Influence of river ecological condition on changes in physico-chemical water parameters along rivers
José Pedro Macedo do Vale Ramião
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Universidade do Minho
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Influência da condição ecológica dos rios sobre as alterações nos parâmetros físico-químicos ao longo dos rios

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Influence of river ecological condition on changes in physico-chemical water parameters along rivers

Abstract

Rivers supports key ecological processes and provides essential benefits to human welfare. Humans have been changing river processes and services by changing riparian land cover, river hydromorphology and by discharging pollutants on rivers. Excessive nutrient loadings have been severely impacting river processes and services, so improve the river capacity to buffer excessive nutrient loadings is determinant to human well-being by reducing the impact of pollutant discharges.

To assess the influence of river water chemistry and hydromorphology on changes in physico-chemical water parameters along rivers, six river segments with different trophic status and channel width were selected in the Ave River watershed (northwestern Portugal). The influence of riparian land cover on river habitat quality and physico-chemical water parameters was assessed by dividing each river segment in stretches based on land cover type, and then comparing habitat quality and changes in physico-chemical water parameters along rivers, among stretches. River segments were classified as mesotrophic (S1-S4), eutrophic (S5) and large (S6), and stretches from each river segment type were analyzed independently. The habitat quality was evaluated using the Fluvial Functional Index (FFI), the HABSCORE (RBP) and the Riparian Forest Quality Index (QBR). Changes in physico-chemical water parameters along rivers were determined measuring differences in ammonium, nitrate, phosphate and oxygen concentrations, and conductivity, pH and temperature between the two sampling sites defining each stretch.

Results demonstrated that stretches with more urban and agricultural land use had worse habitat quality than stretches with more natural land cover, regardless river segment type. Nitrate and phosphate concentration tended to increase along stretches with more agricultural and urban land use, but to decrease along stretches with more natural land cover, in all river segment type. Nitrate concentration decreased more along stretches with higher concentrations of nitrate and greater abundance of macrophytes, suggesting that river water chemistry and photosynthetic organisms have a strong influence on nitrate concentration in rivers.

Overall results demonstrated that i) the conversion of natural riparian areas to human land use can degrade river habitat quality and increase nutrient concentrations in rivers, with consequences for river ecosystem services and their economic value, and ii) changes in physico-chemical water parameters along rivers can be related to water chemistry, biota and hydromorphology of rivers.

Influência da condição ecológica dos rios sobre as alterações nos parâmetros físico-químicos ao longo dos rios

Resumo

Os rios suportam processos ecológicos chave e fornecem benefícios essenciais para o bem-estar humano. Os seres humanos têm vindo a alterar os processos e os serviços que os rios providenciam ao alterarem a cobertura do solo ripário, a hidromorfologia dos rios e por descarregarem poluentes nos rios. Cargas excessivas de nutrientes têm vindo a afectar severamente os processos e os serviços fornecidos pelos rios, pelo que melhorar a capacidade dos rios de moderar o excesso de nutrientes é determinante para o bem-estar humano ao reduzir os impactos das descargas de poluentes.

Para avaliar a influência da composição química da água e da hidromorfologia dos rios nas alterações dos parâmetros físico-químicos da água ao longo dos rios, seis segmentos de rio com diferentes estados tróficos e largura de canal foram seleccionados na bacia do Rio Ave (noroeste de Portugal). O efeito da cobertura do solo ripário sobre a qualidade do habitat e os parâmetros físico-químicos da água do rio foi avaliado dividindo cada segmento de rio em trechos de acordo com o tipo de cobertura de solo, e, posteriormente, comparando a qualidade do habitat e as alterações nos parâmetros físico-químicos da água ao longo dos rios, entre trechos. Os segmentos de rio foram classificados em mesotróficos (S1-S4), eutrófico (S5) e largo (S6), e os trechos de cada tipo de segmento de rio foram analisados de forma independente. A qualidade do habitat foi avaliada utilizando o Fluvial Functional Index (FFI), o HABSCORE (RBP) e o Riparian Forest Quality Index (QBR). As alterações nos parâmetros físico-químicos da água ao longo dos rios foram determinadas medindo as diferenças na concentração de amónia, nitrato, fosfato e oxigénio e na conductividade, pH e temperatura entre os dois locais de amostragem que definiam cada trecho.

Os resultados mostraram que os trechos com mais uso de solo urbano e agrícola tinham pior qualidade de habitat do que os trechos com mais cobertura de solo natural, independentemente do tipo de segmento de rio. As concentrações de nitrato e fosfato tenderam a aumentar ao longo de trechos com maior ocupação de solo agrícola e urbana, mas a diminuir ao longo de trechos com maior ocupação natural, em todo o tipo de segmentos de rio. A concentração de nitrato diminuiu mais ao longo de trechos com concentrações mais elevadas de nitrato e maior abundância de macrófitas, sugerindo que a composição química da água dos rios e os organismos fotossintéticos têm uma forte influência sobre a concentração de nitrato nos rios.

No seu conjunto, os resultados mostraram que i) a conversão de áreas ripícolas naturais para usos de solo humano pode degradar a qualidade do habitat dos rios e aumentar a carga de nutrientes, com consequências para os serviços de ecossistemas de rio e o seu valor económico, e que ii) as alterações nos parâmetros físico-químicos da água ao longo dos rios podem estar relacionadas com a composição química da água, as comunidades biológicas e a hidromorfologia dos rios.

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1. Introduction

1.1. Freshwater resources

1.1.1. Importance and benefits

Freshwater is a vital resource that sustains life and improves human welfare (Postel, 2005). Freshwater only account for 2.5% of the total water in the planet, of which 68.7% is stored in glaciers and 0.0064% flows as rivers (Arthurton et al., 2007). Freshwater ecosystems account for less than 0.01% of the planet surface area but support more than 100000 species (WWF, 2015), almost 6% of the actual described species (Dudgeon et al., 2006), 40% of all fish species (Lundberg et al., 2000), and one-third of the vertebrates (Strayer & Dudgeon, 2010).

Freshwaters such as rivers are able to provide important ecosystem services, including water purification, flood mitigation, erosion control, food and raw materials (Postel & Richter, 2003; Aylward et al., 2005; MA, 2005b). However, the value of freshwater ecosystems is frequently underestimated by failing to consider all the benefits they provide (Postel, 2008; Georgiou & Turner, 2012).

1.1.2. Threats and impacts

The global water cycle and freshwater ecosystems have been impacted due to climate change, pollutant discharges, land use change, habitat fragmentation and degradation and introduction of invasive alien species (Carpenter et al., 2011).

Between 1970 and 2000, the freshwater species index declined 55%, while the marine and terrestrial species index declined 25% (Loh et al., 2005). Since 1900, 123 freshwater animal species were extinct in North America, and future extinction rate of freshwater fauna is predicted to be of 4% per decade, which is five times higher than the rate for terrestrial fauna (Ricciardi & Rasmussen, 1999). Moreover, 10000 to 20000 freshwater species may be already extinct or imperiled due to human activities (Strayer, 2006; Strayer & Dudgeon, 2010).

Biodiversity affects the functioning and services provided by the ecosystems (Hooper et al., 2005; Díaz et al., 2006), so changing freshwater biodiversity can cause unexpected and exceedingly harmful impacts, by changing the provision of food, water, raw and medicinal materials, and the capacity of ecosystems to prevent natural disasters and diseases (Chivian, 2002; Naiman & Dudgeon, 2011; Sala et al., 2009).

1.1.3. Current status and future predictions

One billion people cannot access clean drinking water, 2.6 billion people has bad sanitation conditions (WHO/UNICEF, 2010; UNEP, 2011), 3 million people die every year of water-related diseases (Arthurton et al., 2007), and 80% of the world population is severely exposed to water security threats (Vörösmarty et al., 2010).

The amount of freshwater resources is declining as a result of exceeding and unsustainable withdrawals (Arthurton et al., 2007; Gleick et al., 2010), and changes in precipitation and evapotranspiration regimes (Milly et al., 2005). The total annual withdrawal was estimated to be about 3700 km^3, 71% due to agricultural activities, 21% to industry, and 9% to domestic purposes, which exceed in 5% to 25% the long term freshwater supply (Vörösmarty et al., 2005). Even overexploited, the water provision does not match the actual human demand, considering that 20% of the world population has no appreciable water supply, 65% shares low to moderate provisions, while just 15% live with relative water abundance (Vörösmarty et al., 2005).

Despite actual freshwater scarcity and unequal distribution, most of the current impacts and threats continue to decrease water quality, abundance and availability, causing market prices to raise, and thus provoking more social inequalities, cases of extreme poverty and essential resource deprivation (Arthurton et al., 2007; Vörösmarty et al., 2005). Furthermore, countries where water is more easily obtained do not necessarily present better natural water quality, since water became available to consumers after being expensively treated (Shannon et al., 2008). This means that richer countries are solely offsetting the stressor levels without solving their underlying causes (Vörösmarty et al., 2010). Therefore, ecosystem integrity should be primarily maintained and natural restoration plans preferably adopted (Arthington et al., 2009), rather than promoting technology, that although useful to solve particular problems may have high associated costs (Dearmont et al., 1998) and rarely accounts for ecosystem sustainability (Brauman et al., 2007; Gleick et al., 2014). Moreover, keeping ecosystems healthy contributes to reduce poverty and discrimination, since ecosystem degradation mostly affects the poorest nations that have less technology and economic resources to overcome the problems (Bass, 2006; Carpenter et al., 2006; Kumar, 2010).

Recent studies predict that the world population will reach 9 billion people by 2050 and exceed 10 billion in 2100 (UN, 2011), which will certainly cause a greater food and water demand (Alexandratos & Bruinsma, 2012). Freshwater demand is expected to increase 50% in 2025 in developing countries and 18% in developed countries (Arthurton et al., 2007). Climate predictions suggest that raising temperatures, severe precipitation or a greater variability of weather events will occur and may impact ecosystem integrity by affecting biological communities and altering ecosystem functioning (Sala et al., 2000; Woodward et al., 2010). Extreme precipitation regimes significantly affect river discharge, nutrient concentrations, dissolved oxygen and the capacity of ecosystems to maintain their functions (Lytle & Poff 2004; Poff & Zimmerman, 2010).

Temperature is one of the most relevant factors to freshwater ecosystems, since it influences metabolic activity (Brown et al., 2004; Yvon-Durocher et al., 2010b), nutrient cycles (Yvon-Durocher et al., 2010a), organism body size and individual abundances, with consequences to the structure of the food webs (Perkins et al., 2010). Moreover, climate change can affect freshwater ecosystems by changing the interaction with other pressures, such as invasive species (Rahel & Olden, 2008; Staudt et al., 2013) and nutrient loadings (Feuchtmayr et al., 2009; Fernandes et al., 2014).

Climate change will certainly affect ecosystem functions and services, but uncertainties still exist about the planet sustainability and its capacity to meet future human demands. Predictions demonstrate that measures need to be taken towards a more sustainable, equitable and efficient freshwater use at a global scale (Gleick, 2003). Considering the complexity of ecosystem processes and structures underlying the services provided by freshwater ecosystems, an increase in scientific knowledge is still needed to improve the ecosystem processes and structures allowing humans to survive (Reiss et al., 2009; Cardinale et al., 2012; Byrnes et al., 2014).

1.2. River ecosystems

1.2.1. Sources of organic matter for river functioning

Rivers are sensitive, dynamic and productive ecosystems that support a wide range of processes, functions and life forms (Allan, 1995). River processes and functioning depends on several factors, such as river hydromorphology (Elosegui et al., 2010; Poole, 2010; Elosegui & Sabater, 2013), biota (Wallace & Webster, 1996; Covich et al., 1999) and dominant sources of carbon and energy (Tank et al., 2010).

Organic matter in rivers can derive from autochthonous sources resulting from primary producers, and allochthonous sources resulting from terrestrial inputs of leaves, logs, wood debris or dissolved organic matter (Allan, 1995). Autochthonous organic matter in rivers depends on factors affecting the activity of primary producers, such as nutrient concentration, temperature and light availability, whereas allochthonous organic matter depends on the type and density of riparian vegetation, stream retentiveness, wind, precipitation, land slopes and seasonal fluctuations (Allan, 1995). Allochthonous organic matter includes coarse particulate organic matter (CPOM, > 1mm), fine particulate organic matter (FPOM, <1mm) and dissolved organic matter (DOM, <0,45 µ) (Tank et al., 2010). CPOM includes wood and leaves, whereas FPOM result from CPOM fragmentation, animal feces or physical flocculation of dissolved organic matter (Allan, 1995; Tank et al., 2010).

1.2.2. Leaf litter decomposition

Leaf litter decomposition is a key process in forested headwater streams, and may occur as result of several steps, including leaching of soluble compounds, physical abrasion, microbial decomposition and macroinvertebrate consumption (Allan, 1995; Hieber & Gessner, 2002). When leaves fall into streams, they are often colonized by terrestrial fungi, but fungal biomass on submerged leaves tends to increase due to colonization by aquatic fungi, mainly aquatic hyphomycetes (Bärlocher & Kendrick, 1974; Gessner & Schwoerbel, 1991; Gessner, 1997). Fungal colonization promotes leaf litter decomposition by releasing extracellular enzymes that degrade recalcitrant compounds, such as cellulose, hemicellulose and lignin (Subberkrop et al., 1983; Gessner et al., 2007). Leaf litter is also colonized by bacteria, but they may have lower influence than fungi at early stages of litter decomposition (Hieber & Gessner, 2002; Pascoal & Cássio, 2004; Abelho et al., 2005). Microbial colonization decreases recalcitrant compounds and increases nitrogen and phosphorous content on leaves, enhancing its palatability and nutritional value for invertebrate consumption (Subberkrop et al., 1983; Bärlocher, 1985). Macroinvertebrates can be responsible for decomposing 20 to 73% of the total leaf litter biomass entering the streams (Covich et al., 1999). The physical abrasion together with microbial and invertebrate shredder consumption transforms CPOM into FPOM that is an important food resource for invertebrate collectors (Covich et al., 1999; Graça, 2001).

Leaf litter decomposition is influenced by several factors such as the quantity and quality of leaf litter entering the streams (Lecerf et al., 2007; Ferreira et al., 2006; Fernandes et al., 2013), the diversity and activity of biological communities (Graça, 2001; Duarte et al., 2006), water temperature (Fernandes et al., 2009 and 2014), dissolved oxygen (Medeiros et al., 2009), pH (Mulholland et al., 1992; Dangles et al., 2004) and nutrient concentration (Gulis & Subberkrop, 2003; Gulis et al., 2006; Ferreira et al., 2014). Therefore, leaf litter decomposition rate has been proposed as a metric to assess stream ecosystem functioning (Gessner & Chauvet, 2002; Pascoal et al., 2003; Woodward et al., 2012).

1.2.3. River metabolism

River metabolism refers to the balance between primary production and respiration (P/R), and allows us to assess the relative importance of autochthonous and allochthonous organic matter to river functioning (Young et al., 2008; Griffiths et al., 2013).

Rivers continuously flows from the spring to the sea, with differences between upstream and downstream sections regarding physical and biotic attributes. This led to the development of the river continuum concept (Vannote et al., 1980). In forested headwater streams (1^{st} - 3^{rd} order), rivers are narrow and riparian vegetation is dense, which limits solar penetration and the growth of primary producers. Thus, in forested headwater streams allochthonous organic matter is the primary source of carbon and energy, and metabolism is mainly heterotrophic (P/R<1) due to a prevalence of respiration over primary production (Vannote et al., 1980; Tank et al., 2010). Forested headwater streams are generally characterized by a greater abundance of CPOM and a high prevalence of shredder invertebrates that fragment CPOM into FPOM (Vannote et al., 1980). Headwater streams are vital to maintain the functioning of the whole river basin, since they are critical habitats for macroinvertebrates, fish, amphibians and a range of unique species (Meyer et al., 2007; Clarke et al., 2008), and supply mid and high order streams with sediments, nutrients and biota (Gomi et al., 2002; Wipfli et al., 2007).

As rivers flow downstream (4^{th}–6^{th} orders), they increase their widths, and generally present an autotrophic metabolism (P/R>1) due to higher solar penetration that favors primary production over respiration (Vannote et al., 1980; Tank et al., 2010). Thus, mid-order streams tend to be dominated by grazer invertebrates that feed on primary producers, as well as collectors due to great abundances of FPOM transported from upstream (Vannote et al., 1980; Allan, 1995).

Large rivers have a heterotrophic metabolism (>7^{th} orders) because they have a greater channel depth and turbidity that limits primary production. Large rivers are dominated by invertebrate collectors that feed on FPOM transported from upstream (Vannote et al., 1980; Tank et al., 2010).

Stream metabolism and biological communities can change along seasons (Roberts et al., 2007) because i) leaves generally fall during autumn in temperate regions (Abelho & Graça, 1998) where higher abundance of invertebrate shredders is expected to occur (Graça, 2001); ii) light becomes more intense during spring and summer favoring autotrophic metabolism (Tank et al., 2010; Griffiths et al., 2013); and iii) rivers with high flow and less retention structures facilitate CPOM transport to downstream sites (Webster et al., 1999).

1.2.4. Macroinvertebrates

1.2.4.1. Diversity and functional traits

Macroinvertebrates are determinant to the functioning of freshwater ecosystems by participating in key processes such as carbon and nutrient cycling and by influencing stream habitat (Wallace & Webster, 1996; Covich et al., 1999). Macroinvertebrates participate in leaf litter decomposition, which is one of the main pathways for carbon and energy to enter the stream food webs (Wallace & Webster, 1996; Graça, 2001). Macroinvertebrates influence nutrient availability in streams through their feeding, excretion and burrowing activities (Vanni, 2002; Devine & Vanni, 2002) and serve as food for several aquatic and terrestrial organisms such as fishes and birds (Covich et al., 1999; Prather et al., 2013).

Macroinvertebrates through their structures and activities may act as ecosystem engineers, thereby influencing the diversity and types of in-stream habitats (Wallace & Webster, 1996; Moore, 2006). The shell of some macroinvertebrates can be colonized by benthic organisms and can be used as refuge by several preys, while burrower macroinvertebrates determine in-stream habitats through their activity (Wallace & Webster, 1996; Covich et al., 1999). By structuring the stream physical environment, macroinvertebrates indirectly affect the identity and diversity of streams biota, and so the functions and services that streams provides (Wallace & Webster, 1996; Prather et al., 2013).

Ephemeroptera, Plecoptera, Odonata, Diptera and Trichoptera are among the most representative orders of macroinvertebrates in streams (Graça, 2001). Macroinvertebrates taxa can be classified according to their feeding strategy as: scrapers that graze biofilms; shredders that feed on CPOM; gatherer and filter collectors that feed on FPOM either deposited on the streambed or suspended in the water column, respectively; and predators that prey other animals (Wallace & Webster, 1996; Graça, 2001). Functional feeding groups primarily refer to the strategies to acquire resources more than the food type (Graça, 2001). For instance, some filter collectors are omnivorous and shredders may ingest algae and associated microbiota during CPOM consumption, as well as living macrophytes, especially during spring and summer when the stock of leaf litter is reduced (Wallace & Webster, 1996).

Scrapers influence in-stream nutrient cycling and reduce periphyton biomass (Mulholland et al., 1991; Steinman et al., 1991), but they can also enhance algal growth and nutrient uptake by clearing senescent cells and reducing biofilm thickness (Mulholland et al., 1994).

Shredders tend to be more abundant in streams with greater retention capacities of CPOM, and have a great influence on nutrient dynamics by mineralizing organic matter and affecting microbial decomposer activity and biomass (Mulholland et al., 1985; Cheever & Webster, 2014).

Gatherer collectors are frequently the most abundant feeding group of macroinvertebrates in streams (Wallace & Webster, 1996). They can feed on different resource types like FPOM, algae, fecal material (Wallace & Webster, 1996), or bacteria as it was found for chironomids (Hall, 1995). Gatherer collectors can be responsible to recycle up to 70% of the total nitrogen retained in the streams back to primary producers as excreted ammonium (Grimm, 1988; Wallace & Webster, 1996).

Filter collectors have a variety of strategies to feed on suspended particles (Wallace & Webster, 1996). Some filter collectors like Simuliidae filter fine suspended materials, while others like Hydropsychidae capture larger particles as algae or small invertebrates (Wallace & Webster, 1996). Filter collectors remove particulate organic matter from the water column and have a great influence at local and river network scales, by changing food webs from pelagic to benthic dominated and by decreasing the amount of suspended detritus transported downstream (Covich et al., 1999; Prather et al., 2013). Filter collectors are determinant to improve river water quality by removing particles and contaminants from the water column (Prather et al., 2013). Filter collectors significantly depend on hydrological conditions as well as on the quantity and quality of suspended material (Wallace & Webster, 1996; Miserendino, 2007). In streams with high current velocity and abundant suspended material, filter collectors are frequently the dominant feeding group, since their feeding strategy allows them to capture resources without moving and spending much energy (Wallace & Webster, 1996).

Predators influence food webs through direct consumption of preys or non-lethal effects on the growth, reproduction and fitness of prey populations (Wallace & Webster, 1996). Predator abundance depends on several factors like the presence of prey, the abundance and complexity of prey habitats and refuges (Wallace & Webster, 1996), and the number and type of species they prey (strong or weak interactions) (Soulé et al., 2003; Soulé et al., 2005).

In addition to their feeding strategy, the habitat behavior of macroinvertebrates is also determinant for the influence they have on river ecosystems, especially on nutrient cycling and habitat structuring (Wallace & Webster, 1996; Vanni, 2002). Burrower macroinvertebrates, such as chironomids and oligochaets are particularly important to nutrient exchanges between the stream sediment and the overlaying water because of bioturbation (Svensson, 1997; Stief, 2013). Bioturbation increases ammonium released from the benthos (Fukuhara & Sakamoto, 1987) and sediment oxygenation, which then favor nitrification and phosphorous precipitation and adsorption, and facilitate nitrate to reach anoxic layers where denitrification may occur (Svensson et al., 2001; Tuominen et al., 1999; Stief & De Beer, 2006). Benthic macrofauna tend to increase the amount of dissolved inorganic nitrogen in the water column, because ammonium released from disturbed sediments overlap ammonium and nitrate depletion by higher nitrification and denitrification rates in the presence of benthic macrofauna (Stief, 2013).

1.2.4.2. Macroinvertebrates and river ecological condition

Macroinvertebrates have been used as bioindicators of river ecological condition (Birk et al., 2012; Clapcott et al., 2012) since different macroinvertebrates can have distinct sensitivities to pollutants and to changes in river hydromorphology and riparian land cover (INAG, 2009).

Several biotic indexes have been developed to assess river water quality based on the presence or absence of macroinvertebrate taxa that are sensitive to pollution, mainly eutrophication (Chapman, 1996; Mandaville, 2002). The Biological Monitoring Working Party Index (BMWP) was created in the United Kingdom to assess the water quality based on a score system where the most sensitive taxa of macroinvertebrates (at the family level) are assigned to higher values (Hawkes, 1988). The BMWP was later adapted to the Iberian communities of macroinvertebrates and became known as the Iberian Biological Monitoring Working Party Index – IBMWP (Alba-Tercedor & Sánchez-Ortega, 1988; Alba-Tercedor, 1996).

Although the use of biotic indices have provided reliable indication on river water quality, some authors have suggested the incorporation of functional metrics of river ecological condition, such as river metabolism (Young et al., 2008; Bernot et al., 2010), leaf litter decomposition rate (Pascoal et al., 2003; Lecerf et al., 2006; Woodward et al., 2012) or invertebrate functional traits (Bady et al., 2005; Compin & Céréghino, 2007; Miserendino & Masi, 2010). Information on invertebrate functional traits can aid to infer about sources of energy and carbon, energetic balance of food webs and the diversity of niches and habitats, which can help to detect the causes underlying the observed impacts and improve the assessment on river ecological condition. For instance, riparian deforestation increases light availability and primary producers development in the presence of high nutrient concentrations (Rosemond et al., 2000; Hill et al., 2009), which then led to greater abundances of macroinvertebrate grazers (Wallace & Gurtz, 1986; Miserendino, 2007). Miserendino & Pizzolon (2004) found that the species richness, density and biomass of macroinvertebrates was similar among streams with native riparian vegetation and colonized by exotic trees, but streams with native riparian vegetation had greater shredder abundance and biomass, whereas streams dominated by non-native riparian vegetation had more filter and gatherer collectors. This means that although not affecting species richness, density and biomass, the alteration of native riparian vegetation can alter detrital food webs by decreasing shredder abundance and CPOM fragmentation.

Assessing invertebrate functional traits can also provide important information on ecosystem functioning, since ecosystem productivity and resilience is strongly related to species functions and the diversity of traits (Elmqvist et al., 2003; Griffin et al., 2009). Indeed, some macroinvertebrates with close taxonomic designations can have different feeding and habitat behavior strategies, causing them to differently contribute to ecosystem processes, functions and services (Covich, 1999).

From the analysis of invertebrate functional traits is also possible to infer about the type and diversity of stream habitat, since each feeding strategy is favored by a specific habitat condition (Wallace & Webster, 1996). Perceiving the importance and habitat requirements of each invertebrate functional group can have important implications to stream restoration and management, since habitat modifications that homogenize physical structures can reduce the diversity of traits, and so the processes and functions they underlie (Larsen & Ormerod, 2010).

Therefore, invertebrate functions should also be considered to assess ecosystem condition, rather than just the diversity and sensitivity of invertebrate taxa to pollutants.

1.3. Riparian land cover

1.3.1. Riparian vegetation

Riparian vegetation has a great influence on river functioning by i) reducing water temperature (Johnson & Jones, 2000; Bowler et al., 2012) and solar penetration (Davies-Colley & Rutherford et al., 2004; Davies-Colley et al., 2009), ii) smoothing precipitation and then preventing greater disturbance of hillslope lands (Keim & Skaugset, 2003), iii) increasing river bank stability (Abernethy & Rutherfurd, 2000; Simon & Collison, 2002), iv) determining the quantity and quality of allochthonous organic matter that enters the streams (Dosskey & Bertsch, 1994; Graça & Canhoto, 2006), v) providing wood debris and structuring river channel morphology that determines the availability of terrestrial and in-stream habitats (Gregory et al., 1991; Sweeney et al., 2004), and vi) reducing pollutant loading in streams either by reducing surface runoff (Vidon et al., 2010; Wang et al., 2012), removing pollutants from surface and subsurface flow (Duchemin & Hoghe, 2009; Balestrini et al., 2011; Messer et al., 2012), reducing river bank erosion, and filtering or promoting pollutant processing in streams (Mander & Kimmel, 2007; Ranalli & Macalady, 2010).

Riparian vegetation can reduce the amount of sediments entering the streams from the adjacent lands by reducing runoff and trapping transported sediment (Liu et al., 2008a; Yuan et al., 2009). While doing so, riparian vegetation improves river ecological condition by preventing the input of fine sediment that would increase water turbidity and reduce the diversity of periphyton, macroinvertebrates and fishes (Nakamura & Yamada, 2005), and the input of sand sediments, that would reduce the habitat suitable for fishes and invertebrates by filling the spaces between gravel on the streambed (Nagasaka et al., 2005). Furthermore, by reducing sediment input, riparian vegetation may reduce the amount of phosphorous entering the streams, since it easily adsorbs to soil organic particles (Sekely et al., 2002; McKergow et al., 2003). Riparian buffers can remove up to 90% of sediments in surface flow (Lowrance et al., 1997; Dosskey et al., 2002) but the sediment trapping efficiency of riparian vegetation can be site specific, as it depends on the buffer width, vegetation type, soil properties, slope, runoff flow and the size of leached material (White et al., 2007; Liu et al., 2008a; Yuan et al., 2009). Sediment trapping efficiency appears to be mainly determined by the slope of riparian lands and the riparian buffer width, rather than the vegetation type (Liu et al., 2008a; Yuan et al., 2009).

Riparian vegetation can also reduce sediment inputs by increasing river banks stability and reducing erosion (Dosskey, 2001). Woody vegetation better stabilize hillslope lands from mass failure due to deeper and stronger root systems (Dosskey et al., 2010) whereas herbaceous vegetation better strengths the upper soil layers and does it more quickly than woods (Lyons et al., 2000), so the presence of both herbaceous and woody vegetation has been suggested to better stabilizes river banks (Simon & Collison, 2002).

Riparian vegetation further reduces pollutant inputs in rivers by removing nitrogen (Borin & Bigon, 2002; Lowrance & Shredin, 2005), phosphorous (Reed & Carpenter, 2002; Uusi-Kämppä, 2005) and pesticides (Arora et al., 2010; Otto et al., 2012) from surface and subsurface flow. Riparian vegetation enhances pollutant removal by i) increasing soil organic content, moisture and limiting oxygen availability that promote denitrification (Schnabel et al., 1997; Schade et al., 2001), ii) assimilating and storing nutrient in plant tissues (Mander & Kimmel, 2007; Ranalli & Macaladi, 2010), and iii) increasing litter content in riparian soils and then nutrient uptake by microbial decomposers during the first phases of leaf litter decomposition (Hefting et al., 2005). Riparian vegetation can assimilate up to 350 kg ha^{-1} year^{-1} of nitrogen and 50 kg ha^{-1} year^{-1} of phosphorous (Mander et al., 1997), wherein nutrient assimilation is higher for young and/or fast growing life stages that are developing their leaves and roots where N and P are highly concentrated (Mander & Kimmel, 2007; Dosskey et al., 2010). Furthermore, periodic harvesting can increase nutrient uptake by plants, so if properly managed, riparian buffers can reduce greater amounts of nutrients that otherwise could easily reach the streams (Hefting et al., 2005; Kelly et al., 2007).

Nutrient removal capacity of riparian vegetation can be difficult to predict since it depends on several factors, such as the width, continuity and vegetation type (Weller et all., 1998; Syversen, 2005; Ranalli & Macaladi, 2010), soil properties, water table deep, hydraulic regime, temperature, nutrients residence time and concentration (Lowrance et al., 1997; Willems et al., 1997), and microbial communities, oxygen concentration, moisture and carbon content of riparian soils (Polyakov et al., 2005; Dosskey et al., 2010). Therefore, the establishment of riparian buffer strips needs to be carefully planned to best reduce nutrients and other pollutants inputs (Dosskey et al., 2005; Tomer et al., 2009; Qiu, 2010).

Riparian buffer restoration needs to consider the sites physical conditions, but also the nutrient removal capacity among different vegetation types and/or species (Mander & Kimmel, 2007; Dosskey et al., 2010). Some found higher N removal by forest buffers due to their large roots and total biomass (Hefting et al., 2005), others found higher N removal by herbaceous buffers (Kuusemets et al., 2001), and non-significant differences were also reported between forest and herbaceous buffer strips (Sabater et al., 2003; Syversen, 2005). Indeed, riparian buffers with a combination of herbaceous and woody vegetation are known to have greater nutrient removal capacities than monocultures (Lee et al., 2003; Schultz et al., 2004; Mander et al., 2005).

Riparian vegetation can also affect river water quality by influencing in-stream nutrient processing (Sabater et al., 2000; Sweeney et al., 2004) and by moving stream water to riparian zones through the hyporheic zone, where nutrients can be removed by plant and microbial uptake and denitrification (Schade et al., 2005; Dosskey et al., 2010). Riparian vegetation determines in-stream nutrients processing by affecting stream metabolism through organic matter supply and solar penetration (Sabater et al., 2000). By providing organic matter, riparian vegetation favors microbial decomposers activity and denitrification (Piña-Ochoa & Álvarez-Cobelas, 2006; Dosskey et al., 2010). Microbial decomposers can affect nutrient concentrations in streams by uptaking dissolved inorganic nitrogen (DIN) and soluble reactive phosphorous (SRP) from the water column during microbial colonization and growth, and by releasing inorganic nutrients during organic matter mineralization (Webster et al., 2001; Webster et al., 2009). Riparian vegetation can also limit nutrient uptake by reducing solar penetration and thus photoautotrophic growth and activity (Sabater et al., 2000). Indeed, higher nutrient retention capacities were found in deforested streams due to greater abundance of photoautotrophic organisms (Sabater et al., 2000; Bernhardt et al., 2003).

Forested streams tend to have wider and shallower stream channels with rougher beds, more benthic habitats and lower current velocity than deforested or herbaceous streams (Sweeney et al., 2004; Dosskey et al., 2010). Furthermore, riparian trees provide more coarse debris to the stream channels than herbaceous vegetation, which reduces current velocity and streams bank erosion, and increases the diversity and availability of in-stream habitats for benthic fauna colonization (Sweeney et al., 2004; Dosskey et al., 2010). Therefore, riparian vegetation can also affect nutrient dynamics in streams by changing stream channel morphology and the diversity of in-stream habitats, since nutrients easily adsorb to bed sediments and are assimilated by microbial organisms attached to submerged surfaces (Jarvie et al., 2002a; Sweeney et al., 2004). For example, Cardinale (2011) demonstrated that stream mesocosms with a greater diversity of habitats had more diverse communities of algae and higher uptakes of nitrate than stream mesocosms with a lower diversity of habitats. The importance of community diversity to nutrients uptake had been demonstrating by Bracken & Stachowicz, (2006), who found that the uptake of nitrate and ammonium alone were not affected by algae diversity, but when these two were together, biodiverse assemblages remove 22% more than monocultures, since different algae species complement each other by using different N forms. Therefore, improving riparian vegetation and the diversity of in-stream habitats can help to buffer the impact of excessive nutrient loadings, by allowing more diverse communities to be established in streams (Cardinale, 2011).

1.3.2. Land cover change

Humans have been establishing near by rivers to take advantage from the services they provide, but the conversion of natural riparian areas to human land use has been impacting river processes and services by degrading river hydromorphology, riparian vegetation and by pollutant discharges (Allan, 2004; Carpenter et al., 2011).

The impact of land cover change on freshwaters can be difficult to predict, since it depends on several factors such as soil type, slope, remaining vegetation, historical land use and actual management practices (Gergel et al., 2002; Allan, 2004). Nevertheless, there are some common impacts associated with the conversion of natural riparian areas to human land use such as i) riparian deforestation, ii) nutrients and pollutant inputs, and ii) severe and sudden flow modifications caused by changes in superficial runoff and river channel morphology (Allan, 2004; Cooper et al., 2013).

The presence of urban and agricultural areas in riparian zones has been causing severe impacts on rivers (Carpenter et al., 1998; Carey et al., 2013), but the former have been associated with stronger impacts (Tran et al., 2010; Miserendino et al., 2011).

Agricultural activities tend to establish close to rivers since riparian soils can be more productive and the water for irrigation can be easier obtained. To meet population demands, agricultural activities are generally intensive, which means that soils are continuously used and the application of nutrients and pesticides are common practices (Tilman et al., 2002). As a consequence, agricultural soils tend to have lower quality and organic content, poor production capacities (Tilman et al., 2002) and less permeability (Bharati et al., 2002). In an attempt to overcome the soil low productive capacity, exceedingly amounts of fertilizers and pesticides are frequently used, which cause nutrients to accumulate in soils by surpassing the crops uptake capacity (Hooda et al., 2001; Cassman et al., 2002), and some pests to become resistant to pesticides (Palumbi, 2001; Zhang et al., 2007). Fertilizers and pesticides accumulated in agricultural soils can easily reach and impact rivers, since agricultural soils tend to be less permeable and thus facilitates runoff (Carpenter et al., 1998). The slope of the agricultural areas has a great influence on the impact of agricultural activities on river water quality, since agricultural impacts are mostly associated with non-point sources of pollution (Sliva & Williams, 2001; Pärn et al., 2012).

Phosphorous and nitrogen are commonly used as fertilizers in agricultural fields, but they can be differently transported from upland areas into rivers (Pärn et al., 2012). Phosphorous transport is mainly determined by surface flow and sediment runoff, since phosphorous tends to accumulate in the soils by easily adsorbing to soil particles (Jarvie et al., 2008; Pärn et al., 2012). Nitrate is more prone to leaching, so subsurface flow has a great influence on nitrate transport (Haag & Kaupenjohann, 2001; Pärn et al., 2012). However, nitrate leaching depends on soils properties such as water holding capacity, so surface flow can also determine nitrate transport in less permeable soils (Pärn et al., 2012). Ammonium transport is also affected by surface and subsurface flows, but ammonium is less prone to leaching than nitrate since it easily adsorb to soil particles (Pärn et al., 2012).

The impact of agricultural land cover on river water chemistry and biota has been documented for decades (Miserendino & Pizzolon, 2004; Casalí et al., 2010). However, agroecosystems provides essential benefits like food, bioenergy, pharmaceuticals, and may also produce some other relevant services such as soil and water quality regulation, carbon sequestration and cultural services (Swinton et al., 2007; Power, 2010), but it depends on the intensity and type of activities as on the management practices (Withers & Lord, 2002; Moore & Palmer, 2005). Therefore, sustainable agricultural practices may provide a greater variety of benefits, maintain good soil quality and crop productivity and then allow reducing the environmental and monetary costs associated with fertilizer and pesticide application.

Urban land cover can also impact river functioning and stability, but urbanization generally causes different hydromorphological changes and generates different pollutant types (Walsh et al., 2005). Urbanized areas have a dominance of impervious surfaces and tend to impact river hydromorphology more than agricultural areas (Walsh et al., 2005). This dominance of impervious surfaces cause urban land cover to severely impact river hydrological regime by strongly reducing evapotranspiration, soil infiltration rates and increasing runoff (Walsh et al., 2005).

Moreover, in urbanized areas there are different types of wastes and effluent sources from industrial, wastewater treatment plants or house buildings that can be directly discharged into rivers through sewer pipes, septic system or sanitary overflows (Walsh et al., 2005). Therefore, in urban areas, there is a mixture of point and non-point sources of pollutants, which concentrations and times of discharges on streams and rivers depend more on the rate of waste production than on irrigation or rainfall (Hatt et al., 2004; Carey et al., 2013).

1.4. Eutrophication

Humans have been changing nutrient cycles and increased nitrogen and phosphorous concentrations in ecosystems such as rivers, with consequences for rivers functioning and ecological condition (Chislock et al., 2013). Nutrients increase the activity and biomass of freshwater organisms (Grimm & Fisher, 1986; Francoeur, 2001), but exceeding concentrations and/or chronic exposure can impact freshwater food webs (Smith et al., 2006; Withers & Jarvie, 2008).

Eutrophication is characterized by an excessive growth of photosynthetic organisms associated with high nutrient loadings, and potentially harms freshwater ecosystems by changing food webs and creating hypoxic conditions (Hilton et al., 2006; Chislock et al., 2013). Eutrophication strongly depends on nitrogen and phosphorous concentrations in freshwater ecosystems, but also on other factors such as light penetration, grazer abundance, temperature, water depth or river flow (Dodds, 2007; Feuchtmayr et al., 2009). Eutrophic freshwaters tend to have foul-smelling phytoplankton and high abundance of noxious algae and cyanobacteria that can release neuro and hepatotoxins, reduce solar penetration, decrease oxygen concentration by algae tissues decomposition, and increase photosynthetic rates that deplete inorganic dissolved carbon and raise water pH (Smith et al., 2003; Smith & Schindler, 2009; Chislock et al., 2013).

Eutrophication potentially impact the services provided by freshwater ecosystems such as opportunities for recreation, water abstraction for agricultural, industrial and drinking purposes (Chislock et al., 2013), corresponding to an estimated cost of 2.2 billion dollars per year in USA (Dodds et al., 2008).

1.5. Nutrient dynamics in streams and rivers

Nutrient dynamics in streams have been a topic of major interest since the sixties, due to the anthropogenic impacts that have been changing nutrient concentrations in streams and so the processes and services they provide (Mulholland & Webster, 2010).

The importance of in-stream processes to nutrient concentrations in streams began to be noticed with studies observing i) increased primary productivity and heterotrophic activity after nutrient additions (Stockner & Shortreed, 1978; Elwood et al., 1981); ii) nutrient depletions from headwaters to downstream locations (Hill, 1979) and iii) uptake of added radiotracers along streams (Elwood & Nelson, 1972). Since then, the study of nutrient dynamics in streams has been improved through the upgrade of nutrient addition and radioisotope tracer techniques (Mulholland & Webster, 2010), the establishment of conceptual models for stream studies (Meyer et al., 1988; Stream Solute Workshop, 1990), and the implementation of national scale projects, such as the Lotic Intersite Nitrogen Experiment (LINX I & II), that aimed to assess nitrogen cycling in streams involving simulation modeling and field tracer ^{15}N additions (Mulholland & Webster, 2010).

Although studies have been demonstrating that in-stream processes can modify nutrient concentrations in streams, there is still the need to address i) the contribution of different processes to nutrient removal and the factors regulating such processes (Webster et al., 2003; Valett et al., 2008), ii) the effect of land cover change on nutrient removal capacity of streams (Von Schiller et al., 2008; Sobota et al., 2012) and iii) nutrient dynamics in large rivers (Ensign & Doyle, 2006; Tank et al., 2008).

1.5.1. Nitrogen

Nitrogen is a vital component of life since it makes part of biomolecules such as proteins and DNA that exist in all living organisms (Bernhard, 2010). It can be found in the atmosphere as nitrogen gas (N_2), in inorganic forms like ammonia (NH_3), nitrite (NO_2^-) and nitrate (NO_3^-), and in organic forms as amino acids and acid nucleic (Bernhard, 2010).

Nitrogen is mostly abundant as N_2, which accounts up to 80% of the Earth atmosphere (Vitousek et al., 1997). However, nitrogen tends to limit biological activity as it only becomes available to the organisms after being reduced to ammonia (NH_3) by a select group of prokaryotes containing the enzyme nitrogenase, or by abiotic processes such as lightning and the combustion of fossil fuels (Bernhard, 2010).

Nitrogen in streams can result from N_2 fixation (Marcarelli et al., 2008), geological weathering and soil runoff, precipitation, allochthonous organic matter input and anthropogenic discharges (Mulholland & Webster, 2010). Dissolved inorganic nitrogen (DIN) can be produced in streams by plant leaching, organic matter mineralization and consumer excretion (Mulholland & Webster, 2010). Dissolved inorganic nitrogen (DIN) can be removed from the stream water by i) autotrophs and heterotrophic microbes uptake (Webster et al., 2003; Roberts & Mulholland, 2007), ii) chemical precipitation and adsorption to benthic surfaces and/or suspended particles, iii) immobilization and adsorption to the hyporheic zone (Boulton et al., 2010), and iv) complexation to dissolved organic matter (DOM) (Mulholland & Webster, 2010). Nitrogen can be lost from streams by denitrification, downstream transport, and insect emergence (Mulholland & Webster, 2010).

Nitrification and denitrification processes are important sinks for ammonium and nitrate in streams, respectively (Mulholland et al., 2000; Alexander et al., 2009). Nitrification is an aerobic process where ammonia is first oxidized into nitrite and then into nitrate by chemolithoautotrophic bacteria (USEPA, 2002; Bernhard, 2010). Nitrification is an important process in aquatic ecosystems since ammonia can be toxic for most aquatic organisms (Sawyer, 2008; Bernhard, 2010). Denitrification is an anaerobic process where nitrate is reduced to N_2 in response to organic matter oxidation, and is performed by chemoorganotrophs bacteria that return bioavailable nitrogen to the atmosphere while producing other gases such as nitrous oxide (N_2O), that has a greenhouse effect (Bernhard, 2010).

Nitrification is favored under alkaline conditions and high ammonia and oxygen concentrations in streams (Kemp & Dodds, 2001; USEPA, 2002), while denitrification is favored under anoxic conditions with high nitrate concentration and organic content (Arango et al., 2007; Findlay et al., 2011). However, nitrification and denitrification can occur at the same site, since nitrification provides nitrate to denitrifying bacteria and leads to oxygen consumption that favors denitrification (Kemp & Dodds, 2002; Strauss et al., 2006).

Stream metabolism and rates of biological activity also affect ammonium and nitrate retention in streams, since autotrophs and heterotrophic microbes immobilize dissolved inorganic nitrogen (DIN) from the stream water (Webster et al., 2003; Fellows et al., 2006). Nitrate concentration in streams tends to be minimal in autumn and spring due to higher activity and nutrient demands by microbial decomposers and photosynthetic organisms, respectively (Mulholland, 2004; Roberts & Mulholland, 2007; Goodale et al., 2009). Indeed, some authors suggested that assimilation by plants, algae and microbial decomposers have a greater influence on ammonium and nitrate removal from stream water than nitrification and denitrification (Arango et al., 2008).

Different N forms may be distinctly affected by different biological processes and have different biotic demands, since ammonium uptake strongly depends on both autotrophs and heterotrophic microbes assimilation (Sabater et al., 2000; Hall & Tank, 2003), while nitrate uptake is mostly affected by photoautotrophs assimilation (Mulholland et al., 2006; Hall et al., 2009). Furthermore, nitrate was found to travel longer distances than ammonium before being removed from the water column in either small (Peterson et al., 2001) or large rivers (Tank et al., 2008). This may occur because i) ammonium is generally the preferable N form for both autotrophic and heterotrophic organisms (Dortch, 1990; Hildebrand, 2005), ii) ammonium does not have to be reduced by the organisms prior to incorporation and thus requires less energy to be assimilated (Kemp & Dodds, 2002), iii) nitrification can account for a large fraction of ammonium removal and leads to nitrate production (Mulholland et al., 2000), and iv) ammonium more readily adsorb to mineral surfaces than nitrate (Sabater et al., 2000; Hall et al., 2013).

Ammonium and nitrate retention in streams can be further affected by the stream water chemistry, hydromorphology, and by surface and groundwater exchanges. Nitrification, denitrification and biological assimilation of N increased with higher concentrations of ammonium and nitrate in streams (Dodds et al., 2002), but tended to be less efficient once become nutrient saturated (O'Brien et al., 2007; Mulholland et al., 2008). Longer water residence time and higher ratio of streambed area per total water volume favors ammonium and nitrate retention in streams, by increasing the contact between water and benthic surfaces where most biochemical and physical processes occur (Sweeney et al., 2004; Mulholland et al., 2009). Hyporheic zone can be a source or a sink of nitrate to stream water, depending on whether it is dominated by nitrification and organic matter mineralization or by higher denitrification rates, respectively (Jones & Holmes, 1996; Mulholland & Webster, 2010). Oxygen availability in hyporheic zone determines whether it is a source or a sink of nitrates by defining nitrification and denitrification rates (Jones & Holmes, 1996).

Several studies have been performed in streams dominated by urban and agricultural land use to assess the effect of land cover change on stream capacity to buffer increasing nutrient loadings (Bernot et al., 2006; Hall et al., 2009; Von Schiller et al., 2009). Some have demonstrated that urbanization reduces the stream nutrient retention capacity by i) decreasing fine benthic organic matter and thus microbial activity and nutrient demand (Meyer et al., 2005), ii) saturating nutrient concentrations (Newbold et al., 2006), and iii) decreasing channel complexity and primary producers demand due to algaecide discharges (Grimm et al., 2005).

However, the effect of land cover change on stream nutrient retention capacity is difficult to predict, since it depends on the stream attributes modified by human activities. In this sense, urban streams with high nutrient loadings and turbidity inhibiting photoautotrophs may have lower nutrient retention capacities than forested streams, while urban streams with high photosynthetic activities and moderate nutrient loadings may have higher nutrient retention capacities. Moreover, land cover change may have no effect on stream nutrient retention capacity, since urban and agricultural streams tend to have higher photosynthetic activities caused by riparian deforestation (increase nutrient retention potential) but increased nutrients loadings (decrease nutrient retention efficiency) (Hall et al., 2009).

Most studies on nutrient dynamics have been performed in small streams, where most ammonium and nitrate are thought to be retained in a watershed, since small tributaries account for most of the river networks length and have a higher ratio of streambed area per total water volume relatively to large rivers, which increase the contact between water and benthic surfaces (Alexander et al., 2000; Peterson et al., 2001). However, some studies on large rivers began to emerge and suggest that large rivers have similar uptake velocities of ammonium and nitrate as small streams, which means that large rivers can strongly contribute to nutrient retention in river networks by having long transport distances and water residence times associated with high nutrient loadings (Wollheim et al., 2006; Mulholland et al., 2008; Tank et al., 2008). Mulholland et al. (2008) suggested that the contribution of large rivers versus small streams to nitrate retention depends on nitrate concentration at the headwater streams. When headwaters have low nitrate loadings they have high retention efficiency and thus end up retaining most of nitrate entering the streams (Mulholland et al., 2008). On the other hand, when headwaters have moderate nitrate loadings they have lower retention efficiency and thus export more nitrate to large rivers where it can be retained (Mulholland et al., 2008).

1.5.2. Phosphorous

Phosphorous is also determinant to the organisms since it makes part of vital biomolecules, such as ATP and DNA, and controls key enzymatic reactions and metabolic pathways (Cordell, 2010). Phosphorous can be found in organic or inorganic forms, either dissolved or particulate (Reddy et al., 1999; Hyland et al., 2005). Phosphorous form determines its bioavailability since only dissolved inorganic phosphorous can be used by plants and microbes (Schachtman et al., 1998; Reddy et al., 1999). Phosphorous bioavailability increases with weathering of mineral phosphorous, desorption and mineralization of organic phosphorous, but decreases with precipitation, adsorption and immobilization into organic forms (Reddy et al., 1999; Hyland et al., 2005). Sources of dissolved inorganic phosphorous in streams include organic matter mineralization, consumer excretion, phosphorous releases from the sediments, and anthropogenic discharges (Withers & Jarvie, 2008).

Phosphorous dynamics in streams is largely determined by the stream metabolism and rates of biological activity (Mulholland, 1992; Hoellein et al., 2007). Indeed, concentration of soluble reactive phosphorous (SRP) in streams tends to be lower in autumn and spring due to microbial decomposers and photoautotrophs uptake, respectively (Mulholland & Hill, 1997; Mulholland, 2004). Biological uptake can affect SRP concentration in streams, but the contribution of autotrophs and heterotrophic microbes assimilation to the total SRP uptake depends on the stream structures (e.g. riparian vegetation) determining its dominant processes (Withers & Jarvie, 2008). Sabater et al. (2000) attributed higher retention efficiency of PO_4-P in deforested than in forested streams to higher algae activity and biomass in the former. Mulholland et al. (1985) demonstrated that the presence of shredders decrease SRP uptake by decreasing microbial decomposers biomass.

The retention of dissolved inorganic nitrogen (DIN) and SRP in streams are both determined by biotic demand, but the retention of SRP is more strongly affected by abiotic processes such as adsorption and desorption to/from suspended particles and benthos (Hall et al., 2013). Indeed, Jarvie et al. (2006b) suggested that bed sediment SRP-exchanges had a greater influence on SRP concentrations than biofilms uptake. Some interesting results demonstrated that bed sediment SRP-exchanges can help to maintain a more stable stream ecological condition, since under high SRP concentrations (downstream of sewage effluent discharges) sediments acted as a sink of SRP, while under lower SRP concentrations (effluent P-stripping) sediments acted as an important source of SRP to the stream biota (Jarvie et al., 2005; Jarvie et al., 2006b).

Phosphorous adsorption depends on sediment composition of organic matter, metal hydroxides, calcium and clay (Evans et al., 2004; Wang et al., 2009), but also on the stream water chemistry, namely pH and redox conditions, wherein reducing redox and/or acidic conditions increase SRP concentration in streams (Moore & Reddy, 1994). Photosynthetic organisms then have an indirect effect on SRP concentrations in streams by releasing oxygen and increasing water pH (carbon dioxide removal) during photosynthesis (Dodds, 2003; House, 2003). Some photoautotrophs, such as macrophytes, further affect phosphorous dynamics by reducing the flow rate and acting as substrate for epiphytic colonization (Withers & Jarvie, 2008).

Phosphorous dynamics in streams is also affected by the stream substrate type and hydromorphology (Withers & Jarvie, 2008). Permeable sediments facilitate water infiltration to the hyporheic zone where microbial activity and thus phosphorous demand can be high (Mulholland et al., 1997; Withers & Jarvie, 2008). Longer water residence times and higher ratio of streambed area per total water volume favors SRP retention in streams, by increasing the time and space for adsorption and/or assimilation by biota (Meals et al., 1999; Jarvie et al., 2002b). However, the effect of hydromorphology on phosphorous cycling depends on the stream water chemistry and biota demand (Hall et al., 2002; Doyle, 2003).

Phosphorus dynamics in large rivers has been less studied than the dynamics of nitrogen (Withers & Jarvie, 2008; Hall et al., 2013). Nutrient dynamics is more affected by the processes occurring at the water column in large rivers than in headwater streams, due to a lower ratio of streambed area per total water volume in the former (Withers & Jarvie, 2008). Thus, phosphorous dynamics is mainly affected by bed sediment adsorption and biofilm uptake in headwaters, and by suspended particle adsorption and phytoplankton uptake in large rivers (Withers & Jarvie, 2008).

Recent results suggest that SRP retention in large rivers can be lower than in small headwater streams (Hall et al., 2013). Hall et al. (2013) demonstrated that the ratio of inorganic nitrogen (N) to SRP retention increased with increasing river size, which may be explained by the lower ratio of streambed area per total water volume in large rivers that decrease the surface area to sorption/desorption processes that are determinant to SRP retention. However, Gibson & Meyer (2007) found that SRP retention in large rivers increased with greater amounts of total suspended solids, probably as a result of P adsorption, so phosphorous dynamics in large rivers may also be high, depending on biogeochemical and physical conditions.

1.6. Ecosystem services

1.6.1. The emergence of the Ecosystem services concept

Ecosystem services have been described as the benefits humans derive, directly or indirectly, from ecosystem functions (Costanza et al., 1997), or simply the benefits people obtain from ecosystems (MA, 2005a).

The concern to balance food supply and population size back to the time of ancient Greece (Feen, 1996), but the perception about the ecosystems importance to human well-being is perhaps even older. Population growth, increasing demands and resource scarcity have caused humans to move beyond the idea that ecosystems provide some benefits, to the need for perceiving how benefits could be improved to meet population demands (Daily et al., 1997; EASAC, 2009).

The ecosystem services concept emerged in the seventies (Westman, 1977) to raise public and decision makers awareness about the importance of nature conservancy to human welfare (Gómez-Baggethun et al., 2010), but it began to have a great impact in the nineties, as soon as it was associated with economics and natural capital concepts (Costanza et al., 1997). Since then, significant efforts have been made to integrate the ecosystem services concept into environmental planning (Fisher et al., 2008; Braat & De Groot, 2012) through i) the implementation of several international initiatives such as the Millennium Ecosystem Assessment (MA) and The Economics of Ecosystem and Biodiversity (TEEB), and ii) the development of methods and mapping techniques to assess ecosystem services (Egoh et al., 2012; Martínez-Harms & Balnavera, 2012).

However, the ecosystem services concept is not yet widely used by environmental planners (Egoh et al., 2007; Daily et al., 2009; Anton et al., 2010), which means that more than just developing new methods and mapping techniques, there is a need for simpler and widely useable definitions, classification frameworks and evaluation techniques, that would allow ecosystem services to be readily used in environmental planning (Cowling et al., 2008; Liu et al., 2008b; Trabucchi et al., 2012).

1.6.2. From ecosystems to human welfare

Ecosystems are able to provide several services, which are generally classified as provisioning, regulating, cultural and supporting ecosystem services (MA, 2005a). Provisioning services are the products obtained from ecosystems, such as water, food, raw material, freshwater and medicinal resources; regulating services are the benefits that ecosystems provides by regulating some of their processes, such as carbon sequestration, water purification, waste management and pest control; cultural services are the non-material benefits obtained from ecosystems such as the aesthetic, spiritual and recreational possibilities offered by ecosystems; and supporting services are the processes that allow ecosystems to supply other benefits such as nutrient cycling, soil formation, decomposition or primary production (MA, 2003; Kumar, 2010).

Ecosystem processes and functions underlie the benefits humans derive from nature, and the pathway that leads a service to be provided is generally described as a cascade framework (Haines-Young & Potschin, 2010). The biophysical structures or ecosystem processes help to ensure ecosystem functions that have potential to provide services improving human welfare (Haines-Young & Potschin, 2010; Kumar, 2010). For example, primary production (process) maintains viable fish population (function) that can be used as food (provisioning service), while vegetation cover (structure) moderates water passage (function) that can minimize flood damages (regulation service) (Kumar, 2010). This cascade framework is simple but can improve environmental planning by helping decision makers to perceive the ecosystem structures and processes that needs to be preserved or restored if some services are intended to be maintained or recovered (De Groot et al., 2010; Kumar, 2010).

