the fluorescence for several wavelengths, exciting pigments with different absorption spectra, which are characteristic for defined algal classes. Additionally, as the three pathways of energy conversion are complementary, the fluorescence yield may serve as an indicator of time- and state-dependent changes in the relative rates of photosynthesis and heat dissipation. Besides, two different types of measures can be obtained, as basal fluorescence is biomass-related, whereas fluorescence caused by strong saturation pulses gives information on functioning.

Community-level physiological profiles (CLPPs) evaluate microbial functional diversity (*i.e.* their catabolic fingerprint) by assessing carbon substrate utilization. The CLPPs have been widely implemented with the Biolog™ method mostly in soils (Calbrix et al., 2005). A respirometric technique based on the analysis of the substrate-induced respiration (SIR) of whole-soil samples was investigated with a multiple carbon-source substrate for CLPP by Degens and Harris (1997). However, this method is still tedious, especially when assaying many microbial samples simultaneously in a multitude of bottles that have to be processed separately to measure the release of CO₂ (Campbell et al., 2003). Chapman et al. (2007) developed the MicroResp™ technique, an alternative method that combines the advantages of the Biolog™ technique, using the microplate system and those of the SIR approach with ability to measure CO₂ production during short-term incubation from a whole soil microbial community. The method produces both a measure of basal respiration (water only control) and the measures of responses to different carbon sources of differing chemical complexity (substrate induced respiration). Even if the method was initially designed for soils, it has also been used in river biofilms (Tlili et al., 2011).

Compartment-specific metabolism integrates a small spatial scale (*i.e.* the patch/habitat) and a short time frame (*i.e.* hours to few days) (Table 2). The complexity and cost associated with measuring compartment-specific metabolism using the simplest approach (*i.e.* closed chambers) are intermediate, requiring trained personnel with analytical skills as well as specialized equipment (*e.g.* dissolved oxygen sensor, closed chambers) (Table 2). Compartment-specific metabolism is sensitive to multiple environmental stressors (Table A.1), depending on the compartment studied.

2.3.3. Biomass accrual

Biomass accrual is defined as the gain in biomass of primary producers over time, and is thus strongly related to GPP (Biggs, 1996). The carbon fixed by primary producers is firstly accumulated as biomass and then follows diverse trophic routes (Tank et al., 2010). Therefore, by measuring the biomass accrual of primary producers, we are assessing the base that sustains aquatic food webs. Biomass accrual has been proposed as a functional indicator in some studies (Udy et al., 2006).

To estimate biomass accrual, measurements of biomass should be repeated over time during the growing period. Biomass accrual can be measured in benthic and planktonic compartments, from micro to macro scale, and at individual or community level. Benthic communities can be divided into two major groups: biofilm and macrophytes. Biofilm can be measured by scrapping cobbles (Biggs and Close, 1989), by deploying artificial inorganic substrata (Corcoll et al., 2014), or by taking a core sample from the sediment. For macrophytes, firstly, areal coverage of the plants is estimated for a given reach, and then, samples are harvested for posterior processing (Gücker et al., 2006). Biomass is estimated by measuring chlorophyll-*a*, when the autotrophic compartment cannot be separated from the heterotrophic one, or AFDM, when the autotrophic compartment can be easily separated (Biggs, 1987). Additionally, gene quantification can also be a good proxy of the biomass of producers. Copy numbers of specific gene from archaea, bacteria or microscopic algae can be estimated by quantitative real-time polymerase chain reaction (qPCR) amplification (Merbt et al., 2011).

Biomass accrual integrates the patch/habitat scale and a wide time frame (*i.e.* hours to months/years), depending on the compartment sampled and the approach used (Table 2). Biomass accrual is easy and cheap to measure, except in the case of molecular techniques, which require specialized equipment and a high level of expertise (Table 2). Biomass accrual has been shown to respond to most environmental stressors (Table A.1).