This cascade framework has been considered to estimate the services provided by ecosystems based on their structures (e.g. land cover) (Burkhard et al., 2009; Koschke et al., 2012); to analyze potential trade-offs among benefits that rely on similar functions (Butler et al., 2013; Kandziora et al., 2013), and to perceive the processes and structures that need to be changed to improve or avoid functions that are valued or cause damages, respectively (Kumar, 2010).

Ecosystem services have been evaluated across different landscape scales based on land cover types, since these are potential indicators of the ecosystems structure, so of the functions and services they provide

(Koschke et al., 2012; Burkhard et al., 2014). Land cover based analysis can incur some uncertainties (Hou et al., 2013), but it is a simple method to estimate ecosystem services at large scales with low efforts (Burkhard et al., 2012). Furthermore, by acting as potential indicators of ecosystems structure, land cover allows to estimate how services have changed over time (Lautenbach et al., 2011) and how different management decisions and land use may influence the services provided by the ecosystems (Willemen et al., 2010; Logsdon & Chaubey, 2013; Bateman et al., 2013).

Some ecosystems services rely on the same structures and processes, so using one service can negatively impact the provision of others (Bennett et al., 2009; Kandziora et al., 2013). For instance, timber extraction eliminates vegetation (ecosystem structure) and so most the other functions and services also provided by the ecosystems (e.g. water purification, wildlife support, opportunity for recreation). Decision makers should be aware about the existence of potential trade-offs among ecosystems services and interests, to avoid using some benefits without accounting for the need to maintain the structures that actually support other functions and benefits. The ecosystem services cascade framework can help decision makers to become aware about the need for a sustainable development and a balance between population and ecosystem demands, by elucidating about the pathway that leads a service to be provided and demonstrating that damaging ecosystem structures and overexploiting some of their benefits may cause other services to be lost (Rodríguez et al., 2006; Bennett et al., 2009). Furthermore, assessing the ecosystem processes and functions that ensure the ecosystem services minimizes overestimation of the total economic value of the ecosystems, by allowing us to perceive that the profits from one service can decrease the profits from other services (Ojea et al., 2012; Kandziora et al., 2013).

Despite the existence of common conflicting benefits, such as water quality and crop production, these can be improved at the same site depending on physical and hydrological conditions as well as on management practices (Power, 2010; Qiu & Turner, 2013). Indeed, more sustainable practices that account for ecosystem stability and maintain high levels of biodiversity may improve ecosystem productivity (Hector et al., 1999; Tilman et al., 2002), and the range of benefits they provide (Bai et al., 2011). Little is known about the mechanisms and processes underlying most ecosystem synergies and trade-offs, but such information is decisive to improve environmental management, by helping to identify landscapes where synergies may exist or where conflicts between competing services are more likely to occur (Nelson et al., 2009; Qiu & Turner, 2013).

Ecosystem services are actually the functions considered useful by human societies, which may not include all ecosystem functions (MA, 2003; Kumar, 2010). Therefore, an ecosystem with more functions has a greater potential to provide more benefits, but may not provide more services, since this depends on the place, time and population preferences (Kumar, 2010). Thus, when assessing ecosystem services, geographical differences and the existence of ecosystems with different potential and distinct demands should considered (Kroll et al., 2012; Burkhard et al., 2014), to perceive where the functions are performed and who value or enjoys them (Burkhard et al., 2012; Boithias et al., 2014).

Assessing demands and effects of ecosystem functions further allows us to avoid some damages, since some functions can have a harmful effect (Dunn, 2010). For instance, ecosystem may provide disservices by facilitating the reproduction and dispersal of species that reduce food production and damage human health (Kumar, 2010). Thus, more functions do not actually imply more benefits since some functions can have a negative impact. Most ecosystem disservices result from bad management decisions that changed ecosystem processes and structures underlying vital functions to ecosystem stability (Kumar, 2010). For example, by changing ecosystem structure (habitats), some species may be lost and food webs may become disrupted, which can facilitate pest outbreaks and the proliferation of species that act as vectors of diseases (Chivian & Bernstein, 2004; Sala et al., 2009). The cascade framework is also valid to ecosystem disservices and thus further improves environmental management by allowing us to perceive the ecosystem structures (e.g. construction of impoundments or flow stabilization) that support harmful functions (e.g. contribute to the development of dominant populations of mosquitoes and other vectors of diseases) and thus need to be changed (Kirkman et al., 2011).

Ecosystems are generally valued just considering the benefits they provide, but disservices should also be considered, since they also rely on ecosystems and have an effect on human welfare (Kumar, 2010). For example, urban trees can improve aesthetic view, air quality and reduce building energetic requirements by increasing shade, but can also cause some allergies, facilitate undesired species dispersal, or increase water use for irrigation (Escobedo et al., 2011). Therefore, considering ecosystem disservices may create well informed decision makers and improve the analysis of possible tradeoffs (Lyytimäki & Sipilä, 2009).

Despite the efforts to assess the factors determining ecosystem services supply, it is still difficult to predict how processes, functions, and then services can be affected by one or a set of impacts (Kremen et al., 2005; Kumar, 2010). Thus, there is a need for simple but effective frameworks that can easily communicate with decision makers and assess the factors determining the supply of ecosystem services.

1.6.3. Ecosystem services valuation

Ecosystems services can have an economic, ecological and a social-cultural value (MA, 2003; Kumar, 2010), which have been frequently quantified according to their economic impact (Costanza et al., 1997; De Groot et al., 2012). Putting a price on nature has attracted public and decision makers interest, but has also created some controversial around the ecosystem services concept (Cornell, 2011; Gómez-Baggethun & Ruiz-

Pérez, 2011). Measuring the value of ecosystem services based on their economic impact have been widely criticized due to its anthropocentric view, the existence of non-market value benefits, and of non-consensual valuation techniques (Wegner & Pascual, 2011; Salles et al., 2011). However, estimating the economic value of the ecosystem services has become a common practice (De Groot et al., 2012) with recognizable benefits, by allowing to i) promote well-informed policy decisions (Willemen et al., 2010); ii) determine the total benefits in the same unit of measure (Schröter et al., 2014); iii) assess the contribution of ecosystems to local and global economies (Kumar, 2010); iv) perceive population preferences and willingness to pay (De Groot et al., 2012); v) reallocate fair amounts of money to compensate damaged ecosystem services at certain sites (Price et al., 2012); vi) identify potential financial sources/beneficiaries (Honey-Rosés et al., 2013), or vii) improve benefit: cost ratios of ecosystems investments (Balmford et al., 2002; Acuña et al., 2013).

The economic value of ecosystems should be estimated considering all the services and disservices they provide to perceive how ecosystems really contributes to global economies and human welfare, but the existence of non-market value benefits makes it difficult to estimate the real economic value of the ecosystems (Kumar, 2010; Chan et al., 2012). Some methods have been developed to value non-market benefits (e.g. supporting and cultural services) (Daniel et al., 2012) based on population and expert surveys (Raymond et al., 2009; Scolozzi et al., 2012), the willingness to pay to preserve the ecosystems or the travel costs to reach a certain location (Kumar, 2010; Van Berkel & Verburg, 2014). However, the value of some services cannot be monetarily expressed, such as the freedom of choice or the intrinsic value of the ecosystems, but they should also be considered rather than just the services with an economic impact (MA, 2003; Kumar, 2010).

1.6.4. Estimating the economic value of ecosystems

When assessing the total economic value of ecosystems, the value of the ecosystem services are generally classified according to their use in direct use values, indirect use values, optional values and non-use values (MA, 2003; Kumar, 2010). Direct use values derive from the benefits directly obtained from ecosystems, and include the value of consumable services such as food, raw materials and medicines, and the value of non-consumable services such as social and cultural activities (MA, 2003; Kumar, 2010). Indirect use values results from regulating services such as pest control, pollination or water and climate regulation and can be considered as public services by providing benefits beyond the ecosystem itself (MA, 2003; Kumar, 2010). Optional values refer to the importance humans give to the optional use of a good or a service in the future (MA, 2003; Kumar, 2010). Provisioning, regulating and cultural services may have an optional value if they are intended to be used in the future (MA, 2003; Kumar, 2010). Non-use values do not involve direct or indirect use, as it refers to the value of knowing that future generations will enjoy ecosystem benefits (bequest value), that other person can also enjoy ecosystems at a moment (altruist value), or simply by being aware that resources or species exist (existence value) (MA, 2003; Kumar, 2010).

Several approaches have been developed to estimate the economic value of ecosystem services (De Groot et al., 2002; Farber et al., 2002), which are commonly divided into market valuation, revealed preference or state preference approaches (Kumar, 2010; Liu et al., 2010).

Market valuations include price-based, cost-based and production-based approaches (Kumar, 2010). Price based approaches value ecosystem services according to their market price, so they are restricted to provisioning (e.g. fish), regulation (e.g. pollination) or cultural services (e.g. recreational activities) with a trade value (Kumar, 2010). Cost-based approaches value ecosystem services according to the costs that can be incurred in the absence of those services (Kumar, 2010; Liu et al., 2010). Cost-based approaches include avoided cost methods, where a service is valued according to the costs incurred if that service is no longer available (e.g. costs of floods that are avoided by ecosystems); replacement cost methods, where the services are valued according to the costs to replace them (e.g. water purification using technology), and mitigation or restoration cost methods where a service is valued based on the costs for mitigating the effects caused by the absence of that service (e.g. flood barriers) or for restoring it (Kumar, 2010; Liu et al., 2010). Production-based approaches value a service according to its contribution to the supply of other services that have a market price (e.g. how water purification improves fisheries) (Kumar, 2010; Liu et al., 2010).

Revealed preference approaches include travel cost methods, where an ecosystem is valued according to the population willingness to pay to travel and visit that ecosystem; and hedonic price methods where ecosystems are valued according to their contribution to increase the economic value of their surrounding lands (e.g. lands with clean air, improved water quality and greater aesthetic views can be more expensive) (Chee, 2004; Brander & Koetse, 2011).

State preference approaches include contingent methods, where ecosystem services are valued by asking population about their willingness to pay to improve the supply of an ecosystem service (e.g. willingness to pay for a better water and air quality), or their willing to accept for its loss or to move to a different area (Loomis et al., 2000).

Additionally, a service can be valued using a benefit transfer method, where the value of a service is adapted from a different study in a similar ecosystem or policy context, in order to overcome the lack of information (Boyle et al., 2010; Kumar, 2010).

1.6.5. Ecosystem services provided by freshwaters

Freshwater ecosystems provide several services such as water for domestic, agricultural and industrial use, attenuation of extreme events, water purification, climate regulation or opportunities for recreation and tourism (Postel & Carpenter, 1997; Aylward et al., 2007). The ecosystem services concept has been widely used in freshwater ecosystems to i) improve watershed planning and benefits (Postel & Thompson, 2005; Meyerhoff & Dehnhardt, 2007); ii) identify water suppliers and demanders (Jujnovsky et al., 2012; Keeler et al., 2012); iii) estimate the economic value of water purification service (La Notte et al., 2012; Maes et al., 2012); iv) determine the economic damage of eutrophication (Pretty et al., 2003; Dodds et al., 2008); v) evaluate ratios of benefit: cost of stream restoration programs (Acuña et al., 2013; Honey-Rosés et al., 2013); vi) assess the value and identify the most productive and endangered riparian areas (Pert et al., 2010; Clerici et al., 2014); vii) estimate the costs and benefits of changes in water quality (Koteen et al., 2002), or viii) predict freshwater benefits under future climatic conditions (Bangash et al., 2013; Terrado et al., 2013).

Some studies have elucidated about the real freshwater value and the cost effectiveness of protecting and restoring freshwater ecosystems. La Notte et al. (2012) estimated that the cost to replace the water purification service of the rivers in Mediterranean region was on average of €2167 km^{-1} in 2005 if using constructed wetlands to nitrogen removal. These results may raise human awareness about the real value of freshwater ecosystems and help to improve environmental planning by considering other functions (nitrogen removal) that also provide important benefits (e.g. clean water for drinking, bath or recreational activities). The monetary estimation may further improve environmental planning by allowing weighing the cost effectiveness of some actions that despite providing attractive profits, may also impact river purification capacity and so the costs if water become to be treated artificially.

Acuña et al. (2013) estimated the cost-effectiveness of stream restoration through wood debris addition or riparian tree maturation. The authors found that adding wood debris increased the potential of streams to provide services such as food, water purification, erosion control or the opportunity for recreational and cultural activities, which would increase 10 to 100 fold the monetary benefits provided by the streams and allowed to recover the investment after 15 to 20 years.

Honey-Rosés et al. (2013) demonstrated that the existing riparian forests along the Llobregat River reduced the water treatment costs by maintaining the stream temperature below critical thresholds, since for higher water temperature more expensive equipment would be needed for drinking water treatment. The authors estimated that the existing riparian forests allowed water treatment managers to save €79,000 per year and that restoring riparian forests could allow them to save €57,000-€156,000 per year in drinking water treatment costs. This study was important to demonstrate the economic impact of some overlooked ecosystem functions and by considering possible beneficiaries (water treatment managers).

In addition to specific studies and models on freshwater ecosystems services, some projects have demonstrated that investing on freshwater ecosystems can actually provide benefits (Postel & Thompson, 2005; Slootweg et al., 2008). An analysis of USA water suppliers demonstrated that the water treatment costs in watersheds with at least 60% of forested cover were 3 times lower than in watersheds with a forest percentage of 10% (Postel & Thompson, 2005). Indeed, some cities in USA began to invest on reforesting their watersheds rather than on constructing filtration plants to improve drinking water quality (Postel & Thompson, 2005).

The most famous ecosystem service project was launched in the New York City in 1997 (Ecosystem Marketplace, 2006). At that time, the US Safe Drinking Water Act forced water suppliers to filter their drinking water unless they proved to protect their watershed sufficiently to guarantee water quality standards (Postel & Thompson, 2005; Ecosystem Marketplace, 2006). This required the New York City to invest 6 billion dollars to construct and 300 million dollars annually to maintain filtration plants to supply its 9 million citizens (Postel & Thompson, 2005; Ecosystem Marketplace, 2006). The city then decided to invest 1.5 billion dollars over 10 years to recover the watershed rather than constructing and maintaining filtration plants (Postel & Thompson, 2005; Ecosystem Marketplace, 2006). The money was used to buy and protect riparian areas that were in private ownership, and to create several environmental programs to i) reduce pollutant runoff from farming, ii) improve forestry management and logging practices, and iii) upgrade wastewater infrastructures and techniques. Furthermore, the newly acquired riparian areas were opened to recreational activities (Postel & Thompson, 2005), and the investment was estimated to boosted local economies in 100 million dollars per year from ecotourism and an increase in business and jobs (Ecosystem Marketplace, 2006). After five years of investment, the Environmental Protection Agency waiver the construction of filtration plants in the New York City by considering that the watershed had been sufficiently protected to maintain good drinking water quality (Postel & Thompson, 2005). This demonstrates that investing on nature can be cost effective and allow us to get additional benefits beyond those intended to be recovered. Indeed, by recovering the causes (riparian degradation and pollution discharges) rather than remediating (filtration plants) the problem (bad water quality), the New York City was able to expand the benefits obtained from the investment and at lower cost, by having improved water quality but also the local economies, the availability of recreational areas and the ecosystems stability and capacity to provide some other benefits.

Another famous project about investing and restoring ecosystems was launched in Costa Rica in 1996 to protect the sources of freshwater supply and improve other ecosystem services (Chomitz et al., 1999;

Pagiola, 2002). The government created a fund to pay land owners for protecting the existing forests or reforesting their lands (Postel & Thompson, 2005). The fund was supported by taxes from fossil fuels, grants from the World Bank and the Global Environmental Facility (GEF) and by financial donations from hydropower producers that benefited from forest conservation (Postel & Thompson, 2005). Hydropower producers decided to support the fund because forested watersheds were estimated to provide more 460,000 m^3 of water per year for energy production than deforested or degraded watersheds. The programme became a model of sustainability and demonstrates that investing in nature can be cost effective and provide several benefits. Indeed, forestry protection was estimated to improve freshwater supply but also many other benefits, such as soil conservation, sedimentation control, fisheries protection, carbon sequestration or opportunities for recreation and tourism. The program was also important for demonstrating the need of communicating with stakeholders interested in distinct ecosystems benefits that can support and promote overall ecosystem services.

1.7. Goals and outline of the thesis

Rivers are determinant to human well-being by supporting vital processes and providing essential benefits. Riparian land cover has a major influence on river processes and services by defining river hydromorphology and riparian vegetation structure and type. Humans have been damaging river processes and services by changing natural riparian land cover to human land use, degrading river hydromorphology, and by discharging pollutants on rivers. High nutrient loadings have been impacting river processes and services, so improve the river capacities to buffer excessive nutrient loadings is determinant to human well-being by reducing the impact of pollutant discharges.

This study aims to assess i) the effect of riparian land cover on river habitat quality and physico-chemical water parameters, ii) the hydromorphological elements, physico-chemical water parameters, and biological attributes of rivers that most determine changes in physico-chemical water parameters along rivers, and iii) the economic value of the water purification service provided by rivers, or the monetary costs to restore nitrogen concentration in rivers where it tended to increase.

For that, we measured changes in physico-chemical water parameters and assessed habitat quality along river sections (stretches) with contrasting land use, trophic status and river width. The study was carried out in mesotrophic river segments (section 3.2), ii) a eutrophic river segment (section 3.3), and iii) in a large river segment width (section 3.4). In section 3.2.1 we compared changes in physico-chemical water parameters along stretches with pristine riparian areas and mesotrophic conditions but different habitat quality attributes.

In section 3.5, we assessed the economic value of the water purification service provided by the stretches, or the monetary costs to restore nitrogen concentrations.

In section 4 results are discussed and possible restoration measures and best management practices are presented.

2. Material and methods

2.1. Study scheme

Six river segments with different trophic status and channel width were selected in the Ave River watershed (northwestern Portugal) to assess the effect of river water chemistry and hydromorphology on changes in physico-chemical water parameters along rivers. To assess the effect of riparian land cover on river habitat quality and physico-chemical water parameters, each river segment was divided in stretches based on land cover type, and stretches were further divided in reaches to habitat quality characterization (Figure 1). Habitat quality and changes in physico-chemical water parameters along rivers were then compared among stretches.

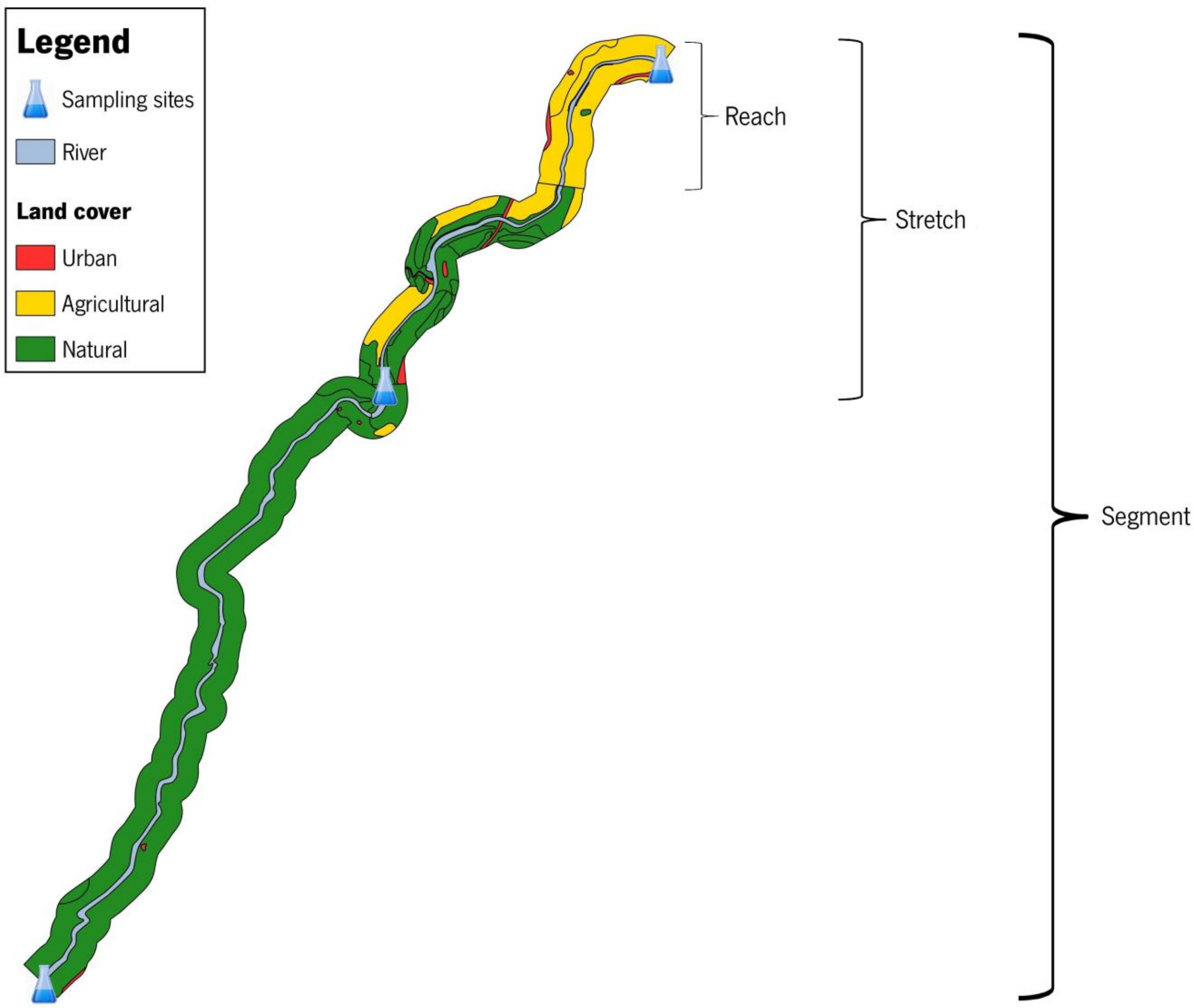

Figure 1. Study scheme: river segment, stretch and reach

River segments were classified as mesotrophic (S1-S4), eutrophic (S5) and large (S6) based on cluster and principal component analysis (PCA) results. Thereafter, stretches from each river segment type were analyzed independently (mesotrophic, section 3.2; eutrophic, section 3.3; and large, section 3.4).

Stretches from river segments classified as mesotrophic were examined as follows: i) all stretches from S1 to S4 to assess the influence of riparian land cover (section 3.2), and ii) stretches dominated by natural riparian areas and with no evidence of anthropic impacts (section 3.2.1), to discriminate the factors that best explain changes in physico-chemical water parameters along rivers, minimizing the effects of pollutant discharges. Additional metrics were used in natural stretches, namely changes in nitrite concentration and data on macroinvertebrate community and leaf litter decomposition, to assess if they could be related to changes in physico-chemical water parameters along rivers.

2.2. Geographic data

Geographic Information Systems (GIS) were used to select sampling sites and characterize landscape patterns and river attributes. Geographic data were mostly manipulated with QGIS 1.8.0 and 2.10.1 (QGIS Development Team), but ArcGIS 10.2 (ESRI, Redlands, CA, U.S.A) was also used to improve results. The coordinate reference system PT-TM06/ETRS89 (European Terrestrial Reference System 1989) was used because it is recommended by the EUREF (European Reference Frame, subcommittee IAG - International Association of Geodesy), and the Direcção-Geral do Território (DGT, 2013).

A Web map with 28 layers georeferencing study sites and portraying landscape patterns and river attributes was created using QGIS Cloud (Sourcepole AG) and is available at http://qgiscloud.com/ZPR/ME_UM. Geographic metadata is available at Appendix 1.

2.2.1. River margins and watercourse

After selecting the river segments and define the stretches and reaches, the sites delimiting them were georeferenced using Bing aerial and Google satellite images in QGIS through the OpenLayers QGIS plugin 1.1.0 (Sourcepole AG). Two satellite sources were used because Bing had a greater resolution but an older reference year than Google.

River margins and watercourse of all stretches and reaches were georeferenced as line type shapefiles using Bing aerial and Google satellite images at a scale of 1:1000 and at 1:500 when needed. The hidcod_25k_ptcont and rios_cdecimal shapefiles as well as field observations and photographs were also used to georeference river margins and watercourse, since river channels with a dense coverage of forest trees were hard to identify just by satellite images. The hidcod_25k_ptcont (1:25000) and rios_cdecimal (1:100000) are shapefiles with the georeferenced rivers and tributaries of mainland Portugal, and were obtained from Atlas da Água that is available at the Sistema Nacional de Informação de Recursos Hídricos (SNIRH) website.

The shapefile for river margins (Appendix 1, River margins) was created to aid land cover design and describe river margin types. River margins were classified based on field observations and photographs, considering 4 types of river margins. River margin types are represented in the maps portraying the stretches (Appendix 2) and are described in Appendix 3.

The shapefile for river watercourse (Appendix 1, River watercourse) was created to improve the georeferencing of rivers relatively to that of hidcod_25k_ptcont and rios_cdecimal shapefiles, and then to accurately determine the lengths of the stretches and reaches using QGIS. River watercourse was georeferenced considering the middle of the wetted channel, since the river segments had different widths. Length values were exported to an excel spreadsheet to compare changes in physico-chemical water parameters along rivers between stretches with different lengths, and to determine the stretches habitat quality.

2.2.2. Land cover

To make a quantitative analysis of riparian land cover along the selected river segments, a shapefile was created using Bing Aerial and Google Satellite images at a scale of 1:1000, tridimensional images from Google Street, field observations and photographs, and available land cover shapefiles for mainland Portugal, namely the Carta de Uso e Ocupação do Solo de Portugal Continental para 2007 (COS2007) (IGEO, 2010) and the CORINE Land Cover for Continental Portugal 2006 (CLC06_PT) (Caetano et al., 2009). COS2007 and CLC06_PT were both used, since COS2007 has a greater detail but the third level of land cover from CLC06_PT was freely available, while just the second level of COS2007 could be consulted for free. COS2007 levels 1 and 2 and CLC06_PT level 3 were obtained from IGEO website.

The design of the land cover shapefile started by creating a baseline polygon shapefile representing 50 meters buffer strip along each river segment side where land cover was analyzed, since it represents an intermediate width used to improve different ecosystem functions (Hawes & Smith, 2005; Siligardi et al., 2007), and it is considered a reasonable extension to define riparian zones (Munné et al. 2003). A standard buffer strip of 50 meters was established for each margin of all river segments, since it is difficult to predict the functional riparian area of each reach.

Independent buffer strips were created for each margin side, since they would also be used to portray the stretches habitat quality that could differ among river banks. QGIS only allowed to create a unique buffer for both sides of a line, so the shapefile with the georeferenced river margins was exported to ArcGIS to create a 50 meters buffer strip for each margin side.

Then, the buffer strips shapefile was separately intersected with COS2007 (land cover classes from level 2) and CLC06_PT, to have the buffer strips divided into land cover types according to COS2007 and CLC06_PT. When confronting the shapefiles that had been intersected with COS2007 and CLC06_PT, with Bing Aerial and Google Satellite images as with field observations and photographs, the number of polygons and the way they were classified did not mirror the actual land cover types. Therefore, an improved shapefile for the riparian land cover at the 6 river segments was created by overlapping the intersected shapefiles with Bing and Google satellite images (scale 1:1000), so the original COS2007 and CLC06_PT polygons for land cover types could be removed, changed or new could be added based on satellite images, as well as field observations and photographs. Polygons for land cover types were further detailed using a scale of 1:500. The land cover shapefile was edited from November 2013 to June 2014, and ended up with 486 polygons, a minimum mapping unit (MMU) of 0.005 hectare, and a scale of 1:1000.

The polygons for land cover type were geometrically revised but also reclassified based on a new classification framework, since the land cover classes from COS2007 fit its original scale and technical procedures, that once different from those here obtained after editing the intersected shapefile, make COS2007 classes to be inappropriate as their criteria ceased to be valid. Furthermore, the land cover classes from COS2007 were defined with the purpose of establishing a reference system for land cover classification independently of their impacts on the river ecological condition. Considering that our study aims to relate the riparian land cover with the river ecological condition at a finer scale, a new land cover classification framework was created (Appendix 4), not only to adjust the criteria of the land cover classes to our work scale, but also to define them according to their impact on rivers. In this sense, COS2007 separates urban continuous fabric into horizontal and vertical according to their height, whereas the new land cover classification framework distinguished urban fabrics by their social context, namely if they are in rural or in highly urbanized areas, since this may significantly affect the amount of pollutants reaching the streams. While doing so, in the new classification framework, even a scarce presence of polygons representing residential buildings in highly urbanized areas (Appendix 4, land cover class 1.1.1.1), indicates that nearby exists a significant population density and/or some related anthropogenic activities, that despite having a low impact on river hydromorphology as they lay beyond the buffer zone (50 m), they may affect the river ecological condition due to a strong human presence and the existence of some plumping systems that may end up discharging some sewage into the streams.

The new land cover classification framework includes original land cover classes but also some other adopted from COS2007 with criteria adjusted to our work scale, as the one referring Green urban areas that belongs to artificial surfaces in COS2007 (class 1.4.1 in COS2007), but to natural and vegetated areas in the new classification framework (Appendix 4, land cover class 3.2.3). In COS2007, the land cover class artificial areas (class 1 in COS2007) include buildings, paved roads, green artificial areas and others related to human society based on the land use. However, paved roads and green urban areas have a different impact on rivers since impervious surfaces can greatly increase runoff. Thus, in the new classification framework, green urban areas belong to the land cover class of natural areas (Appendix 4, land cover class 1) that even including distinct natural land covers, may be more similar to green urban areas regarding river impact than paved roads or buildings. Nonetheless, green urban areas does not have the same influence on river ecosystems as natural forest trees, so the new classification framework includes 5 levels, to have green urban areas closer to bare soils than to forest trees at higher detailed levels.

The land cover classification framework (number of levels, land cover classes definitions and classification criteria) was created based on COS2007 guideline (IGEO, 2010), and standards for agricultural (IFAP, 2007) and forestry definitions (IFN, 2009; ICNF, 2013a, 2013b), so it could be accurate and consistent with reference systems for land cover classification. It has 5 levels and 34 land cover classes at the most detailed level (Appendix 4). Land cover classes up to level 3 were used to portray land cover patterns of the stretches (Appendix 2) but were not included in data analysis, since they are too detailed.

The shapefile with the polygons for land cover type (Appendix 1, Land cover) was used to calculate the relative proportion of land cover along each stretch. The polygons areas were calculated using QGIS, and then the values were exported to an excel spreadsheet where they were manipulated and further used to data analysis. First, for each stretch, the area occupied by each land cover class from level 3 was calculated, and then by each class from levels 1 to 2, by summing the areas of detailed classes that are together at a lower level (e.g. the area of a land cover class from level 2 is the sum of its detailed classes from level 3) (Appendix 4). The area of each land cover class from levels 1 to 3 at a stretch was then divided by the stretch total area, to calculate the relative proportion of each land cover for each stretch. The relative proportion of land cover was used rather than their total area, to fairly compare stretches with different lengths and make the approach for the land cover analysis consistent with that for changes in physico-chemical water parameters along stretches that also considered differences in stretch lengths.

While doing so, it was possible to assign each stretch with its relative proportion of all land cover classes, and then cross this information with that for habitat quality and physico-chemical water parameters.

2.2.3. River flow types and waterfalls

To describe river flow types, a polygon shapefile (Appendix 1, River flow types) was created over that describing land cover, so the former could fit the stream channel by asking QGIS to avoid these two shapefiles to overlap. The shapefile was then divided according to the existing flow types, which were classified based on field observations and photographs, into laminar and turbulent flow.

A point type shapefile was created to georeference the waterfalls that were identified in the field (Appendix 1, River waterfalls). Georeferencing was done using Bing aerial and Google satellite images in QGIS.

River flow types and waterfalls are represented in the maps portraying the stretches (Appendix 2) and are described in Appendix 5 and Appendix 6, respectively.

2.2.4. Ecological and chemical status of rivers under the Water Framework Directive (WFD)

The shapefile of river water bodies (PTINAG_ART13_MRIOS_PTCONT) defined under the Water Framework Directive (WFD) was obtained from InterSIG (InterSIG, 2008). This shapefile had not the classification for the ecological and chemical status assigned to every water body under the WFD in 2010, but the Agência Portuguesa do Ambiente (APA) provided a dbf file containing that information. Both dbf file and the InterSIG shapefile have the single code that identifies each water body at a national scale (MS_CD). Thus, using this common field, the data of ecological and chemical status from the dbf file were incorporated into the shapefile with the georeferenced water bodies.

The resulting shapefile had no information for the water body tributaries, but APA informed that the ecological and chemical status of a defined water body applies to its entire basin. Thus, a polygon shapefile from InterSIG delimiting the area of the water body basins (PTINAG_ART3_BACIAS_PTCONT) and containing the water body single codes (InterSIG, 2006) was intersected with the hidcod_25k_ptcont shapefile, to obtain a new shapefile that included all the tributaries assigned to their water body code. Then, data from the dbf file could also be incorporated into this new shapefile, which allowed to obtain the ecological and chemical status of all tributaries in the Ave River watershed under the WFD in 2010. The shapefiles of water body basins (Appendix 1, Water bodies (WFD)) and river water bodies with data on ecological and chemical status (Appendix 1, Rivers (WFD)) were used to create the map in Figure 2 and some layers of the Web map.

2.2.5. Slope

The slopes at the 50 meters buffer strips were determined using a raster file of slopes from the EPIC WebGIS project, which was based on a digital elevation model (DEM) with a spatial resolution of 25 meters, and contains 7 classes of slopes: 1 (0-3%), 2 (3-5%), 3 (5-8%), 4 (8-12%), 5 (12-16%), 6 (16-25%) and 7 (>25%) (CEAP, 2013a).

The raster file of slopes was first converted to a shapefile format, then restricted to the study area, and finally intersected with the created shapefile for land cover types (section 2.2.2. Land cover), so it was possible to determine the slope of each land cover class within the 50 meters buffer strip. The slope and land use can influence runoff, so the shapefile (Appendix 1, Land cover slope) was used to allow identify the areas where it is more likely to occur (Appendix 2, maps on land cover slope). In this sense, areas with riparian vegetation and buildings are expected to have low runoff, while areas with agricultural activities, bare soils and/or artificial surfaces are expected to facilitate runoff, even though differently (Appendix 1, Land cover slope).

The shapefile was also used to assess if the slope of the agricultural areas would affect changes in physico-chemical water parameters along the stretches with more agricultural lands, namely, along the mesotrophic stretches that were grouped by the cluster analysis once having more agricultural areas (s1A, s3A and s4A) and along the stretch of the eutrophic river segment with more agricultural lands (s5A). For each of these stretches, the area of the agricultural lands with a same slope were summed and divided by the total agricultural area of the stretch. The relative proportion of agricultural areas with a determined slope was then multiplied by the corresponding slope value, and by summing the results for each slope class, it was possible to obtain a weighted value for the slope of the agricultural areas at a stretch. The analysis did not consider artificial/urbanized cover since they mostly refer to buildings, and to point sources of pollution as sewage pipes.

2.2.6. Soil type and ecological value

The soil type and ecological value along each stretch was determined by restricting the shapefile for the soil ecological value created by the EPIC WebGIS project (CEAP, 2013b) to the area referring the 50 meters buffer strips (Appendix 1, Soil ecological value). Unlike slopes, there was no further data manipulation, as the soil ecological values differed among but not within stretches.

The original shapefile (CEAP, 2013b) used national maps for soil types and land capacity to assess soil ecological value based on their productivity and ecological potential. The shapefile has 5 classes for the soil ecological value: 0 (urbanized and water bodies), 1 (very low), 2 (low), 3 (intermediate), 4 (high), and 5 (very high ecological value).

2.3. Selection of river segments and sampling sites

We selected 6 river segments with different trophic status, channel width and riparian land cover, to assess how these attributes could affect changes in physico-chemical water parameters along rivers.

First, COS2007, CLC06_PT, Bing and Google satellite images, hidcod_25k_ptcont and rios_cdecimal were used to identify the water courses that over a determined extension had distinct parts, each one dominated by a determined land cover, wherein the entire length was defined as a river segment, while the parts were defined as stretches (Figure 1). The sampling sites were then established at the upstream and downstream sites of each river segment, but also at internal locations to define the stretches (Figure 1). Similar studies on changes in physico-chemical water parameters along rivers had established sampling sites distanced 1000 to 4500 m (Heidenwag et al., 2001), and 3500 to 7200 m (Vagnetti et al., 2003), so these were the lengths considered to define the stretches, that ended up with 640 to 3500 meters.

River segments with significant influence of tributaries or with the presence of point source of pollutants (e.g. wastewater treatment plants (WWTP)) were preferably excluded, to avoid changes in physico-chemical water parameters that could result from chemical dilution, and to assess the effect of riparian land cover rather than of specific source of impacts. The WWTPs and wastewater discharge sites in the Ave River watershed were located using two shapefiles provided the Agência Portuguesa do Ambiente (APA). Additional information on the WWTPs (INSAAR, 2008a) and wastewater discharge sites (INSAAR, 2008b) were obtained from the shapefiles fields and from INSAAR website.

After selecting a preliminary set of river segments, available data on physico-chemical water parameters from 22 monitoring stations in the Ave River watershed were obtained from SNIRH website. Mean values of ammonium (mg L^{-1} NH_4), nitrite (mg L^{-1} NO_2), nitrate (mg L^{-1} NO_3), phosphorous (mg L^{-1} P), conductivity (µS cm^{-1}), dissolved oxygen (mg L^{-1}), coliforms (most probable number/100 ml) and suspend solids (mg L^{-1}) were calculated for each monitoring station considering data from May 2008 to September 2013. Mean values for the wet season were also calculated considering data for the same period referring the months from October to April. Physico-chemical water parameters were those that would be determined in our study and others that were considered relevant to assess river trophic status, if with more than 3 records after outliers removal.

Data for the mean values were then imported to QGIS and converted to a shapefile (Appendix 1, Monitoring stations) to allow a spatial visualization of the physico-chemical water parameters along the Ave River watershed. Data georeferencing was done using the coordinate values of the monitoring stations available at SNIRH website.

Finally, from a preliminary set of 21 river segments with different dominant land cover (stretches), 6 were selected based on the physico-chemical water parameters in the above mentioned shapefile, so 4 river segments could have moderate nutrient loadings, one could be eutrophic, and the other river segment had a larger width.

After selecting river segments and defining the stretches based on data from monitoring stations and for land cover type, a sampling campaign was conducted to confirm the sampling sites location, physico-chemical water parameters, and land cover. Then, 6 river segments, 15 stretches and 21 sampling sites were defined. Two sampling sites separated by 2 km were further established at the beginning and at the end of a concrete channel (CC) with diverted water from the dam upstream river segment 2, to assess if changes in physico-chemical water parameters along the concrete channel would differ from those occurring along natural streams.

2.4. Study area and river segments characterization

The 6 selected river segments are located in the Ave River watershed (northwestern Portugal), in a landscape dominated by granitic rocks where regosols, anthrosols and cambisols are the predominant soil groups (CEAP, 2013b).

The Ave River watershed has a total area of 1391 km^2 (INAG, 1999) and its spring is located in the Cabreira mountain, at 1260 meters of altitude (INAG, 1999). The mean anual precipitation in the Ave River watershed ranges from 900 to 3900 mm, it is higher near the river spring and 73% falls between October and March (INAG, 1999). At the upper part of the Ave River watershed, where the selected river segments are located, the average temperatue is about 14°C, while the maximum and the minimum average temperatures are about 27°C and 0.4°C, respectively (INAG, 1999).

At the upstream locations of the Ave River watershed, the agricultural activities have a greater economic relevance, while in the towns of Fafe and Guimarães there are significant industrial units dedicated to textile, footwear and leather production, and a considerable urbanization (INAG, 1999).

Some of the water courses in the Ave river watershed are severely modified concerning physico-chemical water parameters, biotic communities, riparian corridors and/or river channel morphologies, especially at the urbanized sites with more industrial activities (APA, 2012). Selho and Vizela rivers have been facing serious environmental problems at least since the nineties (INAG, 1999).

The 6 selected river segments are at the upper part of the Ave River watershed: segments 1 (S1) and 2 (S2) are located nearby the village of Póvoa de Lanhoso, segments 3 (S3) and 4 (S4) nearby the town of Fafe, while segments 5 (S5) and 6 (S6) are nearby the town of Guimarães (Figure 2).

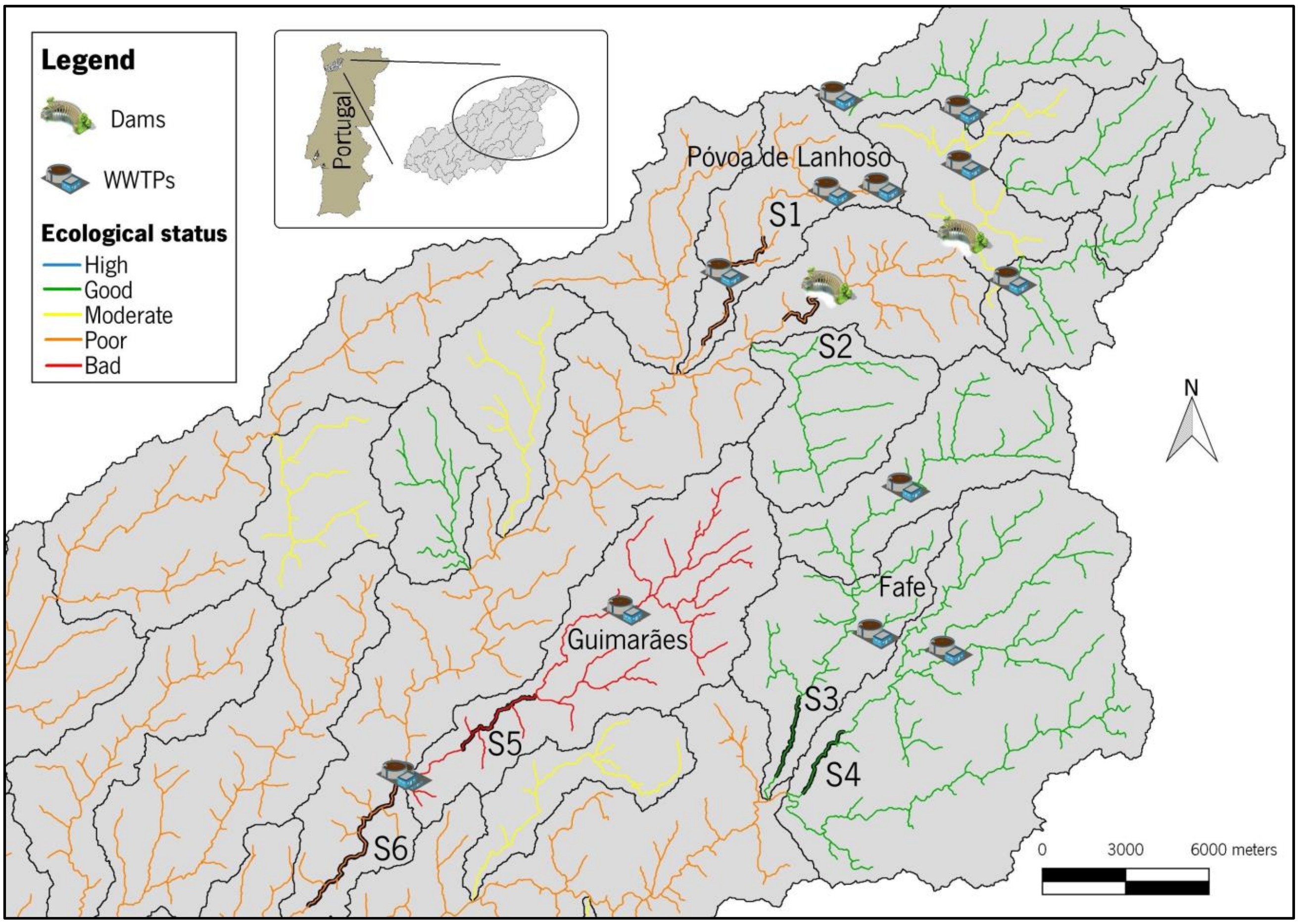

Figure 2. River segments, WWTPs, dams and towns at the upper part of the Ave River watershed. The watershed is divided according to the water body basins, defined under the Water Framework Directive (WFD) (APA, 2012). S1 (river segment 1), S2 (river segment 2), S3 (river segment 3), S4 (river segment 4), S5 (river segment 5), S6 (river segment 6).

River segment 1 (S1) has 6 km and is located in the Pequeno River (PT02AVE0112) (single code of the water body at a European Union scale, used under the Water Framework Directive (WFD)) (Figure 2), a small tributary of the Ave River that has 11 km and a drainage area of 32 km^2 (APA, 2012). In this water body basin, there are 108 inhabitants per km^2, 54.6% of the water is used to agricultural, 37.3% to urban and 7.1% to industrial purposes; 73% of the wastewaters are treated, and 13.2 tons of nitrogen and 2.12 tons of phosphorous are generated per year (APA, 2012). According to the WFD, the Pequeno River had a poor ecological status, a good chemical status, and reduced anthropogenic impacts, but indications of moderate agricultural and hydromorphological pressures (APA, 2012). Upstream from S1, the Pequeno River has pristine riparian areas but there are two small urban wastewater treatment plants (WWTPs) (Figure 2) adopting secondary treatment levels.

River segment 1 (S1) was divided in 3 stretches with distinct riparian land cover and habitat quality attributes. In the first and the second upstream stretches, the riparian soils have a high ecological value and are dominated by anthrosols and regosols, while in the third stretch, the riparian soils have an intermediate ecological value and are dominated by regosols (CEAP, 2013b).

In the first stretch of S1 (s1A), the river crossed a rural environment with sparse habitations, small agricultural areas with orchards, vineyards and livestock to self-consumption, but a significant presence of arable lands especially of forage crops, as corn, not related to intensive practices but to small producers (Appendix 2, s1A). The riverbed was dominated by pebbles and cobbles and there was a mixture of riffles and pools. The river was flanked by constructed rocky walls in a large extension and was evident the presence of pipes that may be used to water extraction or to sewage discharge. Within the first stretch, there is an urban WWTP that was seasonally used adopting secondary treatment levels, and had an annual wastewater volume of 12596 m^3 that were discharged into the stream after treatment (INSAAR, 2008a).

In the first meters of the second stretch of S1 (s1U), the riverbed was dominated by pebbles and cobbles, the river had a turbulent flow as a result of two waterfalls, and there was a small but dense cluster of rural habitations (Appendix 2, s1U). In the remaining part of the second stretch, there was more natural land cover (Appendix 2, s1U), the river had a laminar flow and the riverbed was dominated by silt and sand.

The third stretch of S1 (s1N) was dominated by natural land cover (Appendix 2, s1N) and had distinct flow regimes, depths and sediment types, but for most of its extension, the river was shallow, had a turbulent flow due to the presence of waterfalls, and the riverbed was dominated by pebbles, cobbles, and also by boulders, especially at the final part.

River segment 2 (S2) has 2.7 km and is at the upper part of the Ave River water body PT02AVE0126 (Figure 2), that has 49 km and a drainage area of 145 km^2 (APA, 2012). In this water body basin, there are 514 inhabitants per km^2, 43.1% of the water is used to industrial, 38.4% to urban and 17.9% to agricultural purposes; 97% of the wastewaters are treated, and 81.63 tons of nitrogen and 9.22 tons of phosphorous are generated per year (APA, 2012). This water body was classified as having a poor ecological status, a good chemical status and reduced anthropogenic impacts but indications of high hydromorphological pressures (APA, 2012). The surrounding area in S2 is similar to that described for the Pequeno River, since S2 is also inserted in a rural context with little or non-evidence of urbanization and industrial activities.

Upstream from S2 there are four urban WWTPs and two dams called Guilhofrei and Andorinhas (Figure 2). The four WWTPs used secondary treatment levels and had an annual wastewater volume of at least 274512 m^3 that were discharged into the streams after treatment (INSAAR, 2008a). Guilhofrei and Andorinhas dams began to operate around 1930 and made part of the hydroelectric system in the Ave River watershed (EDP, 2012). River segment 2 is immediately after Andorinhas dam (Figure 2) that is a gravity type dam with 24 meters height and creates a reservoir with 23 ha (EDP, 2012).

In a first part of S2, the riparian soils have a high ecological value and are dominated by anthrosols and cambisols, while in a second part, the riparian soils have an intermediate ecological value and are dominated by regosols (CEAP, 2013b). River segment 2 was not divided in stretches since it had similar land cover and habitat quality attributes. River segment 2 (s2N) was dominated by natural land cover (Appendix 2, s2N), had a low current velocity, a shallow and wide river channel with macrophytes and mosses, and the riverbed dominated by pebbles, cobbles and boulders.

River segment 3 (S3) has 3.48 km and is located in the Vizela River water body PT02AVE0121 (Figure 2), a tributary of the Ave River that has 11 km and a drainage area of 35 km^2 (APA, 2012). In this water body basin, there are 596 inhabitants per km^2, 65.6% of the water is used to urban, 26% to industrial and 5.8% to agricultural purposes; 95% of the wastewaters are treated, and 27.19 tons of nitrogen and 5.51 tons of phosphorous are generated per year (APA, 2012). The results from the WFD indicated that this Vizela River water body had a good ecological and chemical status and reduced anthropogenic impacts (APA, 2012).

Upstream from S3 there are two urban WWTPs, one site of untreated wastewater discharge, and Vizela River passes close to the most urbanized area of the Fafe town before reaching S3 (Figure 2). The WWTPs used secondary treatment levels and had an annual wastewater volume of at least 490810 m^3 that were discharged into the streams after treatment (INSAAR, 2008a).

River segment 3 (S3) was divided in two stretches with distinct riparian land cover and habitat quality attributes. The first stretch has riparian soils with high ecological value dominated by anthrosols and cambisols, while the second stretch has riparian soils with an intermediate ecological value dominated by regosols (CEAP, 2013b). In a primary part of the first stretch (s3A), there were small agricultural areas with corn and vineyards, but considerable density of bush bordering the stream (Appendix 2, s3A). The riverbed was initially dominated by pebbles and cobbles but then by silt and sand, and the river had a laminar flow. In a second part of the first stretch (s3A), the river crossed a golf course and associated facilities (Appendix 2, s3A), wherein the flow was more turbulent and the riverbed had more pebbles and boulders.

In the second stretch of S3 (s3N), the river banks had steep rocky lands with a dense coverage of forest trees (Appendix 2, s3N), the river had a turbulent flow due to the presence of waterfalls and the riverbed was dominated by sand and boulders.

River segment 4 (S4) has 3.1 km and is located in the Ferro River (PT02AVE0120) (Figure 2), a tributary of the Ave River that has 41.5 km and a drainage area of 120 km^2 (APA, 2012). In this water body basin, there are 234 inhabitants per km^2, 68.4% of the water is used to urban, 22.2% to agricultural and 8.8% to industrial purposes; 89% of the wastewaters are treated, and 53 tons of nitrogen and 6.74 tons of phosphorous are generated per year (APA, 2012). According to the WFD, the Ferro River had a good ecological status, and reduced anthropogenic impacts (APA, 2012).

Upstream from S4 there is one urban WWTP, and some of the Ferro River tributaries passes close to the most urbanized area of the Fafe town before reaching S4 (Figure 2). The WWTP used secondary treatment levels and had an annual wastewater volume of 29815 m^3 that were discharged into the stream after treatment (INSAAR, 2008a).

River segment 4 (S4) was divided in two stretches with distinct riparian land cover and habitat quality attributes. The riparian soils in the first stretch have high ecological value and are dominated by anthrosols and regosols, while in the second stretch the riparian soils have an intermediate ecological value and are dominated by regosols (CEAP, 2013b).

In the first stretch of S4 (s4A), there were sparse orchards and vineyards, but extensive lands for crop production (Appendix 2, s4A). The river had a laminar flow and the riverbed was dominated by pebbles and cobbles in the first meters and then by sand and silt. The second stretch of S4 (s2N) had steep rocky lands with a dense coverage of forest trees (Appendix 2, s2N), the river had a turbulent flow due to the presence of waterfalls and the riverbed substrate was dominated by sand and boulders.

River segment 5 (S5) has 4.4 km and is located in the Selho River (PT02AVE0118) (Figure 2), a tributary of the Ave River that has 18.9 km and a drainage area of 60.8 km^2 (APA, 2012). In this water body basin, there are 990 inhabitants per km^2, 58.4% of the water is used to urban, 32.2% to industrial and 8.9% to agricultural purposes, 100% of the wastewaters are treated, and 103.58 tons of nitrogen and 31.46 tons of phosphorous are generated per year (APA, 2012). According to the WFD, the Selho River had a bad ecological status and high urban, agricultural and industrial impacts (APA, 2012).

Selho River flows through a dense urbanized area in the vicinity of the Guimarães town before reaching S5, and there is one urban WWTP located upstream from S5 (Figure 2). The surrounding area along S5 has high urbanized and industrial pressures and intense agricultural practices.

River segment 5 (S5) was divided in 3 stretches with distinct riparian land cover. The riparian soils in the first stretch have very low ecological value and are dominated by regosols and anthrosols, in the second stretch riparian soils are urbanized, while in the third stretch riparian soils have high ecological value and are dominated by anthrosols and cambisols (CEAP, 2013b).

In the first stretch of S5 (s5A), the river banks were eroded and were used for intensive production of vegetables and corn (Appendix 2, s5A). In the second stretch (s5U), the river was narrowed by concrete walls and flanked by road networks and habitations (Appendix 2, s5U). In both stretches, the river had a laminar flow, a riverbed dominated by pebbles and sand, and the river channel was colonized by macrophytes and had some solid wastes. In the third stretch (s5N), most of the river banks were converted into a leisure park that was covered with grass and had some recreational areas (Appendix 2, s5N). In a first part of the third stretch, the river had a turbulent flow, the riverbed was dominated by boulders covered with mosses, and there were some trees between the river and a wall delimiting the park. In a second part, the river had a laminar flow, the river banks were eroded and the substrate was dominated by sand.

River segment 6 (S6) has 6.5 km and is located in the Ave River waterbody PT02AVE0126, 27.6 km downstream from S2 (Figure 2). Although referring to the same water body as S2, river segment 6 flows through a highly urbanized area with industrial pressures, between the towns of Guimarães and Vila Nova de Famalicão.

River segment 6 (S6) is about 300 meters downstream of two WWTPs, called Serzedelo I and Serzedelo II (Figure 2). Serzedelo I began operating in 1997 and despite projected for a population of 100800 (15120 m^3 day^{-1}) (Tratave, 2013a), in 2004 was treating wastewaters from 142013 citizens and had an excess wastewater flow of 66% relatively to that originally planned, which lead to the construction of Serzedelo II (DHVFBO, 2004). Serzedelo II was predicted to start operating in 2007 and to treat wastewater from 170513 citizens, corresponding to 25557 m^3 of wastewater per day (TRATAVE, 2013a). Serzedelo I and II used secondary treatment levels and tertiary treatment to reduce color (TRATAVE, 2013a). During the first half of 2013 (January to June), the Serzedelo WWTPs received wastewaters with 45.1 ± 7.3 mg L^{-1} of nitrogen and 6.3 ± 1.6 mg L^{-1} of phosphorous, of which 380255 ± 25071 m^3 were treated per month by Serzedelo I and discharged in the Selho River with 7.0 ± 1.7 mg L^{-1} of nitrogen and 3.4 ± 1.1 mg L^{-1} of phosphorous, and 704576 ± 100539 m^3 were treated per month by Serzedelo II and discharged in the Ave River with 5.7 ± 1.7 mg L^{-1} of nitrogen and 1.5 ± 0.6 mg L^{-1} of phosphorous (TRATAVE, 2013b).

River segment 6 (S6) was divided in four stretches with distinct riparian land cover and habitat quality attributes. The riparian soils in the first and the fourth stretches have high ecological value and are dominated by anthrosols and cambisols, in the second stretch the riparian soils are urbanized, while in the third stretch riparian soils have an intermediate ecological value and are dominated by regosols (CEAP, 2013b).

The first stretch (s6A) had bush and shrubs, low human presence and some agricultural areas (Appendix 2, s6A), while the second stretch (s6UL) had several textile industrial units on the waterfront and some associated concrete walls (Appendix 2, s6UL). The third stretch (s6N) had a dense coverage of forest trees (Appendix 2, s6N) that despite diverse had some exotic species like *Eucalyptus* spp*.* In the fourth stretch (s6UR), the river banks had more industrial units and habitations as in the second stretch (Appendix 2, s6UR).

The river had a laminar flow in the first (s6A) and the third stretches (s6N), while in the second (s6UL) and the fourth stretches (s6UR) the river had a turbulent flow due to the presence of natural and artificial waterfalls. The riverbed at the first (s6A) and the third stretches (s6N) was dominated by silt and sludge, while at the second (s6UL) and the fourth stretches (s6UR) there were more boulders. All stretches of S6 had several manhole cover close to the river, and the local population reported frequent pollutant discharges from different activities and sources.

2.5. Field and laboratory procedures

2.5.1. River habitat quality

The river habitat quality was evaluated considering three habitat characterization methods, the Fluvial Functional Index (FFI) (Siligardi et al., 2007), the HABSCORE (RBP) (Barbour et al., 1999) and the Riparian Forest Quality Index (QBR) (Munné et al., 2003). Protocols and scoring systems of the habitat characterization methods are in Appendixes 7 and 8, respectively.

According to these methods guidelines, the habitat quality should be evaluated in river lengths smaller than those of the stretches, so the stretches were divided in reaches to habitat quality characterization (Figure 1). Reaches were first defined based on Bing aerial and Google satellite images and on COS2007 and CLC06_PT. Then, reaches were confirmed in the field and adjusted whenever needed, wherein 37 reaches were lastly defined.

Habitat quality data were obtained at the reach level, so they had to be adapted to the stretch level, in which data on land cover and physico-chemical water parameters were analyzed. For that, the score of each habitat quality parameter and habitat characterization method final score in a reach was multiplied by the reach length (m), and further divided by the length of its stretch (m). Finally, the score of all habitat quality parameters and habitat characterization methods final scores in a stretch were calculated by summing the respective reach values. For FFI, the habitat quality is independently evaluated for each margin (Siligardi et al., 2007), so the stretch final score was estimated as the mean of the two margin scores.

To assess the river habitat quality, reaches were prospected for almost their entire length, except for sites that were not accessible. The habitat quality was evaluated by two observers, whenever possible, to get an assessment based on a consensual decision. Then, to improve the habitat quality evaluation, the scores of all habitat quality parameters assigned to each reach were further revised in the laboratory, considering field observations, photographs, habitat characterization method guidelines, riparian vegetation (Aránzazu Prada & Arizipe, 2009; ICNF, 2013c) and macrophytes identification guides (Fonseca et al., 2004; Duarte & Moreira, 2009), as well as Bing aerial and Google satellite images and COS2007 and CLC06_PT shapefiles.

The habitat quality was evaluated considering three times of the year, since some of the habitat quality parameters as those related to riparian vegetation and river flow depends on the season. Reaches habitat quality was mostly assessed in November 2013 and then monitored in March and June 2014, so the final scores of all habitat quality parameters assigned to each reach took into account the season.

The HABSCORE (RBP) method (Barbour et al., 1999) provides two different habitat assessment approaches, one designed for high-gradient or riffle/run prevalent streams (Appendix 7, RBP a)), and another for low-gradient or glide/pool streams (Appendix 7, RBP b)). Based on RBP guideline (Barbour et al., 1999), 23 reaches were assigned to low-gradient streams and 14 reaches to high-gradient streams. The habitat quality parameters assessed for high and low gradient streams are the same expect parameters 2, 3 and 7, which are referred as 2a, 3a and 7a for high gradient streams, and 2b, 3b and 7b for low gradient streams (Appendix 7, RBP).

To complete the FFI habitat quality evaluation, macroinvertebrate communities were prospected (Siligardi et al., 2007). Although the river habitat quality was mostly assessed in November, macroinvertebrates were sampled in June, when the flow conditions allowed the hand net to be used and the river substrates to be prospected. Considering the field observations, 16 sites were selected for macroinvertebrate sampling: four in river segment 1 (S1), one in river segment 2 (S2), three in river segment 3 (S3), two in river segment 4 (S4), one in each stretch of river segment 5 (S5) and three in river segment 6 (S6). Macroinvertebrate community at a sampling site was then assigned to the nearest reaches with similar hydromorphological conditions, since it was not feasible to have one sampling site for each reach.

Benthic macroinvertebrates were sampled with a hand net (60x30 cm; 0.5 mm mesh size) along 1 meter length transects taking into account the existing habitats. Macroinvertebrates on stones and submerged vegetation were also prospected. Samples from each site were placed in zip plastic bags and brought to the laboratory in a cool box. In the laboratory, samples were first preserved in ethanol (97% v/v) and stored at 4°C. Samples were later washed with tap water over a sieve (850 μm), and macroinvertebrates were separated and preserved in ethanol (97% v/v). Macroinvertebrates were identified up to the taxonomic level required in the FFI guideline.

2.5.2. Physico-chemical water parameters

The physico-chemical water parameters were determined for two times in November 2013 in all sampling sites. Sampling had to stop due to laboratory constraints, and was recaptured in March 2014 after a long precipitation period.

All mesotrophic stretches dominated by natural riparian areas (s1N, s2N, s3N and s4N) were sampled for three times in March 2014 during the leaf bag experiment (section 2.5.3), so biological and physico-chemical data could be crossed by referring to a same sampling time. All the other stretches from mesotrophic river segments were just sampled for two times in March. River segments 5 (S5) and 6 (S6) were one time sampled in March and then one time sampled in June, due to a new laboratory constraints occurring in March. Nitrite concentrations were only determined in March in the mesotrophic stretches dominated by natural riparian areas (s1N, s2N, s3N and s4N).

River segments were all sampled early in the morning to minimize differences in water temperature among segments. All mesotrophic river segments (S1-S4) and S6 were sampled from upstream to downstream sampling sites, so stretches from different river segments could be fairly compared. River segment 5 needed to be sampled in a reverse order to facilitate travels among sites

Stream current velocity was determined with a current meter (GO serial #3 17885, G.O. Environmental, Florida, USA). Conductivity, dissolved oxygen concentration, pH, and temperature were determined in situ with field probes (Multiline F/set 3 no. 400327, WTW, Weilheim, Germany). Stream water samples were collected with plastic bottles, transported in a cool box to the laboratory and analyzed within 24 hours. Ammonia (HACH kit, program 385), nitrite (HACH kit, program 371), nitrate (HACH kit, program 353), and phosphate (HACH kit, program 490) concentrations were determined with HACH DR/2000 spectrophotometer (Hach Company, Loveland, CO, USA).

2.5.3. Macroinvertebrate communities and leaf litter decomposition

Macroinvertebrate communities and leaf litter decomposition were analyzed at a representative site of each mesotrophic stretch dominated by natural riparian areas (s1N, s2N, s3N and s4N). To that end, portions of 4g (±0.01) of alder leaves (*Alnus glutinosa* Gaertn.), collected in Autumn 2010, were enclosed in coarse mesh bags (5 mm mesh size; 30x23 cm) to allow free movement of macroinvertebrates and water. Six leaf bags were immersed in each stretch in March 2014, and triplicate leaf bags were retrieved from each sampling site after 8 and 22 days. Each leaf bag was transferred to a zip plastic bag and brought to the laboratory in a cool box. In the laboratory, leaves were washed with tap water over a sieve (850 μm), and macroinvertebrates were separated and preserved in ethanol (97% v/v) within 24 hours. Leaves were dried at 60°C for 48 hours and weighted (±0.01g). Macroinvertebrates were identified to the lowest possible taxonomic level according to Tachet et al. (2010), using a stereo microscope (Leica Zoom 2000). Leaf bags from s3N were vandalized, thus no biological parameters could be determined.

2.5.4. River discharge

To determine the river discharge in the stretches, the existence of tributaries was first checked using the hidcod_25k_ptcont shapefile. Along river segments 2, 3 and 4 there were little or non-evidence of tributaries (Appendix 2, River segments 2, 3 and 4), so in each of them, the river discharge was determined at one site and then the value was assigned to all of its stretches. Some small tributaries existed along the first stretch of S1 (s1A) (Appendix 2, River segment 1), thus the river discharge was determined at its upstream and downstream sampling sites and the mean value between these two was assigned to the first stretch of S1. For the remaining stretches of S1, it was assumed that the river discharge determined at the downstream site of the first stretch remained constant, since no tributaries existed from there (Appendix 2, River segment 1). Along S5 there were also some small tributaries (Appendix 2, River segment 5), so the river discharge was determined at the upstream sampling sites of the first (s5A) and the third stretches (s5N), and the mean value between these two was assigned to each stretch of S5.

The river discharge was determined multiplying the cross-sectional area of the river channel by the length of a defined river section, and then dividing by the time for a float to travel this length (USEPA, 1997). To determine the river discharge in the stretches, the cross sectional area at a selected site was calculated measuring the river depths along a transect from one margin to the other. River discharges were determined in June 2014, when they were lower and thus facilitate the depth measurements.

For each selected site, the river channel depths were measured for every 30 cm from one margin to the other, so for a certain margin distance, it was possible to obtain the correspondent depth. These consecutive depth measurements allowed to divide the cross sectional areas into convex quadrilaterals with at least one pair of parallel sides (consecutive depth measurements), referred as trapezoids. The cross sectional area was then calculated by summing the trapezoids areas based on the trapezoidal rule as a technique to approximate a definitive integral of a function (Cazelais, 2008). The area of a trapezoid was calculated assuming two consecutive depth measures as the bases and the distance that separated them (30 cm) as the height.

After measuring the river depths from one margin to the other at a site, it was four or more times measured the traveled time for a float (leaves with similar size) to reach a defined point located 500, 700 or 10000 centimeters downstream. These lengths were established by defining homogeneous river sections, in which the cross sectional area could be considered constant. Different lengths were used among sites, since they have homogeneous sections with different lengths. Finally, the river discharges were determined by multiplying the cross sectional area of the river channel (cm^2) by the travel distance (cm) and dividing by the float travel time (s).

The river discharge was not determined at S6, since the river had a large channel width and depth that prevented this method to be used.

2.6. Data analysis

Changes in physico-chemical water parameters along a stretch were expressed as the difference between its downstream and upstream sampling sites. Stretches with higher depletions had higher negative values (***Δ*** µg L^{-1}). Changes in physico-chemical water parameters along a stretch were further divided by its length (***Δ*** µg L^{-1} km^{-1}), so stretches could be compared. Changes in physico-chemical water parameters were analyzed considering nutrient concentrations (***Δ*** µg L^{-1}) rather than their total amount (***Δ*** µg), since the latter would cause stretches with greater flow rates to achieve higher depletions. Additionally, the river discharge from which the nutrients amount could be calculated was only determined in June, while the physico-chemical water parameters were determined in different times of the year when the flow had different rates.

To assess the effect of nutrient concentrations on nutrient depletion along the stretches, the values for nutrient concentrations at the upstream sampling site of each stretch were used. To assess the effect of conductivity, pH, and temperature on nutrient depletion, the mean value between the upstream and downstream sampling sites of each stretch were considered, to describe the stretches general condition. For dissolved oxygen, the mean value or a representative value for the stretch dominant condition was used.

For each stretch, there was more than one sample for the physico-chemical water parameters, but just one value for the percentage of each land cover class and habitat quality parameter. Therefore, all the samples of a stretch were associated with its value for the percentage of every land cover class and habitat quality parameter.

Data on physico-chemical water parameters were previously analyzed to outliers identification for river segments classified as mesotrophic (S1-S4), eutrophic (S5) and large (S6) independently, as well as for each individual stretch regarding physico-chemical water parameters and changes in physico-chemical water parameters along the stretches. Data exclusion was based on outlier results and notes from the sampling campaigns.

When S5 was sampled after a severe rainy period (March 2014), the stream flow was higher and the physico-chemical water parameters were completely different from those found in the other sampling dates (November 2013 and June 2014), where the samples were consistent and similar to each other. Thus, statistical analysis for S5 (section 3.3) refers to data from November and June, since it intendeds to elucidate how physico-chemical water parameters commonly changed along the eutrophic stretch (S5).

To assess the physico-chemical water parameters more related to ammonium, nitrate and phosphate depletions just the samples where these had depleted were considered.

For the habitat quality parameters related to river bank erosion (Appendix 7, FFI, parameter 8), vegetation in the wet riverbed (Appendix 7, FFI, parameter 12), pulpy and anaerobic plant detritus (Appendix 7, FFI, parameter 13), and channel alteration (Appendix 7, RBP, parameter 6, and QBR, parameter 4), lower scores means they were more present. Thus, to facilitate interpretation, these habitat quality parameters were analyzed in an inverse order, so stretches with more river bank erosion, vegetation in wet riverbed, pulpy and anaerobic plant detritus and channel alterations could have the highest values. Original scores were used to determine the habitat quality final scores.

The Iberian Biological Monitoring Working Party (IBMWP) (Alba-Tercedor & Sánchez-Ortega, 1988; Alba-Tercedor, 1996) was applied to leaf-associated macroinvertebrates and compared among stretches. Macroinvertebrates were assigned to functional feeding groups and habitat behavior groups based on Barbour et al. (1999). Taxonomic and functional Shannon diversity and richness were calculated using vegan package in R (Oksanen et al., 2013). Evenness was determined using Pielou index. Decomposition rates were determined according to the exponential model $m_t = m_0.e^{-kt}$, where m_t is the remaining leaf dry mass at the time t, m_0 the initial leaf dry mass and k the decomposition rate.

Statistical analyses were all performed using R 3.0.2 (R Foundation for Statistical Computing, Vienna, Austria). Cluster analysis was used to test for similarities among i) river segments regarding physico-chemical water parameters, current velocity and river channel width, and ii) all stretches from mesotrophic river segments regarding their percentage of artificial areas (Appendix 4, land cover class 1), agricultural areas (Appendix 4, land cover class 2) and natural and vegetated areas (Appendix 4, land cover class 3). Cluster analyses were performed using Ward´s hierarquical method and Euclidean distance. Data were previously standardized since variables had different scales and/or variance (Norušis, 2010). The term standardization refers to a numeric data matrix that was centered (subtracting column mean to each column value), and then scaled (dividing each column value by the column standard deviation). Cluster branches were adjusted using dendextend package (Galili, 2015).

Analysis of similarities (ANOSIM) were used to test for significant differences among i) river segment types (mesotrophic, eutrophic and large) regarding physico-chemical water parameters, current velocity and river channel width; ii) the groups of mesotrophic stretches regarding land cover; habitat quality parameters; changes in nutrient concentrations; and changes in conductivity, dissolved oxygen, pH and temperature along the stretches, iii) the mesotrophic stretches dominated by natural riparian areas (s1N-s4N) regarding physico-chemical water parameters; changes in nutrient concentrations and changes in conductivity, dissolved oxygen, pH and temperature, iv) the stretches from the eutrophic river segment (S5) regarding changes in dissolved oxygen, pH and temperature, and v) the stretches from the large river (S6) regarding physico-chemical water parameters; changes in nutrient concentrations; and changes in conductivity, dissolved oxygen, pH and temperature. ANOSIMs were performed using Euclidian distances. Data were previously standardized since variables had different scales and/or variance (Norušis, 2010). ANOSIM were performed considering samples with complete observations as they cannot be done with data containing missing values. Thus, nutrient concentrations and conductivity, dissolved oxygen, pH and temperature were for some separately analyzed, to avoid loss information regarding a parameter just because a missing value existed for another.

One-way ANOVAs (Chambers et al.,1992) were used to test for significant differences in i) physico-chemical water parameters among river segments type, and ii) macroinvertebrate abundance, taxonomic and functional Shannon diversity, richness and evenness among s1N-s4N. ANOVAs were followed by Tukey's post-tests to assess where differences occurred (Yandell, 1997). Data were tested for Gaussian distribution (Shapiro-Wilk test) (Royston, 1995) and homoscedasticity (Bartlett test) (Bartlett, 1937). Kruskal-Wallis tests (Hollander et al., 2013), followed by a Dunn´s test from dunn.test package (Dinno, 2015) were used whenever data did not achieved normal distribution or were not homoscedastic.

SIMPER tests were used to assess the macroinvertebrate taxa and functional groups that were more distinct among s1N-s4N. SIMPERs were performed with Bray-Curtis dissimilarities.

BIOENV was used to assess the land cover that better explained differences in habitat quality parameters in mesotrophic stretches. BIOENV was performed using Euclidean distance, Spearman correlation method, and data for habitat quality previously standardized, since these had different scales (Norušis, 2010). BIOENV was done considering a set of selected land cover (Appendix 4, land cover classes from level 2 and 3) as environmental factors, since the test does not run for a high number of variables.

ANOSIMs, SIMPER and BIOENV were performed using vegan package (Oksanen et al., 2013).

To assess the land cover and the habitat quality parameters that were better represented or more scarce at a stretch or a group of stretches, the values were first standardized and then ordered, not only because they had different scales and/or variance (Norušis, 2010), but also to obtain the values for a stretch or a group of stretches considering the scores for the others.

Principal Component Analysis (PCAs) were performed with standardized data to ordinate i) the 6 river segments according to the physico-chemical water parameters, current velocity and river channel width, ii) the mesotrophic stretches (S1-S4) according to the land cover and habitat quality parameters, and iii) the mesotrophic stretches dominated by natural riparian areas (s1N-s4N) according to the land cover and habitat quality; the physico-chemical water parameters, current velocity and changes in physico-chemical water parameters along stretches; and the relative proportion of the most abundant taxa, functional feeding groups and habitat behavior groups of benthic macroinvertebrates. In PCA with variables that had more than one value for a determined river segment or stretch, these were represented in the PCA plot by their centroids. The PCA of the physico-chemical water parameters, current velocity and river channel width of the 6 river segments was done considering the replicates with complete observations.

Data on physico-chemical water parameters described for natural stretches (s1N-s4N) and then considered to the PCA in section 3.2.1.2 refer to those registered in March 2014, to match with macroinvertebrate data, which were solely collected in March. Data on physico-chemical water parameters of the mesotrophic stretches dominated by natural riparian areas (s1N-s4N) of all campaigns were used to correlation and regression analysis in section 3.2.1.4. Ammonium, nitrite, nitrate, and phosphate concentrations as conductivity and temperature tended to decreased while pH and dissolved oxygen to increase along s1N-s4N, so the values for the first set of parameters were reversed for the PCA, so that higher depletions could be assigned to the highest positive values, to facilitate interpretation.

The PCAs regarding land cover and habitat quality parameters were performed with selected parameters that best distinguished the group of mesotrophic stretches or s1N-s4N.

Data of macroinvertebrates for the PCA were expressed as the percentage of taxa and functional groups in each stretch, so stretches were ordinated according to macroinvertebrate proportion regardless their total abundance (Holland, 2013). Taxa considered for the PCA were the 3 most abundant in each stretch.

Correlations were done using Pearson correlation method and the asymptotic P-values were determined using Hmisc package (Harrel et al., 2014). The graphical displays of the correlation matrixes, also called correlograms, were done with corrgram package (Wright, 2013).

The 3D scatterplots were created using scatterplot3d package (Ligges & Mächler, 2003). Data for changes in ammonium concentration along the stretches were previously reversed, so higher depletions could be assigned to the highest positive values for the scatterplot to be performable.

Linear regression analyses (linear models) were used to test if changes in ammonium and nitrate concentrations along the mesotrophic stretches dominated by natural riparian areas (s1N-s4N) could be related to ammonium and nitrate concentrations, pH or dissolved oxygen. Independent variables were those highly correlated to changes in ammonium and nitrate concentration. Changes in nitrate concentrations predicted by the linear regression model for s5N were obtained using the values registered for nitrate concentration in s5N. Regression plots were created using ggplot2 package (Wickham, 2009).

2.7. Ecosystem services valuation

A replacement cost method was used to estimate the economic value of the water purification service provided by the stretches considering the monetary cost to reduce water nitrogen. For stretches where nitrogen concentration tended to increase it was estimated the monetary cost to restore water nitrogen.

The monetary value to reduce water nitrogen underlies the construction, operation and maintenance of constructed wetlands and was estimated by La Notte et al. (2012), based on an UK inventory of methods to control diffuse pollution and corresponded to 2463 euros per ton of nitrogen removed. A benefit transfer method was used, which means that the existing data were used in a new policy context (Boyle et al., 2010; Liu et al., 2010; Kumar, 2010), since the Portuguese water agencies had no estimation cost for nitrogen removal. Constructed wetlands are not common in Portugal, but they are worldwide applied to purify water from different sources as agricultural and effluent emissions (Kadlec & Wallace, 2009) and to nutrient removal at low concentrations (Vymazal, 2007), as those found in most river segments.

To estimate the replacement cost or the monetary cost to restore water nitrogen, the concentrations of ammonia (NH_3-N) and nitrate (NO_3-N) at the two sampling sites of each stretch (mg L^{-1}) were first multiplied by the stretch flow (dm^3 s^{-1}) to determine the quantity of ammonia and nitrate that was passing at each site per second (mg s^{-1}). To calculate the quantities that were retained or become available along a stretch per second, the differences in the amount of ammonia and nitrate between its downstream and upstream sampling sites were estimated (***Δ*** mg s^{-1}). These differences were then converted to tons and reversed, so that the stretches with nutrient depletions could have the highest positive values. The quantity of ammonia and nitrate that were retained or become available per second at each sample of a stretch were summed, and multiplied by the cost of nitrogen removal (€ ton^{-1}) (La Notte et al. 2012), so that a maximum, minimum and median monetary value could be estimated for each stretch. Monetary costs were then estimated on a daily (€ day^{-1}) or annual basis (€ $year^{-1}$), and further divided by the stretch length (€ $year^{-1}$ m^{-1}).

Nitrite concentration was not used to calculate total nitrogen, since it was just determined in the mesotrophic stretches dominated by natural riparian areas (s1N-s4N). River segment 6 (S6) was not included, since the method used to determine river discharge was not suitable for S6 and the closest hydrometric stations did not provide data on river discharge.

3. Results

3.1. Physico-chemical water parameters of the river segments in the Ave River watershed

The physico-chemical water parameters, current velocity and channel width of the 6 studied river segments in the Ave River watershed are in Table 1.

Table 1. Physico-chemical water parameters, current velocity and channel width of the 6 studied river segments in the Ave River watershed. Data represent mean values ± SD (n=4, except for ammonium and conductivity, n=3). S1 (river segment 1), S2 (river segment 2), S3 (river segment 3), S4 (river segment 4), S5 (river segment 5), S6 (river segment 6).

	River segment					
	Mesotrophic				Eutrophic	Large
	S1	S2	S3	S4	S5	S6
Ammonium ($mg\ L^{-1}\ NH_4^+$)	0.01 ± 0.01	0.01 ± 0.01	0.07 ± 0.06	0.06 ± 0.03	1.01 ± 0.10	0.16 ± 0.09
Nitrate ($mg\ L^{-1}\ NO_3^-$)	3.5 ± 1.1	11.9 ± 0.9	8.3 ± 0.4	6.5 ± 1.9	17.9 ± 3.6	9.0 ± 1.3
Phosphate ($mg\ L^{-1}\ PO_4^{3-}$)	0.01 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.03 ± 0.01	0.21 ± 0.06	0.09 ± 0.05
Conductivity ($\mu S\ cm^{-1}$)	40 ± 2	72 ± 4	79 ± 19	84 ± 6	160 ± 4	146 ± 41
Oxygen ($mg\ L^{-1}$)	11.15 ± 0.79	9.94 ± 0.58	10.53 ± 0.51	10.63 ± 0.22	9.45 ± 0.44	9.79 ± 0.89
pH	6.74 ± 0.24	6.42 ± 0.18	6.69 ± 0.05	6.95 ± 0.09	6.95 ± 0.14	7.11 ± 0.14
Temperature (°C)	11.10 ± 1.06	12.38 ± 1.85	11.35 ± 1.01	11.13 ± 1.11	13.65 ± 2.44	13.59 ± 3.21
Current velocity ($cm\ s^{-1}$)	46 ± 6	11 ± 1	53 ± 5	86 ± 4	70 ± 4	21 ± 1
Channel width (m)	6.60	4.50	6.10	6.30	5.40	26.85

The cluster analysis based on the physico-chemical water parameters, current velocity and channel width of the 6 river segments demonstrated the existence of 3 groups (ANOSIM, R=0.95), which were classified as mesotrophic (S1-S4), eutrophic (S5) and large (S6) (Figure 3).

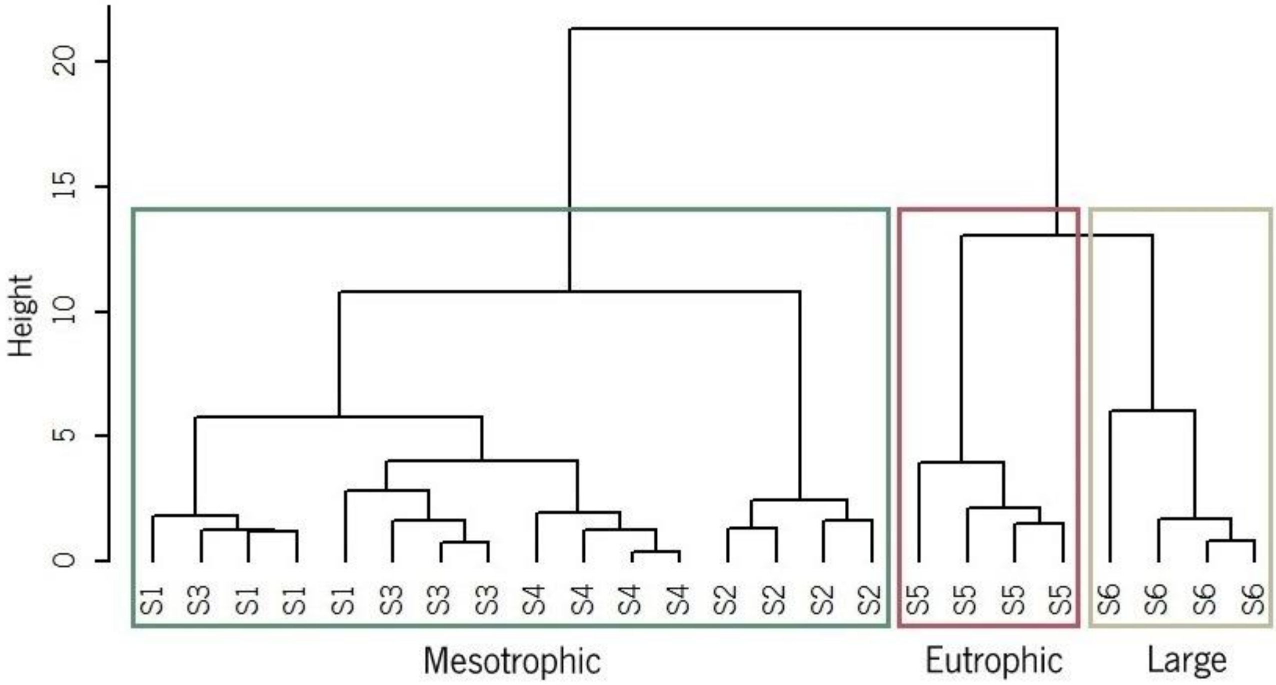

Figure 3. Cluster analysis based on concentrations of ammonium, nitrate and phosphate, conductivity, dissolved oxygen, pH, temperature, current velocity and channel width of the 6 river segments.

The Principal Component Analysis (PCA) of the physico-chemical water parameters, current velocity and channel width of the 6 river segments showed that the Principal Component 1 (PC1) explained 52.7% of the total variance, while PC2 explained 21.3%. PC1 separated mesotrophic river segments (S1-S4) from S5 and S6 based on concentrations of phosphate, ammonium, oxygen and conductivity (Figure 4). PC2 further separated the eutrophic river segment (S5) from S6 based on concentrations of ammonium, nitrate and channel width (Figure 4). PCA corroborated the results of the cluster analysis.

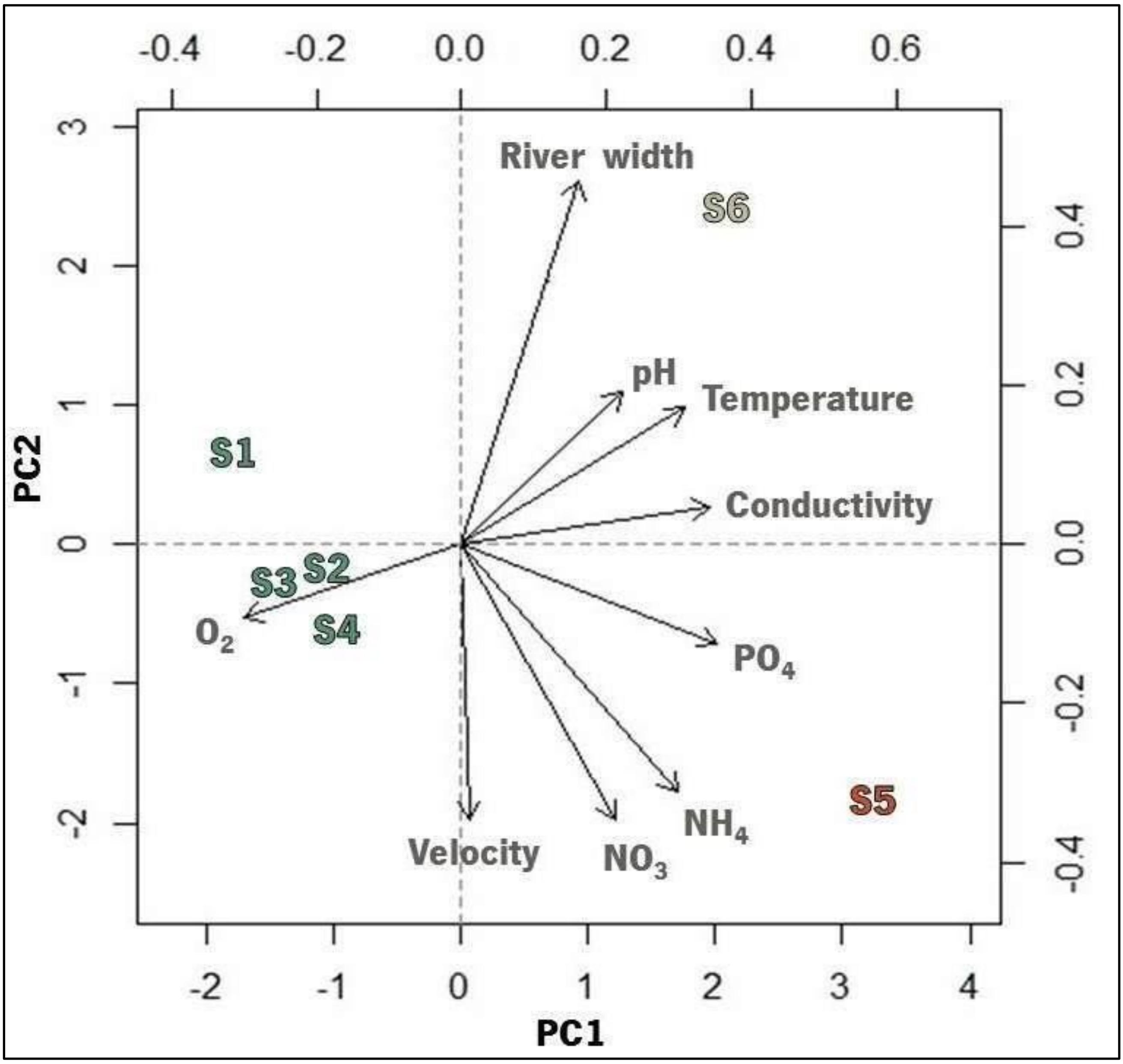

Figure 4. Principal Component Analysis (PCA) of the physico-chemical water parameters, current velocity and channel width of the 6 river segments. The upper and the right axes refer to the variable loadings, while the lower and the left axes refer to the PC1 and PC2 scores.

The eutrophic river segment (S5) had higher conductivity (Dunn´s test, P<0.01) and concentration of ammonium (Dunn´s test, P<0.01), nitrate (Tukey's post-test, P<0.0001), and phosphate (Dunn´s test, P<0.001) than the mesotrophic river segments (S1-S4), but lower dissolved oxygen (Tukey's post-test, P<0.05). River segment 6 (S6) had higher concentration of ammonium (Dunn´s test, P<0.05) and phosphate (Dunn´s test, P<0.01), conductivity (Dunn´s test, P<0.01) and pH (Tukey's post-test, P<0.01) than the mesotrophic river segments, and lower nitrate concentration than S5 (Tukey's post-test, P<0.01) (Table 1). The river width was more than 4 times higher in S6 than in the other river segments.

3.2. Influence of land cover on habitat quality and changes in physico-chemical water parameters along the mesotrophic river segments

The cluster analysis based on the percentage of artificial areas (Appendix 4, land cover class 1), agricultural areas (Appendix 4, land cover class 2) and natural and vegetated areas (Appendix 4, land cover class 3) of the stretches from the mesotrophic river segments (S1-S4), demonstrated the existence of 3 groups (ANOSIM, R=0.98), which were classified as agricultural (s1A, s3A, s4A), urbanized (s1U) and natural (s1N, s2N, s3N, s4N) stretches (Figure 5).

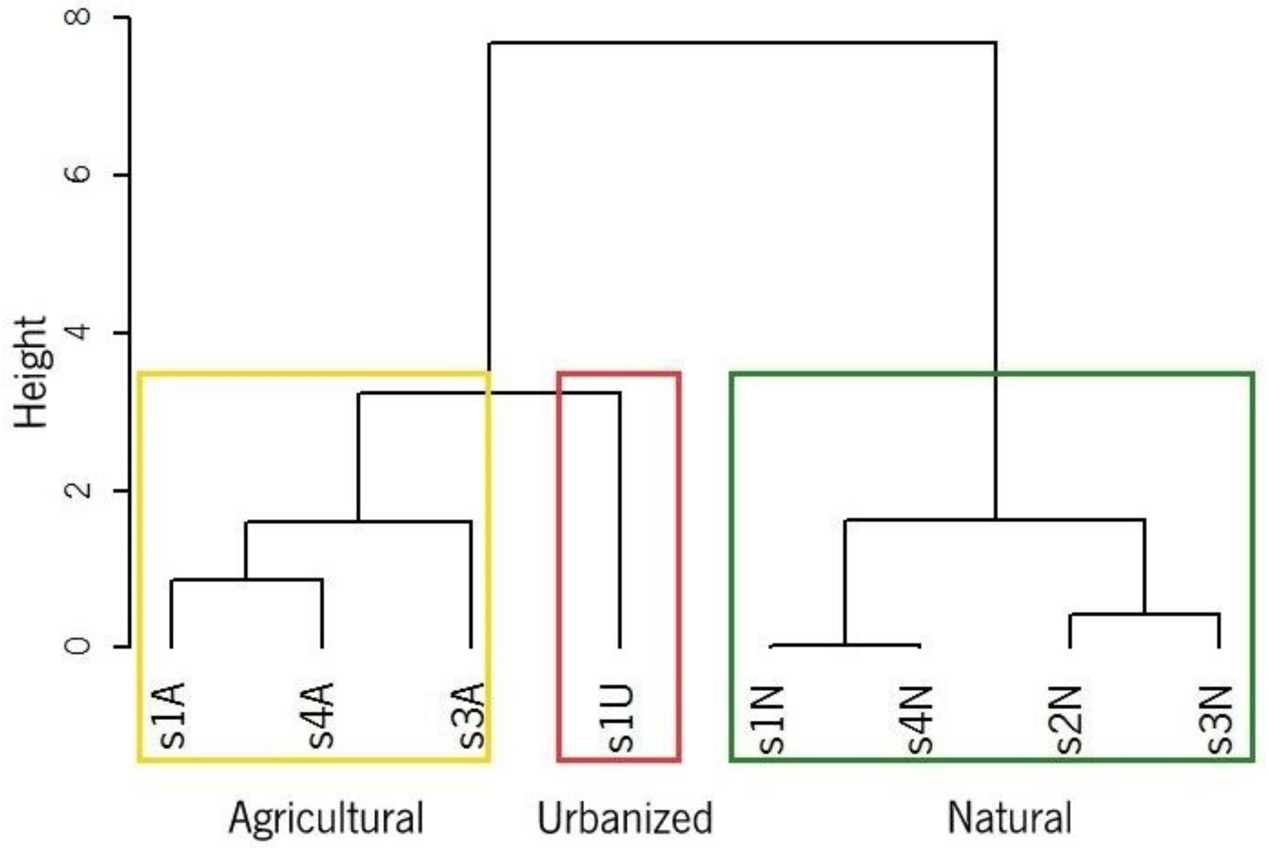

Figure 5. Cluster analysis based on the percentage of artificial areas (Appendix 4, land cover class 1), agricultural areas (Appendix 4, land cover class 1) and natural and vegetated areas (Appendix 4, land cover class 1) of the stretches from the mesotrophic river segments (S1-S4). Numbers indicate the river segment of the stretch (e.g. s1, stretch from river segment 1), while letters indicate the stretch group according to the cluster analysis: A, agricultural, U, urbanized, and N, natural.

Results demonstrated that dense wooded areas (Appendix 4, land cover class 3.1.1) were more abundant in natural stretches and better distinguished natural from agricultural and urbanized stretches, suggesting that this land cover was the more affected by anthropic activities.

Among agricultural stretches, s4A had a greater percentage of agricultural areas (Appendix 4, land cover class 2) and a higher slope of agricultural lands (54.1% and 3.82%, respectively) than s3A (30.6% and 3.71%, respectively) and s1A (44.2% and 3.48%, respectively).

The urbanized stretch (s1U) was dominated by natural and vegetated areas (Appendix 4, land cover class 3 with a coverage of 74.9%) but had more residential buildings (Appendix 4, land cover class 1.1.1) and other artificial surfaces (Appendix 4, land cover class 1.2) than the natural and the agricultural stretches (Appendix 2, s1U).

The groups of mesotrophic stretches also differed regarding their habitat quality attributes (ANOSIM, R=0.57). The habitat quality tended to be higher in natural stretches than in agricultural and urbanized stretches, regardless the habitat characterization method. The habitat quality parameters related to riparian vegetation, namely the width of functional vegetation (Appendix 7, Fluvial Functioning Index (FFI), parameter 3), bank vegetative protection (Appendix 7, HABSCORE (RBP), parameter 9) and riparian cover structure and quality (Appendix 7, Riparian Forest Quality Index (QBR), parameters 2 and 3 respectively) were among the most affected by anthropic activities. River bank erosion (Appendix 7, FFI, parameter 8), morphological and structural diversity of the river channel (Appendix 7, FFI, parameter 9) and channel naturalness (Appendix 7, QBR, parameter 4) were also among the habitat quality parameters that better distinguished agricultural from the other stretches, due to a higher prevalence of erosion, a lower morphological diversity, and the existence of pronounced river channel modifications in agricultural stretches. The vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and the presence of pulpy and anaerobic plant detritus (Appendix 7, FFI, parameter 13) were similar in natural and agricultural stretches, but tended to be greater in the latter. The flooding efficiency (Appendix 7, FFI, parameter 6) was generally improved in natural stretches, although poorly distinguishable from agricultural stretches. However, the low flooding efficiency was caused by embankments in agricultural stretches and by the prevalence of v-shaped valleys in natural stretches. Results further suggested that the presence of more urban areas mostly affected the river channel naturalness (Appendix 7, QBR, parameter 4), the structure and quality of riparian vegetation (Appendix 7, QBR, parameters 2 and 3 respectively) and its continuity (Appendix 7, FFI, parameter 4), but natural stretches tended to have more vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) than the urbanized stretch (s1U).

Results demonstrated that arable lands (Appendix 4, land cover class 2.1.1) and dense wooded areas (Appendix 4, land cover class 3.1.1) were the land cover that best explained differences in habitat quality attributes among the groups of mesotrophic stretches (BIOENV, correlation=0.71).

The Principal Components Analysis (PCA) of the most distinct land cover and habitat quality parameters among the groups of mesotrophic stretches showed that PC1 explained 60.8% of the total variance, while PC2 explained 17.2%. PCA corroborated differences in land cover and habitat quality parameters among the groups of mesotrophic stretches (natural, agricultural and urban) (Figure 6).

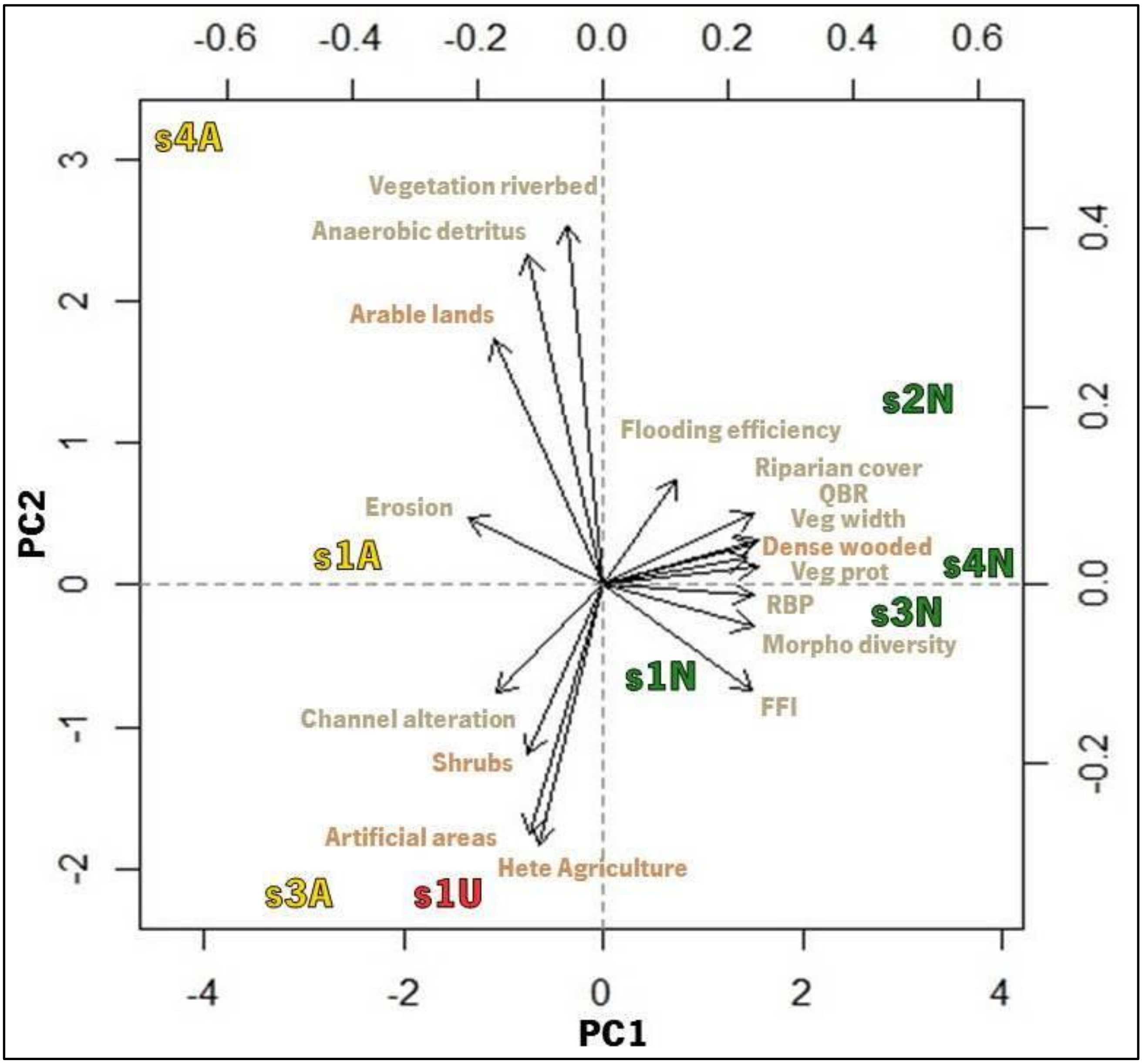

Figure 6. Principal Component Analysis (PCA) of the most distinct land cover and habitat quality parameters among the groups of mesotrophic stretches. The upper and the right axes refer to the variable loadings, while the lower and the left axes refer to the PC1 and PC2 scores. Stretches are distinguished by colors and capital letter according to their group in the cluster analysis (Figure 5). Land cover classes are represented in brown as Arable lands (Appendix 4, land cover class 2.1.1), Dense wooded (Appendix 4, land cover class 3.1.1), Hete Agriculture (Appendix 4, land cover class 2.1.3), Artificial areas (Appendix 4, land cover class 1), Shrubs (Appendix 4, land cover class 3.2). Habitat quality parameters are represented in light grey as Erosion (Appendix 7, FFI, parameter 8), Anaerobic detritus (Appendix 7, FFI, parameter 13), Vegetation riverbed (Appendix 7, FFI, parameter 12), Flooding efficiency (Appendix 7, FFI, parameter 6), Riparian cover (Appendix 7, QBR, parameter 2), QBR (Appendix 7, QBR, final score), Veg. width (Appendix 7, FFI, parameter 3), Veg

prot (Appendix 7, RBP, parameter 9), RBP (Appendix 7, RBP, final score), Morpho diversity (Appendix 7, FFI, parameter 9), FFI (Appendix 7, FFI, final score), and Channel alteration (Appendix 7, QBR, parameter 4).

Changes in nutrient concentrations and conductivity, dissolved oxygen, pH and temperature along the stretches differed among the groups of mesotrophic stretches (ANOSIM, R=0.25 and 0.52 respectively), suggesting that land cover can also affect the river chemistry. Changes in ammonium concentration often decreased along stretches regardless the land cover (Figure 7). Nitrate concentration frequently decreased along natural stretches and increased along agricultural and the urbanized stretch (Figure 7). Phosphate concentration tended to increase along agricultural stretches and to decrease along natural stretches, but changes in phosphate concentration differed less among the groups of mesotrophic stretches than changes in nitrate concentration (Figure 7).

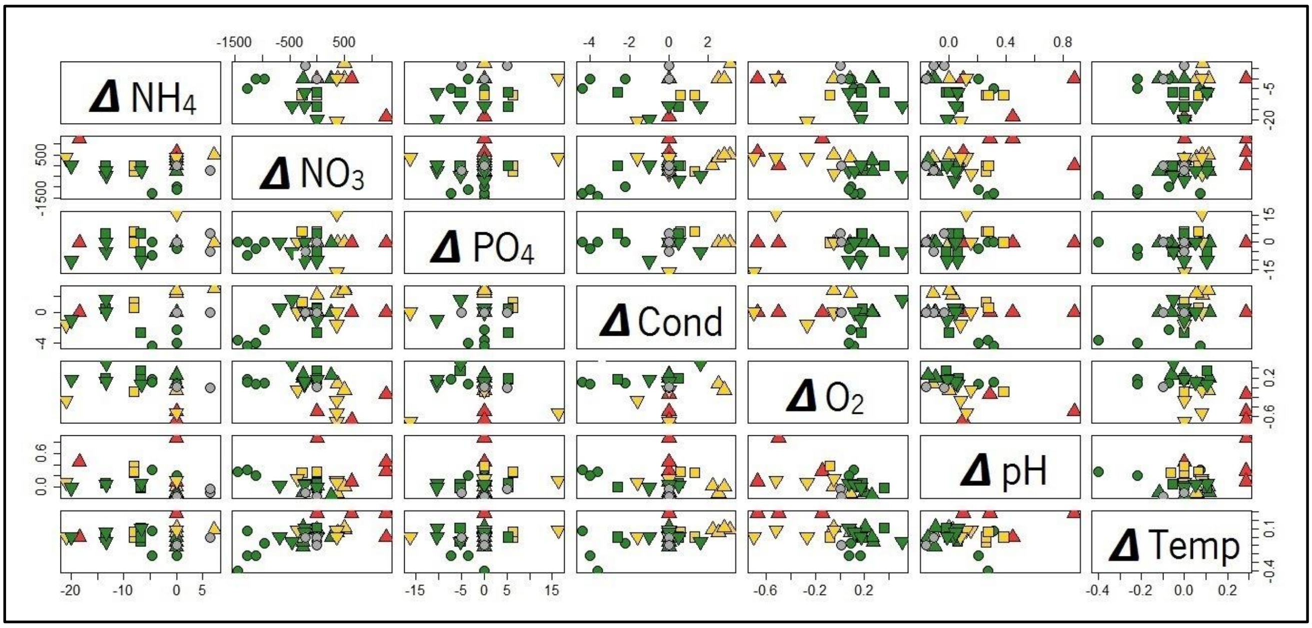

Figure 7. Plot diagram of changes in physico-chemical water parameters along the stretches of the mesotrophic river segments (S1-S4). The values of agricultural stretches are in yellow, urbanized stretch are in red, natural stretches are in green, and the concrete channel is in grey. Stretches from S1 are a triangle pointing up, stretch from S2 is a circle, stretches from S3 are a square and stretches from S4 are a triangle pointing down. Diagonal panel indicates changes along the stretches in ***Δ***NH_4 (NH_4^+ µg L^{-1} km^{-1}), ***Δ***NO_3 (NO_3^- µg L^{-1} km^{-1}), ***Δ***PO_4 (PO_4^{3-} µg L^{-1} km^{-1}), ***Δ***Cond (conductivity µS cm^{-1} km^{-1}), ***Δ***O_2 (dissolved oxygen mg L^{-1} km^{-1}), ***Δ***pH (km^{-1}) and ***Δ***Temp (temperature °C km^{-1}).

Conductivity tended to decrease along natural stretches and to increase along agricultural stretches (Figure 7). Dissolved oxygen increased along natural stretches and decreased along agricultural and urbanized stretches (Figure 7). Temperature and pH increased more along the urbanized stretch (Figure 7). Physico-chemical water parameters had no relevant changes along the concrete channel (Figure 7).

Despite differences among groups, changes in physico-chemical water parameters along the stretches also differed within groups. In agricultural stretches, nitrate concentration and conductivity had stronger increments along s1A, phosphate concentration tended to increase along s3A, oxygen concentration had higher depletions along s4A, and pH increased more along s3A (Figure 7). Changes in physico-chemical water parameters also differed among natural stretches as described in section 3.2.1.2.

Correlation analysis corroborated the observed pattern in Figure 7, since nitrate concentration and conductivity tended to decrease (Pearson correlation= -0.48, -0.46; P= 0.005, 0.02, respectively) and dissolved oxygen to increase (Pearson correlation= 0.66, P= 0.0005) along stretches with more natural riparian areas (Appendix 4, land cover class 3), while an opposite trend was observed along stretches with more agricultural (Appendix 4, land cover class 2) and urban land use (Appendix 4, land cover class 1) (Figure 8). Nitrate concentration, pH and temperature increased more along urbanized stretches (Appendix 4, land cover class 1) (Pearson correlation= 0.50, 0.68, 0.45; P=0.003, <0.001, 0.008, respectively), while conductivity increased more along stretches with more agricultural land (Appendix 4, land cover class 2) (Pearson correlation= 0.46, P= 0.002) (Figure 8).

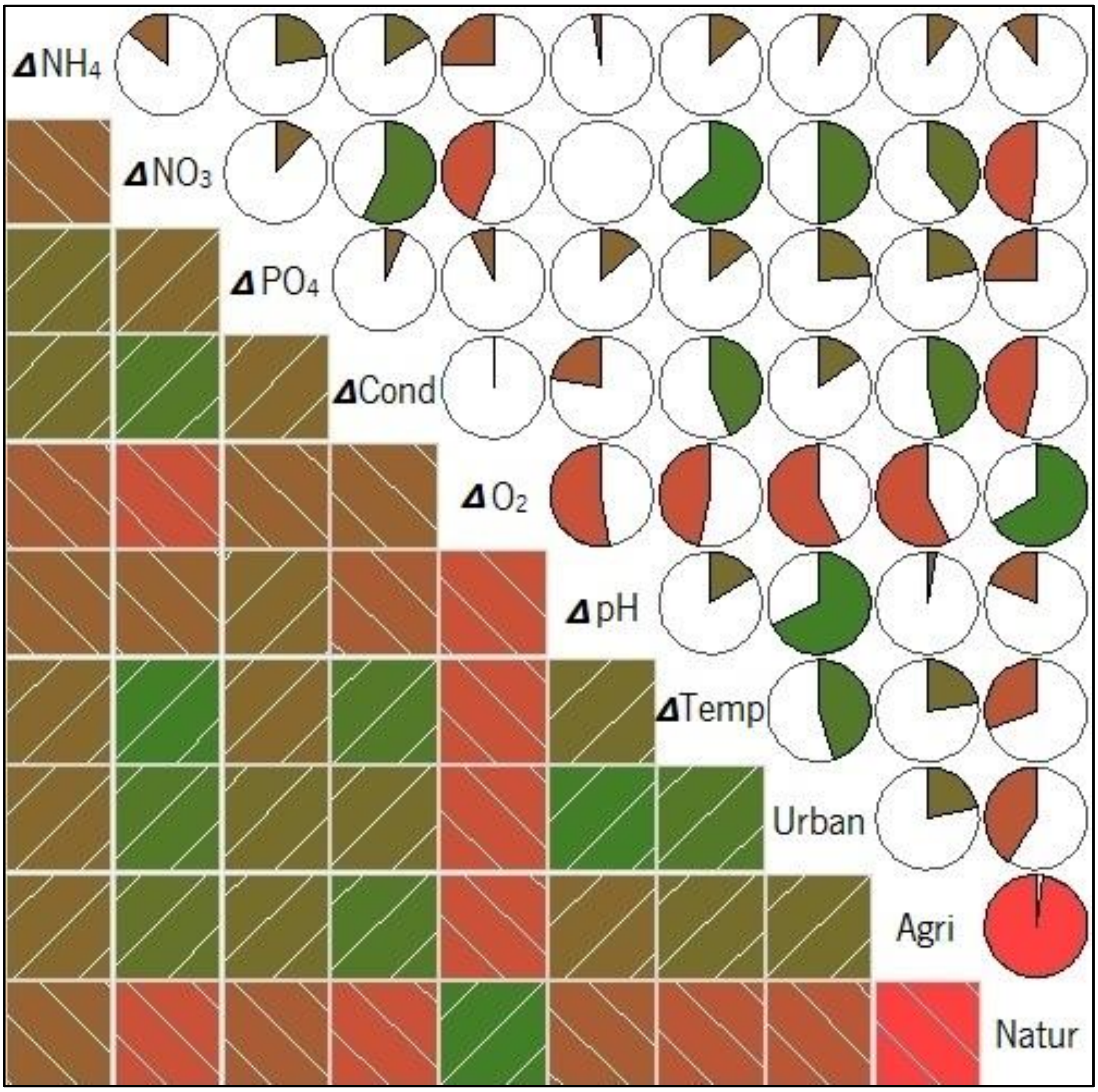

Figure 8. Correlogram of changes in physico-chemical water parameters and land cover along the mesotrophic stretches. Red colour was assigned to negative correlations and green to positive correlations. Panels with red colour and lines leaning to left mean negative correlations; pies with red colour start turning to the left also refer to negative correlations. Diagonal panel indicates changes along the stretches in ***Δ***NH_4 (NH_4^+ µg L^{-1} km^{-1}), ***Δ***NO_3 (NO_3^- µg L^{-1} km^{-1}), ***Δ***PO_4 (PO_4^{3-} µg L^{-1} km^{-1}), ***Δ***Cond (conductivity µS cm^{-1} km^{-1}), ***Δ***O_2 (dissolved oxygen mg L^{-1} km^{-1}), ***Δ***pH (km^{-1}), ***Δ***Temp (temperature °C km^{-1}), and the percentage of artificial areas (Appendix 4, land cover class 1) (Urban), agriculture areas (Appendix 4, land cover class 2) (Agri) and natural and vegetated areas (Appendix 4, land cover class 3) (Natur) of the stretches.

Ammonium depletion was best correlated with ammonium concentration (Pearson correlation= -0.62, P= 0.001) than with dissolved oxygen (Pearson correlation= -0.26, P= 0.2) (Figure 9).