2.4. Pollutant dynamics

2.4.1. Whole-ecosystem dissolved pollutant attenuation

The in-stream attenuation of dissolved pollutants is the consequence of multiple biotic and abiotic processes (*e.g.* biotransformation, photolysis, sorption, hydrolysis, volatilization, oxidation, precipitation) and controls the impact of trace pollutants in aquatic environments (Rivera-Utrilla et al., 2013). Pollutant attenuation is related to the self-purification capacity of running waters and constitutes an ecosystem service itself by reducing pollutant loads to downstream ecosystems and users. Although many of the processes that drive in-stream attenuation are well known, most published studies have been performed in small laboratory-scale set-ups (Kwon and Armbrust, 2006) and usually studying one process (only photolysis, or only biodegradation), thus limiting the viability of translation of the results to field scale. Only a limited number of studies have quantified whole-ecosystem natural attenuation of trace pollutants (Acuña et al., 2015; Kunkel and Radke, 2008; Writer et al., 2011). Methods used for quantitative evaluation of dissolved pollutant attenuation in rivers generally combine conservative tracers or apply Lagrangian sampling, *i.e.* sample a specific parcel of water as it moves downstream (Writer et al., 2011). Some authors (*e.g.* Barber et al., 2006) use conservative substances commonly found in waste, such as boron, gadolinium, carbamazepine or venlafaxine as tracers of wastewater influence. A decrease in the concentration of trace pollutants relative to that of conservative tracers provides an indication of in-stream attenuation. Alternatively, dyes can be added to the effluent to trace its fate, best by combining with other tracers such as calcium or sulphate to account for the effect of dilution, which varies with the hydrological conditions. The Lagrangian sampling is the most rigorous method for linking hydrology and biogeochemical processes (Schwientek et al., 2016). A preliminary conservative tracer study is used to establish travel time between sampling locations, followed by introduction of a pulse of the conservative and trace organic compounds. Integrated samples are collected at each sampling location over a pre-determined interval (based on the preliminary dye study) that represents the time necessary for the introduced tracer mixture to pass each sampling location. The average concentration determined from the integrated composite sample is converted to mass using the measured stream discharge, and in-stream attenuation rate is determined for each compound by assuming first-order irreversible kinetics.

Whole-ecosystem dissolved pollutant attenuation can be measured in running waters of all sizes with homogenous conditions along the reach, provided the reach is sufficiently long (generally with travel times in the same order of magnitude as pollutant half-life). Large rivers with non-homogenous flow or high currents are challenging for sampling and determination of travel time. Stream reaches with rapidly varying discharge as well as large lateral or groundwater inflows are problematic and should be avoided.

In-stream attenuation of dissolved pollutants integrates from reach to segment scale and a time frame from hours to few days (Table 2). The complexity and cost of measuring whole-ecosystem dissolved pollutant attenuation are high. These measurements require expert personnel for field work and especially for laboratory work. In addition, they require specialized equipment, a large team, and intense field and laboratory work (trace analysis of dissolved pollutants). The difficulty of the field measurement increases with the size of the river. The performance of analytical measurements depends on the compounds selected, their number and complexity of analysis using advanced instrumental methods (typically liquid chromatography or gas chromatography coupled to mass spectrometry). It is known that the processes that drive in-stream attenuation depend on river characteristics such as flow, temperature, dissolved oxygen, pH, the hydrological exchange between surface and subsurface compartments (Kunkel and Radke, 2008). However, there is little empirical evidence on the effect of environmental stressors on whole-ecosystem pollutant attenuation (Table A.1).

2.4.2. Compartment-specific dissolved pollutant uptake

This process describes the uptake (abiotic sorption, biotic sorption and bioaccumulation) of pollutants in a particular compartment of the ecosystem, such as sediment, biofilm, invertebrates or fish (Peters et al., 2013; Zenker et al., 2014). *In situ* bioaccumulation tests are used to assess whether sediment-borne contaminants are potentially bioavailable to aquatic biota under field conditions. When measured in separate compartments (water column, sediment, aquatic organisms) it may allow disentangling the specific contribution of a particular compartment to whole-system pollutant dynamics.