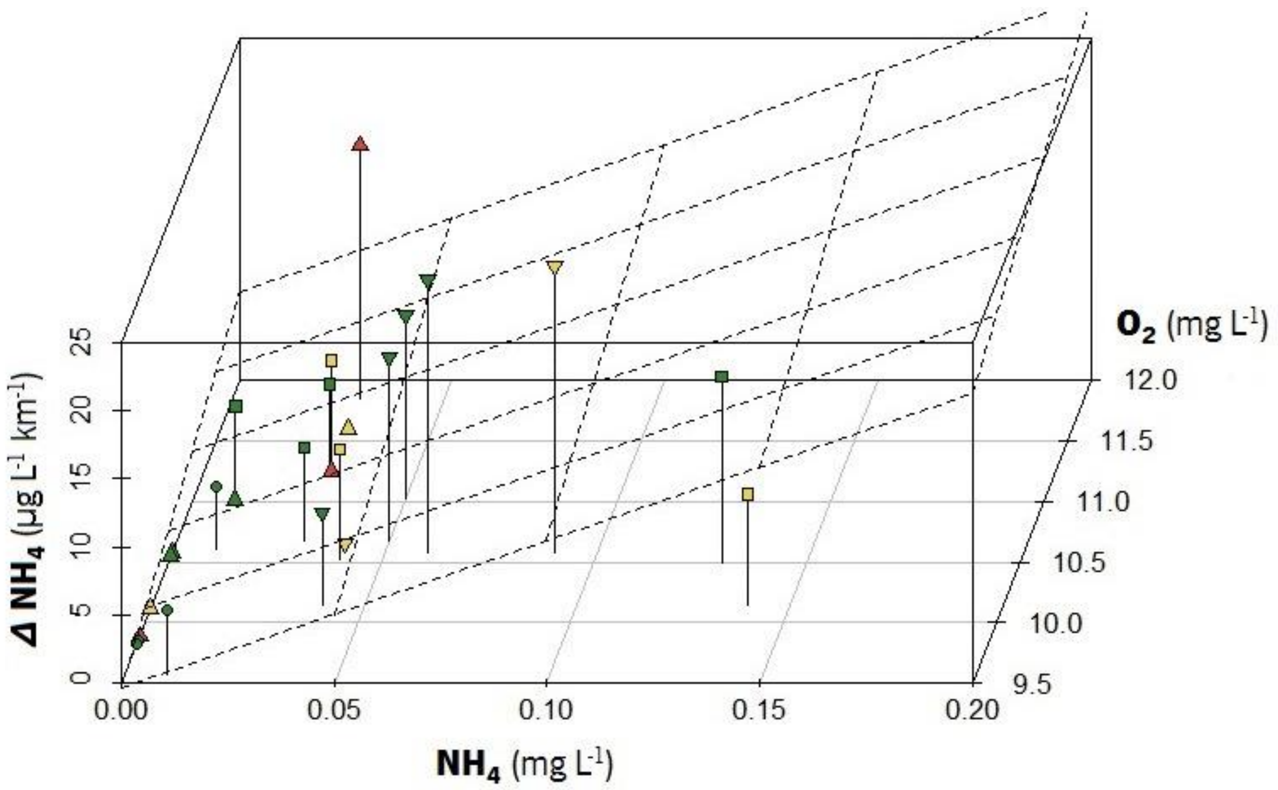

Figure 9. Three dimensional scatterplot of the relationship of changes in ammonium concentration along the mesotrophic stretches (***Δ***NH_4 as the differences in NH_4^+ concentration between sampling sites of a stretch; µg L^{-1} km^{-1}) with ammonium concentration (NH_4 as ammonium concentration at the upstream sampling site of a stretch; mg L^{-1} NH_4^+) and dissolved oxygen (O_2 as the representative value of dissolved oxygen at a stretch; mg L^{-1}). The samples of the agricultural stretches are in yellow, urbanized stretch are in red and natural stretches are in green. Stretches from S1 are a triangle pointing up, stretch from S2 is a circle, stretches from S3 are a square and stretches from S4 are a triangle pointing down.

3.2.1. Factors related to changes in physico-chemical water parameters along the mesotrophic stretches dominated by natural riparian areas

3.2.1.1. Land cover and habitat quality

Natural stretches from S1-S4 (s1N-s4N) had different natural land cover. The stretches s4N and s3N were mostly dominated by dense wooded areas (Appendix 4, land cover class 3.1.1) (Appendix 2, s4N and s3N), while s1N and s2N had more sparse wooded areas (Appendix 4, land cover class 3.1.2), shrubs and herbaceous vegetation (Appendix 4, land cover class 3.2) and open spaces with little or no vegetation (Appendix 4, land cover class 3.3) (Appendix 2, s1N and s2N).

Natural stretches also differed regarding their habitat quality attributes. According to the Fluvial Functioning Index (FFI) the river functionality was very good in s4N, s2N and s3N (score 241, 239, 221, respectively), and very good - good in s1N (score 195). The HABSCORE (RBP) indicated that the habitat quality was optimal in s4N, s3N and s2N (score 175, 166, and 164, respectively) and suboptimal in s1N (score 135). According to the Riparian Forest Quality Index (QBR), the riparian habitat had good quality in s2N, s4N and s3N (score 86, 85 and 84, respectively), and fair quality in s1N (score 59).

Although all stretches had an overall good habitat quality, natural stretches had different improved attributes. The habitat quality parameters best represented in s1N than in the other natural stretches were the flow stability (Appendix 7, FFI, parameter 5), the macrobenthic community (Appendix 7, FFI, parameter 14), and the lack of erosion (Appendix 7, FFI, parameter 8); in s2N were the abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12), the presence of pulpy and anaerobic plant detritus (Appendix 7, FFI, parameter 13), and the flooding efficiency (Appendix 7, FFI, parameter 6); in s3N were the frequency of riffles and channel sinuosity (Appendix 7, RBP, parameter 7a), the presence of functional vegetation (Appendix 7, FFI, parameter 2) and the absence of stream channel modifications (Appendix 7, RBP, parameter 6); and in s4N were the total riparian cover (Appendix 7, QBR, parameter 1), the variety of riverbed habitats and retention structures (Appendix 7, FFI, parameter 7), and the hydromorphological diversity of the stream channel (Appendix 7, FFI, parameter 11).

The Principal Components Analysis (PCA) of the natural land cover and the most distinct habitat quality parameters among the natural stretches showed that PC1 explained 70.1% of the total variance, while PC2 explained 22.9% (Figure 10).

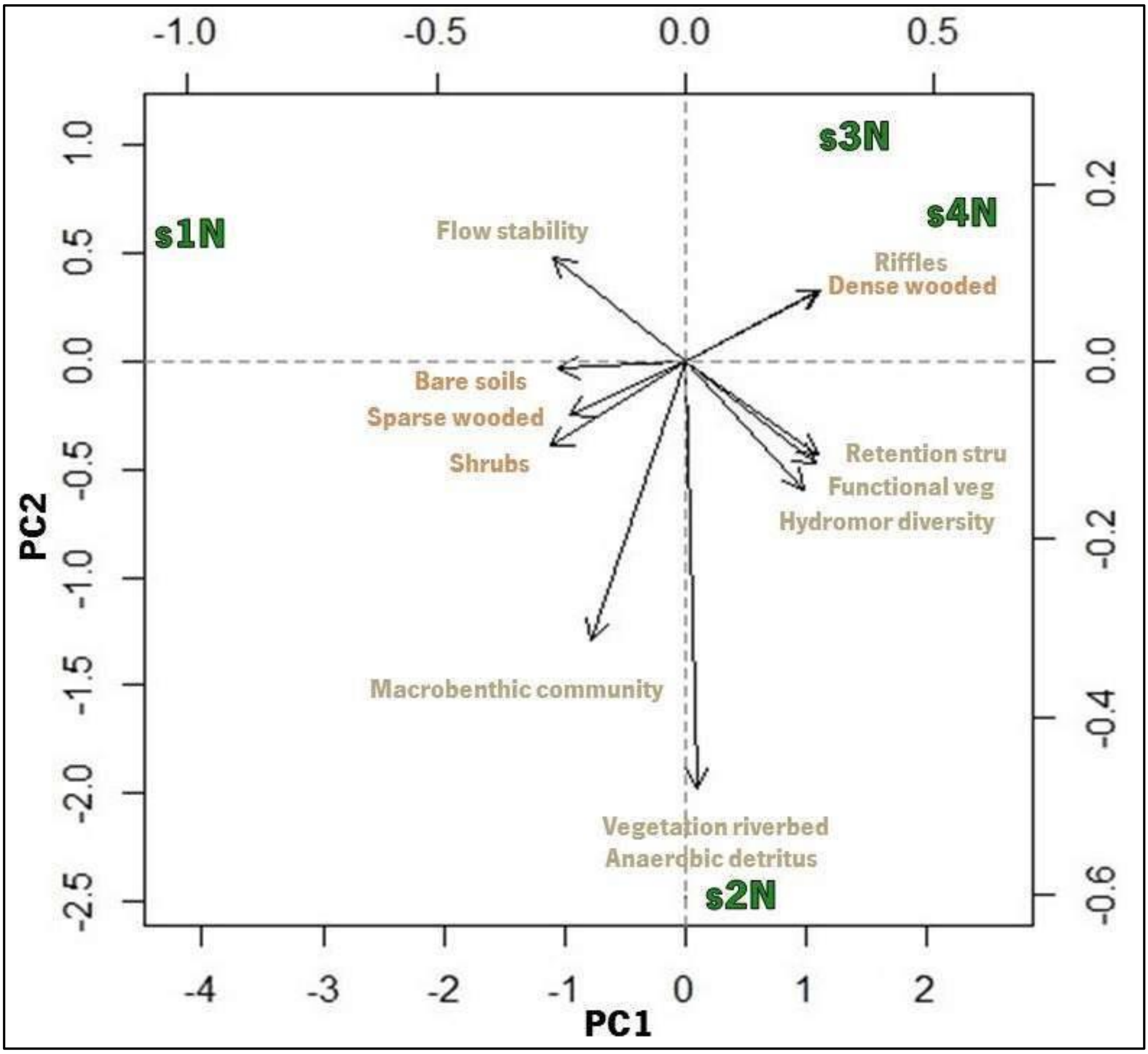

Figure 10. Principal Component Analysis (PCA) of the natural land cover and the most distinct habitat quality parameters among the natural stretches. The upper and the right axes refer to the variable loadings, while the lower and the left axes refer to the PC1 and PC2 scores. Land cover classes are represented in brown as Dense wooded (Appendix 4, land cover class 3.1.1), Shrubs (Appendix 4, land cover class 3.2), Sparse wooded (Appendix 4, land cover class 3.1.2) and Bare soils (Appendix 4, land cover class 3.3). Habitat quality parameters are represented in light grey as Flow stability (Appendix 7, FFI, parameter 5), Riffles (Appendix 7, RBP, parameter 7a), Retention stru (Appendix 7, FFI, parameter 7), Functional veg (Appendix 7, FFI, parameter 1), Hydromor diversity (Appendix 7, FFI, parameter 11), Anaerobic detritus (Appendix 7, FFI, parameter 13), Vegetation riverbed (Appendix 7, FFI, parameter 12), and Macrobenthic community (Appendix 7, FFI, parameter 14).

PC1 and PC2 scores were higher in s4N and s3N than in s1N and s2N (Figure 10), corroborating the existence of improved habitat quality parameters related to riparian vegetation, a greater prevalence of riffles and channel sinuosity, a higher hydromorphological diversity, a better variety of riverbed habitats and retention structures and more dense wooded areas in s4N and s3N, where vegetation in the wet riverbed and pulpy and anaerobic plant detritus were scarce. The stretch s2N had the highest negative scores for PC2 (Figure 10), reflecting its greater abundance of vegetation in the wet riverbed and of pulpy and anaerobic plant detritus. The stretch s1N had higher negative scores for PC1 and low positive scores for PC2 (Figure 10), due to its great flow stability and well-structured macroinvertebrate community (Appendix 7, FFI, parameter 14), but also because s1N had worst habitat quality parameters related to riparian vegetation than s3N and s4N, and lower abundance of vegetation in the wet riverbed and of pulpy and anaerobic plant detritus than s2N.

3.2.1.2. Physico-chemical water parameters

Natural stretches differed in their physico-chemical water parameters (ANOSIM, R=0.75), changes in nutrient concentrations (ANOSIM, R=0.77), and changes in conductivity, dissolved oxygen, pH and temperature (ANOSIM, R=0.56).

The Principal Components Analysis (PCA) of the physico-chemical water parameters, current velocity and changes in physico-chemical water parameters along the natural stretches showed that PC1 explained 37.8% of the total variance, while PC2 explained 26.2%. PCA demonstrated that the natural stretches had different physico-chemical water parameters, current velocity, as well as depletions of nutrient concentrations, conductivity and temperature, and increments of pH and dissolved oxygen (Figure 11). The stretch s2N had higher nitrate concentration and temperature but lower pH, dissolved oxygen and current velocity, while s3N and s4N had higher concentration of ammonium and phosphate, conductivity, pH, dissolved oxygen and current velocity (Figure 11). The stretch s1N had low nutrient concentrations and conductivity, but high dissolved oxygen (Figure 11). Nitrate concentration, conductivity and temperature decreased more along s2N, ammonium and phosphate concentration decreased more along s3N and s4N, while nitrite concentration had stronger depletions along s1N (Figure 11). Oxygen concentration had higher increments along s1N while pH along s2N (Figure 11).

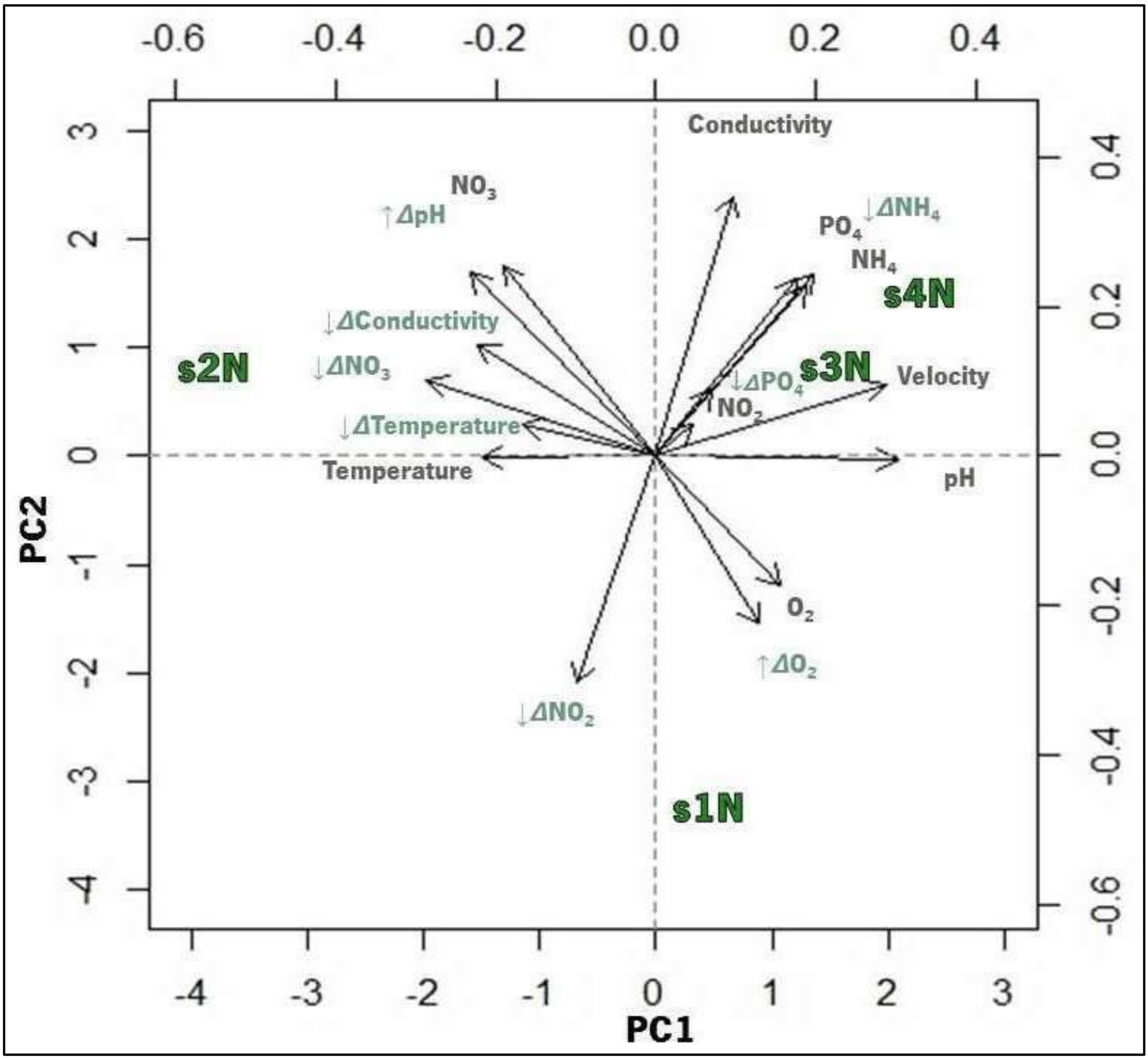

Figure 11. Principal Component Analysis (PCA) of the physico-chemical water parameters, current velocity, depletions of nutrient concentrations, conductivity and temperature (↓***Δ***), and increments of pH and dissolved oxygen (↑***Δ***) along the natural stretches. The upper and the right axes refer to the variable loadings, while the lower and the left axes refer to the PC1 and PC2 scores.

3.2.1.3. Macroinvertebrate communities and leaf litter decomposition

Metrics on macroinvertebrate communities and leaf litter decomposition are in Table 2. The Iberian Biological Monitoring Working Party (IBMWP) index increased by the following order: s2N < s1N < s4N. The ecological water quality according to IBMWP was very good in s4N, good in s1N and poor in s2N. Leaf decomposition rate was higher in s4N than in s1N and s2N (Table 2).

Table 2. Metrics on macroinvertebrate communities and leaf litter decomposition of the natural stretches. Data represent mean values ± SD (n=3), except for leaf decomposition rate, and IBMWP (Iberian Biological Monitoring Party). s1N (natural stretch from river segment 1), s2N (natural stretch referring river segment 2), s4N (natural stretch from river segment 4).

	River stretch		
	s1N	**s2N**	**s4N**
IBMWP	91	30	117
Decomposition rate (k) (day^{-1})	0.026	0.025	0.055
Abundance (N)	30 ± 3	13 ± 1	165 ± 21
Taxonomic diversity (H´)	2.21 ± 0.27	1.23 ± 0.15	2.27 ± 0.06
Taxonomic richness (S)	12 ± 3	5 ± 1	19 ± 1
Taxonomic evenness (J)	0.89 ± 0.01	0.77 ± 0.09	0.77 ± 0.02
Feeding diversity (H´)	1.20 ± 0.08	0.78 ± 0.11	1.53 ± 0.01
Feeding richness (S)	4 ± 1	3 ± 0	6 ± 1
Feeding evenness (J)	0.82 ± 0.02	0.71 ± 0.10	0.89 ± 0.06
Habitat behavior diversity (H´)	1.42 ± 0.19	0.90 ± 0.02	0.95 ± 0.04
Habitat behavior richness (S)	5 ± 1	3 ± 0	4 ± 1
Habitat behavior evenness (J)	0.92 ± 0.05	0.82 ± 0.01	0.71 ± 0.10

Macroinvertebrate abundance and taxonomic richness were higher in s4N, intermediate in s1N and lower in s2N (Tukey's post-test, P<0.05) (Table 2). Taxonomic diversity was lower in s2N than in s4N and s1N (Dunn´s test, P<0.05) (Table 2). Taxonomic evenness did not differ among stretches (one-way ANOVA, P>0.05) (Table 2).

Feeding diversity was higher in s4N, intermediate in s1N and lower in s2N (Tukey's post-test, P<0.05) (Table 2). Habitat behavior diversity was higher in s1N than in s2N and s4N (Tukey's post-test, P<0.05) (Table 2). Feeding richness was higher in s4N than in s1N and s2N (Dunn´s test, P<0.05), feeding evenness was higher in s4N than in s2N (Tukey's post-test, P<0.05), while habitat behavior evenness was higher in s1N than in s4N (Tukey's post-test, P<0.05) (Table 2). Habitat behavior richness did not differ among stretches (Kruskal-Wallis test, P>0.05) (Table 2).

Hydropsyche sp., *Ephemerella* sp., and *Calopteryx* sp., were the most abundant taxa in s1N; *Caenis* sp., and Tanypodinae in s2N, and Tanypodinae, *Hydropsyche* sp., and *Potamopyrgus* sp. in s4N. Tanypodinae, *Hydropsyche* sp., and *Potamopyrgus* sp. were in higher number in s4N and best distinguished it from s1N and s2N (SIMPER).

Filter and gatherer collectors were the dominant functional feeding groups in s1N; gatherer collectors and predators in s2N, and scrapers and predators in s4N. Clingers and sprawlers were the most abundant habitat behavior groups in s1N; sprawlers and burrowers in s2N, and burrowers and clingers in s4N. Scrapers, burrowers and predators were more abundant in s4N and best distinguished it from s1N and s2N (SIMPER).

The Principal Components Analysis (PCA) of the relative proportion of the most abundant taxa, functional feeding groups and habitat behavior groups of benthic macroinvertebrates demonstrated that PC1 explained 47.2% of the total variance while PC2 explained 31.5%. PCA corroborates the existence of different dominant taxa and functional groups of benthic macroinvertebrates among the natural stretches (Figure 12).

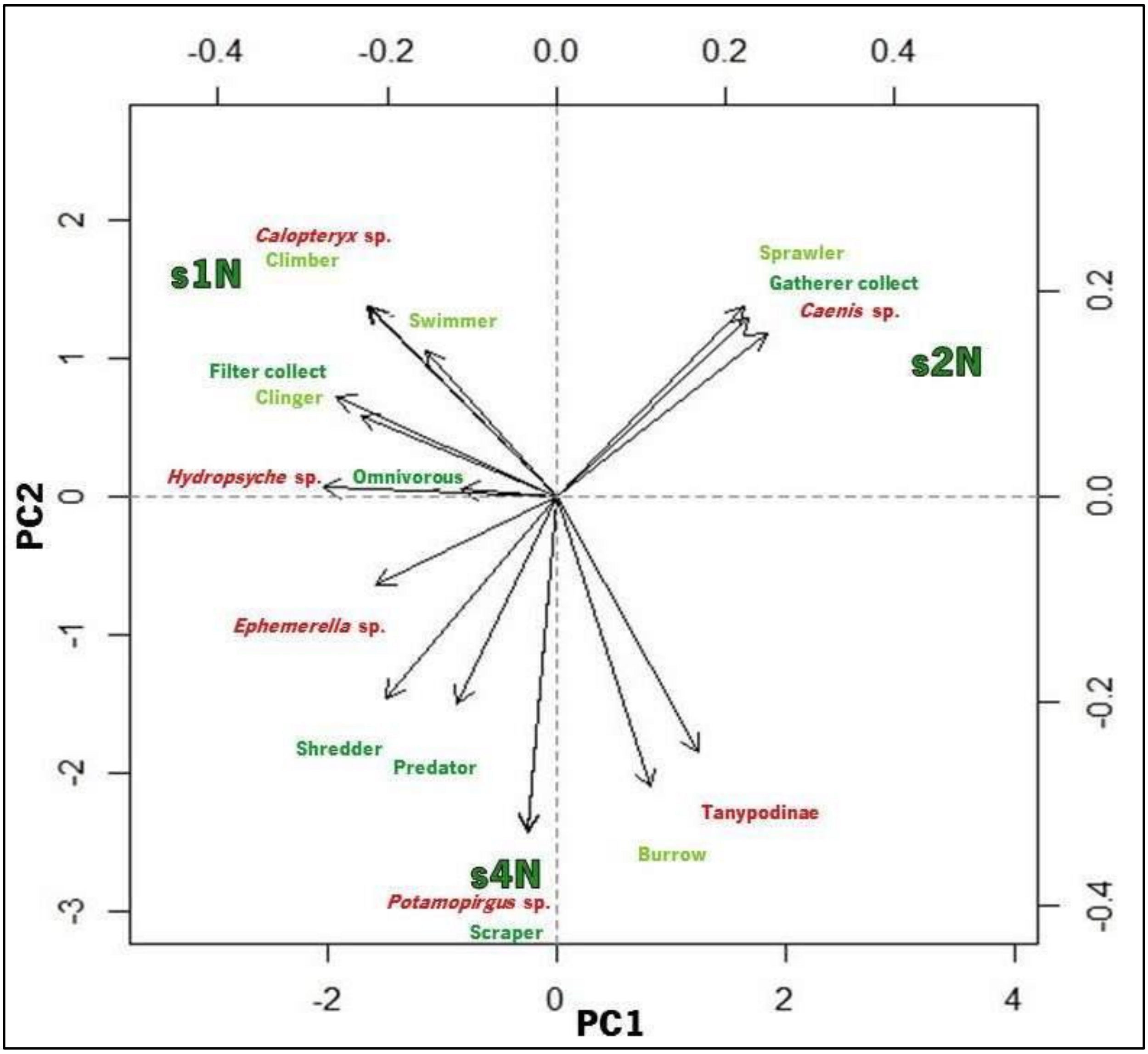

Figure 12. Principal Component Analysis (PCA) of the relative proportion of the most abundant taxa (red), functional feeding groups (dark green) and habitat behavior groups of benthic macroinvertebrates (light green). The upper and the right axes refer to the variable loadings, while the lower and the left axes refer to the PC1 and PC2 scores.

3.2.1.4. Main factors explaining changes in physico-chemical water parameters

Changes in ammonium concentration along the natural stretches were mostly correlated with the frequency of riffles and channel sinuosity (Appendix 7, RBP, parameters 7a and 7b; Pearson correlation=-0.75, $P<0.01$), the variety of riverbed habitats and retention structures (Appendix 7, FFI, parameter 7; Pearson correlation= -0.62, $P<0.05$), the hydromorphological diversity of the stream channel (Appendix 7, FFI, parameter 11; Pearson correlation=-0.57, $P<0.03$), and the presence of functional vegetation (Appendix 7, FFI, parameter 1; Pearson correlation=-0.50, $P<0.06$). Changes in nitrite concentrations were positively correlated with the frequency of riffles and channel sinuosity (Appendix 7, RBP, parameters 7a and 7b; Pearson correlation=0.82, $P<0.01$) and the presence of functional vegetation (Appendix 7, FFI, parameter 1; Pearson correlation=0.76, $P<0.01$). Changes in nitrate concentrations were strongly correlated with the abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12; Pearson correlation=-0.91, $P<0.001$) and pulpy and anaerobic plant detritus (Appendix 7, FFI, parameter 13; Pearson correlation=-0.91, $P<0.001$). Changes in phosphate concentrations were poorly correlated with the habitat quality attributes.

Changes in dissolved oxygen were positively correlated with changes in conductivity (Pearson correlation= 0.72, $P=0.01$) (Figure 13), negatively correlated with vegetation in the wet riverbed (Appendix 7, FFI, parameter 12; Pearson correlation= -0.48, $P=0.08$), and were not correlated with the frequency of riffles and channel sinuosity (Appendix 7, RBP, parameter 7a and 7b; Pearson correlation= 0.00, $P=0.99$) nor with the coverage of dense wooded areas (Appendix 4, land cover class 3.1.1; Pearson correlation= 0.00, $P=0.99$). Changes in water pH were strongly correlated with the presence of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12; Pearson correlation=0.86, $P<0.0001$) and functional riparian vegetation (Appendix 7, FFI, parameter 1; Pearson correlation=0.65, $P<0.05$).

Ammonium depletion was higher with higher concentration of ammonium (Pearson correlation= -0.78, $P=0.0006$), conductivity (Pearson correlation= -0.76, $P=0.0065$), current velocity (Pearson correlation= -0.69, $P=0.04$), and water pH (Pearson correlation= -0.55, $P=0.07$) (Figure 13). Nitrite depletion was higher with lower conductivity (Pearson correlation= 0.83, $P=0.002$) and current velocity (Pearson correlation= 0.57, $P=0.1$), but nitrite depletion was not correlated with nitrite concentration and dissolved oxygen (Pearson correlation= -0.08, -0.04; $P=0.8$, 0.8) (Figure 13). Nitrate depletion was higher with higher concentration of nitrate (Pearson correlation= -0.81, $P<0.0001$) and temperature (Pearson correlation= -0.59, $P<0.05$), and lower current velocity (Pearson correlation= 0.76, $P=0.004$), pH (Pearson correlation= 0.75, $P=0.002$) and dissolved oxygen (Pearson correlation= 0.61, $P=0.01$) (Figure 13). Phosphate depletion was higher with higher concentration of phosphate (Pearson correlation= -0.49, $P=0.04$) (Figure 13).

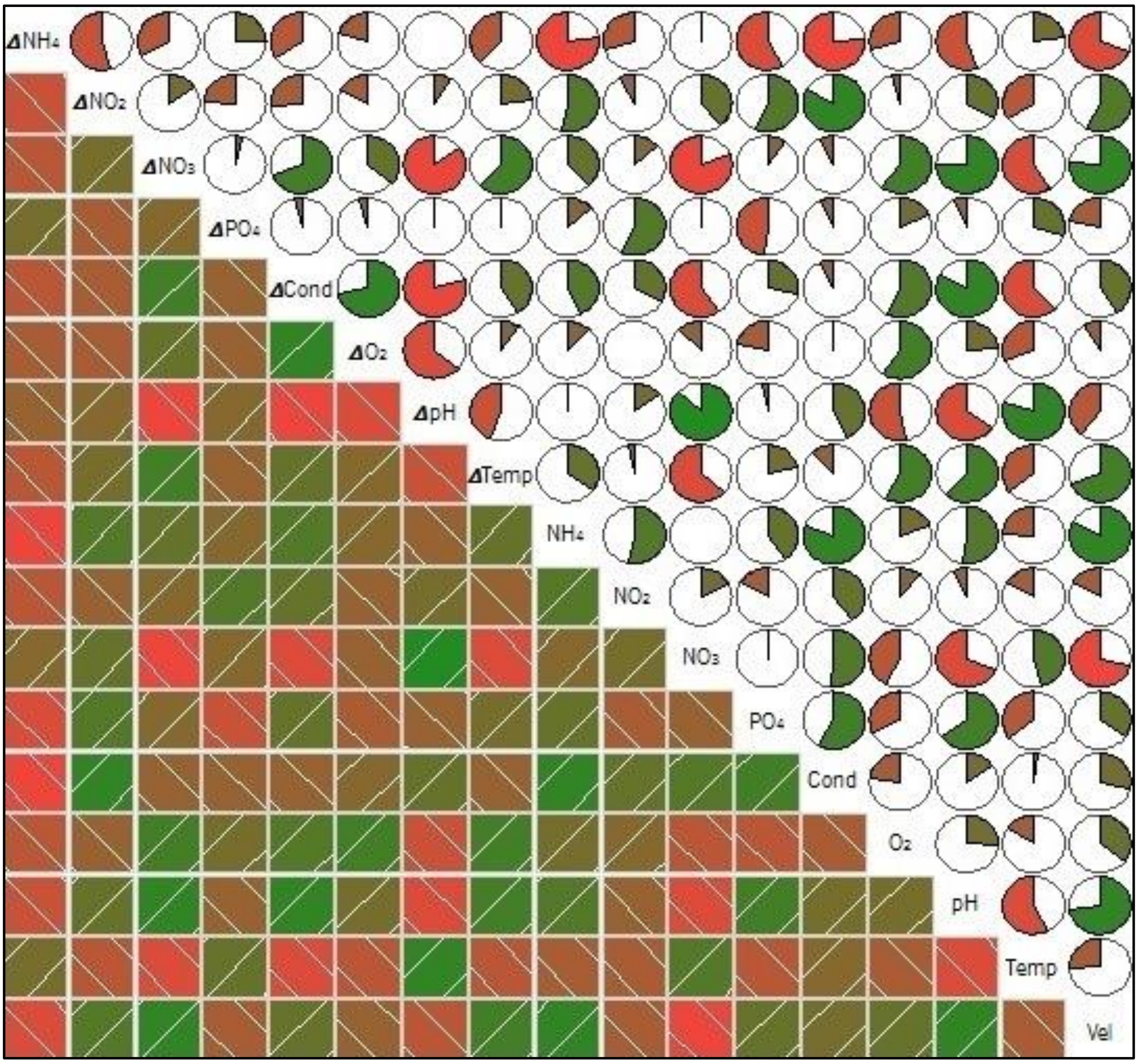

Figure 13. Correlogram of changes in physico-chemical water parameters along the natural stretches, nutrient initial concentrations and representative values of conductivity, dissolved oxygen, pH, temperature and current velocity. Red colour was assigned to negative correlations and green to positive correlations. Panels with red colour and lines leaning to left mean negative correlations; pies with red colour start turning to the left also refer to negative correlations. Diagonal panel indicates changes along the stretches in **Δ**NH_4 (NH_4^+ µg L^{-1} km^{-1}), **Δ**NO_2 (NO_2^- µg L^{-1} km^{-1}), **Δ**NO_3 (NO_3^- µg L^{-1} km^{-1}), **Δ**PO_4 (PO_4^{3-} µg L^{-1} km^{-1}), **Δ**Cond (conductivity µS cm^{-1} km^{-1}), **Δ**O_2 (dissolved oxygen mg L^{-1} km^{-1}), **Δ**pH (km^{-1}), **Δ**Temp (temperature °C km^{-1}); nutrient initial concentrations of NH_4 (mg L^{-1} NH_4^+), NO_2 (mg L^{-1} NO_2^-), NO_3 (mg L^{-1} NO_3^-), PO_4 (mg L^{-1} PO_4^{3-}); and representative values of Cond (conductivity, µS cm^{-1}), O_2 (mg L^{-1}), pH, Temp (temperature, °C), and Vel (current velocity, cm s^{-1}).

Regression analysis corroborated that ammonium depletion was related to ammonium concentration (Adjusted r^2=0.57, P<0.001) but not to pH and dissolved oxygen (Adjusted r^2=0.23, 0.01; P=0.08, 0.3, respectively). However, stretches had different ammonium depletions for similar initial concentrations, suggesting that other factors may have also determined changes in ammonium concentrations along the natural stretches (Figure 14).

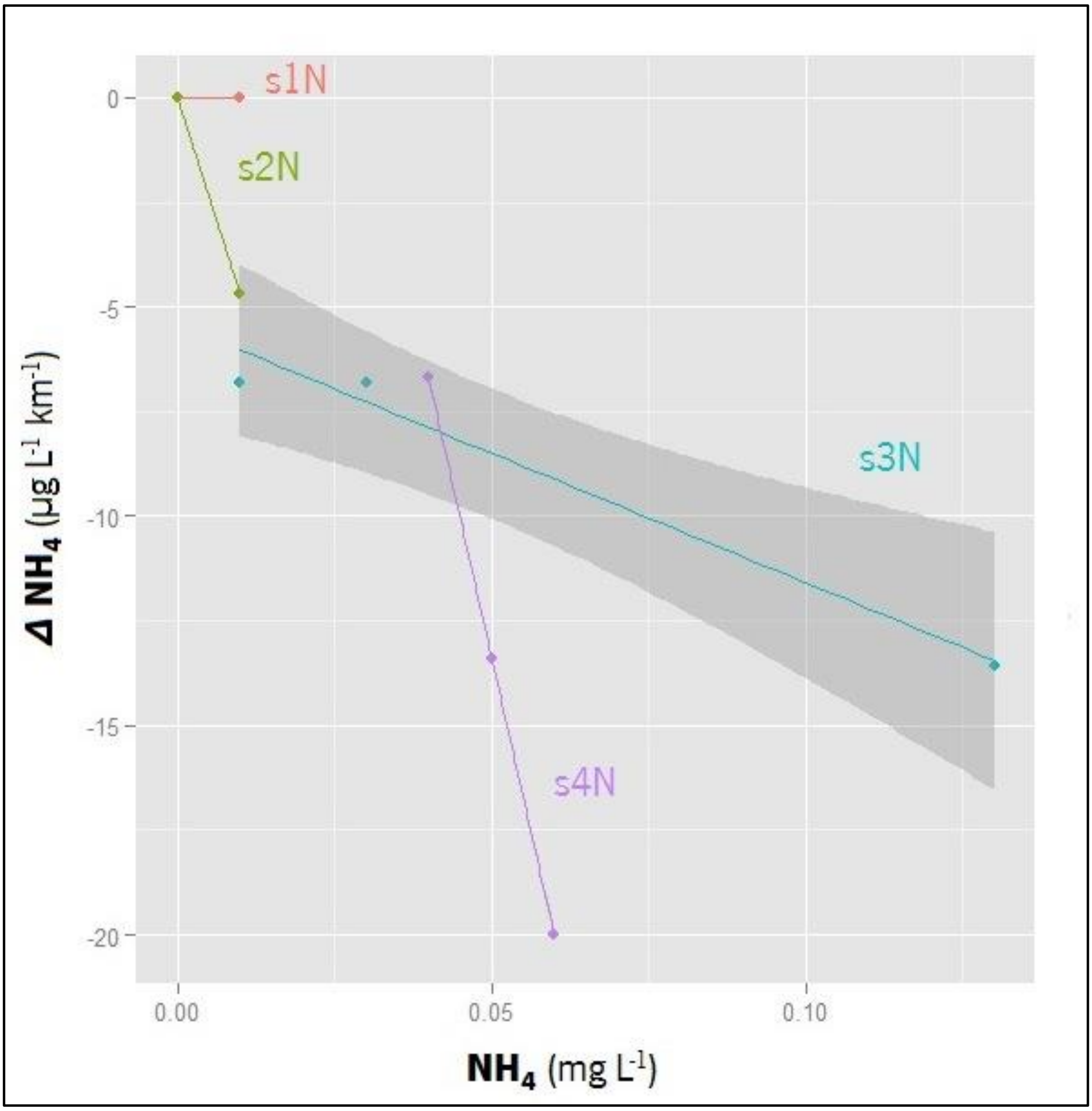

Figure 14. Regression plot of the relationship between ammonium concentration and its depletion along the natural stretches.

Regression analysis corroborated that nitrate depletion was more related to nitrate concentration (Adjusted r^2=0.63, P<0.001) than to pH (Adjusted r^2=0.52; P=0.002) or dissolved oxygen (Adjusted r^2=0.32, P=0.013). However, nitrate concentration did not changed along s1N and s3N regardless its initial concentration, and decreased more along s2N for similar or lower nitrate concentrations in the stream (Figure 15), suggesting that other factors may have also determined nitrate depletion rather than just its initial concentrations.

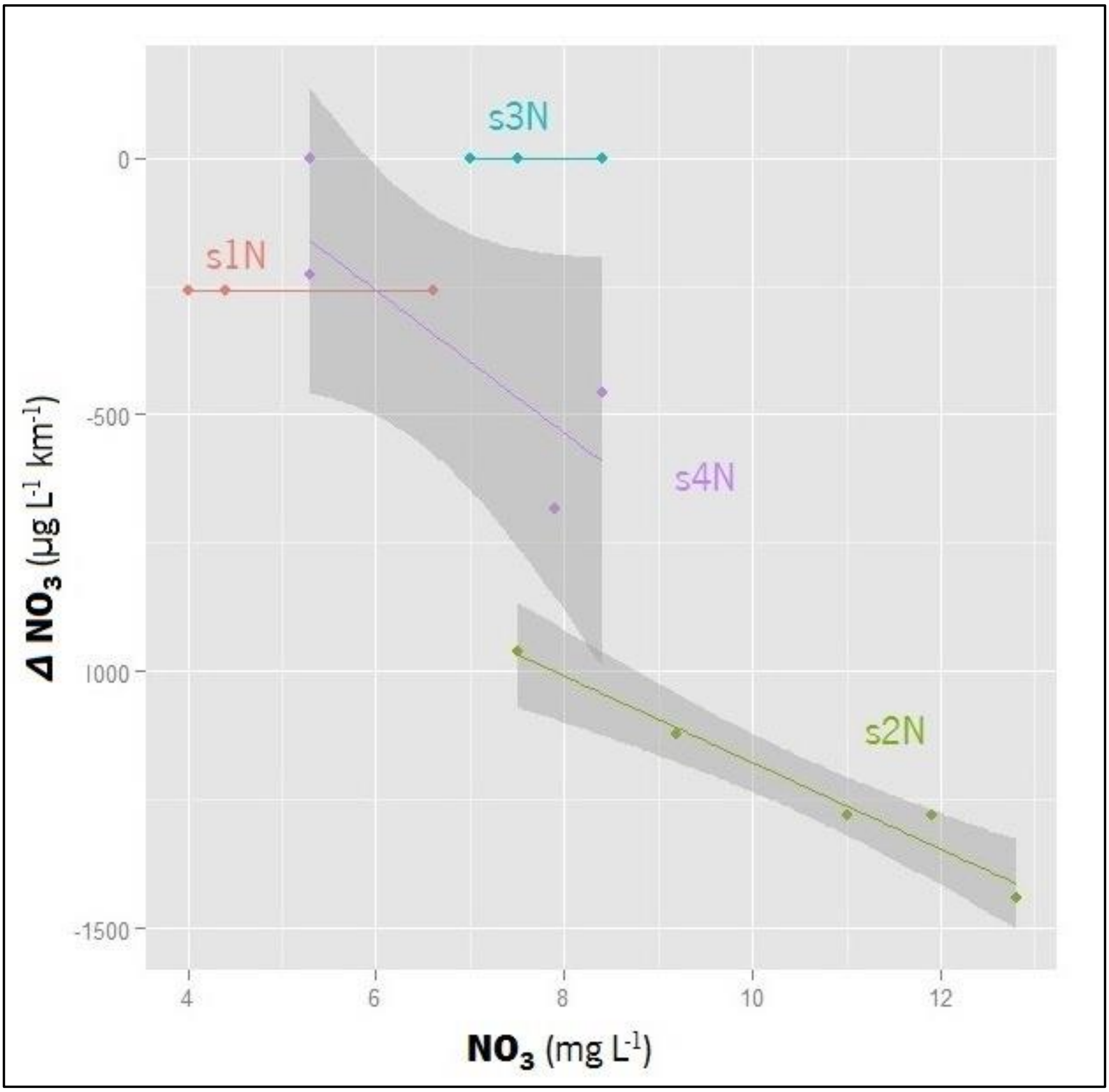

Figure 15. Regression plot of the relationship between nitrate concentration and its depletion along the natural stretches.

3.3. Influence of land cover on habitat quality and changes in physico-chemical water parameters along the eutrophic river segment

The eutrophic river segment (S5) included three stretches with different land cover. The first upstream stretch (s5A) was dominated by arable lands (Appendix 4, land cover class 2.1.1 with a coverage of 90.6%) (Appendix 2, s5A), the second stretch (s5U) by residential and laboral buildings (Appendix 4, land cover classes 1.1.1 and 1.1.2 with a coverage of 32.6% and 12.6% respectively) (Appendix 2, s5U), and the third stretch (s5N) by green urban areas (Appendix 4, land cover class 3.2.3 with a coverage of 63%) (Appendix 2, s5N). The slope of the agricultural areas in s5A was about 2.8%.

All stretches of S5 had poor habitat quality. According to FFI, the river functionality was fair in s5A (score 71) and poor in s5N and s5U (scores 50 and 36, respectively). The HABSCORE (RBP) indicated that all stretches of S5 had marginal habitat quality (scores 96, 83 and 80 for s5A, s5U and s5N, respectively), and QBR that all stretches of S5 had extremely degraded riparian habitats (scores 10, 5 and 0 for s5A, s5N and s5U, respectively). The stretches s5A and s5U had higher abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12), s5U had more pulpy and anaerobic plant detritus (Appendix 7, FFI, parameter 13), and s5N had better habitat quality parameters related to riparian vegetation, namely bank vegetative protection (Appendix 7, RBP, parameter 9). The habitat quality parameters more affected in s5A than in the other stretches of S5 were the river bank stability (Appendix 7, RBP, parameter 8), the continuity of functional vegetation (Appendix 7, FFI, parameter 4) and the bank vegetative protection (Appendix 7, RBP, parameter 9); in s5U were the riparian vegetative zone width (Appendix 7, RBP, parameter 10), the hydromorphological diversity of the stream channel (Appendix 7, FFI, parameter 11) and the channel flow status (Appendix 7, RBP, parameter 5); and in s5N were the variety of riverbed habitats and retention structures (Appendix 7, FFI, parameter 7), the conditions of the river substrate (Appendix 7, RBP, parameter 2b), and the diversity of pools (Appendix 7, RBP, parameter 3b).

Ammonium concentration strongly decreased along s5N but high depletions were also registered along s5U and S5A (Figure 16). Nitrate and phosphate concentrations had high depletions along s5N but high increments along s5A and s5U (Figure 16). Phosphate concentrations had more frequent increments along s5A while nitrate severely increased along s5U (Figure 16). The stretch s5U had the highest increments of nitrate, far greater than those of the urbanized stretch from the mesotrophic river segments (s1U), where urbanization was not so severe (14.7% of artificial areas (Appendix 4, land cover class 1) in s1U and 65.7% in s5U).

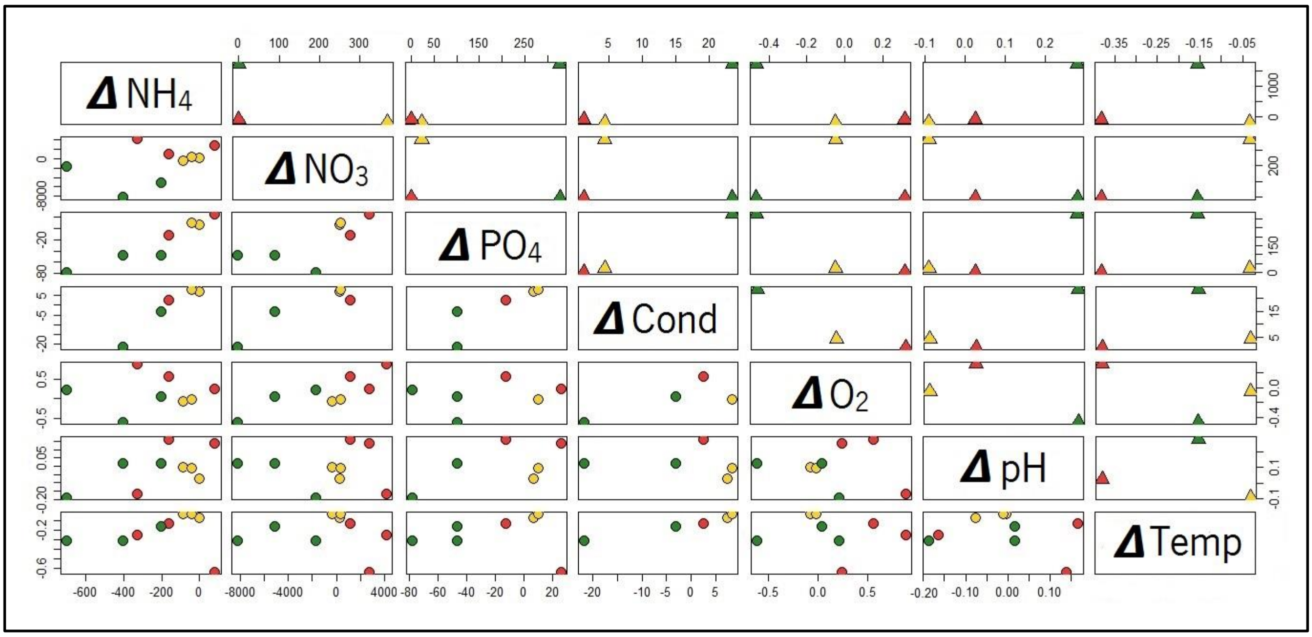

Figure 16. Plot diagram of changes in physico-chemical water parameters along the stretches of the eutrophic river segment (S5). The lower panel refers to changes in physico-chemical water parameters during ordinary conditions, while the upper panel refer to the sample collected after a severe rainy period. The values of s5A are in yellow, s5U are in red and s5N are in green. Diagonal panel indicates changes along the stretches in ***Δ***NH_4 (NH_4^+ µg L^{-1} km^{-1}), ***Δ***NO_3 (NO_3^- µg L^{-1} km^{-1}), ***Δ***PO_4 (PO_4^{3-} µg L^{-1} km^{-1}), ***Δ***Cond (conductivity µS cm^{-1} km^{-1}), ***Δ***O_2 (dissolved oxygen mg L^{-1} km^{-1}), ***Δ***pH (km^{-1}) and ***Δ***Temp (temperature °C km^{-1}).

In S5, nitrate depletion increased with higher concentration of nitrate (Pearson correlation= -0.90, P= 0.09), especially along s5N. Nitrate depletions were higher along s5N (Figure 16) than along the natural stretches of the mesotrophic river segments (Figure 7), and nitrate depletions predicted for s5N by the linear regression model of nitrate depletion and its initial concentration (-3153, -2676, -1953 µg L^{-1} km^{-1}) (section 3.2.1.4) underestimated those obtained along s5N (-8237, -5148, -1716 µg L^{-1} km^{-1}).

Changes in dissolved oxygen, pH and temperature along stretches did not differ among the stretches of S5 (ANOSIM, R=0.08) due to a great internal variability (Figure 16). However, dissolved oxygen and pH increased more along s5U, conductivity decreased along s5N but increased along s5A and s5U, and temperature severely changed between sampling sites of all stretches (Figure 16).

Results suggested that changes in physico-chemical water parameters along the stretches of S5 during more frequent and severe precipitation events might be different from those obtained (Figure 16, upper panel). When S5 was sampled after a long term precipitation period in March, the stream flow was very high, nutrient concentration and conductivity were lower, oxygen concentration was increased, and changes in physico-chemical water parameters along the stretches were different from those commonly recorded, since s5N had the highest ammonium, phosphate, conductivity and pH increments, s5U had high ammonium depletion, while s5A had high ammonium depletion but high nitrate and phosphate increments (Figure 16, upper panel).

3.4. Influence of land cover on habitat quality and changes in physico-chemical water parameters along the large river segment

The four stretches of the large river segment (S6) had different land cover. The first upstream stretch (s6A) was dominated by natural areas (Appendix 4, land cover class 3 with a coverage of 76%), mostly sparse vegetated (Appendix 4, land cover class 3.3.1 with a coverage of 19.9%), but had the higher percentage of arable lands (Appendix 4, land cover class 2.1.1 with a coverage of 19.1%) (Appendix 2, s6A). The second stretch (s6UL) had a greater presence of laboral buildings (Appendix 4, land cover class 1.1.2 with a coverage of 15.1%) (Appendix 2, s6UL), while the third stretch (s6N) was dominated by natural areas (Appendix 4, land cover class 3 with a coverage of 97.1 %), especially dense wooded areas (Appendix 4, land cover class 3.1.1 with a coverage of 70.9%) (Appendix 2, s6N). The fourth stretch (s6UR) had the highest coverage of residential buildings (Appendix 4, land cover class 1.1.1 with a coverage of 9.8%), but also some areas with laboral buildings (Appendix 4, land cover class 1.1.2 with a coverage of 8.5%) (Appendix 2, s6UR).

Stretches from S6 also differed regarding their habitat quality attributes. According to FFI, the river functionality was very good in s6N (score 224), good in s6A (score 163) and fair in s6UL and s6UR (scores 97 and 88, respectively). The HABSCORE (RBP) indicated that the habitat quality was optimal in s6N (score 156), suboptimal in s6A (score 121) and marginal in s6UL and in s6UR (scores 99 and 97 respectively). According to QBR, the riparian habitat had good quality in s6N (score 90), poor quality in s6A (score 53) and very bad quality in s6UL and s6UR (scores 20 and 19, respectively).

The habitat quality parameters best represented in s6N than in the other stretches of S6 were the width of the functional vegetation (Appendix 7, FFI, parameter 3), the total riparian cover (Appendix 7, QBR, parameter 1) and the continuity of functional vegetation (Appendix 7, FFI, parameter 4).

The physico-chemical water parameters did not significantly differ among the stretches of S6 (ANOSIM, R= -0.28), due to a great variability between sampling times. The most pronounced differences were observed in June, when the flow and the oxygen concentration were reduced while nutrient concentrations, conductivity, pH and temperature were increased.

Changes in nutrient concentrations and conductivity, dissolved oxygen, pH and temperature along stretches did not differ among the stretches of S6 (ANOSIM, R= 0.19 and 0.16, respectively) (Figure 17). However, conductivity and temperature decreased more along s6A; s6UL had the most pronounced phosphate increments; s6N was the only with no nutrient increments and the highest phosphate depletions, while s6UR had more frequent increments of nitrate (Figure 17).

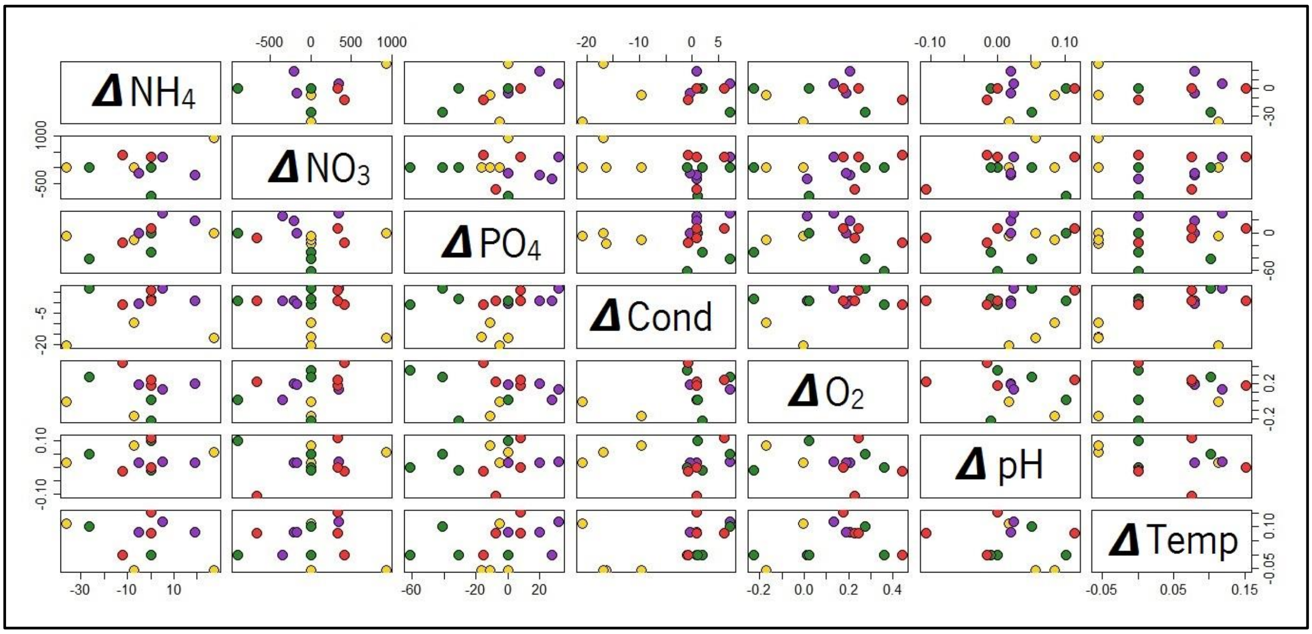

Figure 17. Plot diagram of changes in physico-chemical water parameters along the stretches of the large river segment (S6). The values of s6A are in yellow, s6UL are in purple, s6N are in green and s6UR are in red. Diagonal panel indicates changes along the stretches in ***Δ***NH_4 (NH_4^+ µg L^{-1} km^{-1}), ***Δ***NO_3 (NO_3^- µg L^{-1} km^{-1}), ***Δ***PO_4 (PO_4^{3-} µg L^{-1} km^{-1}), ***Δ***Cond (conductivity µS cm^{-1} km^{-1}), ***Δ***O_2 (dissolved oxygen mg L^{-1} km^{-1}), ***Δ***pH (km^{-1}) and ***Δ***Temp (temperature °C km^{-1}).

Correlation analysis corroborated that phosphate concentration decreased more or slightly increased along stretches with more natural riparian areas (Appendix 4, land cover class 3) (Pearson correlation= -0.71, P= 0.002) than with more urban (Appendix 4, land cover class 1) (Pearson correlation= 0.63, P= 0.008) or agricultural land uses (Appendix 4, land cover class 2) (Pearson correlation= 0.53, P= 0.04).

Ammonium depletion increased with higher concentration of ammonium but lower dissolved oxygen (Pearson correlation= -0.61, 0.74; P= 0.08, 0.02 respectively). Nitrate depletion increased with higher dissolved oxygen but not with nitrate concentration (Pearson correlation= -0.47, -0.04; P= 0.14, 0.9 respectively). Phosphate depletion increased with higher concentration of phosphate and pH but not with dissolved oxygen (Pearson correlation= -0.58, -0.55, 0.08; P= 0.06, 0.09, 0.8 respectively).