Pollutant uptake by biofilm, plankton, macroinvertebrates or fish is typically measured by enclosing the organisms in chambers or bottles, and comparing their pollutant concentration at the beginning and at the end of the incubation (Arnot and Gobas, 2006; Van Geest et al., 2010). The incubations can be performed in the river or in the laboratory under constant or varying pollutant concentrations. Chamber characteristics such as size, shape and material depend on the species to be studied. In the laboratory, temperature and water circulation are kept controlled.

Compartment-specific dissolved pollutant uptake integrates a small spatial scale (*i.e.* the patch/habitat) and a wide time frame (*i.e.* from hours to weeks), depending on the compartment studied (Table 2). The complexity and cost associated with these measurements are high, mainly due to the chemical analyses of sediment/biota samples (Table 2). As in the case of whole-ecosystem dissolved pollutant attenuation, there is little empirical evidence on the effect of environmental stressors on compartment-specific dissolved pollutant uptake (Table A.1). However, it is known that sorption to sediments is influenced by temperature and sediment characteristics (*i.e.* particle size and organic matter content). On the other hand, in real situations the opposite process (pollutant resuspension) should be taken into consideration as well. In the case of aquatic organisms, uptake levels depend on factors such as their lipid content, individual size or life stage (Arnot and Gobas, 2006). The octanol-water partitioning coefficient (Kow), a measure of hydrophobicity, is often used to explain bioaccumulation and sorption (Mackay and Fraser, 2000).

2.4.3. Solid and adsorbed pollutant degradation

Many pollutants are found in running waters in solid or adsorbed form (Eerkes-Medrano et al., 2015; Gross and Kalra, 2002), and their degradation is an important ecosystem function. Solid and adsorbed pollutant degradation encompasses a variety of biotic and abiotic processes giving rise to heterogeneous patterns across the surface of the material. These patterns cannot be investigated using conventional analytic methods that only render an “average” picture of the changes that occurred in the sample form (Creclius et al., 2014). This is particularly the case when biotic processes are involved.

In this context, several approaches based on matrix-assisted laser desorption/ionization (*i.e.* MALDI-TOF MS and MALDI-TOF Imaging) are currently being developed (Rivas et al., 2016a; Rivas et al., 2016b). Specifically, the latter provides a rapid and efficient tool to study 2D spatial variations in the chemical composition of a polymer probe exposed to different aquatic environments. The sample is scanned in two dimensions at a preselected spatial resolution while the mass spectrum is recorded. Thus, the spatial distribution of a large amount of compounds (starting polymer fragments and its transformation products) is analyzed simultaneously without destroying the sample. It allows obtaining images based on single mass/charge ions or treating the whole spectral information using appropriate image processing tools.

The measurements using MALDI-TOF MS and MALDI-TOF Imaging integrate a small spatial scale (*i.e.* the patch/habitat), but cover a wide time frame (*i.e.* from days to several months), depending on the ambient conditions (Table 2). The complexity and costs associated with these measurements are high, because expert personnel, expensive equipment and intensive work are needed for laboratory preparation, equipment operation and image processing (Table 2). There is no

empirical evidence yet on the response of this process to environmental stressors (Table A.1).

2.5. Community dynamics

2.5.1. Invertebrate drift

Drift is the downstream transport of invertebrates with the water flow (Brittain and Eikeland, 1988; Waters, 1972). Invertebrates can drift both passively (when high flow events shear them from the substrate, or when dislodgement occurs while moving around) and actively (in search for food or different substrate, or to escape predators). As invertebrates affect nutrient cycling and energy flow in stream communities, drift can have important consequences for ecosystem functioning.

Drift is easily sampled in most streams by using drift nets (Elliott, 1970; Smock, 2006) set in the water for specified periods of time. Drift samples are commonly taken every few hours over a 24-hour period, as drift often shows diel variation. Data are often quantified as drift density (*i.e.* the number of invertebrates drifting per 100 m³ of water) or drift rate (*i.e.* the number of invertebrates passing a sampling point per hour).

Drift measurements integrate from patch/habitat to reach scale and from hours to days. Measuring drift is of intermediate complexity: data are easy to obtain and analyze, and sample processing requires taxonomic expertise only if species richness and/or composition are of interest, but not to determine total drift density. Measurements become more difficult as the size of the river increases (Table 2). The cost associated with drift measurements is generally intermediate because some specialized equipment is needed (*e.g.* drift net, stereomicroscope) (Table 2). Costs may increase if hiring taxonomic experts is required. Drift is sensitive to multiple environmental stressors that affect both hydromorphology and physicochemistry of rivers (Table A.1).

2.5.2. Secondary production

Secondary production is the generation of heterotrophic biomass through time (Huryn and Wallace, 2000). This process integrates density, biomass, individual growth rate, reproduction, survival and development time of individuals in a population. Secondary production is an ecologically significant variable as it estimates the role of a species in the transfer of nutrients and energy across the food web (Dolbeth et al., 2012). Here we focus on the secondary production of invertebrates.

There are two main approaches to estimate secondary production: a) the instantaneous growth method, and b) the size-frequency method (Benke and Huryn, 2006). The instantaneous growth method calculates secondary production from estimates of biomass and individual instantaneous growth rates (Benke and Huryn, 2006). The application of this method requires cohorts to be differentiated (*i.e.* synchronic life cycles). For each sampling time, size-frequency plots are constructed, and body mass is estimated. Instantaneous growth rate is estimated from the average body mass values in consecutive samplings, assuming an exponential growth rate. Production between consecutive sampling dates is calculated as the product between average biomass and instantaneous growth rate. Annual secondary production is the sum of monthly production values.

The size-frequency method does not require differentiating cohorts and, thus, it can be used to estimate secondary production of species with asynchronous life cycles (Benke and Huryn, 2006). It requires a repeated sampling program covering an entire year and estimates the secondary production from the distribution of body-size classes for that year. Body-size classes are established following development stages or categories of arbitrary size and regular range. Using body-mass distribution plots, the average survival curve for a hypothetical cohort is calculated. Then, average annual production is estimated as the sum of biomass lost between consecutive body size classes multiplied by the number of body mass categories considered and divided by the life cycle length.

Spatially, measurements of secondary production integrate the reach scale, whereas temporally, these measurements integrate from weeks to years (Table 2). Secondary production is usually estimated on an annual scale. The complexity and cost associated with these measurements are high (Table 2). Samples are easy to obtain, but some species are difficult to identify and secondary production can be tricky to determine. It requires a long sampling time and identifying invertebrates at the species level requires expertise (Table 2). In addition, some specialized equipment (e.g. sampling nets, stereomicroscope) is required. The complexity and cost increase with river size. Macroinvertebrate secondary production is sensitive to a range of natural and anthropogenic stressors (Table A.1).

2.5.3. Fish migration

Migration is defined as the regular, synchronized, repeated movement of animals from one place to another to feed, to reproduce, to avoid predators, to reach specific habitats for overwintering or to avoid hydrological extremes (Binder et al., 2011; Lucas et al., 2001). Fish migration usually occurs to reproductive (spawning), feeding or refuge sites, and can be either large-scale or small-scale migration (within a catchment). Many fish migrate, although the most conspicuous migrations are associated with species alternating marine and freshwater habitats. Migration forms a part of the regular behaviour of many fish species and is crucial for the survival of fish populations. Fish migration can be an important measure of the success of river connectivity restoration (Tummers et al., 2016).