3.5. Ecosystem services and benefits

Monetary costs to restore ammonia and nitrate concentrations or to replace the water purification service provided by the stretches are in Table 3. In stretches with more agricultural and urban land use money would be spent to restore ammonia and nitrate concentrations, while in stretches with more natural and vegetated areas money would be spent to replace ammonia and nitrate depletions (Table 3).

Replacement costs differed among natural stretches. The stretch s4N had the highest replacement costs (€ $year^{-1}$) among the mesotrophic stretches, even though lower than s5N (Table 3). Differences in replacement costs among the natural stretches were more pronounced when costs were estimated accounting for the stretches length (€ m^{-1} $year^{-1}$) since s5N had the lowest length (Table 3). However, in s5N a great amount of money would be spent to restore ammonia and nitrate concentrations during severe precipitation events (Table 3).

Urbanized stretches had the highest monetary costs, especially the most urbanized stretch (s5U) (Table 3). Results further demonstrated that stretches with more agricultural and urban land use might have high replacement costs, such as s3A (Table 3).

Table 3. Monetary costs to restore ammonia and nitrate concentrations or to replace the water purification service provided by the stretches of the mesotrophic (S1-S4) and eutrophic river segments (S5). Negative values indicate money to restore ammonia and nitrate concentration, while positive values indicate money to replace the water purification services provided by the stretches as ammonia and nitrate depletions.

River stretch	Income	(€ m^{-1} $year^{-1}$)	(€ $year^{-1}$)	(€ day^{-1})
s1A	Maximum	0	0	0
	Minimum	-8	-27543	-75
	Median	-6	-22953	-63
s1U	Maximum	0	0	0
	Minimum	-28	-19389	-53
	Median	-20	-14057	-39
s1N	Maximum	6	9695	27
	Minimum	-6	-9695	-27
	Median	6	9695	27
s2N	Maximum	2	6398	18
	Minimum	2	4266	12
	Median	2	5759	16
s3A	Maximum	11	17396	48
	Minimum	0	0	0
	Median	1	1581	4
s3N	Maximum	2	3163	9
	Minimum	1	1581	4
	Median	1	1581	4
s4A	Maximum	11	13472	37
	Minimum	-11	-13472	-37
	Median	-10	-12125	-33
s4N	Maximum	21	40416	111
	Minimum	2	4042	11
	Median	8	16166	44
s5A	Maximum	15	45954	126
	Minimum	-7	-20424	-56
	Median	-5	-15318	-42
	Severe precipitation*	5	15318	42
s5U	Maximum	-13	-10212	-28
	Minimum	-71	-56165	-154
	Median	-71	-56165	-154
	Severe precipitation*	6	5106	14
s5N	Maximum	223	142966	392
	Minimum	96	61271	168
	Median	135	86801	238
	Severe precipitation*	-135	-86801	-238

4. Discussion

4.1. Influence of land cover on habitat quality and changes in physico-chemical water parameters along the mesotrophic river segments

Stretches with more agricultural and urban land use had worse habitat quality, higher nutrient increments and higher oxygen depletions than stretches with more natural riparian areas. Therefore, the conversion of natural riparian areas to human land use may impact river habitat quality and water chemistry, with consequences for river functioning and services. This was expected because agricultural and urban activities tend to degrade river hydromorphology and discharge pollutants on rivers (Allan, 2004; Walsh et al., 2005).

Agricultural and urban stretches had worse habitat quality mostly because riparian vegetation was degraded (Figure 6). This may severely impact river functioning and services because riparian vegetation ensures key ecological processes (Dosskey et al., 2010). Agricultural and urban stretches had more erosion and lower morphological diversity than natural stretches, which might be due to riparian deforestation, especially of forest trees, since these can reduce bank erosion and increase the diversity and availability of in-streams habitats by providing more coarse debris and promoting wider channels (Sweeney et al., 2004; Dosskey et al., 2010). Moreover, stream channels in most agricultural and urban stretches were embanked, which reduced the availability of in-stream habitats and increased erosion at the bends where embankments were not present. Therefore, biotic communities in these stretches are expected to be less diverse because of sediment inputs and a low diversity of in-streams habitats (Sweeney et al., 2004; Nakamura & Yamada, 2005). This may reduce key ecological processes in streams such as nutrient retention that was found to be higher in the presence of more diverse communities colonizing different habitats (Cardinale, 2011). Furthermore, more diverse communities can be more productive and resilient to stressors (Cardinale et al., 2012; Loreau & Mazancourt, 2013), so the services and stability of streams in agricultural and urban stretches may be compromised.

Riparian vegetation has a great influence on stream chemistry so changes in physico-chemical water parameters along stretches may have been influenced by riparian vegetation. Oxygen concentration decreased more along agricultural and urban stretches (Figure 8), especially along s1U and s4A (Figure 7). Oxygen concentration decreases with increasing conductivity and temperature (Mesner & Geiger, 2010), so oxygen depletions along s1U may have been related to its high increments of temperature (Figure 7). Temperature increased along s1U probably because of its low coverage of dense wooded areas (Appendix 4, land cover class 3.1.1) and may significantly affect ecosystem functioning by changing organisms metabolism (Brown et al., 2004; Yvon-Durocher et al., 2010b), nutrient cycles (Yvon-Durocher et al., 2010a), and/or the structure of food webs (Perkins et al., 2010). Conductivity and temperature remain constant along s4A (Figure 7), and the river had a laminar flow and evidence of organic matter accumulation, so oxygen depletions along s4A may have been related to organic matter mineralization.

Nutrient concentrations in streams may increase as a result of natural processes, such as organic matter mineralization and nutrients desorption from bed sediments, and this may improve organism activity and river functioning. However, in agricultural and urbanized stretches, nutrient increments were frequent and higher than in natural stretches (Figure 7), which suggests they may have been influenced by human activities discharging pollutants on rivers, increasing organic matter mineralization, enriching groundwaters with nitrogen and phosphorus or increasing nutrient stored in bed sediments that were further released to the water column.

Nitrate concentrations increased more than phosphate along agricultural and urban stretches (Figure 7 and Figure 8) probably because phosphate is mainly transported by surface runoff whereas nitrate is more prone to leaching (Pärn et al., 2012), and samples were collected during dry weather conditions when surface runoff was expected to be low. This agrees with previous findings that groundwater provides the major source of nitrate inputs to rivers whereas surface runoff is the major source of phosphates (Jarvie et al., 2008 and 2010). However, groundwater nutrients and their interactions with stream water were not assessed and nutrient increments may have also resulted from point sources of pollutants and in-stream processes.

Phosphate increments were rare and generally occurred along the stretches of S3 (s3A and s3N) (Figure 7). This suggests that phosphate increments in S3 were related to natural processes rather than human activities, since it did not depend on riparian land use. However, phosphate increments were more frequent in s3A than in s3N (Figure 7), and s3A crossed a golf course and associated facilities (Appendix 2, s3A) where some tubes were found close to the river and grass seemed to be fertilized and watered throughout the year, so phosphate increments along s3A may have also resulted from these human activities.

The highest increments of nitrate occurred along s1U (Figure 7), and may have occurred because of pollutant discharges from human activities. The habitations at s1U were sparse but most had private areas for agricultural activities and were in the waterfront of the stream, which may have facilitated pollutant discharges. This agrees with Jarvie et al. (2010) who found that even sparse and isolated groups of houses are sufficient to cause high nutrient inputs.

Nitrate concentration also increased along agricultural stretches (Figure 8), especially along s1A (Figure 7). The stretch s1A had lower percentage and slope of agricultural areas than s4A but several pipes immerged into the stream and an urban wastewater treatment plant (WWTP). Previous studies reported high nutrient loadings in streams downstream from WWTPs (Ahearn et al., 2005; Gücker et al., 2006), so point sources of pollutants such as effluents from the WWTP might have contributed to the high nitrate increments along s1A, either directly or indirectly via sediment enrichment with nutrients that were further released maintaining high concentrations in the water column (Haggard et al., 2005; Jarvie et al., 2010).

Conductivity increased more along agricultural stretches, especially along s1A, but remained constant along s1U (Figure 7). This was not expected because conductivity increases in the presence of dissolved inorganic compounds such as ammonium, nitrate and phosphate and with increasing temperature (EPA, 1997; Wang & Yin, 1997) and s1U had higher nitrate and temperature increments than s1A (Figure 7). Effluents from WWTPs and point sources of pollutants significantly increase conductivity because of the presence of many dissolved compounds (Haggard et al., 2005). Therefore, point sources of pollutants may have actually contributed to nutrient increments along s1A.

Changes in water pH were strongly correlated to urban land cover (Figure 8) and increased more along s1U, but high increments were also detected along s3A, s4A and s2N (Figure 7). Stream pH can increase due to natural processes such as photosynthesis and/or to human products increasing alkaline conditions, such as lye (Mesner & Geiger, 2010). Therefore, pH may have increased along s2N and s4A due to high abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) (Figure 6) and along s3A and s1U due to human activities, since the latter had no signs of photoautotrophs but close human activities. This suggests that the habitations at s1U and the golf course and associated facilities at s3A may have actually influenced river chemistry and thus contributed to their nitrate and phosphate increments (Figure 7).

Changes in ammonium concentration were unexpected since it is a waste product of animal metabolism but tended to decrease along stretches regardless the land use (Figure 7). This may have occurred because i) pollutant inputs were mostly nitrate and phosphorous fertilizers and/or laundry detergents that commonly use phosphate, rather than sewage discharges, and ii) ammonium in stream water can be retained within a few meters by nitrification or biota uptake, since ammonium is the preferable N form for both photoautotrophs and heterotrophic microbes (Mulholland et al., 2000; Peterson et al., 2001).

Ammonium depletion was best correlated to ammonium concentration than to dissolved oxygen (Figure 9). This was likely because ammonium uptake rate increases with increasing concentrations of ammonium in streams (Dodds et al., 2002), mostly because of photoautotrophs and heterotrophic microbes assimilation (Hall & Tank, 2003; Arango et al., 2008), which also depend on other factors such as temperature or nutrient concentrations (Roberts & Mulholland, 2007; Fernandes et al., 2014). However, nitrification can be an important sink for ammonium in streams (Mulholland et al., 2000), so higher oxygen concentrations may improve ammonium retention, as suggested by our results (Figure 9). Therefore, oxygen depletions along the agricultural and the urbanized stretches (Figure 7) may have impacted ammonium retention in streams.

Ammonium is toxic for most aquatic organisms especially under high pH (Sawyer, 2008), which means that oxygen depletions together with pH increments occurring along some agricultural and the urbanized stretches (Figure 7) might severely impact stream ecosystems by reducing ammonium uptake and increasing its toxicity.

Ammonium depletion did not differ among the groups of mesotrophic stretches (Figure 7) probably because ammonium concentration was generally low. Streams dominated by agricultural and urban land use may have low nutrient retention capacities because of high nutrient loadings (Newbold et al., 2006), but nutrient retention in agricultural streams may be high because of high metabolism associated with an increase in nutrient loadings, light penetration and consequently photoautotrophs activity and biomass (Bernot et al., 2006; Von Schiller et al., 2008). The agricultural stretches had higher abundances of vegetation in the wet riverbed and a lower coverage of riparian vegetation than the natural stretches (Figure 6), so the former may have higher nutrient retention capacities as they were not expected to be nutrient saturated. Although nutrient retention is a key ecosystem service in streams, high nutrient loadings associated with a lower coverage of riparian vegetation in agricultural stretches may increase the risk of eutrophication and cause some other vital services to be lost. Therefore, improve nutrient retention in streams while promoting good habitat quality and natural nutrient loadings may increase the set of benefits provided by the streams.

Changes in physico-chemical water parameters were not relevant along the concrete channel (Figure 7), probably because nutrient concentrations were low and point or non-point sources of pollutants were not likely to occur. Nitrate concentrations were lower whereas oxygen concentrations were higher at the concrete channel than at s2N (not shown), which was not expected since the water comes from the same reservoir. The higher oxygen concentrations at the concrete channel may be explained by its turbulent flow whereas the higher nitrate concentrations at s2N may have been related to the small tributary that existed between the reservoir and s2N (Appendix 2, s2N). The low nutrient concentrations at the concrete channel may have limited nutrient depletions.

Nutrient retention in the concrete channel is expected to differ from natural stretches, because of differences in hydromorphology and habitat quality attributes. Izagirre et al. (2013) compared nutrient uptake between streams and diversion canals from hydropower plants, such as the concrete channel, and found that nutrient uptake was similar among streams and diversion canals, but the latter had higher ammonium uptake probably because of more stable substrate and uniform current that could favor the growth of primary producers such as filamentous green algae and mosses. However, the concrete channel had a turbulent flow and no riparian vegetation, which may limit photoautotrophs despite increasing light penetration and may reduce the provision of alloconthounous organic matter to leaf associated microbes. Moreover, the concrete channel had more than 2 meters deep and frequent changes in discharge that may limit the establishment of epiphytic organisms. Therefore, nutrient retention in the concrete channel is expected to be lower than in natural stretches because high nutrient retentions are mostly related to photoautotrophs and heterotrophic microbes assimilation (Arango et al., 2008; Hall et al., 2009). Andorinhas and Guilhofrei reservoirs were eutrophic at least in the nineties (INAG, 1999), so allow more water to flow in natural streams and improve their nutrient retention capacity may reduce eutrophication and increase the services provided by the streams.

4.1.1. Factors related to changes in physico-chemical water parameters along the mesotrophic stretches dominated by natural riparian areas

Ammonium, nitrate and phosphate depletion along the natural stretches were more related to their initial concentration in streams than to other physico-chemical water parameters (Figure 13). An increase in nutrient depletion with increasing concentrations in streams was expected because nutrients uptake is highly dependent on stream concentration and tends to be higher under elevated than at lower concentrations in streams (Dodds et al., 2002). This may be due to an increase in biota assimilation, nitrification and denitrification rates under elevated nutrient concentrations, even though the process efficiency may decline as stream concentration increases (O'Brien et al., 2007; Mulholland et al., 2008). Low nutrient concentrations were found to limit nutrient uptake in streams (Hoellein et al., 2007), which might be the case in s1N where ammonium, nitrate and phosphate concentrations were low (Figure 11).

Nitrate depletion was more related to nitrate concentration than ammonium and phosphate depletion to ammonium and phosphate concentration in streams, respectively, as suggested by other authors (Hoellein et al., 2007; Hall et al., 2013). However, we found a broader range of nitrate concentrations relatively to that of ammonium and phosphate, which may explain the higher correlation between nitrate depletion and its initial concentration in streams (Figure 13).

Results suggested that most stretches were not nitrogen saturated, as they responded linearly to increasing concentrations of ammonium (Figure 14) and nitrate (Figure 15). The lack of response of some stretches to increasing concentrations of ammonium and nitrate (Figure 14 and Figure 15), and the existence of stretches with higher depletions under similar or lower concentrations of nitrogen suggested that other factors may have also affected ammonium and nitrate depletions along the natural stretches.

In our study, ammonium depletion was higher along s4N and s3N, which had increased ammonium concentrations and higher pH and dissolved oxygen (Figure 11). Nitrification is an important sink for ammonium in streams and may account up to 19% of the total ammonium uptake (Dodds et al., 2000; Mulholland et al., 2000). Nitrification is favored under alkaline conditions and high ammonium and oxygen concentrations in streams (Kemp & Dodds, 2001 and 2002), which may explain the highest ammonium depletions along s4N and s3N.

As nitrification depends on pH and dissolved oxygen, it may be influenced by the river hydromorphology and metabolism. For instance, greater current velocities favor oxygen dissolution, and photosynthesis leads to an increase in water pH and dissolved oxygen (Mesner & Geiger, 2010). Our results demonstrated that ammonium depletion was further related to current velocity and the habitat quality parameters that prevailed in s3N and s4N, such as the variety of riverbed habitats and retention structures (Appendix 7, FFI, parameter 7), the hydromorphological diversity of the stream channel (Appendix 7, FFI, parameter 11), and the presence of functional vegetation (Appendix 7, FFI, parameter 1), that may improve oxygen concentration and thus nitrification rates.

Changes in ammonium concentration were correlated to pH but not to dissolved oxygen (Figure 13). This may have occurred because: 1) oxygen concentration was high in s1N where low ammonium concentration may have inhibited ammonium depletion; 2) ammonium depletion may have also been affected by other processes less dependent on dissolved oxygen such as biota uptake and adsorption to riverbed or suspended particles.

We found that nitrite depletion was not related to nitrite concentration and dissolved oxygen (Figure 13), which was not expected since nitrite oxidation is highly dependent on oxygen (Bernhard, 2010). This can be related to the lower number of replicates, and the reduced range of nitrite concentrations. However, nitrite depletion tended to be higher at s1N and s2N, where nitrite concentrations and dissolved oxygen were lower than at s3N and s4N (Figure 11). This may have occurred because streams with higher ammonium concentration, such as s3N and s4N, tend to accumulate more nitrite as a result of ammonium oxidation (Von der Wiesche & Wetzel, 1998).

Nitrate depletion was higher along s2N (Figure 11). Denitrification may have contributed to the highest nitrate depletions along s2N, because it is favored under anoxic conditions with longer water residence times and increasing concentrations of nitrate (Alexander et al., 2009; Klocker et al., 2009), and s2N had the lowest oxygen concentrations, reduced current velocity and the highest concentrations of nitrate (Figure 11). The positive correlation of nitrate depletion with its initial concentration and the positive correlation with current velocity and dissolved oxygen (Figure 13) suggest that denitrification may have contributed to decrease nitrate concentrations along some natural stretches. Therefore, the lowest nitrate concentration and the highest ammonium and oxygen concentrations at s3N and s4N may have inhibited denitrification and improved nitrification rates, which may have increased nitrate concentrations and then reduce its depletion along s3N and s4N (Figure 11).

The greatest abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) in s2N may have reduced denitrification rates, because photoautotrophs increase oxygen concentration in streams as a result of photosynthesis (Dodds, 2003; Mesner & Geiger, 2010). Indeed, previous findings demonstrated that stream substrates dominated by diatoms and algae had lower denitrification rates because they were generally oxygen saturated, while benthic habitats with more fine particle organic matter (FPOM) had high denitrification rates under increasing concentrations of nitrate, as they limit oxygen penetration to the lower anoxic zones where denitrification generally occurred (Kemp & Dodds, 2001 and 2002). However, photosynthetic organisms can also improve denitrification rates by increasing benthic organic content and/or nitrification rates that supply denitrifying bacteria with nitrate (Findlay et al., 2011).

Nitrification and denitrification can be important sinks for ammonium and nitrate in streams and so they may have contributed to ammonium and nitrate depletions along the natural stretches, but biota uptake is known to dominate dissolved inorganic nitrogen (DIN) retention in streams (Hall & Tank, 2003; Arango et al., 2008), which may have been the case in our study.

Ammonium retention tends to be improved with increasing rates of gross primary production (GGP) and community respiration (CR) (Webster et al., 2003), but nitrate retention is mostly controlled by the rates of GPP (Fellows et al., 2006; Hall et al., 2009). Several authors have demonstrated the importance of GPP and CR to ammonium retention and of GPP to nitrate uptake: Sabater et al. (2000) found that ammonium uptake was high in both forest and deforested streams because of high activity of heterotrophic microbes and algae biomass, respectively; Hall & Tank (2003) demonstrated that GPP and CR explained 82% of the variation in ammonium uptake velocity, while CR did not controlled nitrate uptake velocity and GPP explained 75% of its variation; Mulholland et al. (2006) demonstrated that nitrate uptake rate was 2 to 3 times higher at the midday and 50% greater at sunny than at overcast days because of photoautotrophs assimilation; Roberts & Mulholland (2007) found lower nitrogen concentrations in spring and autumn due to higher rates of primary productivity and heterotrophic respiration, respectively, but variation in nitrate concentration was explained in 91% by the rates of GPP and the highest downstream declines of nitrate were observed in spring, when GPP was increased. This suggests that autotrophs assimilate both nitrate and ammonium, while heterotrophs are more likely to immobilize ammonium (Hall & Tank, 2003; Roberts & Mulholland, 2007). Our results agree with previous findings since nitrate depletion was higher along s2N where vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) was highly abundant, whereas ammonium depletion was higher along s4N (Figure 11) where leaf litter decomposition had the highest rates (Table 2). Moreover, nitrate concentration in all mesotrophic river segments was lower in early spring whereas ammonium concentrations were similar between sampling times (not shown), suggesting a strong influence of photoautotrophs on nitrate concentration.

Nitrate is generally used by autotrophs and heterotrophic microbes when ammonium cannot meet N demand (Mulholland et al., 2000; Tank et al., 2008), so the low ammonium concentration and the greatest abundances of vegetation in the wet riverbed may have forced photoautotrophs to assimilate nitrate in s2N because it was highly available, while the higher concentration of ammonium in s3N and s4N may have allowed photoautotrophs and heterotrophic microbes to use ammonium before assimilating nitrate. This may have contributed to the highest nitrate depletions along s2N and the highest ammonium depletions along s3N and s4N (Figure 11).

Roberts & Mulholland (2007) found that nitrate concentrations tended to increase but ammonium concentrations tended to decline downstream during summer, probably because of high nitrification rates increasing nitrate concentrations and lower rates of primary productivity and community respiration resulting from forestry canopy closure in later spring (Hill et al., 2001; Roberts et al., 2007) and low availability of leaf litter in streams during summer. Therefore, the lower nitrate depletion along s3N and s4N, even when presenting similar or higher concentrations of nitrate than s2N (Figure 15) may be due to their higher coverage of dense wooded areas (Appendix 4, land cover class 3.1.1) limiting GPP.

Since s2N had a high abundance of vegetation in the wet riverbed, it was expected to have a great N demand, so ammonium depletions along s2N may be higher than at s3N and s4N under similar concentration of ammonium in stream. Indeed, when ammonium concentration was higher than 0 at the upstream sampling site of s2N, ammonium concentration was always equal to 0 at its downstream sampling site (not shown), suggesting that s2N may be a potential hotspot for both nitrate and ammonium uptake.

Results demonstrated that phosphate depletion was correlated to its initial concentration in streams but not to other physico-chemical water parameters (Figure 13) or habitat quality attributes. This may have occurred because phosphate concentration was generally low and there was a short range of phosphate concentrations that may have limited greater phosphate depletions. The stretches s3N and s4N had the highest phosphate concentrations in streams and then the highest phosphate depletions (Figure 13). This agrees with previous findings where phosphate uptake increased with higher concentrations of phosphate (Bernot et al., 2006). Phosphate concentrations at the stretches s1N and s2N were virtually null so phosphate depletion was probably limited by its low availability. However, increasing phosphate uptakes have been associated with the presence of stream autotrophs such as algae and macrophytes (Sabater et al., 2000; Bernot et al., 2006), so the stretch s2N may have high phosphate depletions under increasing concentrations of phosphate.

Although the highest phosphate concentration may have contributed to the highest phosphate depletions along s3N and s4N, it may have also resulted from their higher oxygen concentrations and pH, since phosphate precipitation and adsorption are generally higher under alkaline and/or oxidized conditions (Moore &

Reddy, 1994; Dodds, 2003). Indeed, macrophytes and periphyton were scarce in s3N and s4N and SRP-exchanges can have a greater influence on SRP concentrations than biological uptake (Jarvie et al., 2006b), so phosphate adsorption and/or precipitation may have contributed to phosphate depletions along s3N and s4N. However, changes in phosphate concentration were not correlated to pH or dissolved oxygen (Figure 13), which may have occurred because: 1) pH and oxygen concentration were high in s1N where the low phosphate concentration may have limited phosphate depletion; 2) small increments of phosphate occurred along s3N where pH and oxygen were high; and 3) phosphate concentration also decreased along s2N where pH and oxygen were lower, probably as a result of photoautotrophs assimilation.

Phosphate depletions along s2N may be more related to photoautotrophs assimilation than to phosphate precipitation and/or adsorption, since pH and dissolved oxygen were low in s2N. However, periphyton photosynthesis locally increases pH and saturate oxygen concentration near the sediment surface (Dodds, 2003), so phosphate adsorption and precipitation may have also contributed to phosphate depletions along s2N because of its high abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12).

Macroinvertebrate communities and leaf litter decomposition also differed among the natural stretches, which may have been related to differences in physico-chemical water parameters and hydromorphology and may have affected changes in physico chemical water parameters along the natural stretches. Leaf decomposition rate was higher in s4N than in s1N and s2N (Table 2). Leaf decomposition rate is higher under moderate than low or high nutrient concentrations and is generally co-limited by nitrogen and phosphorous in streams (Woodward et al., 2012; Ferreira et al., 2014). Therefore, leaf litter decomposition may have been limited by the low nitrogen and phosphate concentrations in s1N and by the low phosphate concentrations in s2N. Furthermore, the highest coverage of dense wooded areas (Appendix 4, land cover class 3.1.1) in s4N and the lowest oxygen concentrations in s2N (Figure 11) may explain differences in shredder abundance and then on leaf decomposition rate among the natural stretches (Table 2).

Nutrient retention in streams can be high during autumn due to N and P immobilization by leaf associated microbes (Webster et al., 2003; Mulholland et al., 2004). The killing of sediment microbes with chloride significantly decreased P retention (D'angelo et al. 1990) and lower nutrient uptake was found after leaf and wood removal (Webster et al. 2001), suggesting that heterotrophic microbes immobilization is relevant to nutrient retention in streams. Therefore, nutrient uptake by leaf associated microbes may have contributed to the highest ammonium and phosphate depletions along s4N because of its higher leaf decomposition rates. However, leaf colonizing microbes were not assessed in this study and the highest leaf decomposition rate at s4N may have occurred because of its turbulent flow (physical abrasion) and/or highest shredder abundance. Shredders may enhance nutrient retention in streams by removing senescent cells and increasing microbial activity (Cheever et al., 2011), but may also reduce nutrient retention by reducing leaf microbial biomass more than increase their activity (Mulholland et al., 1985). Therefore, the highest shredders abundance at s4N may have actually reduced its nutrient depletions.

Leaf litter decomposition may have been related to microbial decomposition in s2N since shredders were absent in s2N and its current velocity was low. Nitrogen immobilization in leaves increase with nitrogen concentration and temperature in stream water (Fernandes et al., 2014), so N and P immobilization by leaf associated microbes may have contributed more to nutrient retention in s2N than in s4N and s1N, since s2N had the highest nitrate concentration and temperature despite lower decomposition rate. However, phosphorous concentration in s2N was reduced which may limit the activity of leaf associated microbes and then nitrogen uptake (Cheever & Webster, 2014). Therefore, as nitrate retention in streams is mostly affected by photoautotrophs uptake and macrophytes were highly abundant in s2N, the highest nitrate depletions along s2N were probably caused by photoautotrophs uptake.

The abundance and diversity of taxa and feeding groups of macroinvertebrates were higher in s4N than in s1N and s2N, probably because of its higher abundance of dense wooded areas (Appendix 4, land cover class 3.1.1) (Figure 10) that may have increased the provision of allochthonous organic matter to macroinvertebrate community. Leaf fragmentation by shredders in s4N may have increased gatherer collectors and then predator abundance, such as Tanypodinae that are burrower macroinvertebrates (Figure 12). Bioturbation may have reduced nitrogen depletions along s4N by increasing ammonium and nitrate releases from the sediments more than nitrification and denitrification rates (Stief, 2013).

Scrapers were more abundant in s4N, which was not expected because of its higher abundances of dense wooded areas (Appendix 4, land cover class 3.1.1) (Figure 10). This may have occurred because leaf bags were placed in spring before canopy closure when light was highly available. Furthermore, the stretch s4N had high current velocity and a turbulent flow that limit the development of macrophytes and favored periphyton and epiphytic diatoms (Withers & Jarvie, 2008), which are known to be used by scrapers as food resource. The existence of scrapers at s4N suggests that photoautotrophs could be present and may have improved nutrient retention in s4N, especially of nitrate. However, scrapers may improve nutrient retention by clearing senescent cells and decreasing biofilm thickness (Mulholland et al., 1994), but may also reduce nutrient retention by reducing periphyton biomass (Mulholland et al., 1991; Sabater et al., 2002). Furthermore, Roberts & Mulholland (2007) reported an increase in ammonium concentration during spring, probably because of grazers excretion associated with high algae biomass. Therefore, scrapers may have actually decreased nitrogen and phosphorous retention in s4N.

Macroinvertebrate abundance and diversity were low in s2N, where *Caenis* sp. was the dominant taxa and gatherer collectors the dominant feeding group (Figure 12). The dams upstream from s2N began to operate in 1930 and at least from the nineties they had bad water quality for fish and recreational activities and eutrophic conditions (INAG, 1999), which may have impacted macroinvertebrates diversity, water quality (Table 2) and allowed greatest abundances of macrophytes in s2N. The Kuparuk River was fertilized with phosphorous for more than sixteen years to assess the response of stream communities and processes to nutrient enrichment (Slavik et al., 2004). After 8 years of phosphorous fertilization, mosses coverage in the Kuparuk River increased from 5% to 50%, increasing ammonium uptake up to 400% (Wollheim et al., 2001) and the abundance of gatherer collectors, such as chironomids and *Ephemerella* sp. that feed on FPOM trapped by bryophytes (Slavik et al., 2004). At s2N nutrient loadings may be high for decades, macroinvertebrate community was dominated by *Caenis* sp. (gatherer collectors from Ephemeroptera order such as *Ephemerella* sp.), and macrophytes and mosses almost cover the entire stream channel at the site where leaf bags were placed in s2N, so nutrient uptake may also be high in s2N because of its high abundance of macrophytes and mosses.

Filter collectors were more abundant in s1N probably because it had more waterfalls located upstream that may have increase the amount of suspended material. Filter collectors may have a great influence on nutrient dynamics in streams by removing particulate organic matter and redistributing nutrients within the water column (Wallace & Webster, 1996; Prather et al., 2013). Nutrient depletions along s1N are expected to be low regardless biota demand and stream dominant processes because of its low nutrient concentrations.

Results demonstrate that differences in physico-chemical water parameters and biota among the natural stretches may have caused distinct ammonium, nitrate and phosphate depletions, but differences in hydromorphology may also explain differences in nutrient depletions among the natural stretches. The stretch s2N had longer water residence times and higher ratio of streambed area per total water volume, which may favor nitrogen and phosphate adsorption and assimilation by biota, nitrification and denitrification, and nutrient exchanges with the hyporheic zone (Sweeney et al., 2004; Mulholland et al., 2009). This corroborate that s2N may be a hotspot for nutrient retention. However the effect of hydromorphology on nutrient dynamics in streams depends on physico-chemical water parameters and biota demand (Hall et al., 2002; Doyle, 2003). Indeed, despite the optimal hydromorphological conditions, the stretch s2N may have lower ammonium and phosphate depletions because of its lower water pH and dissolved oxygen that limit nitrification and phosphorous adsorption. However, the longer water residence time and the sparse vegetation at s2N may have contributed to the high abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) that may largely improve nitrogen and phosphate retention. The stretches s3N and s4N were not expected to have higher abundances of macrophytes even with increasing concentrations of nitrogen and phosphate, since they have a turbulent flow and a dense coverage of forest trees (Appendix 4, land cover class 3.1.1). This may reduce nutrient retention in s3N and s4N but may also reduce the risk of eutrophication.

Results further suggest that changes in water pH, dissolved oxygen and conductivity along stretches could be related to the hydromorphology and biota of the natural stretches. Changes in water pH were highly correlated with the presence of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and functional riparian vegetation (Appendix 7, FFI, parameter 1). This was expected because plants remove carbon dioxide from the water during photosynthesis, which increases water pH (Mesner & Geiger, 2010). Furthermore, pH increased more along s2N (Figure 11) and changes in water pH had a strong negative correlation with changes in nitrate concentrations (Figure 13). This may have occurred because: 1) denitrification can increase pH by releasing OH^- ions (Rivett et al., 2008), and ii) nitrate assimilated by plants is first reduced to ammonium prior to incorporation into aminoacids, which produces OH^- ions that are excreted and causes the surrounding medium to become alkaline (Xu et al., 2012). This strongly suggests that nitrate depletions along s2N may have resulted from denitrification and/or photoautotrophs assimilation and that s2N is a hotspot for nitrate retention.

Water pH had lower increments along s3N and s4N (Figure 11) and decreased along s1N. This may be due to the highest ammonium depletions along s3N and s4N and the highest nitrite depletions along s1N, since hydrogen ions released during nitrification increases water acidity (USEPA, 2002). However, this is not sufficient to support the influence of nitrification in ammonium and nitrite depletions along s3N and s4N, and s1N, respectively, since lower pH increments may have also resulted from lower abundances of macrophytes, reduced nitrate depletions, as well as other factors influencing water pH, such as the highest decomposition rate in s4N that is known to increase water acidity (Mesner & Geiger, 2010).

Changes in conductivity also differed among the natural stretches and decreased more along s2N (Figure 11). This may have occurred because nitrate depletions along s2N were higher than ammonium and phosphate depletions along s3N and s4N. Furthermore, the stretch s2N had lower pH and the highest pH increments (Figure 11), which may have contributed to its higher conductivity depletions since this is lower in neutral conditions (Leveling, 2002). Changes in ammonium concentration were negatively correlated to changes in conductivity (Figure 13), which was not expected because conductivity increases in the presence of dissolved inorganic compounds, such as ammonium, nitrate and phosphate (USEPA, 1997). Ammonium decreased more along s3N and s4N where pH increments created alkaline conditions, which may have increased conductivity and caused it to be negatively correlated with changes in ammonium concentration.

Changes in oxygen concentrations were positively correlated with changes in conductivity (Figure 13), negatively correlated with vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and were not correlated with the frequency of riffles and channel sinuosity (Appendix , RBP, parameter 7a), nor with the coverage of dense wooded areas (Appendix 4, land cover class 3.1.1). This was not expected because salty water holds less oxygen, riffles favor water oxygenation, and vegetation releases oxygen during photosynthesis and decrease water temperature by increasing shade, which increases oxygen concentration (Dosskey et al., 2010; Mesner & Geiger, 2010). Oxygen concentration increased more along s1N (Figure 11) where vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and dense wooded areas (Appendix 4, land cover class 3.1.1) were scarce, riffles (Appendix 7, RBP, parameter 7a) were less present (Figure 10) but waterfalls were more abundant and may have increased oxygen concentration by increasing flow turbulence. This may explain oxygen correlations (Figure 13) and the highest oxygen increments along s1N (Figure 11).

Results demonstrated that changes in physico-chemical water parameters along natural stretches can be related to water chemistry, biota and hydromorphology of streams. Nutrient depletions may have resulted from biota uptake, nitrification and/or adsorption in s3N and s4N, but mainly from photoautotrophs assimilation in s2N. High abundance of mosses and macrophytes may increase nutrient retention in s2N but may also increase eutrophication and impact other vital benefits. Therefore, streams should be managed and restored accounting for all services and disservices they provide.

4.2. Influence of land cover on habitat quality and changes in physico-chemical water parameters along the eutrophic river segment

Stretches of S5 had high nutrient loadings and low habitat quality, regardless the land use. This was expected in S5, since human activities have been degrading hydromorphology and increasing pollutant loadings in the Selho River for decades (INAG, 1999; APA, 2012).

Nitrate and phosphate concentrations and conductivity had high increments along s5A and s5U but high depletions along s5N (Figure 16). This suggests that leisure parks can provide more than just cultural services. Nutrient increments along s5A and s5U were likely due to agricultural and urban activities, respectively, either by point or non-point sources of pollution, nutrient enrichment of bed sediments and groundwater, or decomposition of organic matter in stream. Surface runoff may contribute to increase nutrient concentrations along s5A, since agricultural fields were regularly irrigated, bank vegetative protection (Appendix 7 RBP, parameter 9) was extremely degraded and bank stability (Appendix 7, RBP, parameter 8) was compromised. Therefore, phosphate increments may have been more frequent along s5A (Figure 16) because surface runoff and erosion can dominate phosphorous transport (Withers & Lord, 2002; Pärn et al., 2012). However, agricultural areas in s5A had a low slope, which limit surface runoff.

The stretch s5A had high abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) that increases pH and oxygen concentration (Mesner & Geiger, 2010) but pH and oxygen decreased along s5A (Figure 16). Agricultural and urban streams tend to have high organic and nutrient inputs that increase microbial decomposers activity reducing oxygen concentration and increasing stream acidity (Cooper et al., 2013). Lima-Fernandes et al. (2014) reported high leaf decomposition rates a few meters upstream from s5A, so high organic matter decomposition may have decreased pH and dissolved oxygen and increased nutrient concentrations along s5A.

Conductivity is more related to point rather than diffuse sources of pollutants, since the former have more dissolved whereas the latter more suspended materials (Wang & Yin, 1997). The replacement of native vegetation by crops can shallower groundwater table and increases stream salinity (Cooper et al., 2013). Therefore, the highest increments of conductivity along s5A (Figure 16) suggest that point sources and/or groundwater nutrients may have also contributed to its nutrients increments. Point sources of pollutants were just detected near s5U but they are likely to exist and contribute to nutrient increments along s5A. Agricultural activities in s5A are intensive for decades, so groundwater and bed sediments are expected to be nutrient enriched and probably contribute to increase nutrient concentrations along s5A (Jarvie et al., 2005 and 2006b). Furthermore, within s5A there is a small tributary that can be a source of nutrient from nearby agricultural fields.

Point rather than diffuse sources of pollutants are expected to dominate nutrient inputs in s5U, since the stream was delimited by concrete walls that prevented erosion and runoff and the houses in the waterfront of the stream had some pipes connected to the stream channel and private areas for agricultural activities (Appendix 2, s5U). Furthermore, within s5U there was a small tributary with a very bad smell that may have been nutrient enriched, since it cross a heavily urbanized area. This agrees with previous findings that wastewaters are the major source of nitrogen and phosphorous in urbanized streams (Ortiz et al., 2008). Therefore, nutrient inputs in s5U can have a great impact on stream ecological condition since point sources of pollutants have more dissolved compounds than diffuse sources and may occur throughout the year, including spring and summer where runoff is reduced but the low flow and high solar penetration increases the risk of eutrophication (Jarvie et al., 2006a; Edwards & Jarvie, 2008; Jarvie et al., 2008). Groundwater is not expected to be a relevant source of nutrients in s5U since land use changes tends to decrease groundwater inputs to streams (Cooper et al., 2013) and impervious surfaces dominates in s5U.

Nitrate concentrations increased more along s5U than along the other stretches of S5 (Figure 16) and the urbanized stretch of the mesotrophic river segments (Figure 7) (s1U). This was expected because nutrient inputs often increases with increasing human development and nitrate fluxes are generally higher than ammonium or phosphate fluxes (Cooper et al., 2013).

Nitrate and phosphate depletions were higher along s5N (Figure 16) than along the natural stretches of the mesotrophic river segments (Figure 7). This was likely due to higher abundances of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and higher nutrient loadings increasing nutrient uptake rates in s5N (Dodds et al., 2002). Nitrate concentrations in S5 were lower in late spring despite an increased in ammonium and phosphate concentrations (not shown), suggesting a great influence of photoautotrophs on nitrate concentrations. Furthermore, denitrification rates are expected to be high in S5 because of higher nitrate concentrations, lower dissolved oxygen and current velocity (Inwood et al., 2007; Findlay et al., 2011) and because urban and agricultural streams are likely to have high organic loadings (Cooper et al., 2013). Therefore, nutrient retention may also be high in s5A and s5U despite lower than the nutrient increments, since both stretches had more vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and lower current velocity than s5N. The stretch s5U had more vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) and the highest pH and oxygen increments (Figure 16), probably because photosynthesis increases water pH and oxygen concentrations and nitrate uptake by plants increases water pH. This suggest that vegetation in the wet riverbed (Appendix 7, FFI, parameter 12) had a great influence on stream chemistry along s5U and then probably on nutrient retention. However, s5U cross a heavily urbanized area where strong alkaline pollutants, such as lye, are likely to be discharged into the stream.

Nitrate and phosphate concentrations had high depletions along s5N, but ammonium concentration decrease along all stretches of S5 (Figure 16). Lima-Fernandes et al. (2014) found that N immobilization in leaves was higher in the Selho River, a few meters from s5A, than in streams with lower nitrogen and phosphorous concentrations. This may explain ammonium depletions along all stretches of S5 (Figure 16) since ammonium fluxes are generally lower than those of nitrate (Ackerman & Schiff, 2003), and ammonium is the preferable N form for photoautotrophs and heterotrophic microbes (Mulholland et al., 2000; Peterson et al., 2001).

Although high nutrient loadings and exceedingly growth of macrophytes may increase nutrient retention in stretches of S5, they may also impact other vital services. Furthermore, high nutrient loadings and low diversity of in-stream habitats may decrease the efficiency of nutrient retention in stretches of S5 (Mulholland et al., 2008; Cardinale, 2011).

Temperature was quite different between all sampling sites of S5 (Figure 16), especially of s5U, probably because riparian canopy was virtually absent in all stretches of S5 but s5U had more impervious surfaces and isolated pools. Results denoted temperature depletions along the stretches of S5 (Figure 16) but it may refer to temperature increments since S5 was sampled from down to upstream sampling sites. Therefore, S5 may easily become eutrophic during summer and spring because of high nutrient loadings and light availability, as suggest by the its exceedingly abundance of vegetation in the wet riverbed (Appendix 7, FFI, parameter 12). Furthermore, S5 may be vulnerable to increasing stream discharge because changes in physico-chemical water parameters along the stretches of S5 were quite different when it was sampled after a severe precipitation event (Figure 16, upper panel). Although the stretch s5N had high nutrient depletions when the stream discharge was reduced (Figure 16, lower panel), it had the highest ammonium, phosphate, conductivity and pH increments when S5 was sampled after a severe precipitation event (Figure 16, upper panel). This was expected because sewage and runoff inputs often peaks during and/or after storm discharges (Cooper et al., 2013). When the stream discharge was increased, most of the river banks that were eroded in s5N become submerged and collapse and the pipes close to stream were quite active. The stream water was almost entirely black, had a lot of suspended wastes, and ammonium and pH had the highest increments, suggesting wastewater inputs. Therefore, pollutant loadings in s5N may increase during periods of high discharge because of sewerage overflow and pollutant inputs from eroded banks.

Nitrate and phosphate concentrations remained constant along s5U after the severe precipitation event, and pH had lower increments (Figure 16, upper panel). This was likely due to impervious side walls in s5U preventing erosion and runoff and an increase in discharge diluting nitrogen and phosphorous concentrations. The stretch s5A had the highest phosphate increments with increasing discharge, probably because surface runoff and bank erosion were more likely to be high. This suggests that surface runoff and bank erosion may influence nutrient inputs in s5A because of its low bank stability (Appendix 7, RBP, parameter 8) and bank vegetative protection (Appendix 7, RBP, parameter 9).

Results demonstrated that streams in urbanized areas may have low habitat quality and high nutrient loadings, and despite the high nutrient retention, they may be less resistant to changing climate conditions and provide less benefit.

4.3. Influence of land cover on habitat quality and changes in physico-chemical water parameters along the large river segment

River segment 6 had high nutrient loadings (Table 1) probably because it is downstream polluted tributaries and a few meters downstream the Serzedelo WWTPs (Figure 2). Effluents from WWTPs can be the major source of nitrogen and phosphorous for rivers downstream WWTPs, accounting for more than 50% of nitrogen and phosphorous loads (Cooper et al., 2013). Moreover, effluents from WWTPs can significantly increase river discharge, temperature and conductivity (Haggard et al., 2005). Therefore, the Serzedelo WWTPs can have a great influence on water chemistry of S6 because of their high effluent volume (TRATAVE, 2013b). Conductivity and temperature decreased more along the first stretch of S6 (Figure 17) probably because of effluent dilution from the Serzedelo WWTPs.

The physico-chemical water parameters and discharge of S6 were quite different between sampling times, probably because of the existence of many tributaries and some dams upstream from S6 (Figure 2). Furthermore, there was a weir within s6UR that was used at least in the past to electricity production and a site within s6UL to water abstraction and sludge discharge from industrial activities, causing the river to become almost dry during the summer according to local population. When S6 was sampled in early June, nutrient concentrations, conductivity, pH and temperature were much higher whereas oxygen concentration and discharge were much lower than at the other sampling times, and most of the river substrates were exposed. Therefore, river processes and functioning in S6 may be compromised because of frequent and severe changes in water chemistry and hydrology.

Stretches with more urban areas had worse habitat quality and higher nutrient increments than stretches with more natural riparian areas (Figure 17) suggesting that human activities may impact river hydromorphology and change water chemistry even in rivers with high discharge. Locals informed that wastewaters were discharged within s6UL, at the same site where water was abstracted for industrial activities referring a fishing track, and the stretches s6UL and s6UR had a strong human pressure because of industrial units and habitations at the waterfront of the river (Appendix 2, s6UL and s6UR). Rivers downstream from WWTPs tend to have more benthic and suspended organic material and nutrients accumulated in bed sediments maintaining high water column concentrations (Gücker et al., 2006; Jarvie et al., 2006b). Furthermore, Gibson & Meyer (2007) suggested that ammonium increments along a large urban river downstream from a WWTP, such as S6, were likely due to organic matter mineralization. The stretch s6A had a muddy substrate and oxygen decreased along s6A (Figure 17), suggesting high organic carbon accumulation and mineralization. Therefore, nutrient increments may be more related to point sources of pollutants in s6UL and s6UR, and to organic matter mineralization and/or nutrient releases from bed sediments in s6A. Groundwater inputs may also contribute to increase nutrient concentrations along s6A since agricultural activities are developed for a long time, but the existing bushes and forest trees bordering the river and the low slope in s6A may limit surface runoff (Appendix 2, s6A). In s6UL and s6UR, surface runoff may be an important source of pollutants because of the presence of impervious surfaces close to the river. Furthermore, close to the weir in s6UR, the right bank was eroded, had a high slope and was used for pasture and garbage deposition (Appendix 2, s6UR), so surface runoff may significantly impact water chemistry and the diversity of in-stream habitats with increasing precipitation. Small tributaries existed within all stretches of S6, but their influence on physico-chemical water parameters is expected to be minimal because S6 had a high discharge.

Nitrate concentration increased more along s6UR, whereas phosphate concentrations along s6UL (Figure 17), probably because s6UL had more industrial and commercial facilities where detergents and cleaning products incorporating phosphate are frequently use. This agrees with previous findings that phosphate fluxes increase more with increasing commercial and industrial land use than with increasing residential and mixed urban (Ackerman & Schiff, 2003).

The stretch s6N was dominated by dense wooded areas (Appendix 4, land cover class 3.1.1) and had better habitat quality than the other stretches of S6, mostly because of improved habitat quality parameters related to riparian vegetation. The highest coverage of dense wooded areas (Appendix 4, land cover class 3.1.1) may have reduced pollutant inputs and then nutrient increments along s6N (Figure 17), and may also provide other important services such as air quality regulation and habitat provision for terrestrial and aquatic fauna. This suggests that a dense coverage of forest trees may prevent high pollutant inputs in rivers even those crossing heavily urbanized areas.

Phosphate concentration had the highest depletions along s6N but also decreased along s6A (Figure 17). Gibson & Meyer (2007) quantified nutrient uptake in a large urban river receiving effluents from a WWTP and found lower nitrogen and phosphorous uptake relatively to other less modified streams, but phosphorous uptake increased with increasing suspended solids and temperature. Therefore, phosphate depletions along s6A and s6N (Figure 17) may have been due to phosphorous adsorption, since both stretches had a laminar flow and more suspended material than s6UL and s6UR. Moreover, S6 had high oxygen concentration and pH (Table 1) and phosphate depletion increased with higher concentration of phosphate and pH. This suggests that phosphorous adsorption may have influenced phosphorous depletions since oxidized and/or alkaline conditions increases phosphorous adsorption and precipitation (Moore & Reddy, 1994; Doods, 2003).

Ammonium often decreased along the stretches of S6 regardless the land use (Figure 17), as in mesotrophic and eutrophic river segments. Tank et al. (2008) found that nitrate to ammonium demand increased as river size increased whereas Hall et al. (2013) found an opposite trend. Therefore, for large rivers there is no consensus about the preferable N form. However, an increase in suspended materials and/or a heterotrophic metabolism in large rivers are expected to reduce the ratio of nitrate to ammonium demand, since ammonium retention can also be influenced by abiotic processes while nitrate retention is dominated by photoautotrophs assimilation (Tank et al., 2008). Ammonium had the highest depletions along s6A and s6N

(Figure 17), so it may have been related to ammonium adsorption to suspended materials. Ammonium depletion was higher with lower dissolved oxygen, suggesting that nitrification poorly contributed to ammonium depletion. This agrees with previous findings that nitrification is a minor sink for ammonium in large rivers (Gibson & Meyer, 2007). Hall et al. (2013) found lower demand of phosphorous compared to nitrogen as stream size increased, which may not be the case in our study since phosphorous retention depends more on adsorption to suspended material than nitrogen retention (Withers & Jarvie, 2008; Jarvie et al., 2010), and suspended material seemed to be abundant in s6A and s6N. Nitrate depletions were frequent along s6UL (Figure 17), which was not expected since it was likely to have more pollutant inputs and had a turbulent flow that may limit phytoplankton growth and activity. Nitrate concentrations in S6 were lower in late spring despite an increased in ammonium and phosphate concentrations (not shown), such as in mesotrophic and eutrophic river segments, suggesting a strong influence of photoautotrophs on nitrate concentrations regardless river size or trophic condition.

River segment 6 is expected to have lower nutrient uptake velocities than most mesotrophic river segments because of high nutrient loadings from the Serzedelo WWTPs and frequent fluctuations in discharge that may reduce biofilm development on river substrates. However, nutrient retention cannot be compared between S6 and the other river segments because this study did not account for nutrient addition techniques and consecutive measurements that would allow determining nutrient uptake velocity to normalize the effect of depth and current velocity on nutrient retention (Stream Solute Workshop, 1990). Assess nutrient retention in large rivers may improve water quality management by allowing us to perceive the role of large rivers in nutrients export to downstream ecosystems. Therefore, additional studies including nutrient addition techniques and consecutive measurements are expected to provide important findings.

4.4. Ecosystem services and benefits

Results demonstrated that a great amount of money would be spent to replace ammonia and nitrate depletions of most natural stretches, and that a great amount money would be spent to restore ammonia and nitrate concentrations of most agricultural and urban stretches (Table 3). Results for monetary costs mirror changes in nitrogen concentrations along the stretches and includes weakness as follows: i) monetary costs to remove nitrogen may be different in our study area and among stretches, since constructed wetlands may not be functional near some stretches; ii) nutrient depletion may have resulted from dilution and/or nutrient retention that did not remove nitrogen; iii) changes in nutrient concentrations were measured in November and March whereas stream discharges were measured in June; and iv) nutrient concentrations in most stretches were low so there was no need for nitrogen removal. Furthermore, we did not account for all ecosystem services and disservices contributing to the total economic value of the stretches. However, results for changes in nitrogen concentrations expressed as monetary costs may easily communicate with public and decision makers and raise people awareness about the real ecosystem value and cost effectiveness of protecting and restoring ecosystems. This is determinant for effectively restoring ecosystems since public opinion influences decision makers and determines the success of nature conservancy by protecting or degrading restored ecosystems.

The stretch s3A had high replacement costs (Table 3) despite the urban and agricultural land uses, suggesting that common competing interests such as provisioning and regulating services can coexist depending on management practices and site specific conditions. The stretch s5U had the highest monetary costs during ordinary conditions, whereas s5N had the highest replacement costs during ordinary conditions but a great amount of money would be spent in s5N to restore ammonia and nitrate concentrations during severe precipitation events (Table 3). This may encourage decision makers to restore stream hydromorphology, treating wastewater and improving WWTP treatment levels for improving streams benefits, whereas results for changes in nitrogen concentration would hardly attract public and decision makers interest. Efficiently communicating our results is as important as producing good scientific results, since these will succeed if demanded and increase people benefits.

4.5. Challenges and opportunities for restoring rivers processes and services

Agricultural and urban stretches in all river segment types had worse habitat quality than natural stretches mostly because of riparian deforestation and/or the presence of non-functional vegetation. Furthermore, agricultural and urban stretches had more river bank erosion, lower hydromorphological diversity, higher nutrient increments and more oxygen depletions than natural stretches, probably because of riparian deforestation. Therefore, improving riparian vegetation may allow to recover most services that are being lost by reducing sediments and nutrient inputs and river bank erosion, increasing the diversity of in-stream channel or improving river functioning and biota by providing organic material and influencing light penetration. This may affect nutrient retention in streams by changing photoautotrophs and heterotrophic microbes assimilation and the diversity of in-stream habitats, but results are difficult to predict since an increased in nutrient retention because of heterotrophic microbes and improved in-stream habitats may be offset by a reduction of photoautotrophs. Nevertheless, riparian vegetation is likely to improve many other services and avoid eutrophication.

Diverse riparian buffers with herbaceous, shrubs and riparian trees are expected to be more effective in stabilizing river banks and reducing erosion as nutrient and sediment inputs (Mander et al., 2005; Mander &

Kimmel, 2007). However, the effectiveness of riparian vegetation in improving river functioning and stability also depends on vegetation width and continuity, slope, hydrology or soil properties (Lowrance et al., 1997; Yuan et al., 2009). Although sediment and nutrient retention efficiency depends on site specific conditions, riparian buffers with 3-6 meters width can significantly reduce the amount of pollutants from agricultural runoff (Lim et al., 1998; Syversen et al., 2005; Yuan et al., 2009), so establishing riparian buffers as narrow as 3-6 meters may help to reduce pollutant inputs in most agricultural and urban stretches. Riparian buffers are expected to have higher nutrient retention capacities if including younger and/or periodically harvested plant species (Hefting et al., 2005; Kelly et al., 2007).

Riparian buffers width depends on the stream order and floodplain type as well as on the functions intended to be restored (Hawes & Smith, 2005). Riparian buffers of 3 meters width have been shown to effectively increase bank stabilization, whereas buffers of 50 meters width may be required to protect aquatic habitats (Hawes & Smith, 2005; Dosskey et al., 2012). Moreover, riparian buffer width may differ among sites depending on soil properties, slope, land use, or stream order and floodplain type, since riparian functional areas are expected to be wider in large rivers with broader floodplains and lower slope (Hawes & Smith, 2005; Holmes & Goebel, 2010), so in S6 relatively to the other river segments.

Riparian buffers on low order streams can me more effective in improving water quality than wider buffers at large rivers already polluted (Hawes & Smith, 2005). Furthermore, headwater streams depend more on riparian vegetation than large rivers since allochthonous organic matter is the primary source of carbon and energy in headwater streams, where riparian canopy has a greater influence on light penetration and water temperature (Tank et al., 2010). Therefore, although results suggest that the presence of more dense wooded areas (Appendix 4, land cover class 3.1.1) may avoid greater nutrient inputs in s6N, riparian buffers may be more effective if first implemented upstream, to improve headwater functioning and avoid high nutrient loadings when the Ave River reach S6.

Narrow but continuous riparian buffers can be more effective in protecting rivers from runoff than fragmented buffers of greater width, since runoff can be channelized and reach the rivers through the buffer gaps (Reed & Carpenter, 2002; Hawes & Smith, 2005). Footpaths for river access improve the services provided by rivers such as recreational activities, but they should be as narrow and widening as possible to reduce runoff (Hawes & Smith, 2005).

Leaf litter decomposition increases with increasing the number of leaf species in oligotrophic streams but not in eutrophic streams, where leaf litter decomposition depends more on the quality of leaf species (Lima-Fernandes et al., 2015). Therefore, more diverse riparian buffers are expected to increase leaf litter decomposition in mesotrophic river segments (S1-S4) but not in S5. The effect of leaf litter diversity on leaf litter decomposition is primarily related to the identity of the leaf species (Swan & Palmer, 2006; Lecerf et al., 2007), so riparian buffers with more key riparian species such as *Salix* spp., *Alnus glutinosa* (L.) Gaertn., and *Fraxinus angustifolia* Vahl. (Aránzazu Prada & Arizipe, 2009) are more likely to improve river functioning and stability in all river segment types.