Fish migration is commonly assessed by direct monitoring of fish movements and generally comprises the use of capture-dependent methods such as electrofishing, fish traps and nets, or the blocking method (Zale et al., 2012). Capture-independent methods (visual observation, video recording, automatic fish counters, telemetry and hydro-acoustic sonar) are also applicable in specific conditions.

Fish migration measurements integrate the river segment scale, and a time frame from weeks to months/years (Table 2). The complexity of this measurement is high, because it requires taxonomic expertise and specific fieldwork skills (Table 2). The associated cost is also high mainly due to the need for specialized equipment (e.g. electrofishing gear, traps, nets) and a large team of specialized personnel (Table 2). Complexity and cost increase with river size. The measurement of fish migration can be automated via installation of equipped fish passes (Table 2). Historic data can be used to follow temporal changes (Table 2). Fish migration is affected by many environmental stressors, especially those disrupting longitudinal connectivity, altering the natural flow regime and degrading hydromorphology (Table A.1).

2.5.4. Recolonization

Recolonization is the process whereby organisms become re-established in disturbed habitats from which they had previously disappeared, for any reason such as floods, droughts or toxic spills (Mackay, 1992). Here we focus on fish and invertebrates, although recolonization can be also measured for other types of organisms, including microbes and plants. In the case of fish, recolonization mainly depends on hydrologic barriers (Detenbeck et al., 1992). In the case of invertebrates, recolonization mainly depends on drift from upstream sites, on animals moving short distances by crawling or swimming, and on hyporheic and aerial sources (Mackay, 1992). Recolonization is a relevant process for the ecosystem because it strongly affects the communities and the associated processes. This process can be useful to assess, for example, river restoration success (Baumgartner and Robinson, 2017).

Fish recolonization can be quantified by monitoring methods such as electrofishing (Zale et al., 2012). Invertebrate recolonization is commonly measured with trays or baskets, with individual stones, or from benthic samples. They are placed into a stream at regular time intervals, collected at the end of the experiment, and number of organisms and species composition over time are determined (Smock, 2006). If the direction of colonization is of interest, upstream and downstream traps

that have only one opening can be used (Williams and Hynes, 1976). In larger streams, baskets can be used instead of trays, which are placed on the substrate and later collected using a dredge (Anderson and Mason, 1968). Data are commonly quantified as invertebrate densities, i.e. number of invertebrates per unit area (or volume for baskets in large rivers). An alternative method to trays consists in using natural stones found onsite. Individual stones are easier to manipulate than substrate trays, although they may not be representative of the whole invertebrate community (Doeg and Lake, 1981). Briefly, stones are removed from the stream and their surfaces are brushed with a scrubbing brush to remove all invertebrates and biofilm. Afterwards, the stones are left to dry, their surface area is estimated, and they are marked with paint and returned to the stream. Then, randomly selected stones are sampled on several sampling occasions. Sampling can be done by placing a D-framed net immediately downstream of the stone or a specific sampling device (Lake and Doeg, 1985).

Measurements of recolonization integrate from patch to reach scale, and a time frame from weeks to years (Table 2). The complexity and cost associated with the simplest approach (i.e. invertebrate recolonization) are intermediate, requiring trained personnel and specialized equipment (e.g. samplers, stereomicroscope) (Table 2). Complexity and costs are higher for fish than for invertebrate recolonization, and increase with river size. The rate of recolonization can be altered by environmental stressors that affect the distance to sources of colonists, the barriers to organism dispersal and the species-specific dispersal abilities (Table A.1).

2.5.5. Insect emergence

Emergence is the process by which adult flying insects leave the water to search for a mate in land (Ballinger and Lake, 2006; Gratton and Zanden, 2009). Insect emergence can show the abundance, health and production of aquatic populations. Additionally, it can affect terrestrial predators such as spiders, bats and swallows, and can also directly affect human well-being, when it reaches nuisance levels (Ibáñez et al., 2008).