Less permeable surfaces of clay or concrete, nude soils and/or areas used for agricultural or logging activities in steeper lands are more prone to impact rivers by surface runoff (Hawkes & Smith, 2005), so they should be primary reforested or ceases to be used for human activities (Appendix 2, maps on land cover slope). Sediment trapping efficiency of riparian buffers is reduced as sediments size decreases and with increasing slope (>10%), so establishing riparian buffers on steep areas and/or with small particle sizes may be ineffective or require wider buffers to reduce sediment runoff (Liu et al., 2008; Yuan et al., 2009). Highest sediment trapping efficiencies were found for riparian buffers of 10 meters width on areas with a 9% slope, but the sediment trapping efficiency may not significantly improve when buffers have more than 10 meters (Liu et al., 2008). Therefore, an effective restoration needs to account for the site specific conditions and the functions intended to be restored, since under-sized buffers and/or in riparian areas with non-profitable conditions may provide inadequate protection for water bodies, while over-sized buffers may be more expensive and remove lands for production resulting in greater economic losses but non-additional ecological benefits. The maps on slope and land use (Appendix 2, maps on land cover slope) and the Web map we created can help to define restoration priorities, by providing data on land cover and slope, habitat quality and changes in physico-chemical water parameters along the stretches (Appendix 1). In this sense, livestock practices in s6UR with a high slope (16-25%) (e.g. right bank near the waterfall) (Appendix 2, maps on land cover slope) should be primarily moved to a different location and/or riparian buffers should be established where the low slope (8-12%) may increase the buffer trapping efficiency.

Passive restoration (recover riparian forests and allow natural inputs of wood) is preferable used than active restoration (adding wood to the stream channel) because it avoids non-natural amounts or diversity of wood in stream channels and is generally more cost effective, but active restoration has been increasingly used as an important interim measure (before riparian forest became mature and increasing natural wood inputs) to improve the hydromorphological and ecological condition of rivers and streams (Kail et al., 2007; Antón et al., 2011; Acuña et al., 2013). Moreover, active restoration can improve stream functioning in densely populated areas and when stream banks are on private own avoiding passive restoration measures (Kail & Hering, 2005). Therefore, adding dead wood to most mesotrophic and eutrophic river segments may also improve the diversity and availability of in-stream habitats, nutrients cycling and erosion control (Acuña et al., 2013). Most restoration projects used fixed wood structures but non-fixed woods has a lower economic cost and may have a greater ecological relevance since wood dynamics is important to channel morphology and biota (Kail et al., 2007). The cost effectiveness of active restoration is expected to decrease with increasing channel slope and stream power and to be lower in large river segments such as S6, since dead wood can be less stable and have a minor effect on hydrology (Kail et al., 2007). Furthermore, dead wood should be placed upstream from urban and agricultural areas and downstream from natural lands to avoid flood damages. Therefore, the maps on slope and land use (Appendix 2, maps on land cover slope) and the Web map we created with data on land cover and river flow type (Appendix 1) may help to establish active restoration priorities.

Pollutant concentrations of untreated wastewaters are higher than that of effluents from WWTPs (Carey et al., 2013; Tratave, 2013b), so discharges of untreated wastewaters should be primarily eliminated and wastewaters previously treated. However, effluents from WWTPs can significantly impact rivers, especially those with low discharge (Haggard et al., 2005; Gücker et al., 2006). Tertiary treatment levels for wastewaters include physical, chemical and biological processes to remove suspended and dissolved material that remains in the water after secondary treatment levels, and are preferably used for effluents discharged in streams more sensitive to pollution, more likely to become eutrophic or with a poor dilution capacity (Correia, 2007; INSAAR, 2011). The stretch s1A is a small stream with a low discharge and locals informed that fish populations are trying to be recovered at S1 and that the stream is used for recreational activities. Therefore, if the WWTP at s1A continues to be used, its effluents should be discharged into a stream with a higher discharge or its treatment levels should be improved to avoid impact fish recovery and the services provided by the stream, such as opportunities for recreational activities. Both Serzedelo WWTPs have a high effluent volume but effluents from Serzedelo II are discharged in the Ave River whereas effluents from Serzedelo I are discharged a few meters upstream in the Selho River (Tratave, 2013a), which has a lower discharge. Therefore, discharging both effluents in the Ave River may avoid impacting the Selho River while causing similar impacts on the former.

River segment 6 had high nutrients loadings, probably because of the Serzedelo WWTPs and untreated wastewater discharges. Effluents from the Serzedelo WWTPs are within the legal limits to be discharged in rivers (Law decree no 236/98 of 1 August), so managers of WWTPs are not actually obligated to improve wastewater treatment levels. Therefore, laws may first need to be revised if we intend to improve the ecological condition of rivers.

The Ave River is frequently used for fishing and there is a fishing track in s6UL. However fishermen informed us that fish cannot survive in the Ave River and needs to be introduced, probably because of pollution. Furthermore, leisure parks in the waterfront of the Ave River (S6) may be less frequented because of water pollution. Therefore, the costs of improving wastewater treatment levels as the sewerage systems to avoid untreated wastewater discharges may be compensated by an increased in leisure and fishery activities.

Some interesting measures have been adopted to reduce the impact of effluents from WWTPs on ecosystems, such as using the effluents (reclaimed water) to urban (e.g. clean pavements and irrigate public parks), agricultural (e.g. irrigate crops) and industrial activities (e.g. cooling systems, paper production, chemical and textile industry) (USEPA, 2012; Carey et al., 2013). Effluents from WWTPs are not commonly used in Portugal mostly because of the higher treatment costs and local distrust concerning environmental and human health problems of using reclaimed water (Marecos do Monte & Albuquerque, 2010). Effluents from WWTPs have been mostly used in USA, such as in Florida where a dual distribution system for both potable and reclaimed water was implemented to improve ecological and economic incomes by taxing both systems. There is a range of treatment options depending on the use of reclaimed water, but using treated wastewater implies higher treatments costs to improve water quality (Marecos do Monte & Albuquerque, 2010; USEPA, 2012). The cost effectiveness of reclaimed water can be improved by adapting treatment levels to its use requirements and by using it to close human activities to reduce the distribution costs (Marecos do Monte & Albuquerque, 2010). The Serzedelo WWTPs have a high effluent volume and are surrounded by agricultural fields and industrial units that may fit their water demands with reclaimed water. However, effluents from the Serzedelo WWTPs are just used in the WWTPs facilities to clean pavements and equipment or to dilute reagents. Therefore, become using and taxing reused water may allow compensating its higher treatment costs, reduce the rates for agricultural and industrial activities and the impacts on the Ave and Selho rivers.

Global agriculture production increased as a result of fertilizer and pesticide inputs that also impacted other vital ecosystem services (Tilman et al., 2002). Human demand is expected to increase in the near future so increasing agriculture efficiency while reducing its environmental impact is a major challenge. Agriculture efficiency depends on management practices as on soil properties and productive capacity (IUSS Working Group WRB, 2014). In this study area, most agricultural areas were in soils with high ecological value dominated by anthrosols and regosols, except in S5 where agricultural soils had very low ecological value and in S3 and S6 where agricultural soils were dominated by anthrosols and cambisols (CEAP, 2013b). Cambisols are among the most productive soils on earth for food production whereas regosols are extensive in eroding lands and mountains terrain and are more frequently irrigated because of its low moisture holding capacity (IUSS Working Group WRB, 2014). Therefore, agricultural areas in regosols should primarily move to another location since they are more likely to be less productive and require more water and fertilizer inputs. Agricultural areas in S5 have very low ecological value likely because of long term used for intense agricultural practices, which then probably make them less productive and requiring more water and fertilizers.

Furthermore, agricultural areas in S5 are in a heavily urbanized area with high road traffic and industrial activities that may impact air quality and then crops safety and quality. Therefore, moving agricultural activities of S5 to another location is expected to improve the functioning and services provided by the Selho River as agricultural efficiency and productivity.

Agricultural efficiency and impacts on ecosystems also depend on the management practices such as the rate and timing of fertilization (Cassman et al., 2002; Tilman et al., 2002). At the beginning of the 21th century only 30-40% of nitrogen and 40% of phosphorous fertilizers applied in agricultural fields were taken up by crops, but the efficiency of maize fertilization in the United States increased up to 36% in 21 years as a result of public education, research development, soil testing and selection for higher productive capacities, and/or an improvement in the time and rate of fertilization (Tilman et al., 2002). Therefore, rather than just changing agricultural land use there is also a need to change agricultural practices. In this sense, cultivating more diverse set of crops species, applying fertilizers near the plant roots, and fertilizing during active plant growth periods and more regularly than using greater amounts at each time may improve agriculture efficiency and reduce the environmental and economic costs of fertilizers application (Tilman et al., 2002). Furthermore, establishing riparian buffers to reduce runoff may provide habitat for pollinators and pest control species, increase soil fertility and moisture content, and then favor agriculture productivity and reduce the need for fertilizers, pesticides and irrigation systems (Tilman et al., 2002).

4.6. Conclusions and future perspectives

Results demonstrate that land cover influences river functioning and services by influencing water chemistry and habitat quality attributes. Therefore, adopting best management practices, restoring riparian forest and river channel, and monitoring how these measures influence river functioning and stability are determinant to assess the best measures to improve river processes and services.

This study provides good indications about the influence of water chemistry, biota and hydromorphology on changes in physico-chemical water parameters along rivers, but it does not include proper techniques to study nutrient dynamics in rivers and streams, such as conservative tracers, radioisotopes or nutrient additions (Stream Solute Workshop, 1990), that would allow us to assess the effect of dilution, nutrient accumulations in all major ecosystem compartments, nutrient retention in low enriched streams, or to compare nutrient dynamics among rivers with different current velocity and depth by using standard metrics such as the uptake velocity. Therefore, additional studies including proper techniques are expected to provide interesting findings because of differences in chemical, biological and hydromorphological attributes among the stretches of mesotrophic, eutrophic and large river segments. Furthermore, assessing nutrient retention in rivers with such different attributes can improve river management by allowing us to perceive the structures and processes that improve nutrient retention in rivers and streams. Moreover, assessing water purification and other services provided by rivers, as well as their economic, ecological and social impact, can improve environmental planning by attracting public and decision makers interest, and by allowing us to find out cost effective measures that will optimize ecosystem contribution to environmental sustainability and human welfare.

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Appendix 1. Geographic metadata

Theme	Influence of river ecological condition on changes in physico-chemical water parameters along rivers
Coordinate reference system	ETRS89/PT-TM06
Datum	ETRS89 (European Terrestrial Reference System 1989)
Ellipsoid	GRS80
Projection	Transverse Mercator
Latitude of origin	39° 40' 05'',73 N
Longitude of origin	08° 07' 59'',19 W
EPSG	3763
Units	meters (m)
Working scale	1:1000
Elaboration data	December 2014 to October 2015

Dams

Layer georeferencing the dams upstream from river segment 2 (S2). Dams were georeferenced using Bing aerial and Google satellite images in QGIS through the OpenLayers QGIS plugin 1.1.0 (Sourcepole).

Legend

WWTPs

Layer georeferencing the wastewater treatment plants (WWTPs) upstream and within river segments. It was created based on two shapefiles provided by the Agência Portuguesa do Ambiente (APA).

Legend

River water sampling sites

Layer georeferencing the river water sampling sites.

Layer fields

- **SITE_ID** – sampling site identifier. “Ss” refers to sampling site; the first number identifies the river segment whereas the second number identifies the sampling site, from up to downstream sampling sites (e.g. Ss1.2 – second sampling site of river segment 1; Ss2.3 – third sampling site of river segment 2).
- **RIVER_SEGM** – river segment of the sampling site (S1 – river segment 1; S2 – river segment 2, etc.).

Legend

River discharge

Layer georeferencing the sites for river discharge determination.

Layer fields

- **SITE_ID** – river discharge site identifier. “Rd” refers to river discharge; the first number identifies the river segment and the letter the stretch where river discharge was determined (e.g. Rd1.N – river discharge determined in the natural stretch of river segment 1 (S1); Rd4.A – river discharge determined in the agricultural stretch of river segment 4 (S4)).
- **RIVER_SEGM** – river segment of the site (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the site. Number indicates the river segment whereas letter the stretch where river discharge was determined (e.g. s1N – natural stretch of river segment 1 (S1); s4N – natural stretch of river segment 4 (S4)).

Legend

Leaf bags experiment

Layer georeferencing the sites where leaf bags where placed.

<u>Layer fields</u>

- **SITE_ID** – sampling site identifier. Leaf bags were placed at the third river water sampling site in river segment 4 (4.3), and downstream the first and the third river water sampling sites in river segment 2 (2.11) and 1 (1.31), respectively (see metadata of "SITE_ID" field in "River water sampling sites" to additional information on numbers meaning).
- **RIVER_SEGM** – river segment of the sampling site (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the sampling site. Number indicates the river segment whereas letter the stretch where the sampling site is located (e.g. s1N – natural stretch of river segment 1 (S1); s4N – natural stretch of river segment 4 (S4)).

<u>Legend</u>

River waterfalls

Layer georeferencing the river waterfalls identified in the field. River waterfalls classification is available at Appendix 6.

<u>Layer fields</u>

- **WF_ID** – river waterfall identifier. "Wf" refers to waterfall; the first number identifies the river segment, the letter identifies the stretch, whereas the second number identifies the waterfall, from up to downstream sampling sites (e.g. Wf3A1 – first waterfall in the agricultural stretch of river segment 3 (S3); Wf4N2 – second waterfall in the natural stretch of river segment 4 (S4)).
- **RIVER_SEGM** – river segment of the waterfall (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the waterfall. Number indicates the river segment whereas letter the stretch where waterfall is located (e.g. s1N – natural stretch of river segment 1; s4N – natural stretch of river segment 4).
- **WF_TYPE** – river waterfall type (Appendix 6).

Legend

River watercourse

Layer georeferencing the river watercourse of the river segments. River watercourse was divided according to reaches, to allow data analysis at the lowest working level (reach).

Layer fields

- **RIVER_SEGM** – river segment of the river watercourse (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the river watercourse. First number indicates the river segment whereas letter the stretch of the river watercourse (e.g. s1N – river watercourse of the natural stretch from river segment 1 (S1)).
- **REACH** –reach of the river watercourse. First number indicates river segment, the letter identifies the stretch, whereas the second number identifies the reach of the river watercourse, from up to downstream sampling sites (e.g. r5.1A – river watercourse of the first reach in the agricultural stretch of river segment 5; r2.4N - river watercourse of the fourth reach in the natural stretch of river segment 5).
- **LENGTH** – length of the river watercourse (meters).

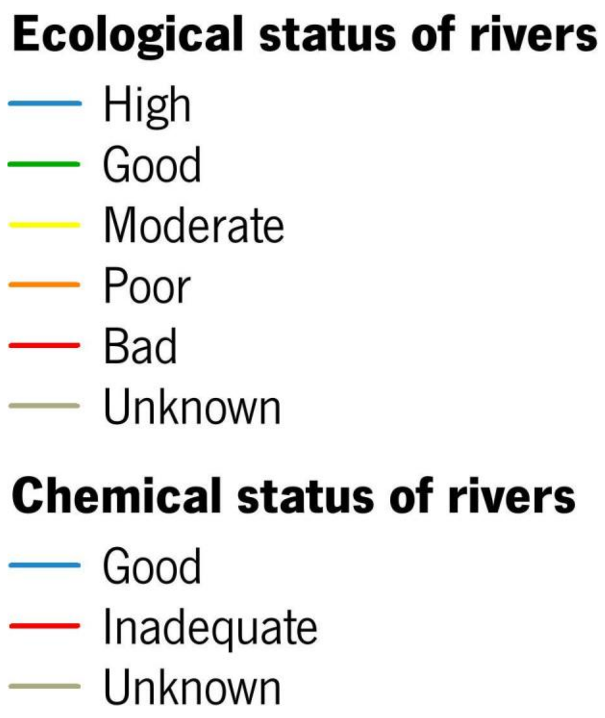
Legend

Ecological and chemical status of rivers

Layers portraying the ecological and chemical status of rivers in the Ave River watershed under the Water Framework Directive (WFD). Layers were created based on the hidcod_25k_ptcont (1:25000) shapefile from Atlas da Água, the shapefiles for river water bodies (PTINAG_ART13_MRIOS_PTCONT) and water body basins (PTINAG_ART3_BACIAS_PTCONT) from InterSIG, and information on ecological and chemical status of water bodies provided by the Agência Portuguesa do Ambiente (APA), as described in section 2.2.4.

Ecological and chemical status of rivers are portrayed by the colors of the WFD to portray ecological and chemical status of river water bodies. Layer style does not distinguish modified and artificial from natural water bodies.

Legends



River margins

Layer portraying the river margins type. River margins classification is available at Appendix 3. Layer was divided according to reaches, to allow data analysis at the lowest working level (reach).

Layer fields

- **ID** – single number of each river margin type in every reach.
- **RIVER_SEGM** – river segment of the river margin (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the river margin. First number indicates the river segment whereas letter the stretch of the river margin (e.g. s1N – river margin of the natural stretch of river segment 1 (S1)).
- **REACH** –reach of the river margin. First number indicates the river segment; the letter identifies the stretch, whereas the second number identifies the reach of the river margin, from up to downstream sampling sites (e.g. r5.1A – river margin of the first reach in the agricultural stretch of river segment 5 (S5); r2.4N - river margin of the fourth reach in the natural stretch of river segment 2 (S2)).
- **MARG_SIDE** – side of the river margin (r – river margin of the right river bank; l – river margin of the left river bank (considering direction from up to downstream)).
- **LENGTH** – length of the river margin (meters) in the correspondent reach.

Legend

Broad margins

V-shape and deep valleys

Constructed rocky margins

Constructed impervious margins

River flow types

Layer portraying the river flow types. River flow types classification is available at Appendix 5. Layer was divided according to reaches, to allow data analysis at the lowest working level (reach).

Layer fields

- **ID** – single number of each river flow type in every reach.
- **RIVER_SEGM** – river segment of the river flow type (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the river flow type. First number indicates the river segment whereas letter the stretch of the river flow type (e.g. s1N – river flow type in the natural stretch of river segment 1 (S1)).
- **REACH** –reach of the river flow type. First number indicates the river segment, the letter identifies the stretch, whereas the second number identifies the reach of the river flow type, from up to downstream sampling sites (e.g. r5.1A – river flow type in the first reach of the agricultural stretch of river segment 5 (S5); r2.4N - river flow type in the fourth reach of the natural stretch of river segment 2 (S2)).
- **AREA** – area of the river flow type in the river section (m^2).

Legend

Laminar

Turbulent

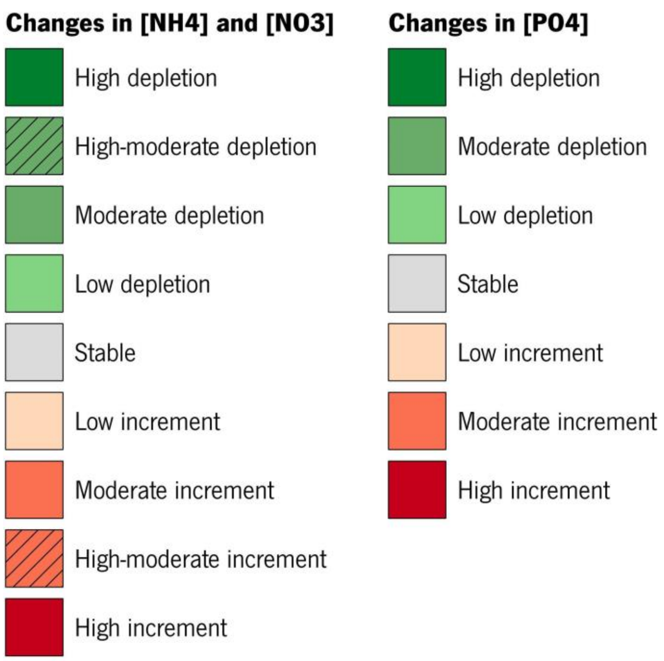
Changes in [NH4], [NO3] and [PO4]

Layers portraying changes in ammonium, nitrate and phosphate concentration along the stretches in ***Δ*** µg L^{-1} km^{-1} NH_4^+, ***Δ*** µg L^{-1} km^{-1} NO_3^- and ***Δ*** µg L^{-1} km^{-1} PO_4^{3-}, respectively. Changes in ammonium and nitrate concentration are portrayed as follow: High depletion, High-moderate depletion, Moderate depletion, Low depletion, Stable, Low increment, Moderate increment, High-moderate increment, and High increment. Changes in phosphate concentration are portrayed as follow: High depletion, Moderate depletion, Low depletion, Stable, Low increment, Moderate increment, and High increment. Classes were created based on mean values for changes in ammonium, nitrate, and phosphate concentration along the stretches (n=5 for s2N and s4N; n=3 for s5A, s5U and s5N; and n=4 for the other stretches).

Layers fields

- **RIVER_SEGM** – river segment of the stretch where changes in physico-chemical water parameters were recorded (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch where changes in physico-chemical water parameters were recorded. Number indicates the river segment whereas letter the stretch (e.g. s1N – natural stretch of river segment 1 (S1); s4N – natural stretch of river segment 4 (S4)).
- **LENGTH –** length of the stretch (km).
- **AREA –** area of the stretch (river section) (m^2).

Legends



Changes in [NH4], [NO3] and [PO4] (preci)

Layer portraying changes in ammonium, nitrate and phosphate concentration along the stretches of river segment 5 (S5) after a severe precipitation period (n=1). Layer fields and legend are the same as for “Changes in [NH4], [NO3], [PO4]”. “Changes in [NH4], [NO3], [PO4] (preci)” is above “Changes in [NH4], [NO3], [PO4]” to allow visualize how physico-chemical water parameters changed after a severe rainy period, relatively to ordinary conditions.

Habitat quality (FFI) (RBP) (QBR)

Layers portraying the river habitat quality at the reach level according to the Fluvial Functioning Index (FFI), the HABSCORE (RBP), and the Riparian Forest Quality Index (QBR). Layers styles were created based on those used by the habitat characterization method to portray river habitat quality.

<u>Layers fields</u>

- **ID** – single number of each river bank (50 meters buffer strip).
- **RIVER_SEGM** – river segment of the river bank (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the river bank. First number indicates the river segment whereas letter the stretch of the river bank (e.g. s1N – river bank on the natural stretch of river segment 1).
- **REACH** –reach of the river bank. First number indicates the river segment; the letter identifies the stretch, whereas the second number identifies the reach of the river bank, from up to downstream sampling sites (e.g. r5.1A – river bank of the first reach in the agricultural stretch of river segment 5 (S5); r2.4N - river bank of the fourth reach in the natural stretch of river segment 2 (S2)).
- **MARG_SIDE** – side of the river bank (r – right river bank; l – left river bank (considering direction from up to downstream)).
- **AREA** – area of the river bank.

Legends

Habitat quality (FFI)	Habitat quality (RBP)	Habitat quality (QBR)
Excellent	Optimal	Riparian habitat in natural condition
Excellent-very good	Suboptimal	Some disturbance, good quality
Very good	Marginal	Disturbance important, fair quality
Very good-good	Poor	Strong alteration, poor quality
Good		Extreme degradation, bad quality
Good-fair		
Fair		
Fair-poor		
Poor		

Land cover (L1) (L5)

Layers portraying the land cover (levels 1 (L1) and 5 (L5) (Appendix 4)) at the 50 meter buffer strips of the river segments. Land cover classes and classification framework are available at Appendix 4. Layers were divided according to reaches, to allow data analysis at the lowest working level (reach).

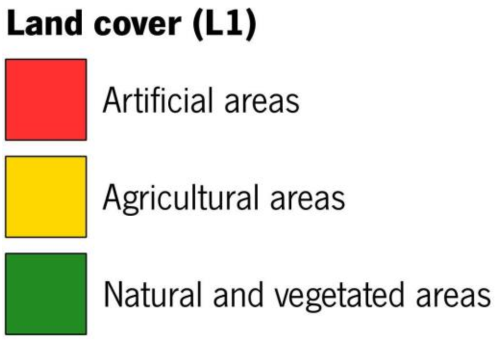
Layers fields

- **ID** – single number of each polygon.
- **RIVER_SEGM** – river segment of the land cover (polygon) (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the land cover (polygon). First number indicates the river segment whereas letter the stretch of the land cover (polygon) (e.g. s1N – land cover in the natural stretch of river segment 1 (s1)).
- **REACH** –reach of the land cover (polygon). First number indicates the river segment, the letter identifies the stretch, whereas the second number identifies the reach of the land cover (polygon), from up to downstream sampling sites (e.g. r5.1A – land cover (polygon) in the first reach of the agricultural stretch of

river segment 5 (S5); r2.4N - land cover (polygon) in the fourth reach of the natural stretch of river segment 2 (S2)).

- **MARG_SIDE** –river bank side of the land cover (polygon) (r – land cover on the right river bank; l – land cover on the left river bank (considering direction from up to downstream)).
- **AREA** – area of the land cover (polygon).

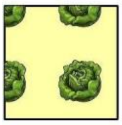
Legends

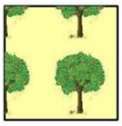


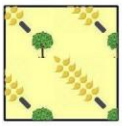
Land cover (L5)

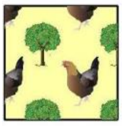
- Continuous residential buildings in highly urbanized areas
- Discontinuous residential buildings in highly urbanized areas
- Continuous residential buildings in rural areas
- Discontinuous residential buildings in rural areas
- Industrial units
- Wastewater treatment facilities
- Agricultural facilities
- Comercial and touristic facilities
- Abandoned buildings
- Buildings with a seasonal use
- Highly impervious surfaces
- Dump sites
- Sport fields
- Other recreational areas
- Other moderately impervious surfaces

 Grain and forage crops

 Vegetable crops

 Orchards and vineyards

 Arable lands with permanent crops

 Complex cultivation patterns

 Livestock


Dense mixture of broad-leaved species


Dense mixture of broad-leaved with coniferous species

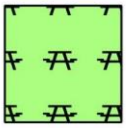

Sparse mixture of broad-leaved species

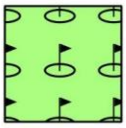

Sparse mixture of broad-leaved with coniferous species


Herbaceous vegetation

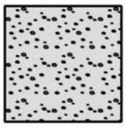

Dense shrubs


Sparse shrubs


Leisure parks


Golf courses


Sparse vegetated areas


Bare rocks


Dirt paths

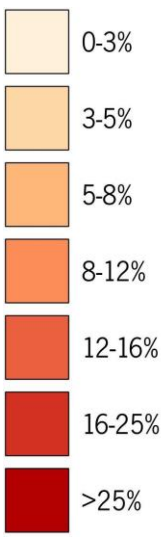
Slope

Layer portraying the slope at the 50 meters buffer strips of the river segments. Layer was divided according to the land cover classes of level 5 (Appendix 4), since it was created by converting a raster file of slopes (CEAP, 2013a) to a shapefile format, and then intersecting it with the shapefile for land cover, as described in section 2.2.5.

Layer fields

- **ID** – single number of each polygon.
- **RIVER_SEGM** – river segment of the polygon (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the polygon. First number indicates the river segment whereas letter the stretch of the polygon (e.g. s1N – polygon in the natural stretch of river segment 1 (S1)).
- **REACH** –reach of the polygon. First number indicates the river segment; the letter identifies the stretch, whereas the second number identifies the reach of the polygon, from up to downstream sampling sites (e.g. r5.1A – polygon in the first reach of the agricultural stretch of river segment 5 (S5); r2.4N - polygon in the fourth reach of the natural stretch of river segment 2 (S2)).
- **MARG_SIDE** –river bank side of the polygon (r – polygon on the right river bank; l – polygon on the left river bank (considering direction from up to downstream)).
- **AREA** – area of the polygon.

Legend

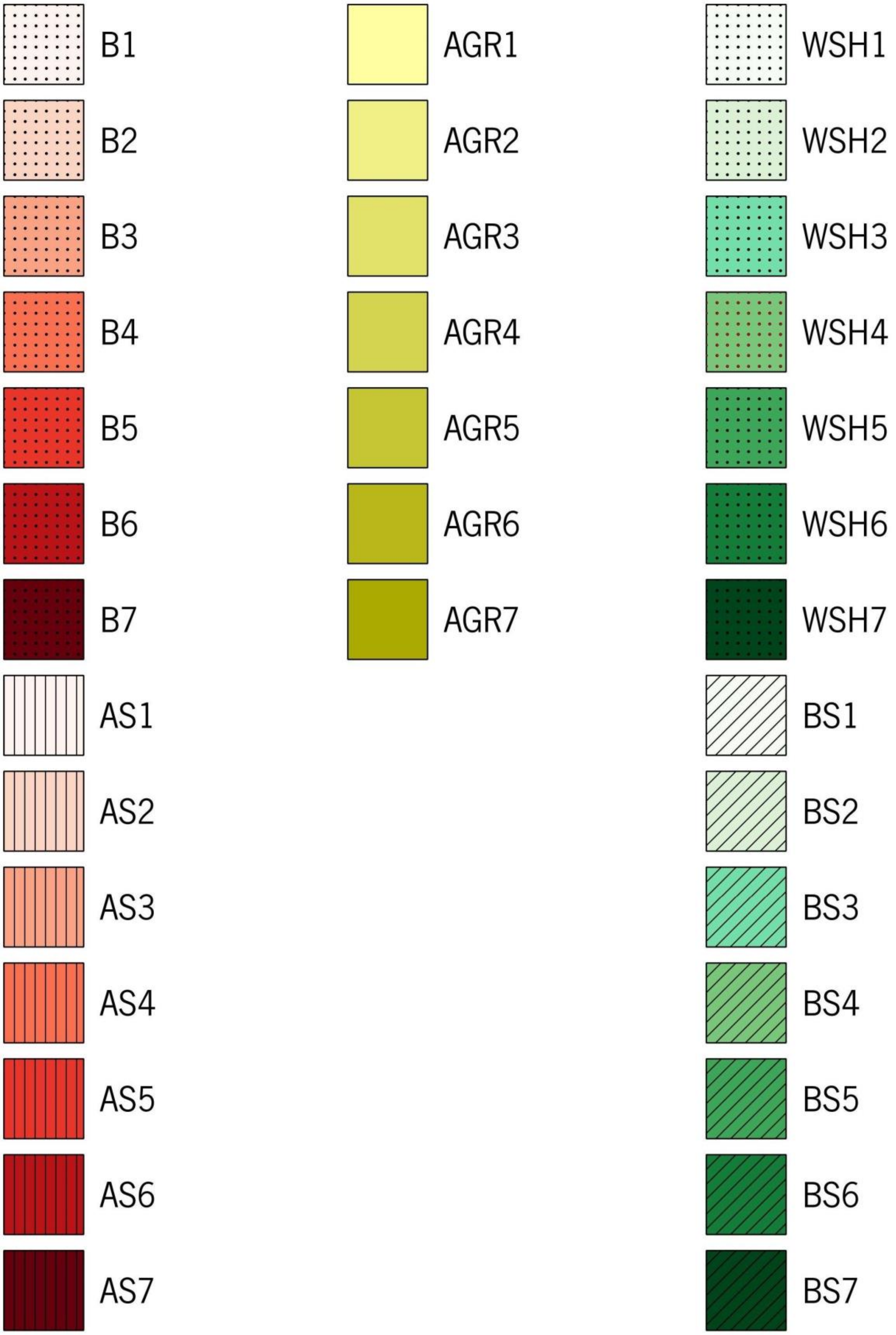


Land cover slope

Layer with intersected information for land cover and slope at the 50 meters buffer strips of the river segments. It was created by intersecting the shapefiles for land cover and slope, as described in section 2.2.5.

Legend distinguishes land cover type as follow: B (Buildings (Appendix 4, land cover class 1.1)), AS (Artificial surfaces (Appendix 4, land cover class 1.2)), AGR (Agricultural areas (Appendix 4, land cover class 2)), WSH (Woody, shrubs and herbaceous vegetation (Appendix 4, land cover classes 3.1 and 3.2)), and BS (Bare soil (Appendix 4, land cover class 3.3)); and slope as: 1 (0-3%), 2 (3-5%), 3 (5-8%), 4 (8-12%), 5 (12-16%), 6 (16-25%) and 7 (>25%) (e.g. AGR3 refers to agricultural areas with a slope of 5-8%). Legend distinguishes i) major land cover types by colors as: artificial (red), agricultural (yellow) and natural (green); ii) land cover from the same major land cover type by fill patterns (e.g. artificial surfaces are portrayed by red polygons with line patterns whereas buildings are portrayed by red polygons with point patterns); and iii) polygons with the same land cover (e.g. artificial surfaces) but different slopes by a color ramp (e.g. artificial surfaces with low slope are portrayed by polygons with slight red color and line patterns, whereas artificial surfaces with high slope are portrayed by polygons with intense red color and line patterns). Six land cover were selected based on their influence to runoff.

Legend


B1
B2
B3
B4
B5
B6
B7
AS1
AS2
AS3
AS4
AS5
AS6
AS7
AGR1
AGR2
AGR3
AGR4
AGR5
AGR6
AGR7
WSH1
WSH2
WSH3
WSH4
WSH5
WSH6
WSH7
BS1
BS2
BS3
BS4
BS5
BS6
BS7

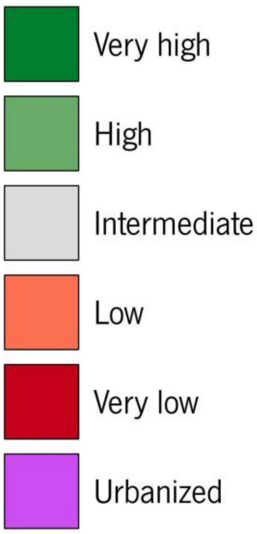
Soil ecological value

Layer portraying the soil type and ecological value at the 50 meter buffer strips of the river segments. It was created based on the shapefile for the soil ecological value from the EPIC WebGIS project (CEAP, 2013b), as described in section 2.2.6.

<u>Layer fields</u>

- **ID** – single number for each polygon.
- **RIVER_SEGM** – river segment of the polygon (S1 – river segment 1; S2 – river segment 2, etc.).
- **STRETCH** – river stretch of the polygon. First number indicates the river segment whereas letter the stretch of the polygon (e.g. s1N – polygon in the natural stretch of river segment 1 (S1)).
- **MARG_SIDE** –river bank side of the polygon (r – polygon on the right river bank; l – polygon on the left river bank (considering direction from up to downstream)).
- **AREA** – area of the polygon.

<u>Legend</u>



Water body basins

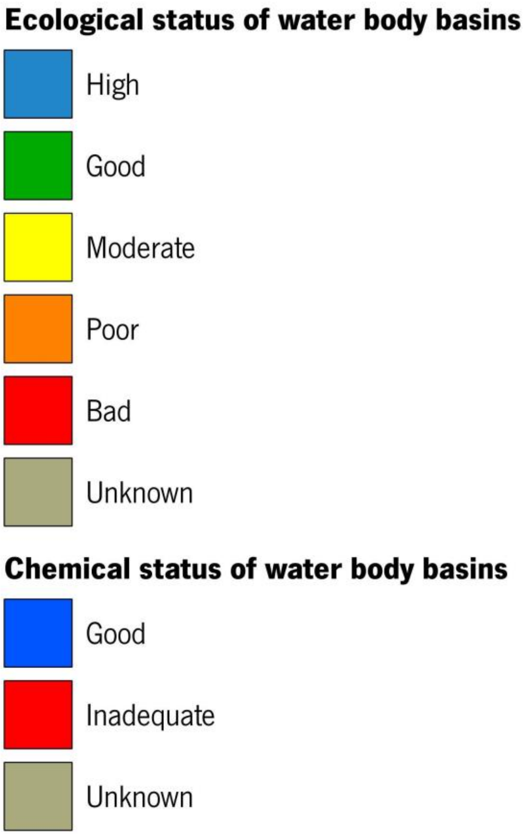
Layer portraying the water body basins in the Ave River watershed under the Water Framework Directive (WFD) (InterSIG, 2006).

Ecological and chemical status of water body basins

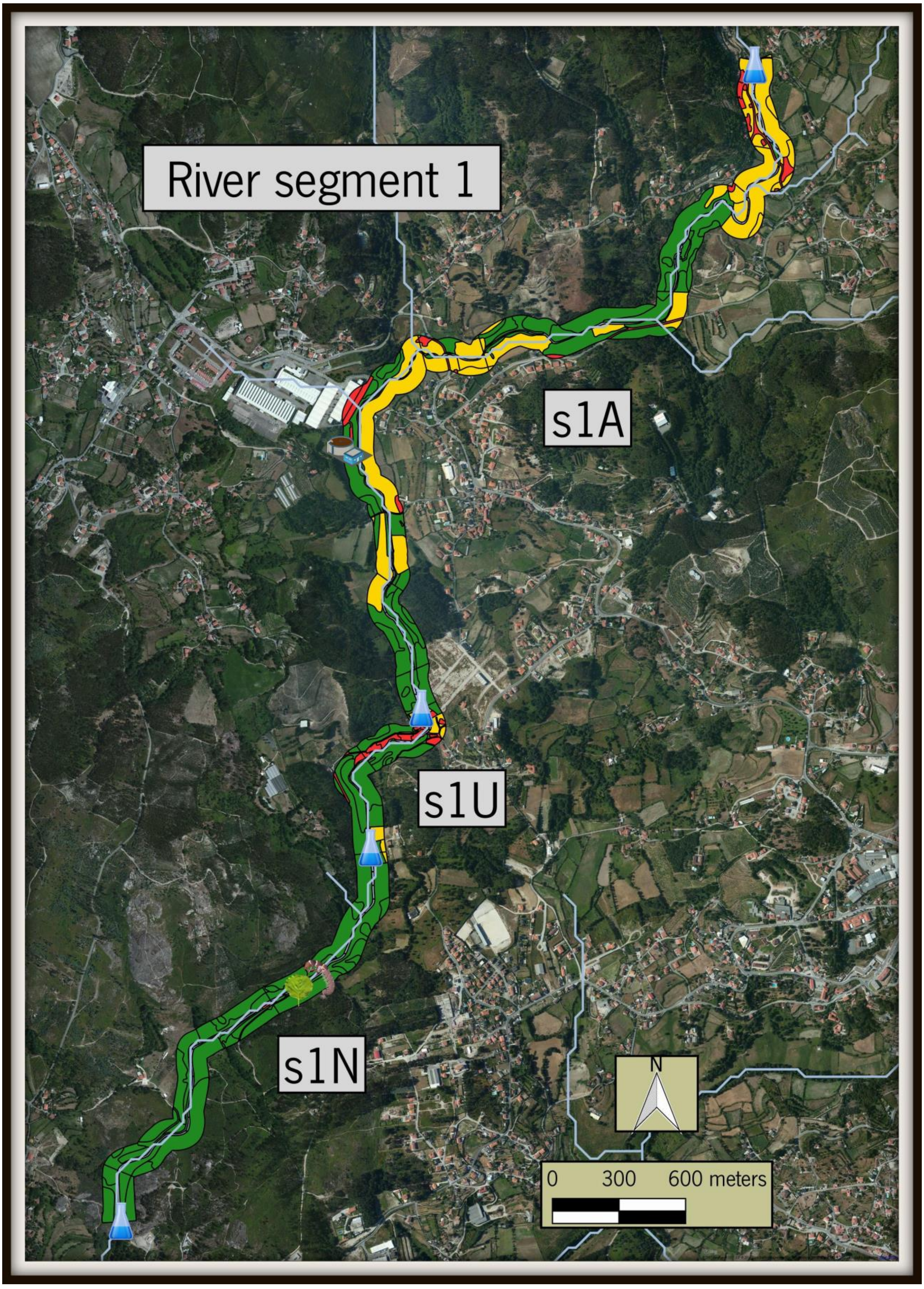
Layer portraying the ecological and chemical status of the water body basins in the Ave River watershed under the Water Framework Directive (WFD). Layers were created based on the shapefile for the water body basins (PTINAG_ART3_BACIAS_PTCONT) from InterSIG, and information on ecological and chemical status of water bodies provided by the Agência Portuguesa do Ambiente (APA), as described in section 2.2.4.

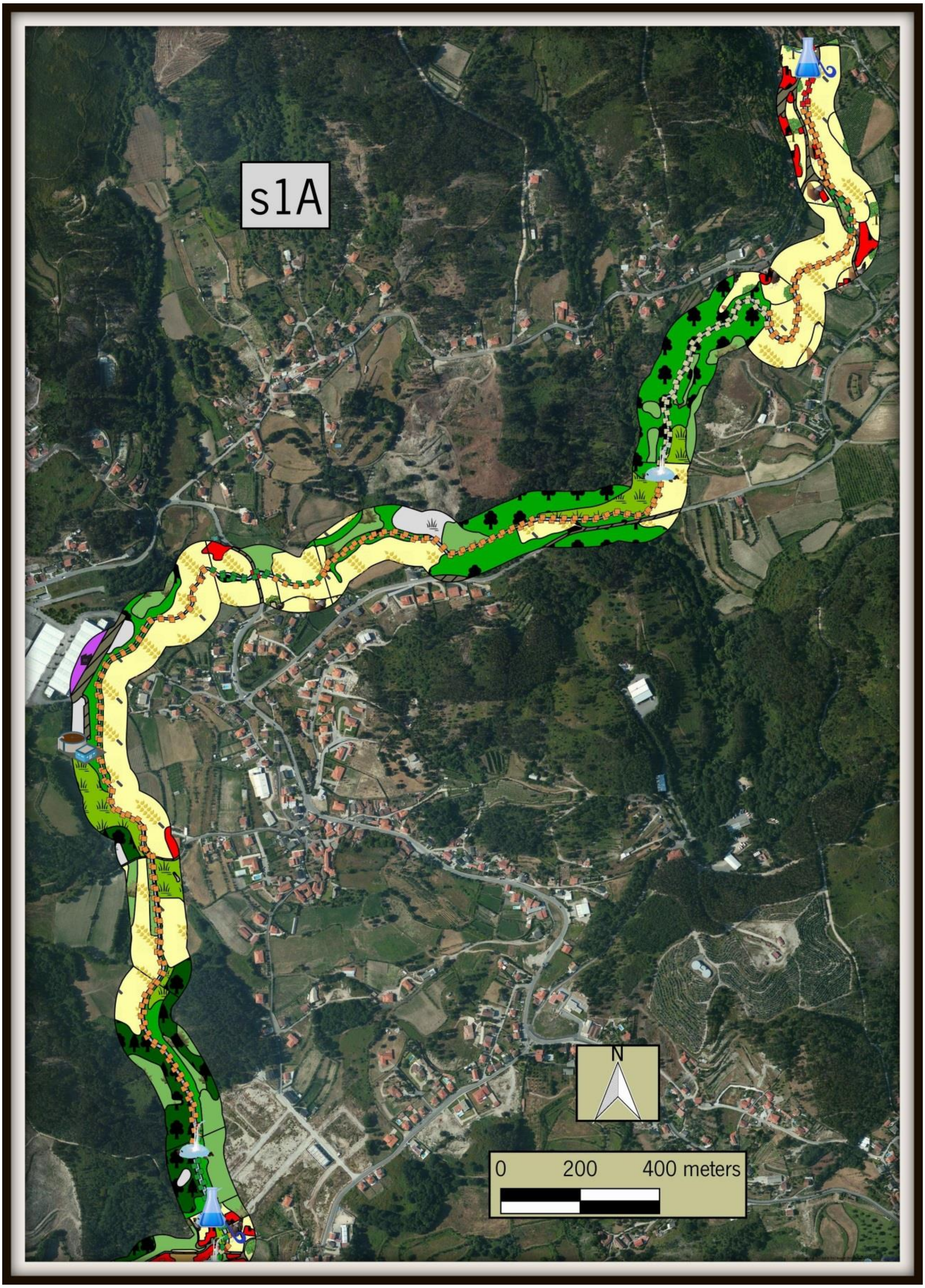
Ecological and chemical status of water body basins are portrayed by the colors of the WFD to portray ecological and chemical status of water bodies.

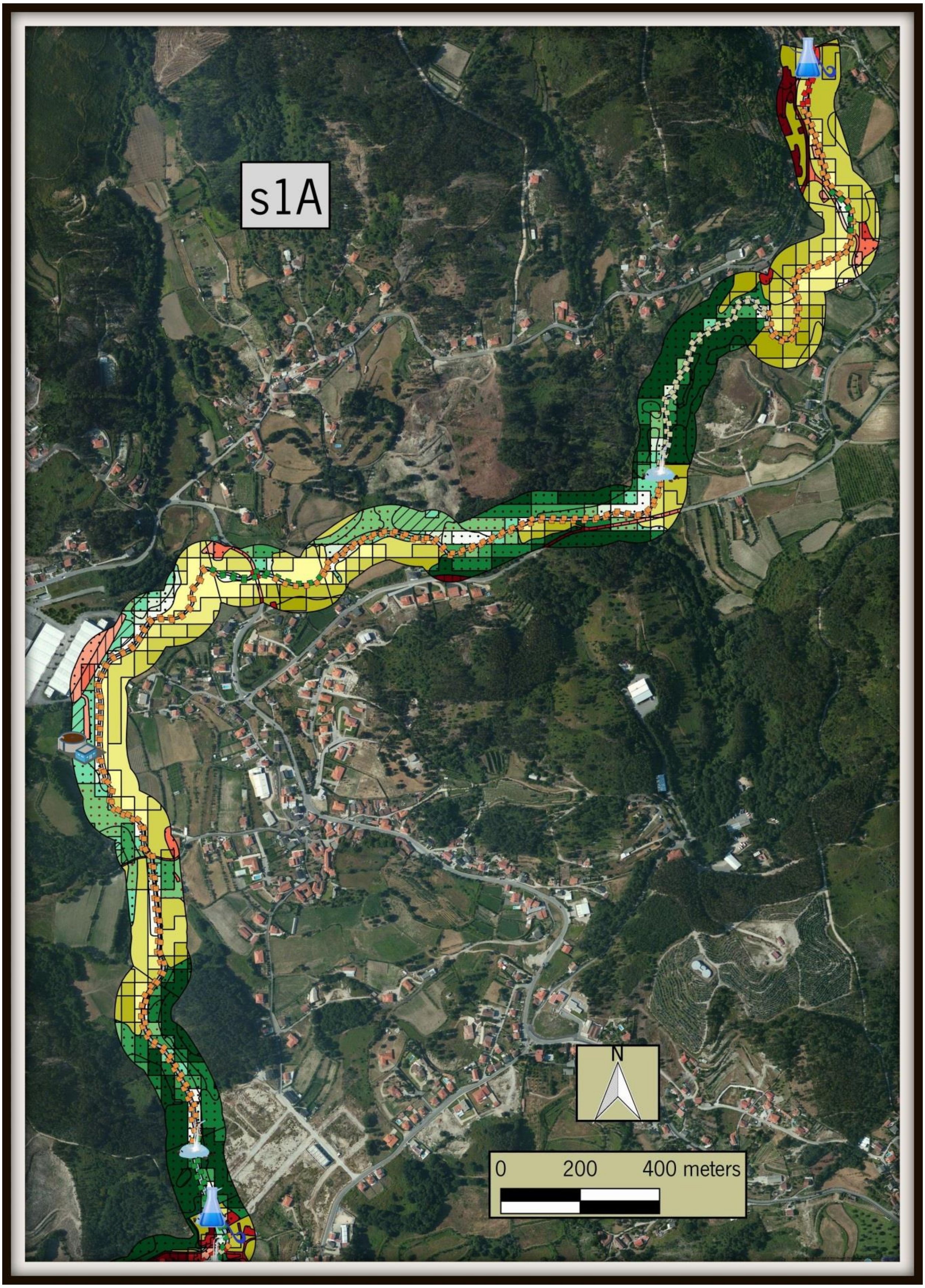
<u>Legend</u>

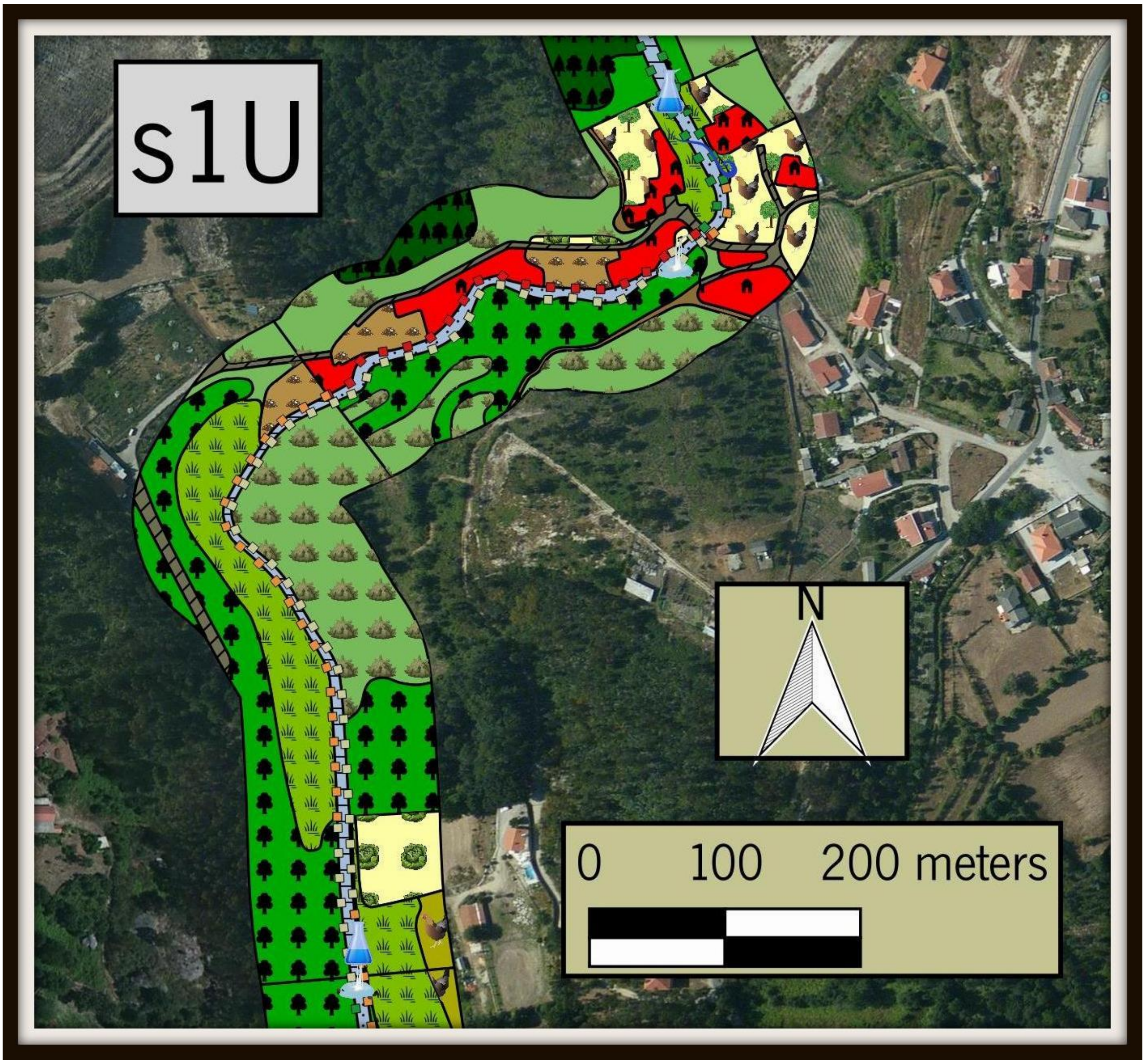


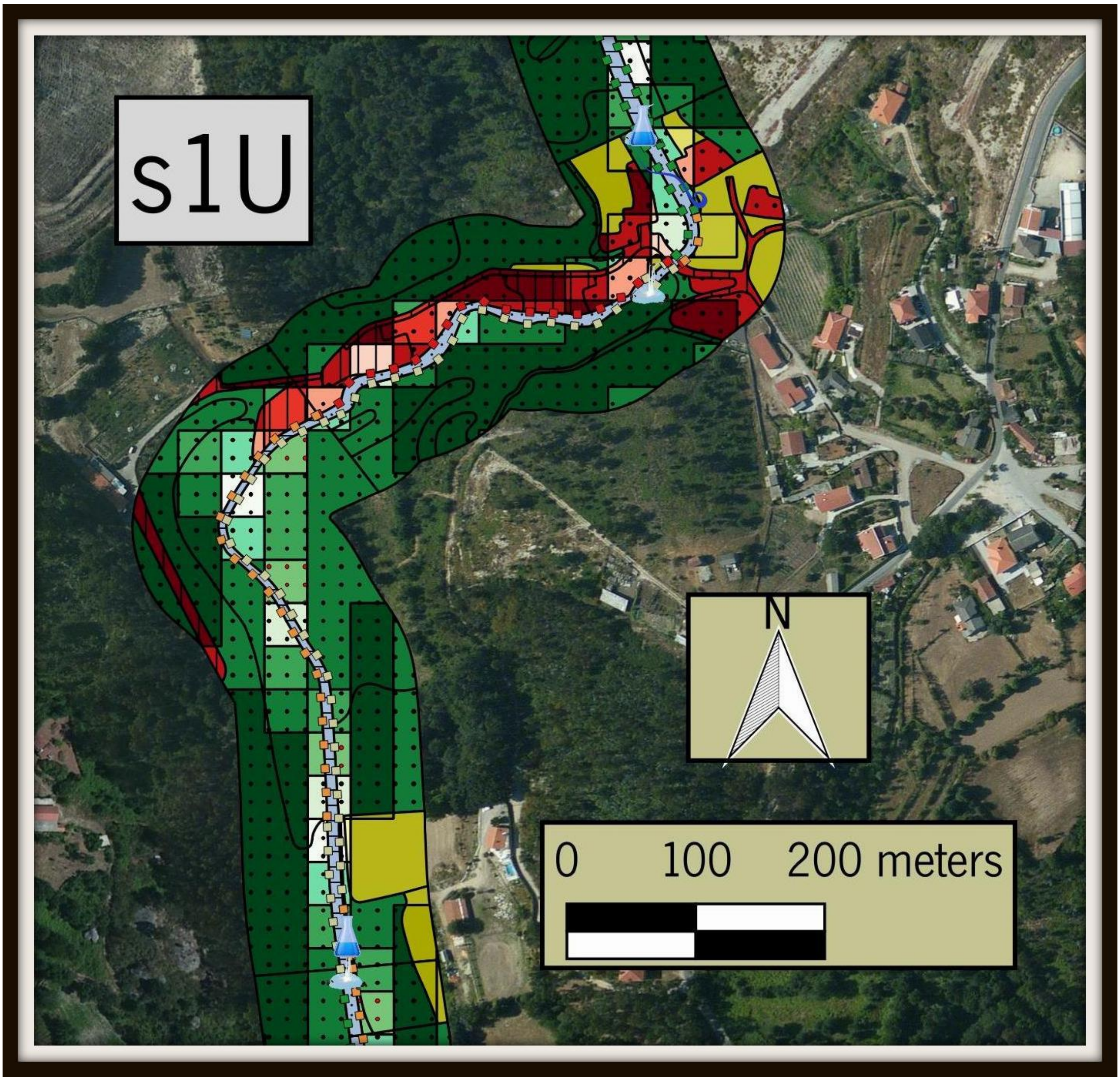
Appendix 2. Maps portraying river segments and stretches