Emergence can be measured with traps typically consisting of pyramidal mesh tents enclosing a certain area (Smock, 2006). A bottle partly filled with a preservative is attached to the apex of the trap. Emergence traps can be anchored to the streambed or placed floating in the water and fixed with metal bars driven into the sediments. The amount of time the traps are set in place depends on the objective of the study: only night emergence (to compare with bat activity, for instance), several days (when we expect rapid changes in environmental conditions or we are interested in describing peak emergence rates), or weeks (when the emergence rates are small and we are not interested in the description of life cycles but in the total emergence rate for a certain period).

Measurements of emergence integrate from patch to reach scale, and a time frame from hours to weeks (Table 2). The complexity of these measurements is intermediate: samples are easy to obtain, identifying the invertebrates involves medium/high taxonomic skills, and data processing is easy (Table 2). The complexity of measuring emergence increases in high currents, where setting the traps is challenging. The associated cost is intermediate because some specialized equipment is necessary (e.g. emergence traps, stereomicroscopes) (Table 2). Emergence is the outcome of the energetic balance of insects throughout their larval life and thus, any environmental stressor that can affect the larvae can affect the rate of emergence (Table A.1).

2.5.6. Consumption and related physiological processes

Consumption (= ingestion) rate is the quantity of food an animal eats per time unit. It can affect growth, production and survival of individuals. Part of the amount of food consumed (C) is digested and assimilated (A) and part is egested as feces (F). The assimilated food ($A = C - F$), in turn, is excreted as chemical waste products (U), respired for metabolism (R), or invested in growth (g) and reproduction (r). Thus,

animal production (P) = $A - U - R$ (Benke and Huryn, 2006). Consumption rates can be used to estimate the amount of matter and energy a consumer takes from a particular trophic level. When information about the bioenergetics of the consumer is available, this can be used to estimate how much energy it is able to transfer to higher trophic levels. Here, we focus on invertebrates, which cover a wide range of feeding strategies (Cummins, 1973).

Invertebrate consumption rates can be estimated either in the field or in the laboratory. Field trials offer more realistic estimates but are prone to failure because of unpredictable events such as floods or vandalism (Lamberti et al., 2006). Consumption rates can be estimated at the species level, but also for a functional feeding group. Other processes such as assimilation, egestion, excretion, respiration, growth or reproduction require controlled experiments in the laboratory. The general approach consists in isolating individuals in small enclosures, and adding pre-weighed amounts of food. Extra sets of enclosures without the consumer but with food are used as controls to measure changes in the amount of food due to other causes. Consumption is then calculated as the difference of food mass between the beginning and the end of the experiment, to which the change in control enclosures is subtracted. The consumption rate can be expressed as mass consumed per individual or per invertebrate mass over time (Canhoto et al., 2005). Enclosures can be set both in the field and in the laboratory (Peckarsky, 2006).

Laboratory controlled experiments in closed cages or microcosms can also be used to measure other physiological processes (Hauer and Resh, 2006; Naylor et al., 1989): egestion rate (collecting feces from the enclosures); excretion rate (by measuring the increase of nitrogen, phosphorus or ammonium in water microcosms); respiration rate (by means of respirometry); and growth rate (by estimating the increase of body mass during the experiment).

Consumption and related physiological processes integrate the patch and, to a lesser extent, the reach scale as well as a time frame from hours to weeks (Table 2). In general, daily consumption and growth rates are used, but field and laboratory experiments can be extended in time (rarely >2–3 months). Egestion, excretion and respiration processes are usually measured in minutes, hours or days. The complexity of measuring these processes is intermediate (Table 2). Consumption rate and other physiological processes require abundant replication and rigorous control. The little information published about assimilation, egestion, excretion and respiration of aquatic invertebrates are a handicap for these approaches. Overall, the complexity and costs associated with these measurements are intermediate, because a certain level of laboratory skills and specialized equipment (e.g. stereomicroscope, analytical balance, respirometer) are necessary (Table 2). Invertebrate consumption and related physiological processes are affected by a wide range of environmental stressors that directly or indirectly influence invertebrate physiology (Table A.1).