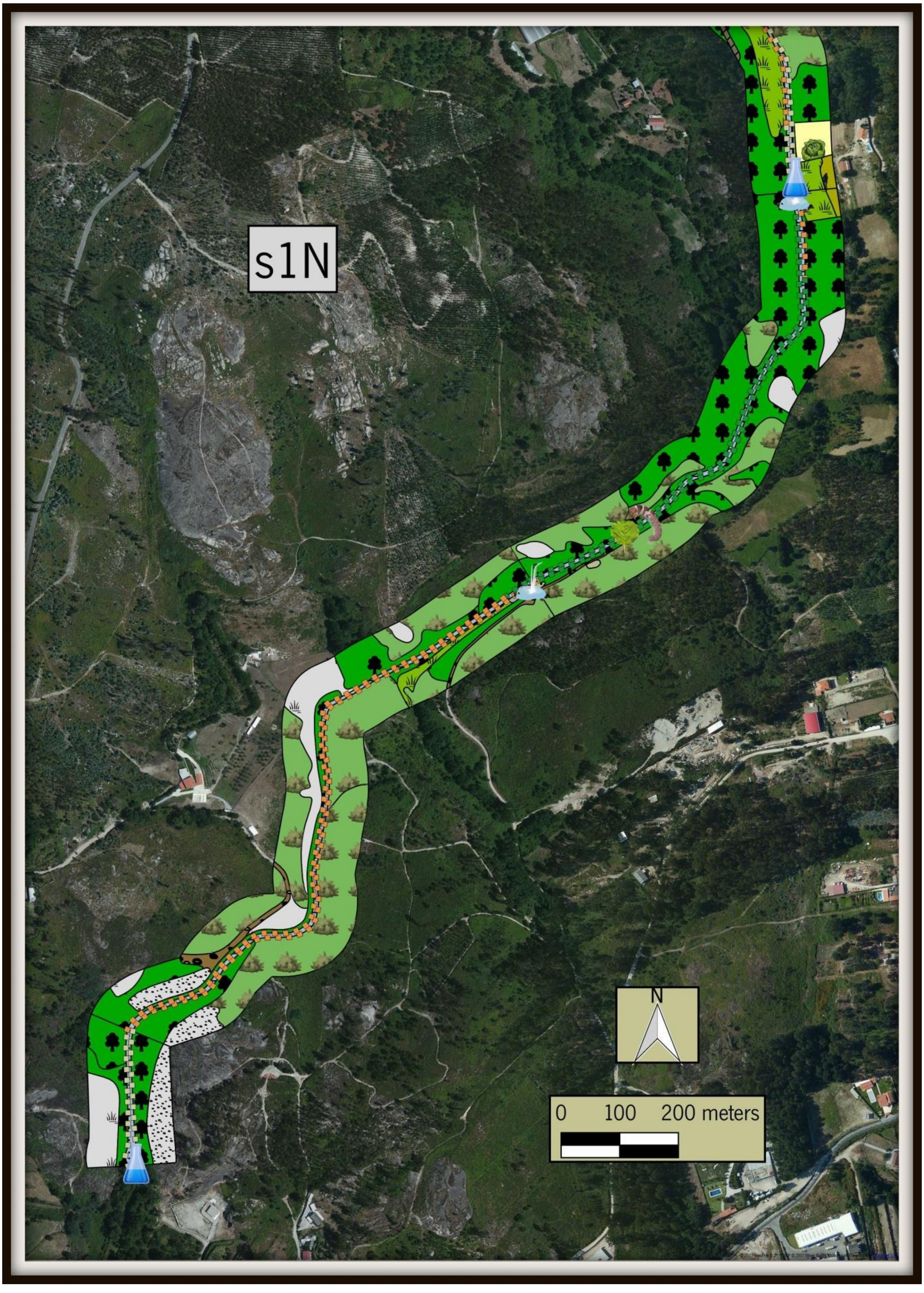


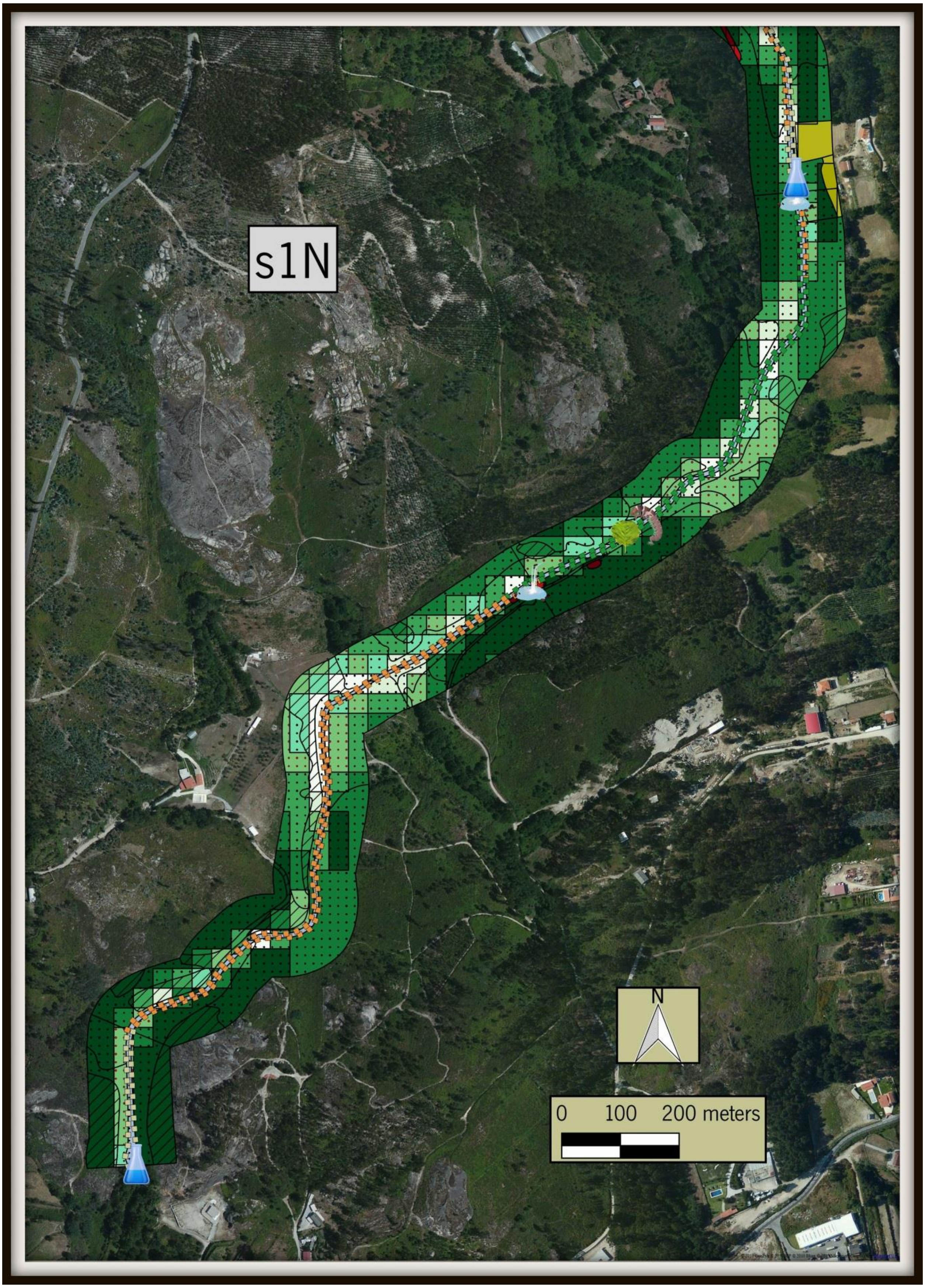

s1A
N
0
200
400 meters

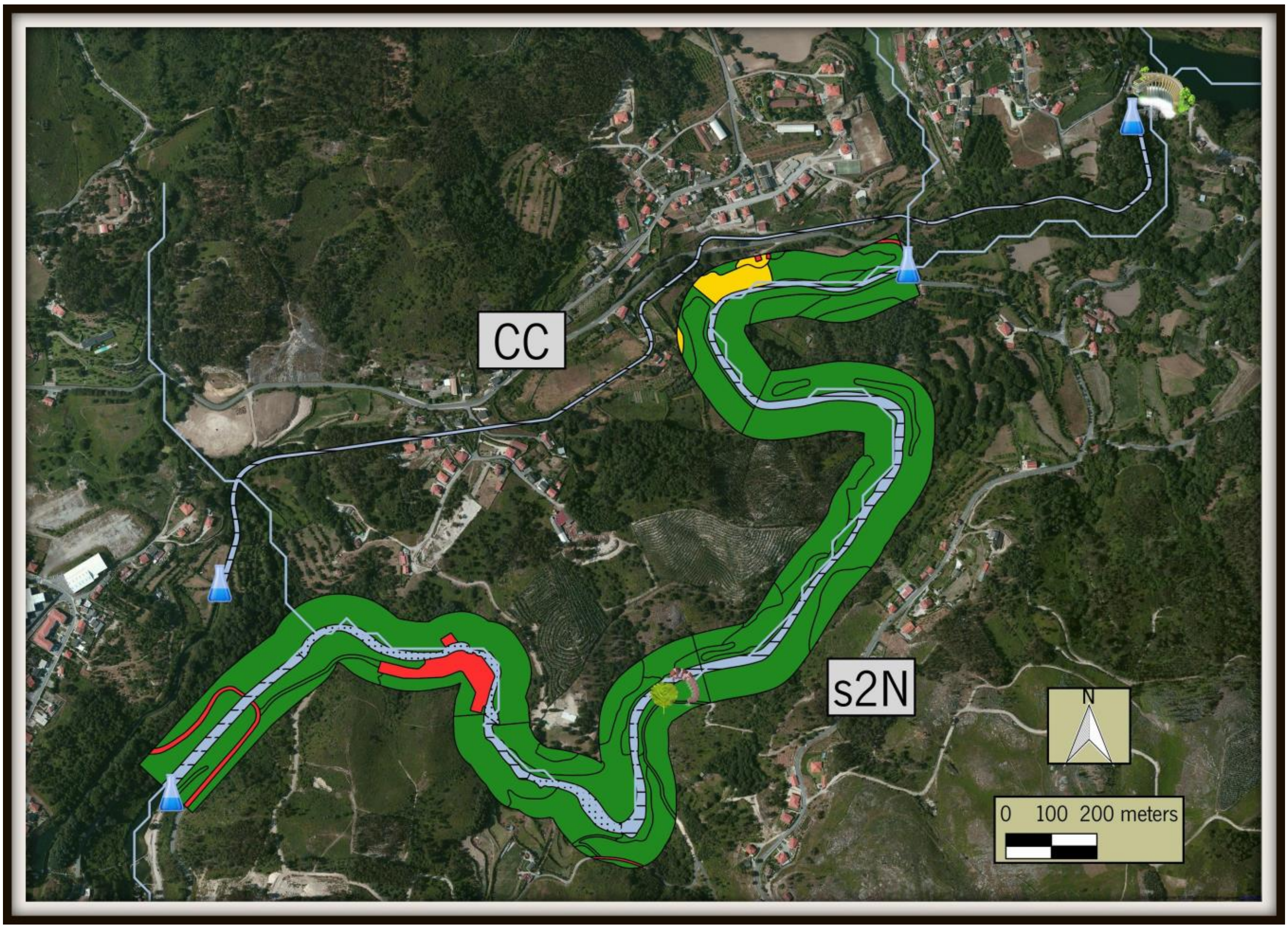

s1A
N
0 200 400 meters

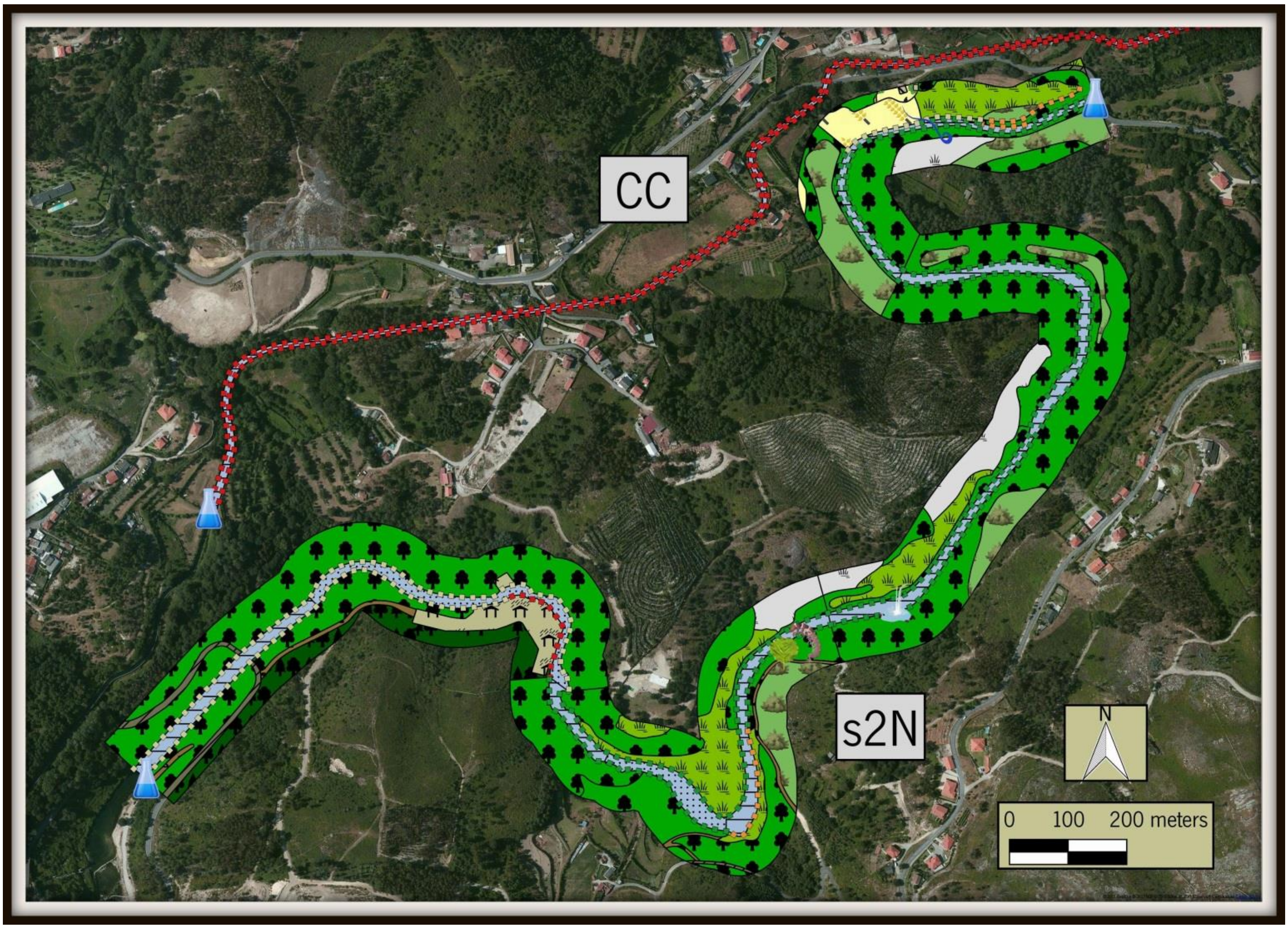

s1U
N
0 100 200 meters

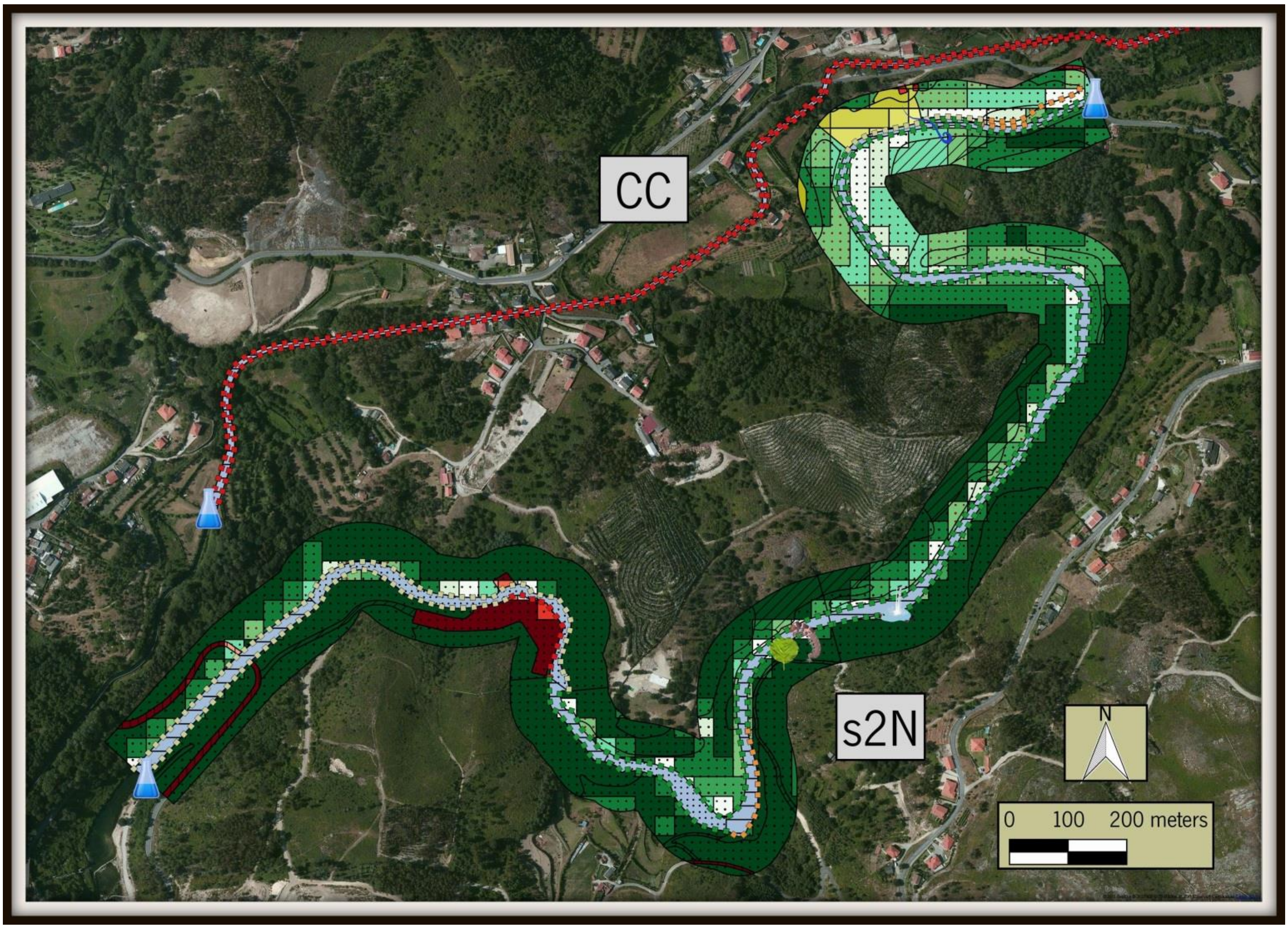

s1U
N
0 100 200 meters

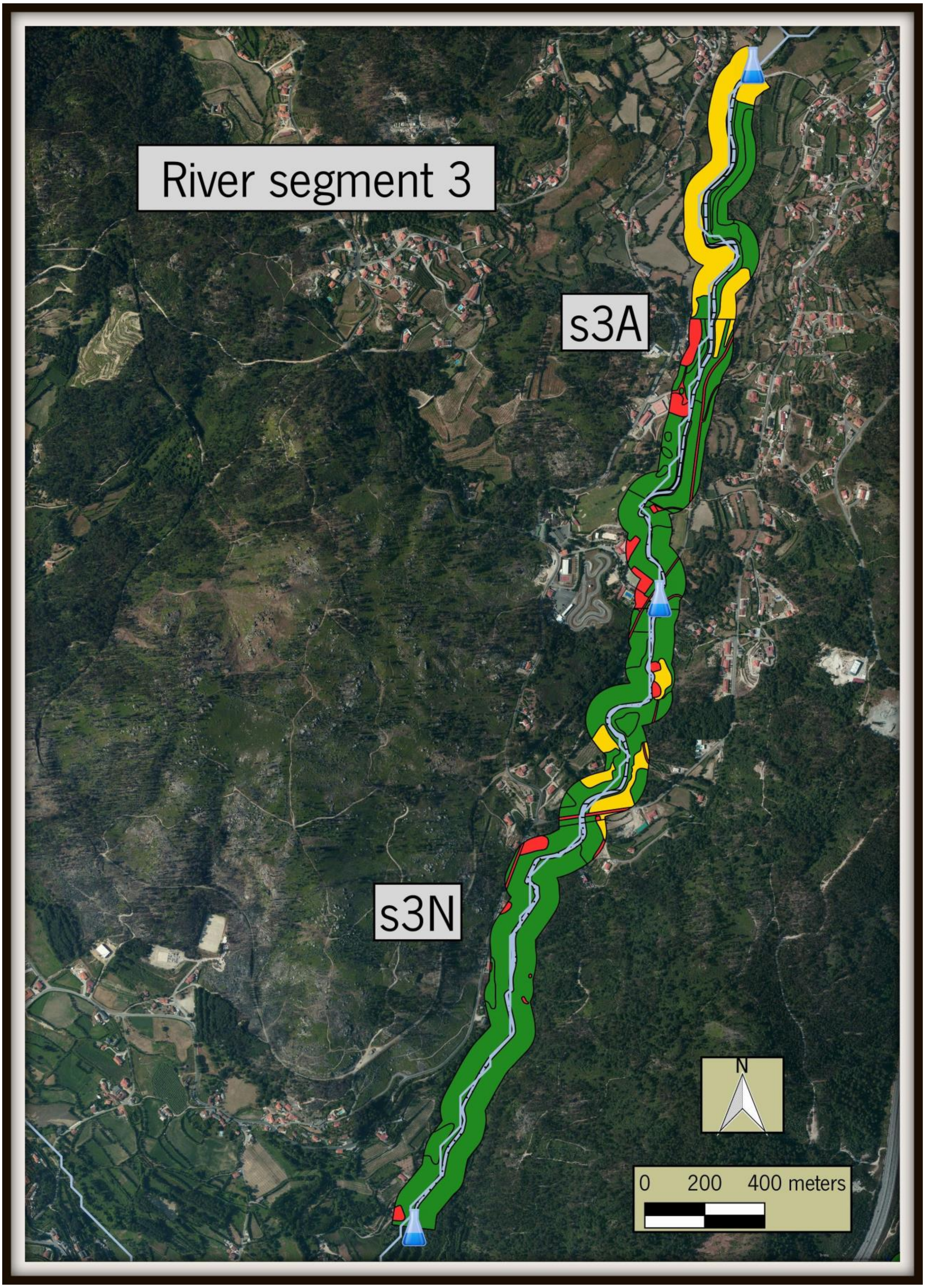

s1N
N
0 100 200 meters

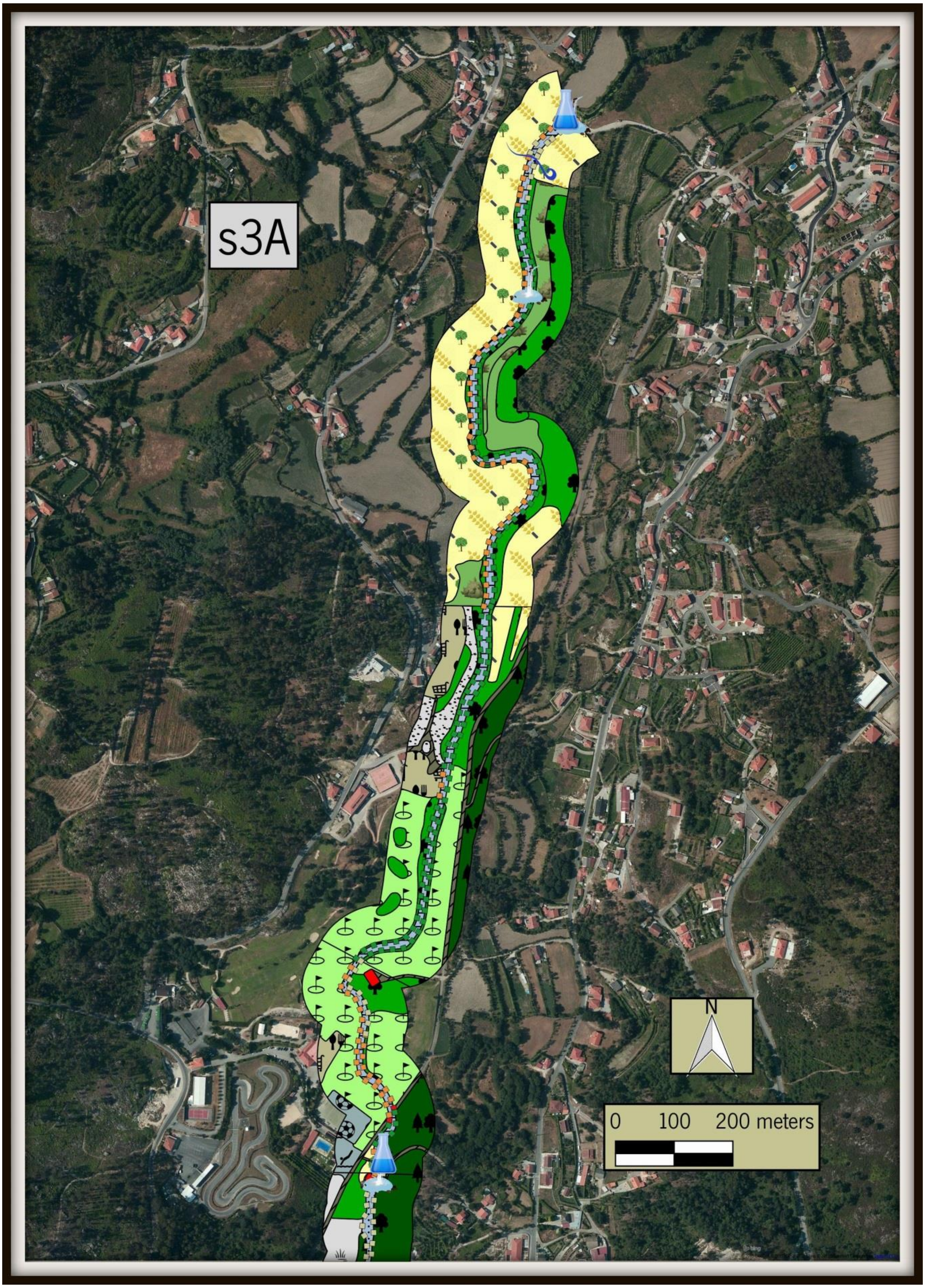

s1N
N
0 100 200 meters

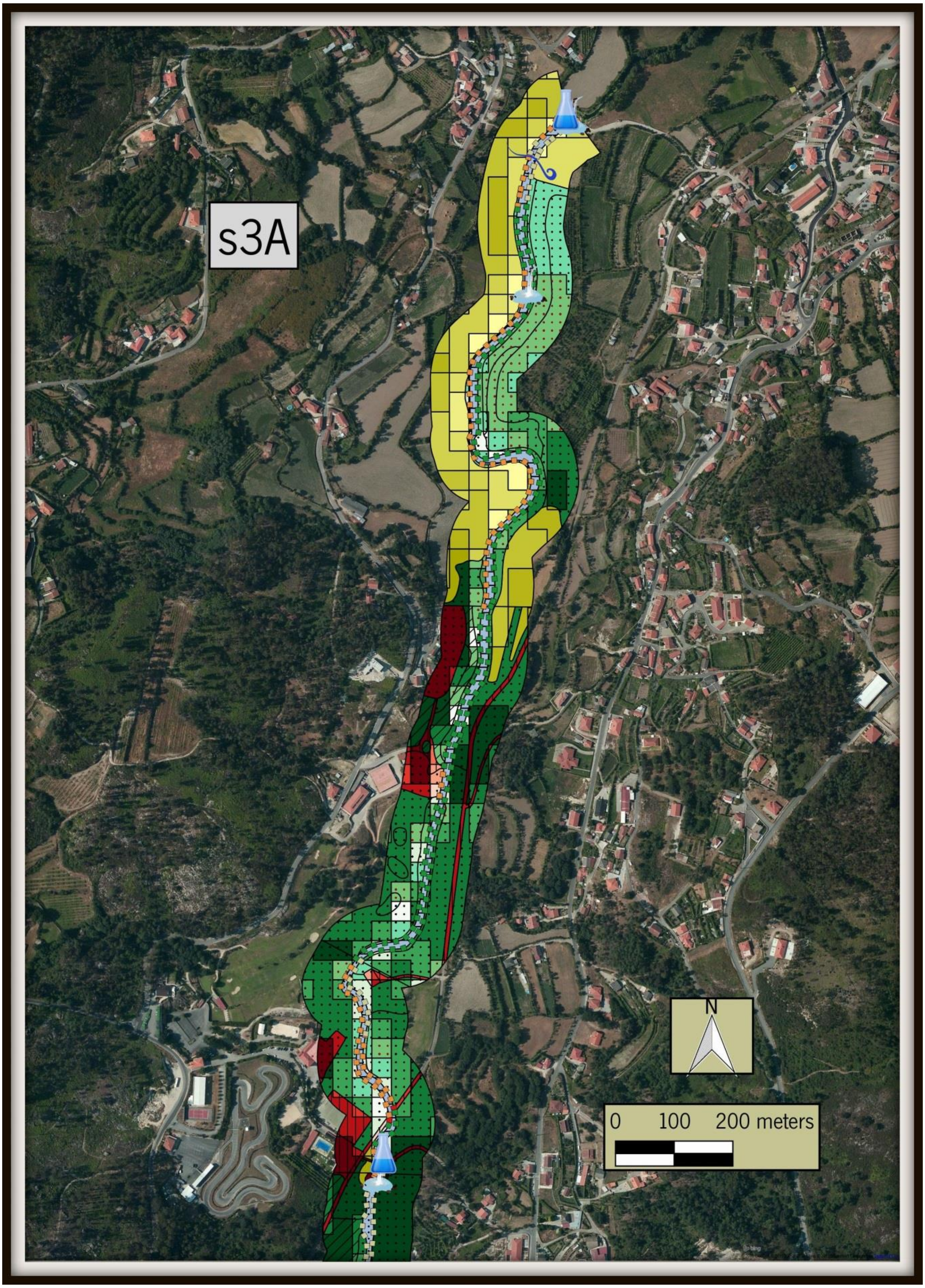

CC
s2N
N
0 100 200 meters

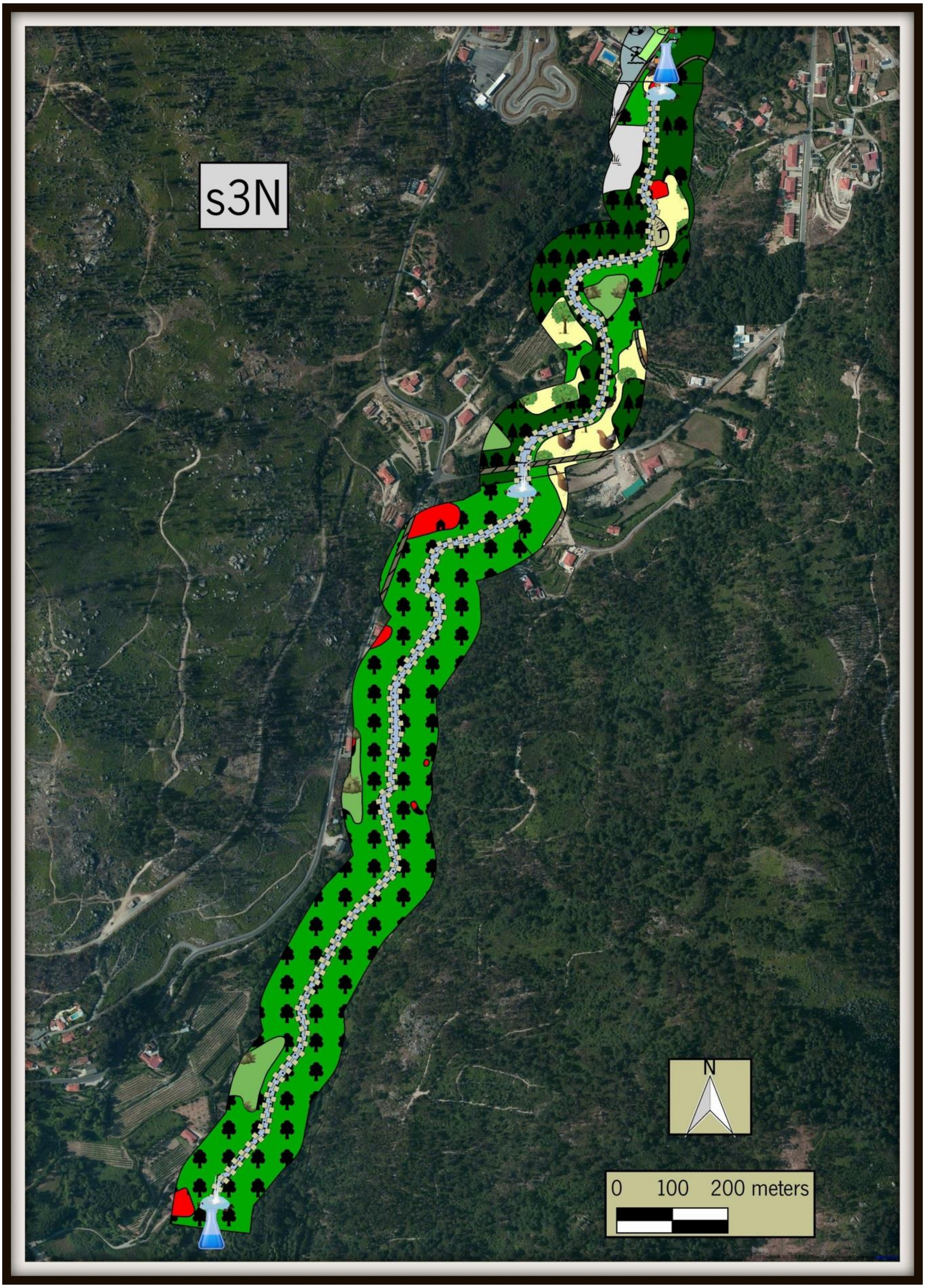

CC
s2N
N
0 100 200 meters

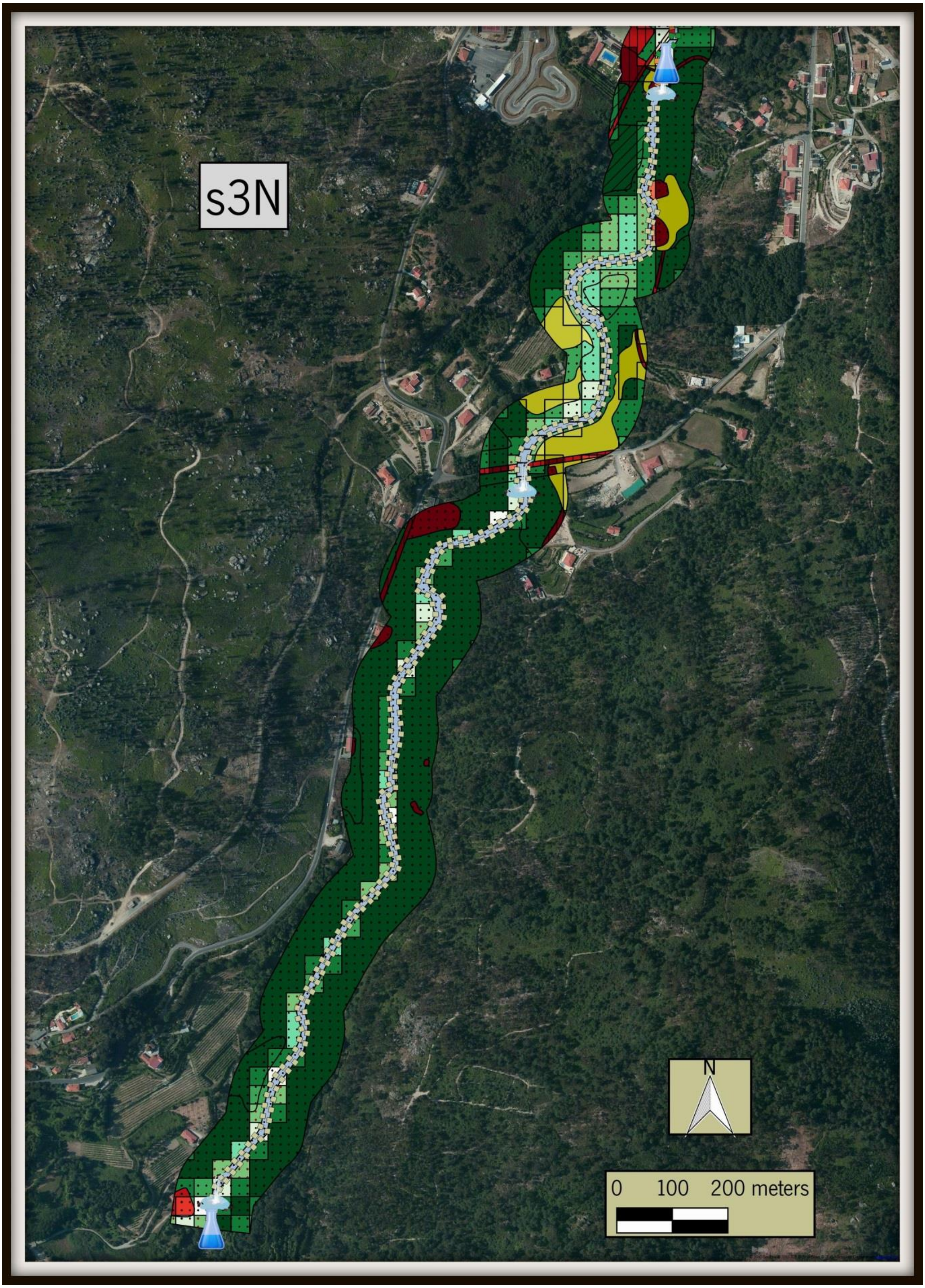

CC
s2N
N
0 100 200 meters

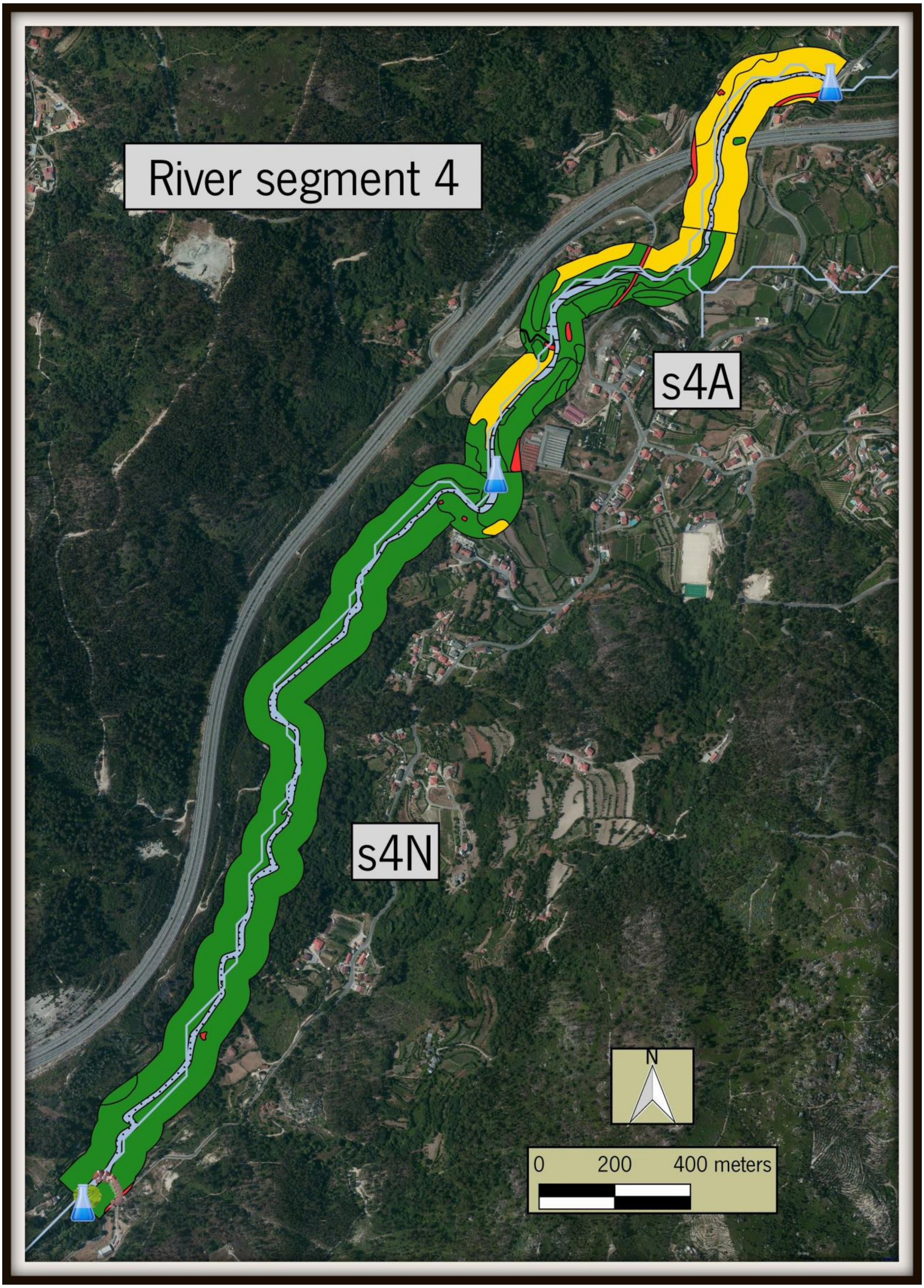

River segment 3
s3A
s3N
N
0 200 400 meters

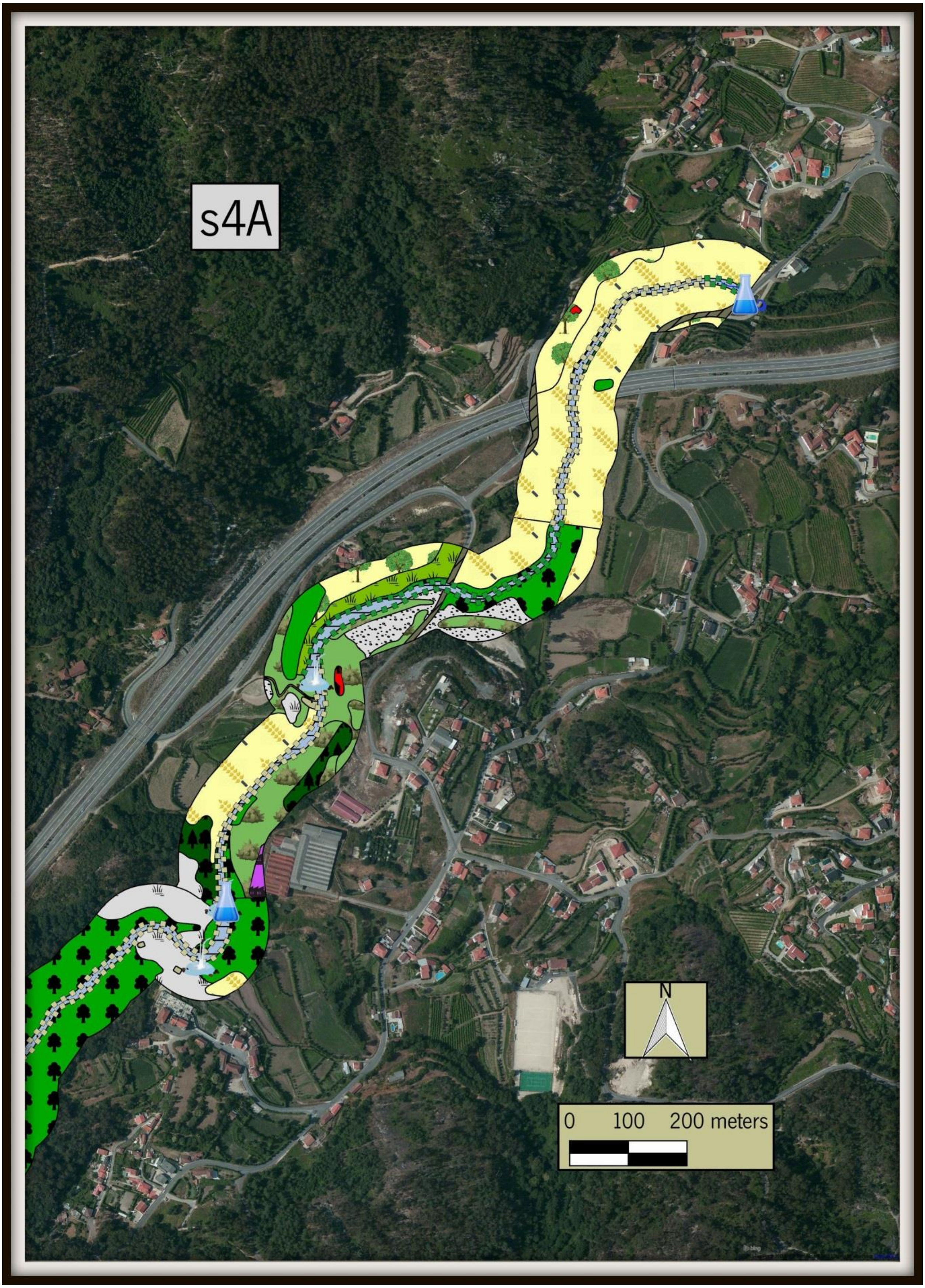

s3A
N
0 100 200 meters

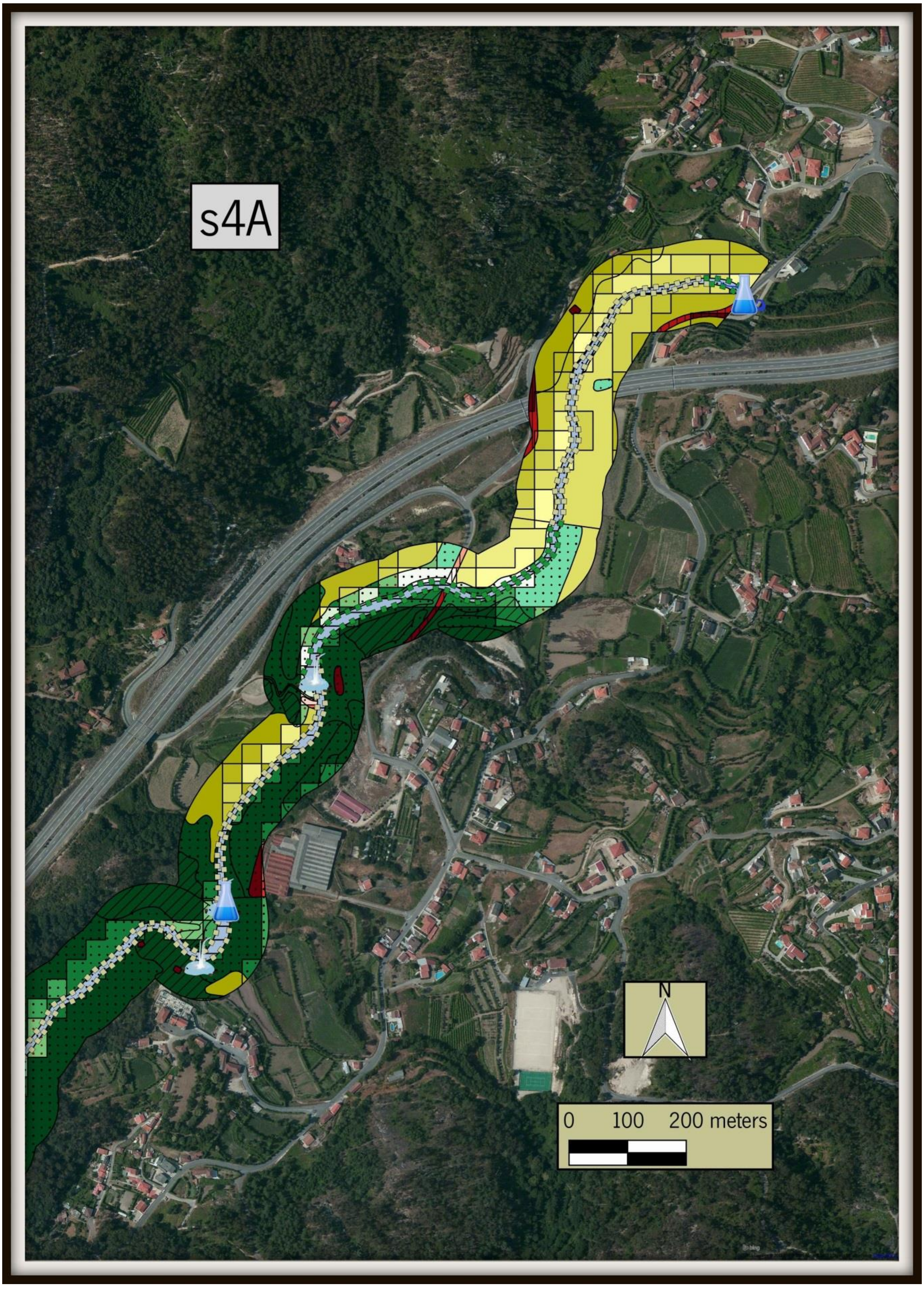

s3A
N
0 100 200 meters

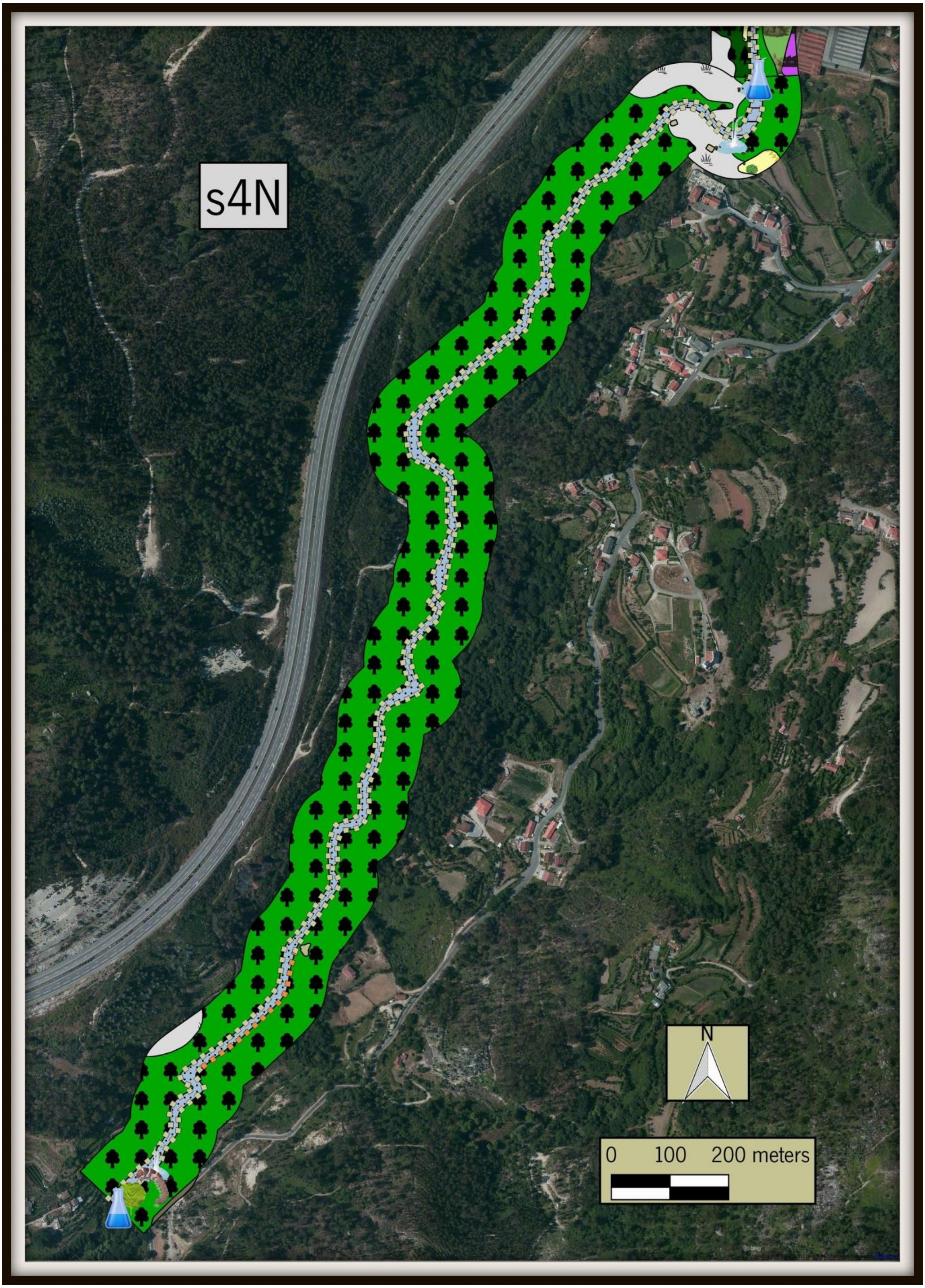

s3N
N
0 100 200 meters

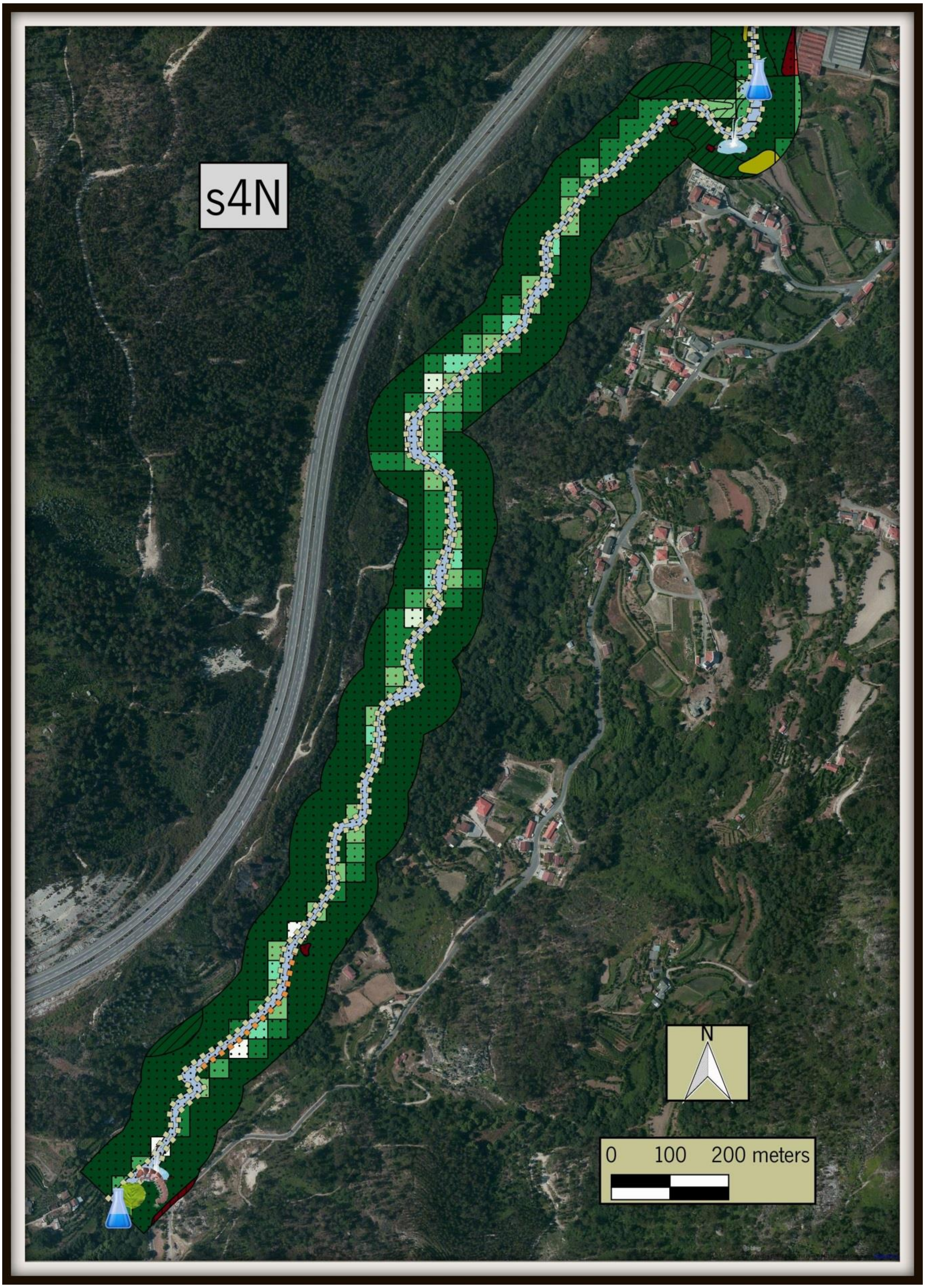

s3N
N
0 100 200 meters

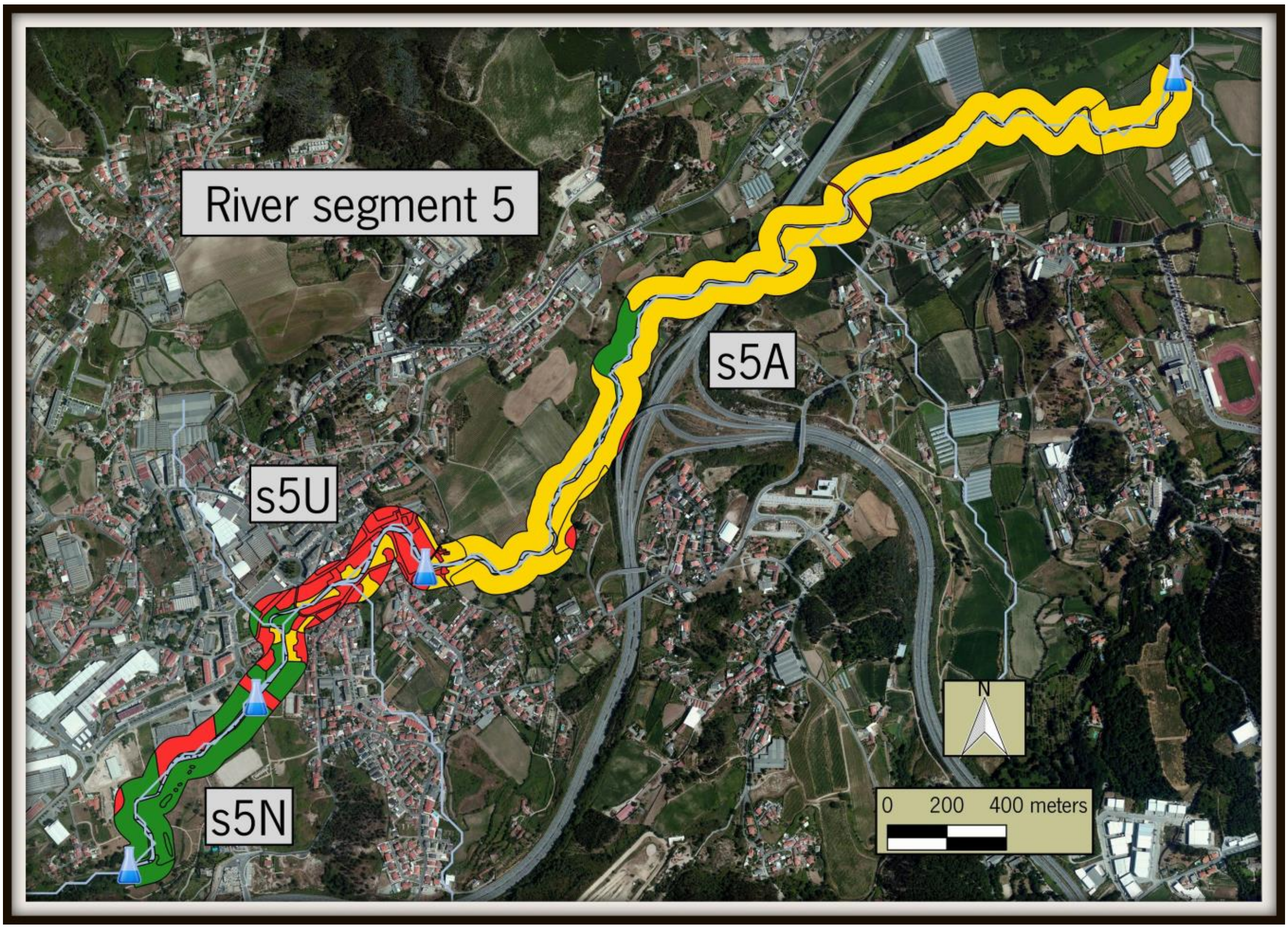

River segment 4
s4A
s4N
N
0
200
400 meters

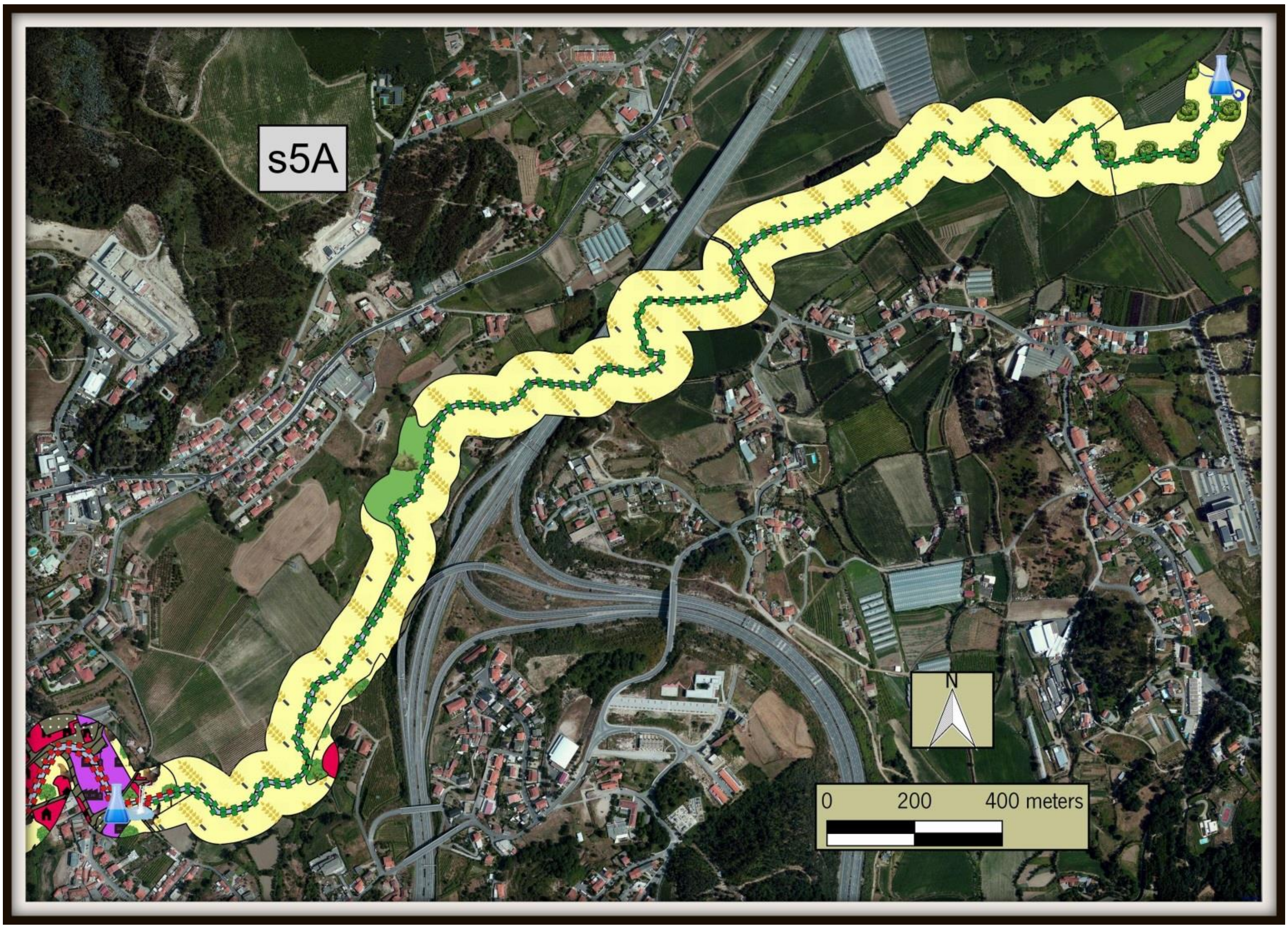

s4A
N
0 100 200 meters