3. Strengths, limitations and future developments

The synthesis presented here adds to other works developed during the last years for the measurement of structural and functional variables in rivers (e.g. Birk et al., 2012; Elozegi and Sabater, 2009; Hauer and Lamberti, 2006). The major contribution of our synthesis is that it compiles and classifies key processes in a way that provides scientists and managers with key information on the significance of the process, how it can be approached, and a set of criteria (e.g. scale, complexity, cost) to seek the most appropriate measures to be applied in a particular study. Moreover, it facilitates comparisons among different processes and helps to identify areas where more research is needed. Thereby, it should help filling an important knowledge gap and facilitate a more comprehensive assessment and understanding of the ecology of river ecosystems. Nonetheless, we are also aware that as any other synthesis, our work is non-exhaustive, due to several reasons.

Firstly, our synthesis does not include all possible processes and environmental stressors. We have selected biologically-mediated

processes that are representative of different categories (i.e. from auto-ecology to biogeochemistry) and relatively easy to apply. We have also focused on stressors that are commonly found as a consequence of human pressures and that show general effects on river ecosystem functioning. Some stressors, such as invasive species, have been excluded because their effects are very difficult to generalize and are highly dependent on the location and the biology of each particular species. More physical not strictly biologically-mediated processes (e.g. meander migration, hydraulic retention) as well as other particular stressors could be incorporated in future syntheses.

Secondly, our synthesis does not explicitly quantify the response of each process to a given environmental stressor. As mentioned in the introduction, functional processes have been much less studied than structural variables in rivers, and thus, there is still little information on many stressor-process interactions (e.g. factors governing pollutant attenuation). However, there are many situations in which both scientists and managers can be interested in direct measurements of river ecosystem functioning. These may range from measuring the effects of a channel reconfiguration work on nutrient retention to assessing the efficiency of actions taken to reduce the emergence of a pest species. Our synthesis offers a broad overview on the main processes that can be measured in rivers and the rationale behind them, on the main potential and limitations of each process, as well as on the environmental context in which we can expect the processes to show a response. Future studies will likely attempt to quantify stressor effects on river ecosystem functioning through meta-analysis or similar approaches (Koricheva et al., 2013).

Finally, we intentionally refrain from developing threshold values or indicators of functional impairment or river ecological status. Although there are tentative guidelines for some of the processes included in our synthesis (e.g. Bunn et al., 2010; Gessner and Chauvet, 2002; Young et al., 2008), functional indicators of ecological status are still poorly developed. Yet, we hope this synthesis will advance their development and implementation by fostering the use of functional measures in scientific studies and river monitoring. Similarly, translating processes into ecosystem services that economically quantify their value should additionally encourage the incorporation of functional measures in river monitoring, conservation, restoration and mitigation programs (Acuña et al., 2013; Vermaat et al., 2015).

For allowing future improvement and expansion, the present synthesis is incorporated to the GLOBAQUA toolbox, developed in the frame of the EU-project GLOBAQUA (Navarro-Ortega et al., 2015). This toolbox is openly available on the Internet (<http://www.globaqua-project.eu/en/content/Toolbox.50>) and will be updated continuously through our own inputs and suggestions as well as new ideas and corrections from other users.

To sum up, despite being a cornerstone for management and conservation, ecosystem functioning is seldom measured except for some purely scientific purposes. The synthesis presented here provides both scientists and managers with a compilation of a wide array of ecosystem processes in running waters that cover different scales and environmental stressors, as well as a set of criteria to choose the most appropriate measure for each study. This synthesis should facilitate measuring different ecosystem processes in many locations, thereby promoting knowledge on the response of ecosystem functioning to environmental stressors. This is an essential step towards the improvement of future river management as well as to the design management programs that consider ecosystem functioning more explicitly.

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Appendix A. Supplementary data

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