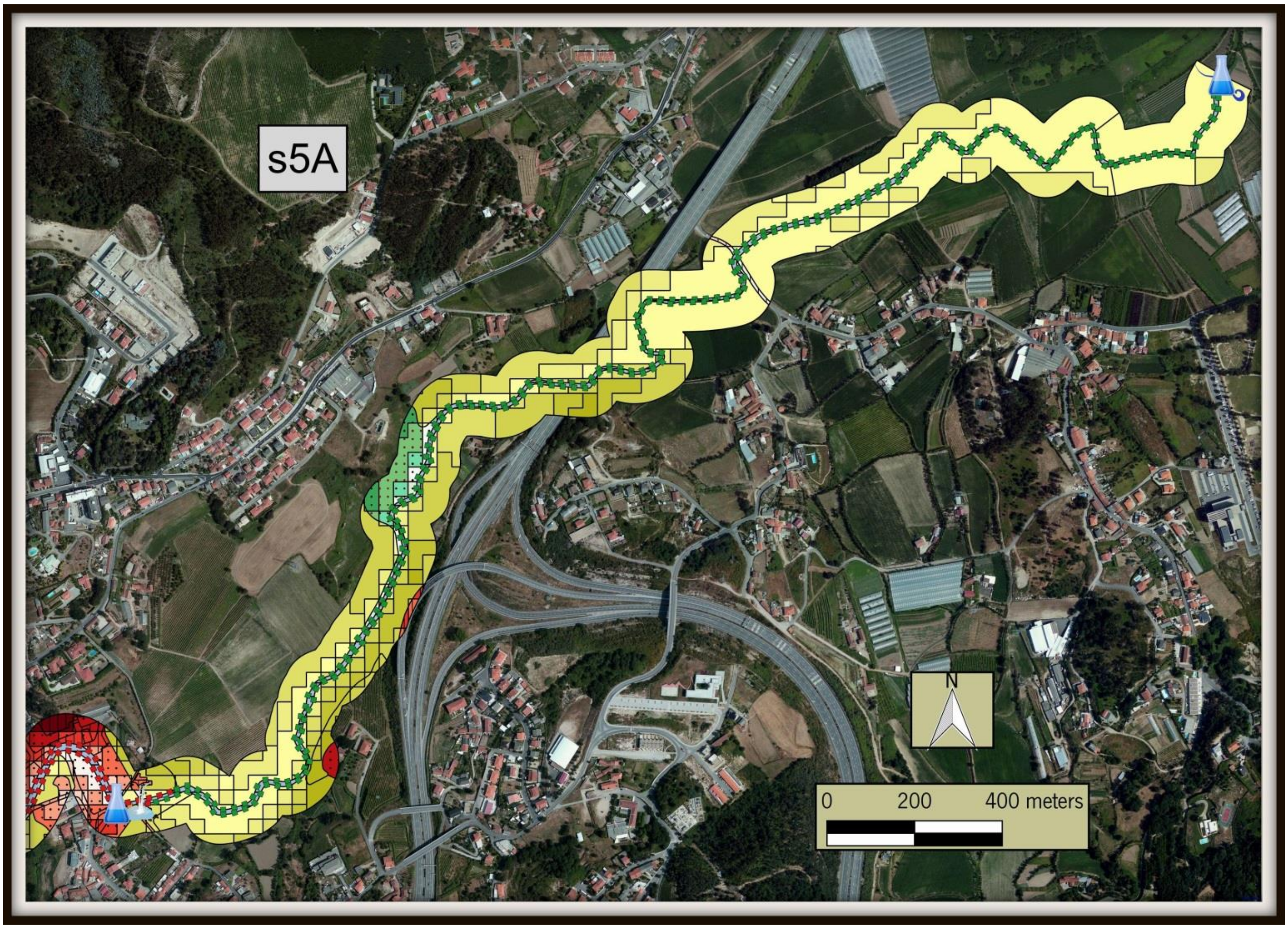

s4A
N
0 100 200 meters

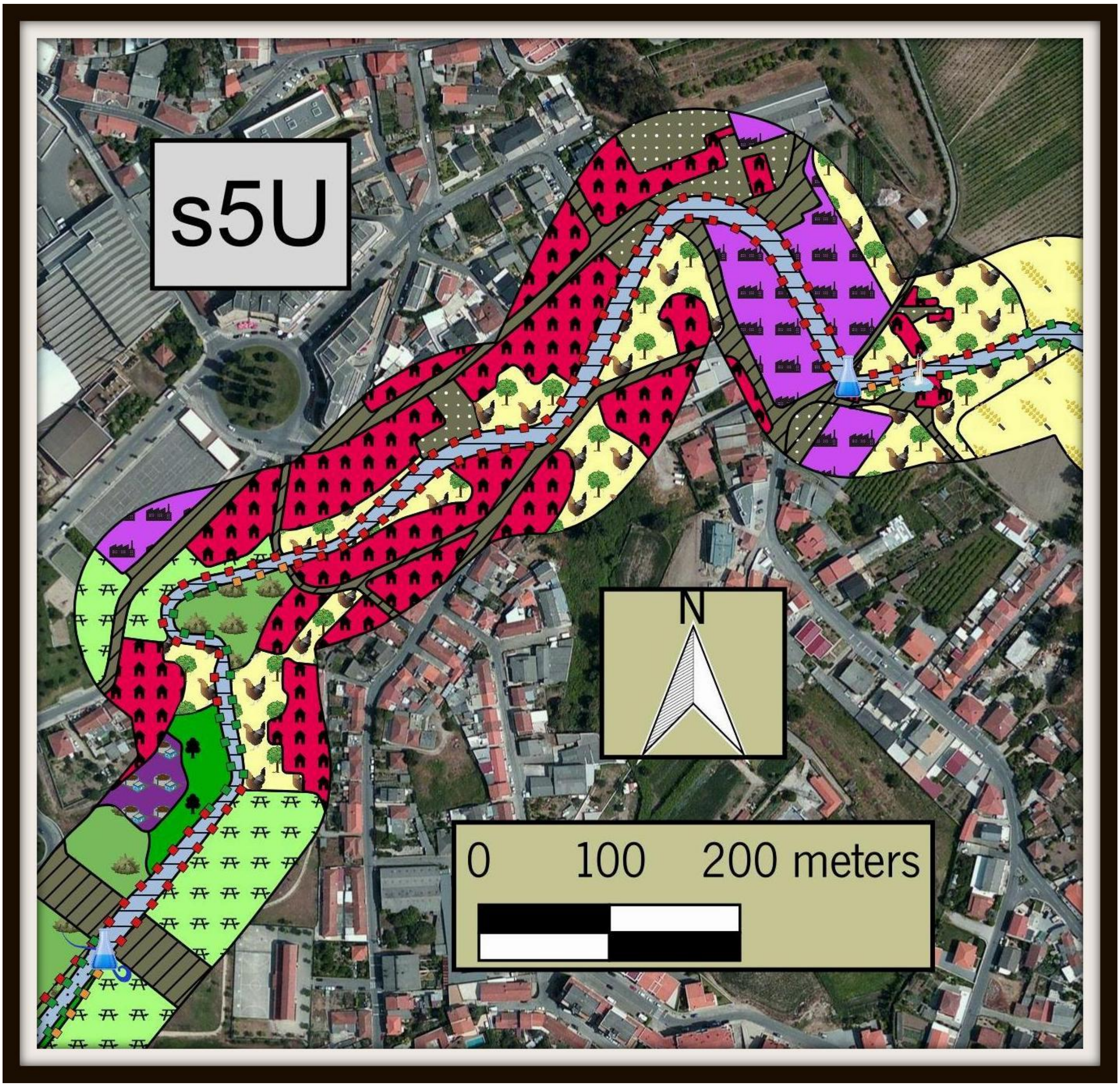

s4N
N
0 100 200 meters

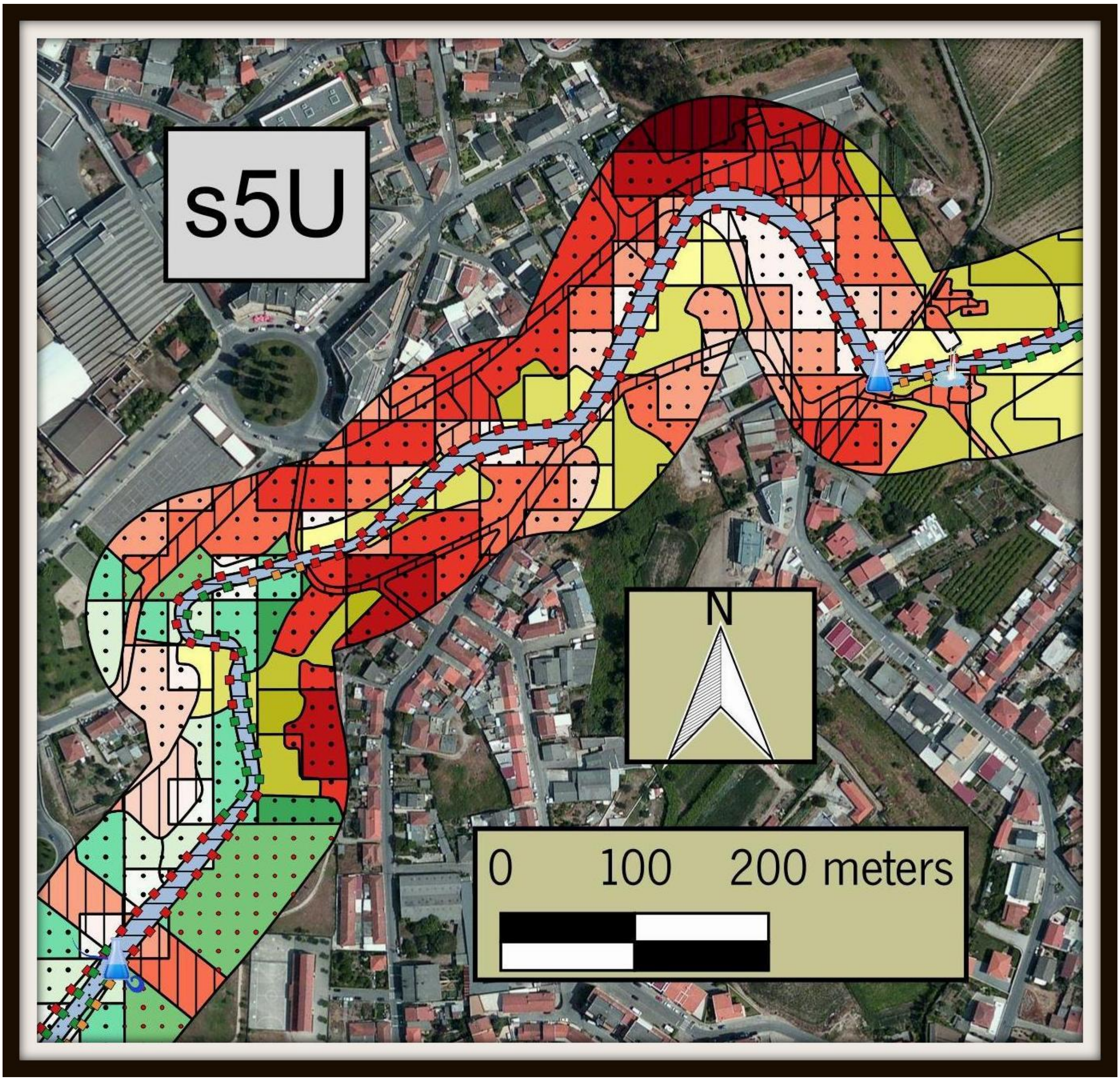

s4N
N
0 100 200 meters

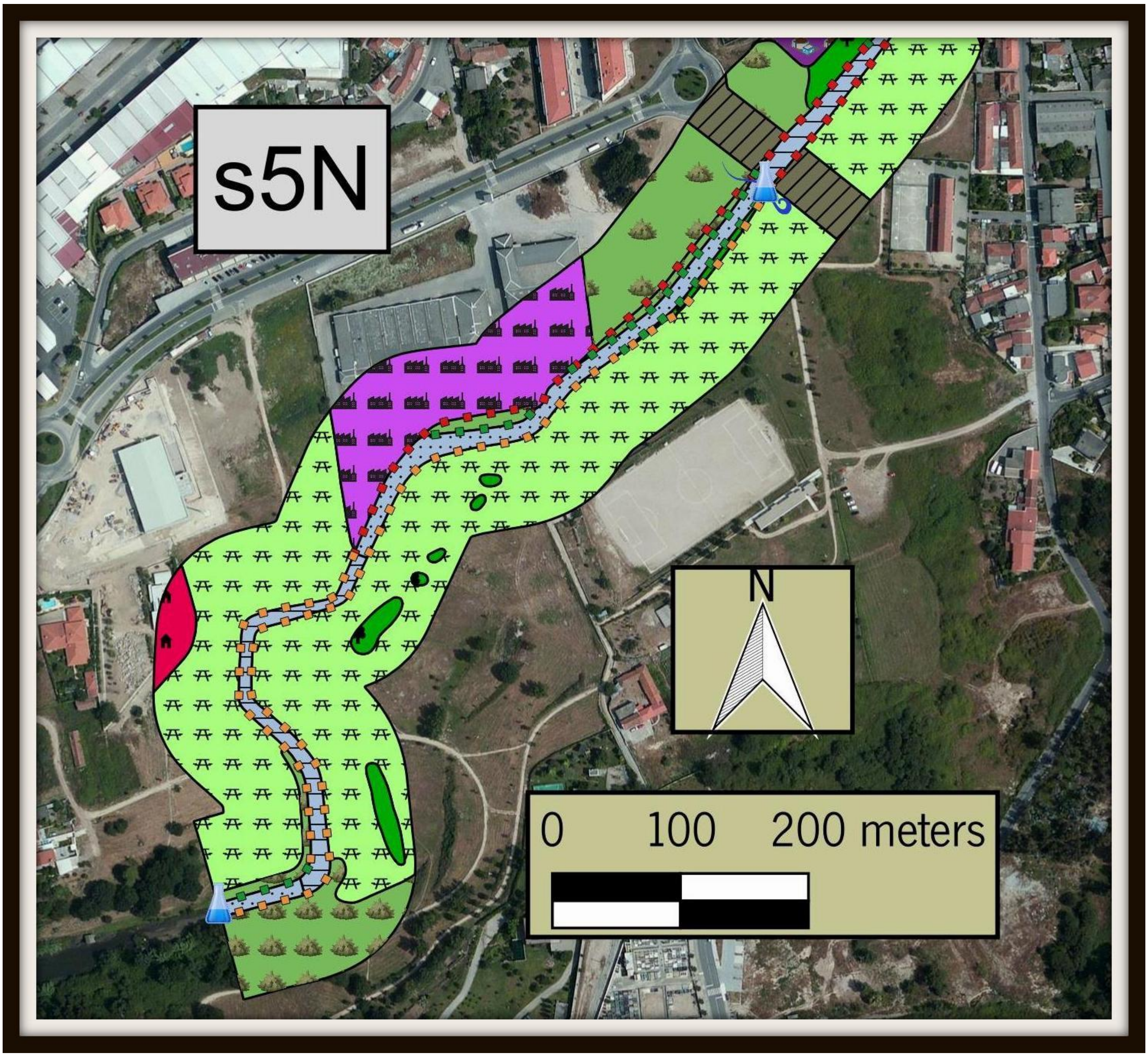

River segment 5
s5A
s5U
s5N
N
0 200 400 meters

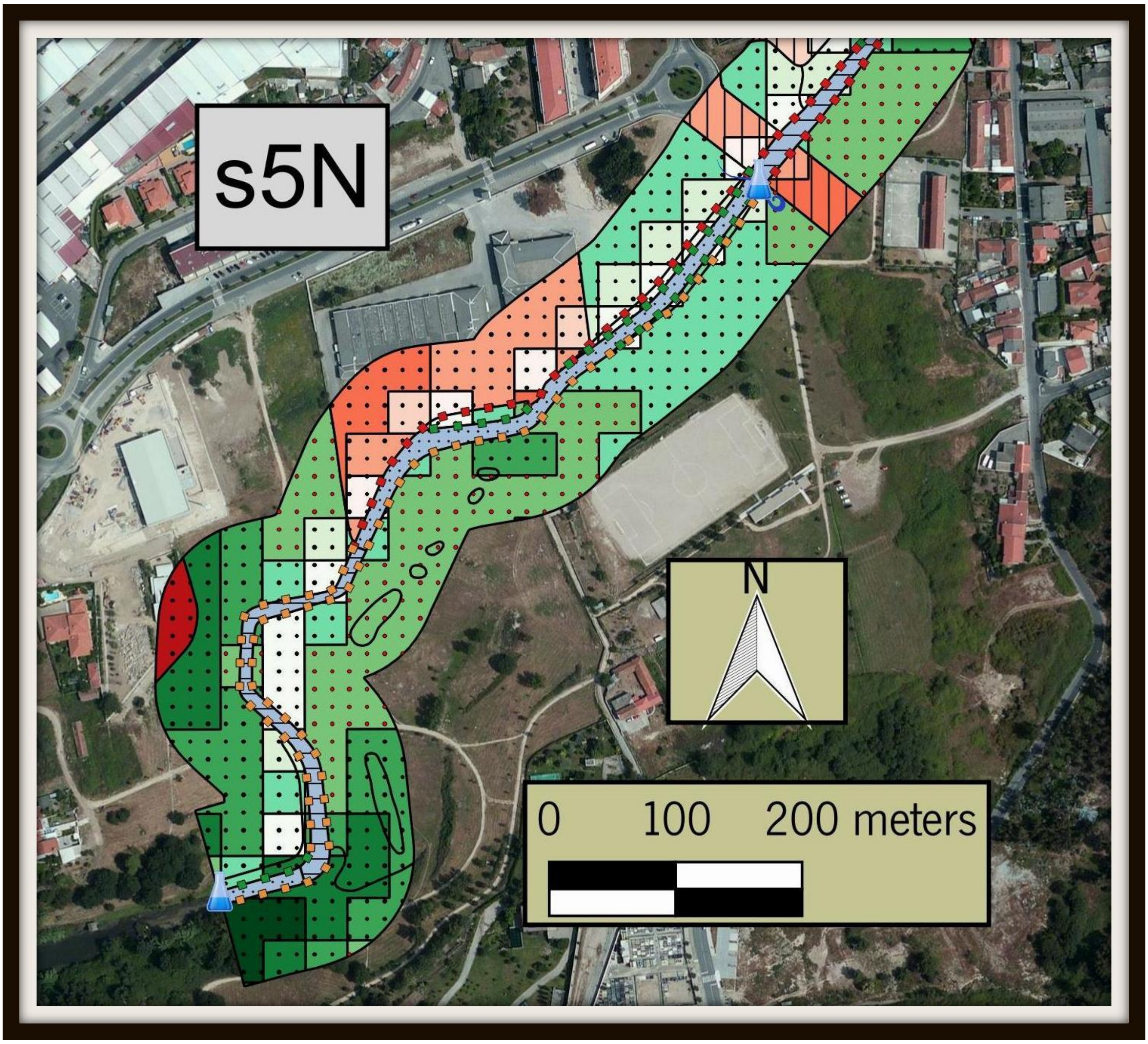

s5A
N
0
200
400 meters

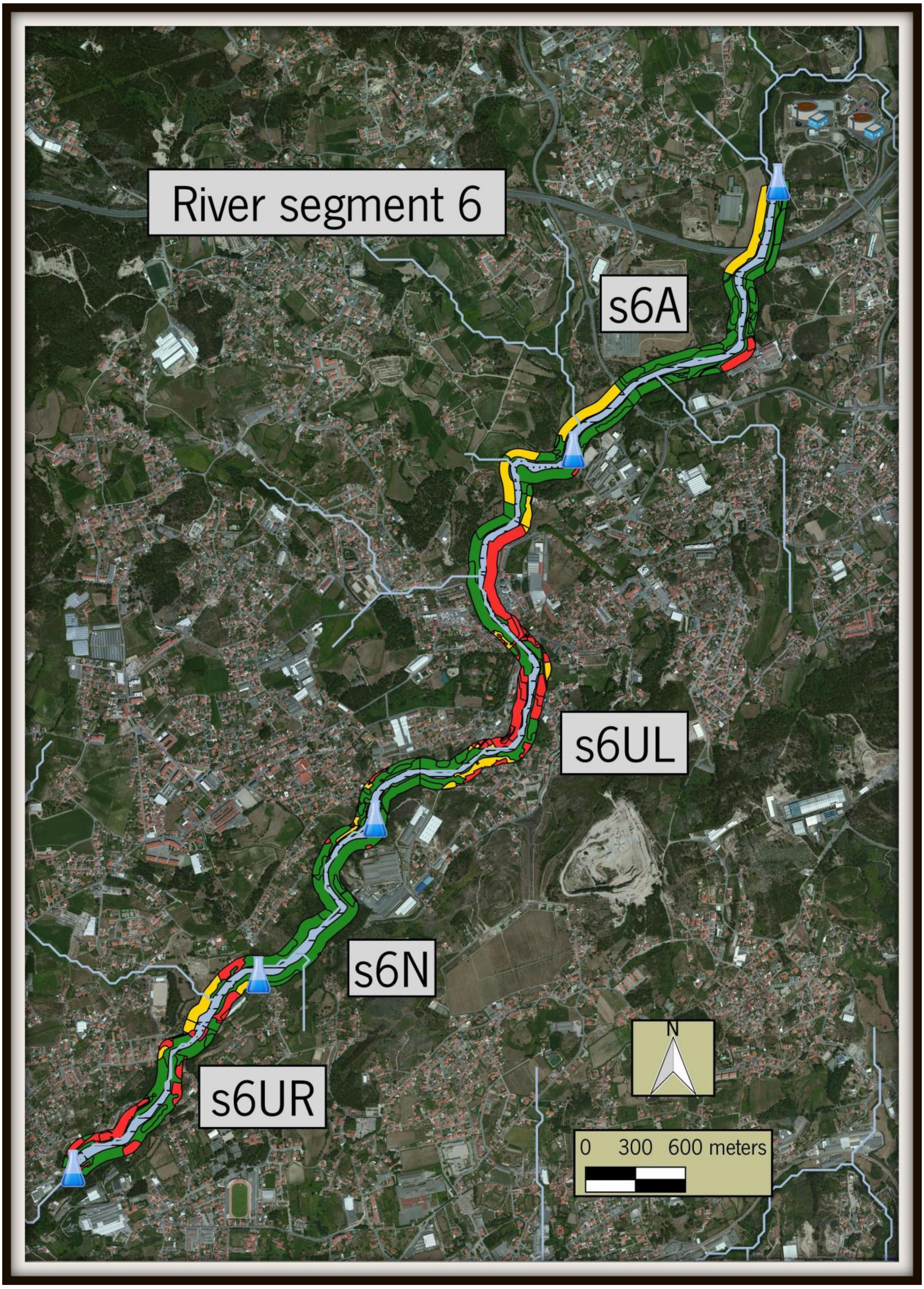

s5A
N
0 200 400 meters

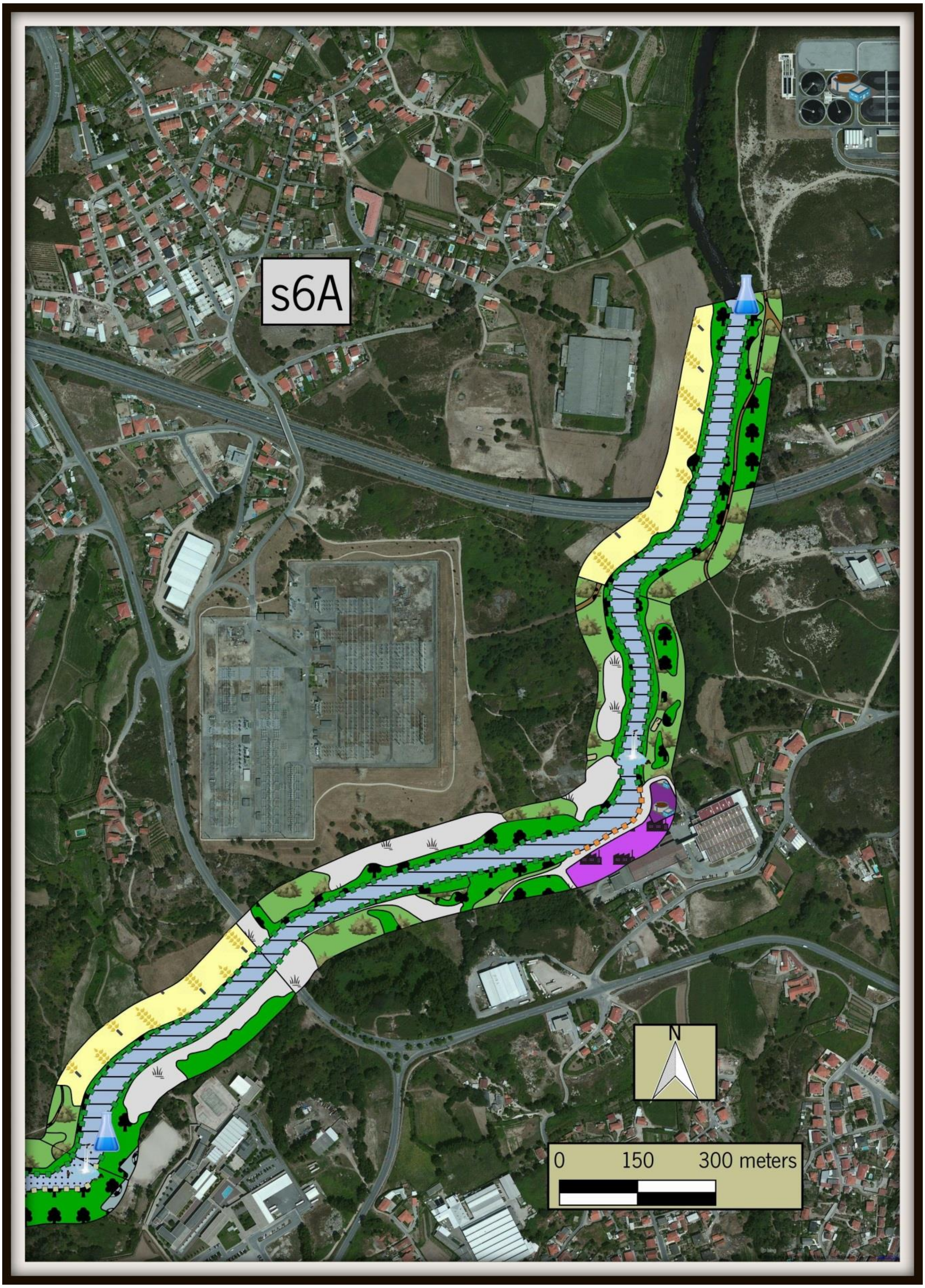

s5U
N
0 100 200 meters


s5U
N
0 100 200 meters

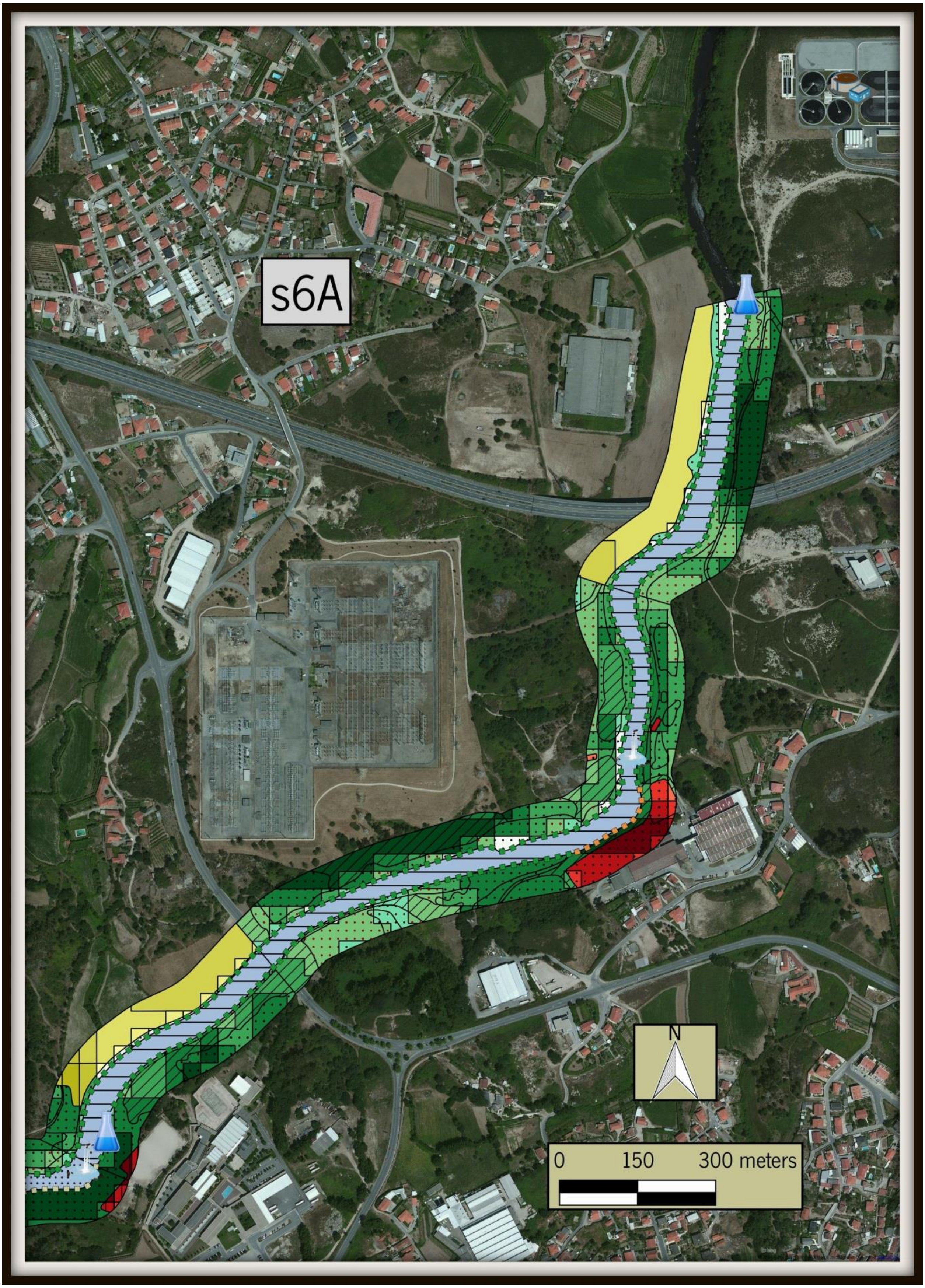

s5N
N
0 100 200 meters

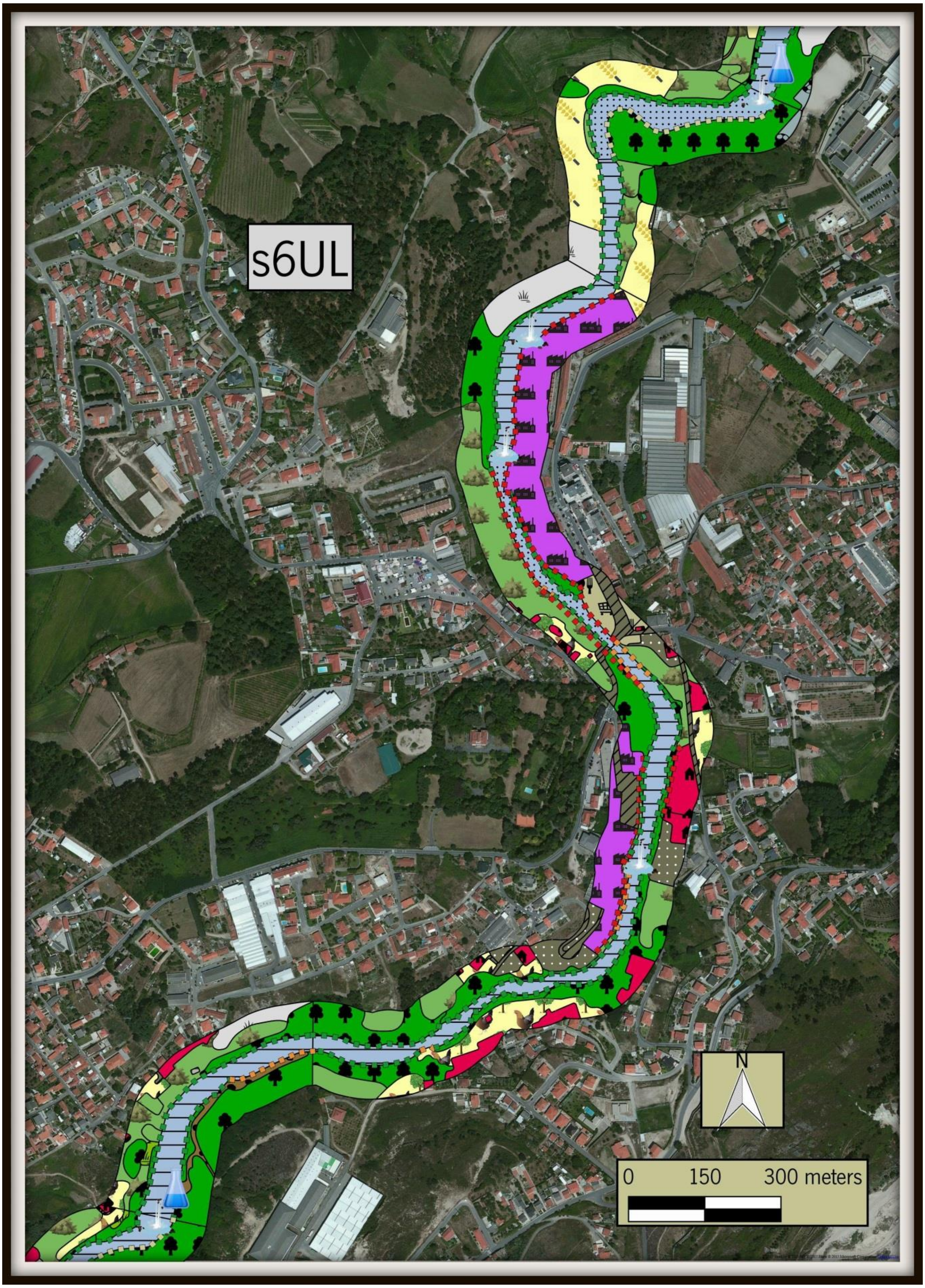

s5N
N
0 100 200 meters


River segment 6
s6A
s6UL
s6N
s6UR
N
0 300 600 meters



s6A
N
0
150
300 meters


s6A
N
0
150
300 meters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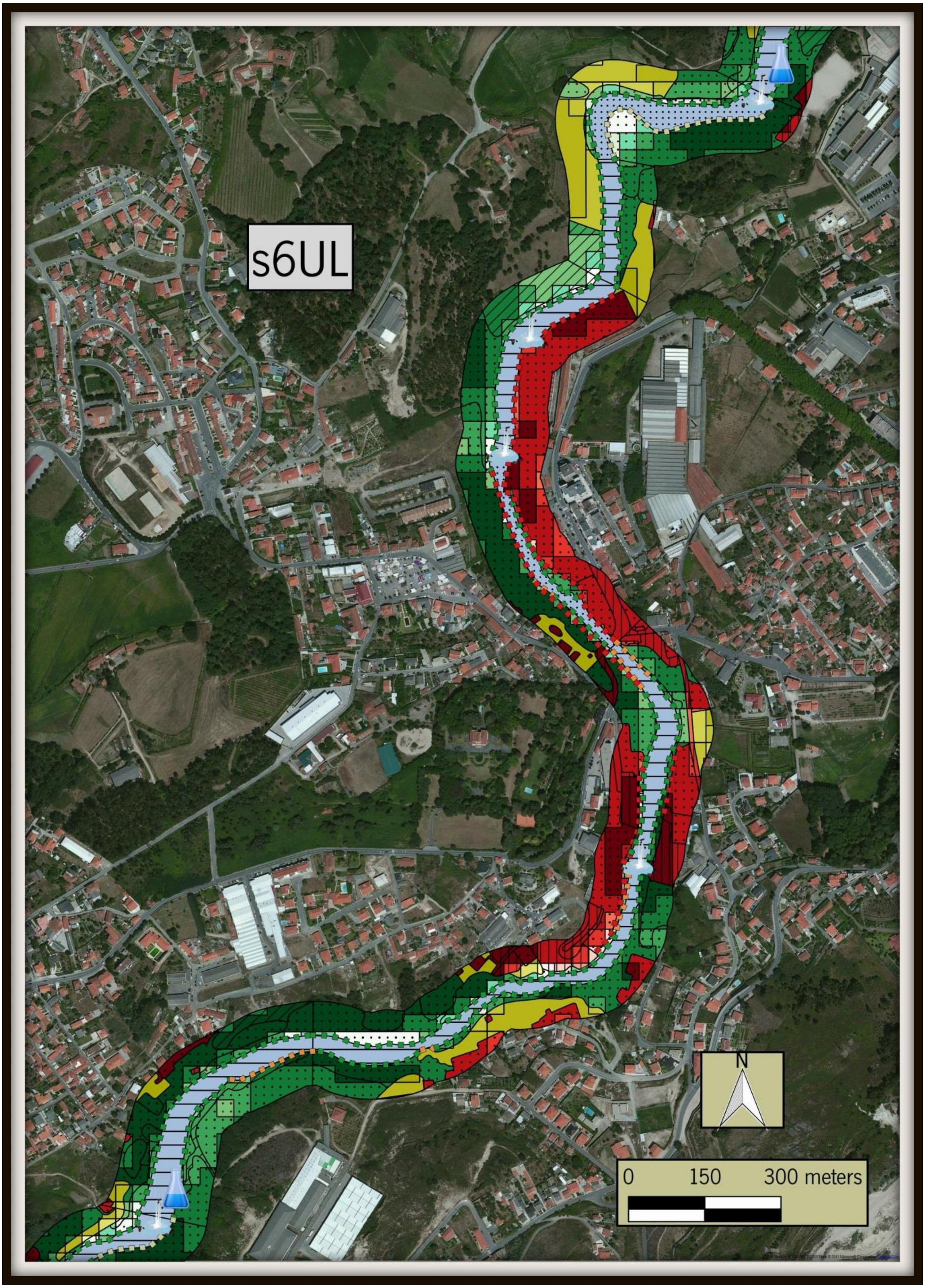
300 meters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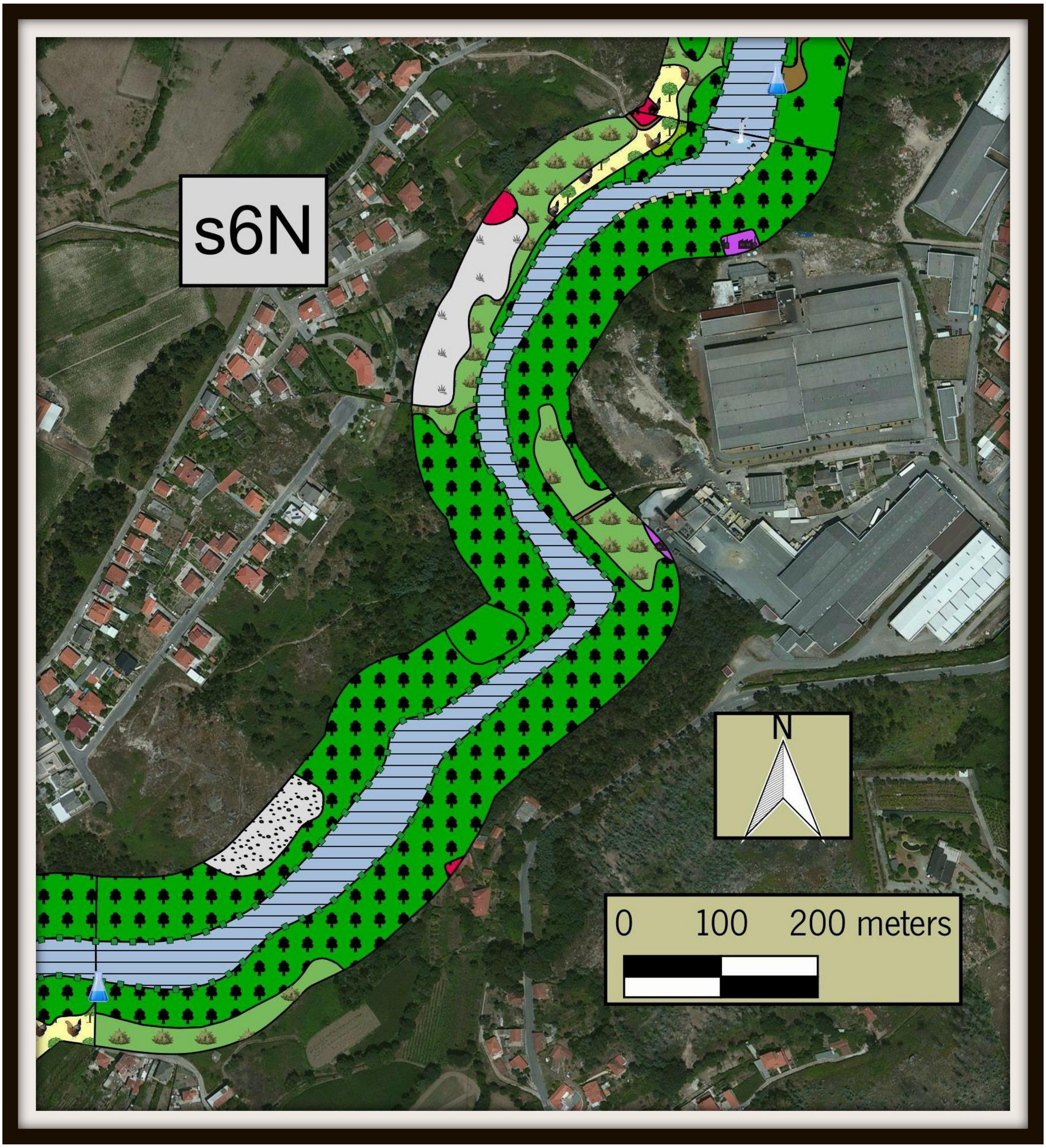
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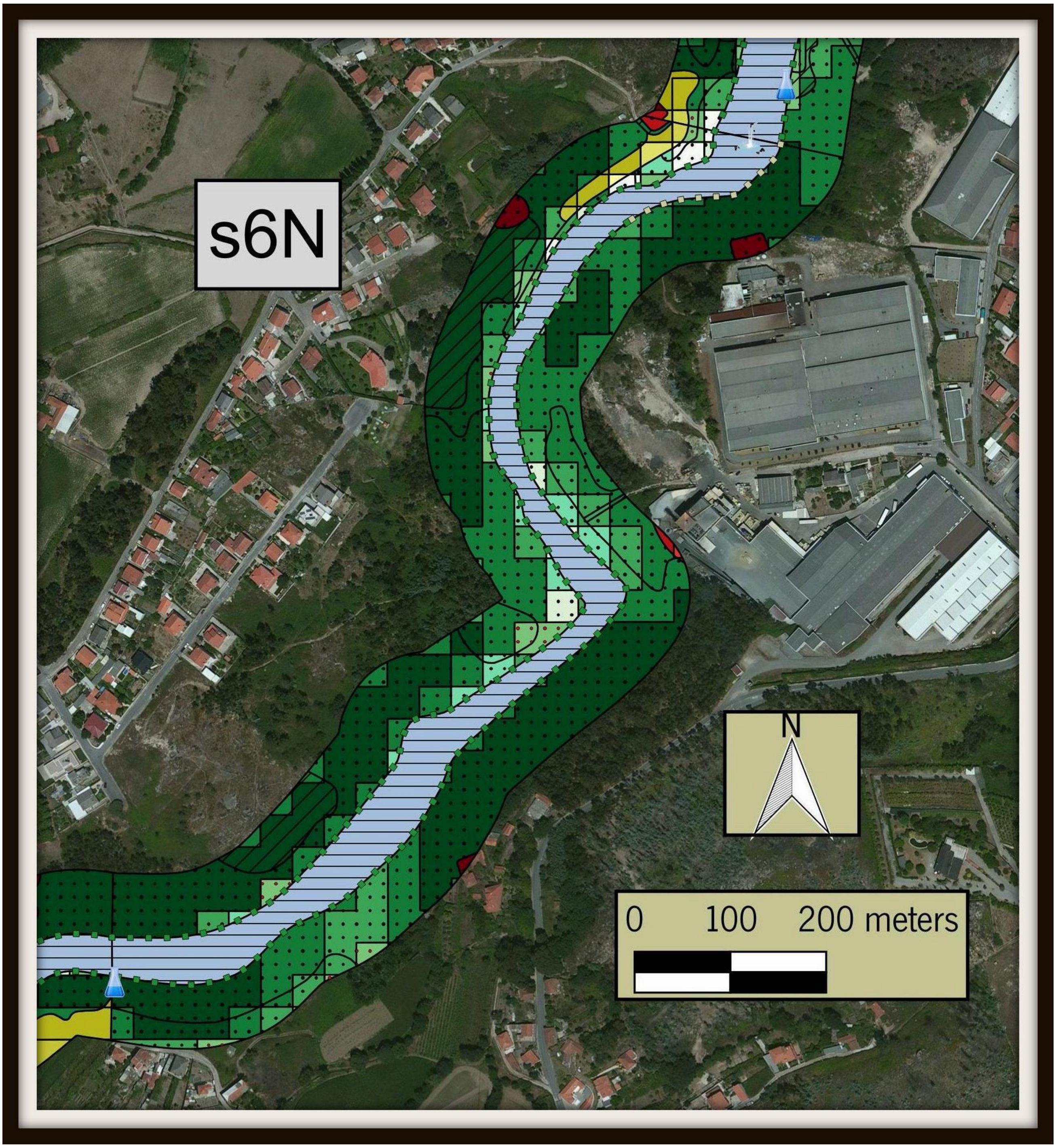
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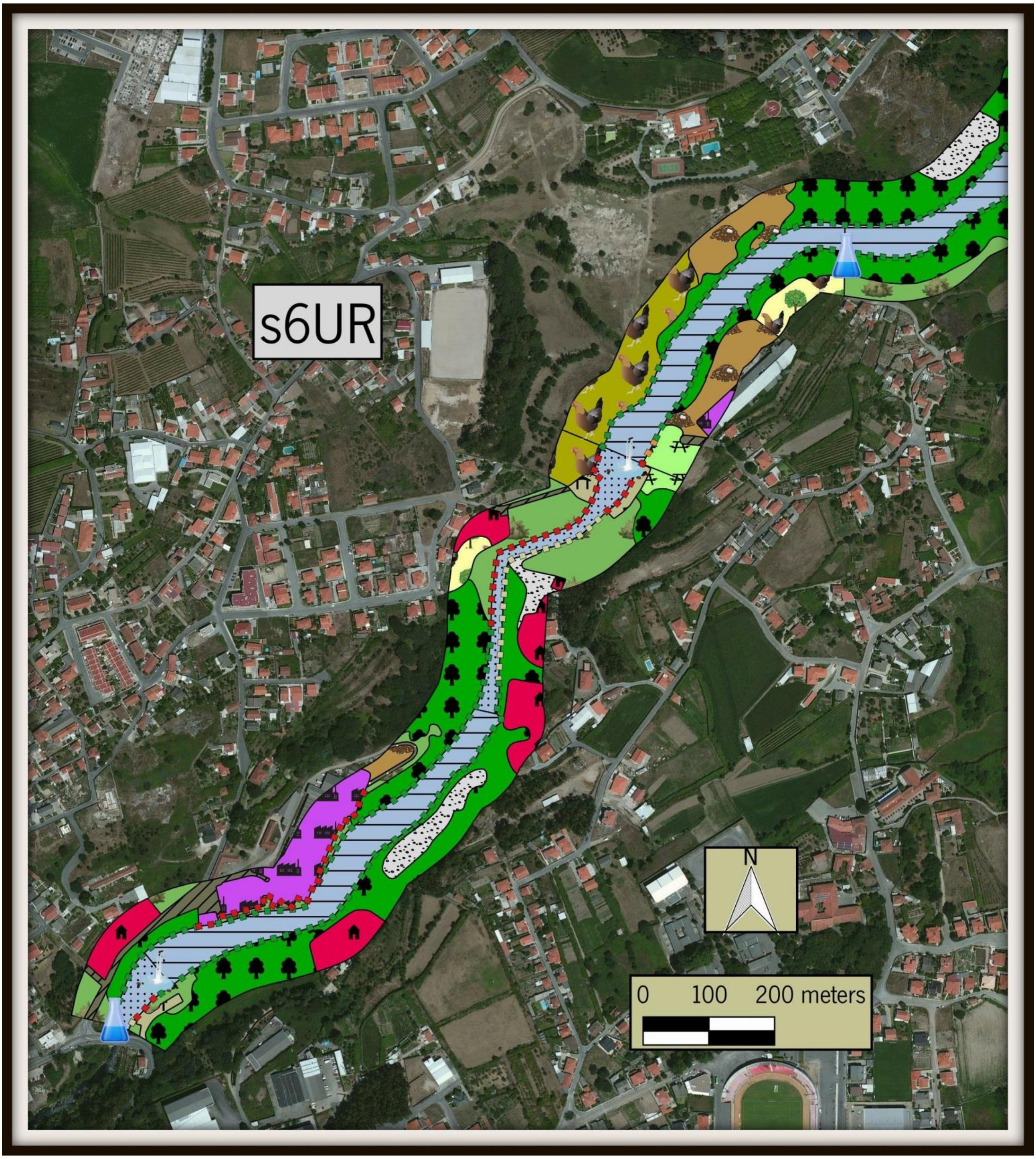
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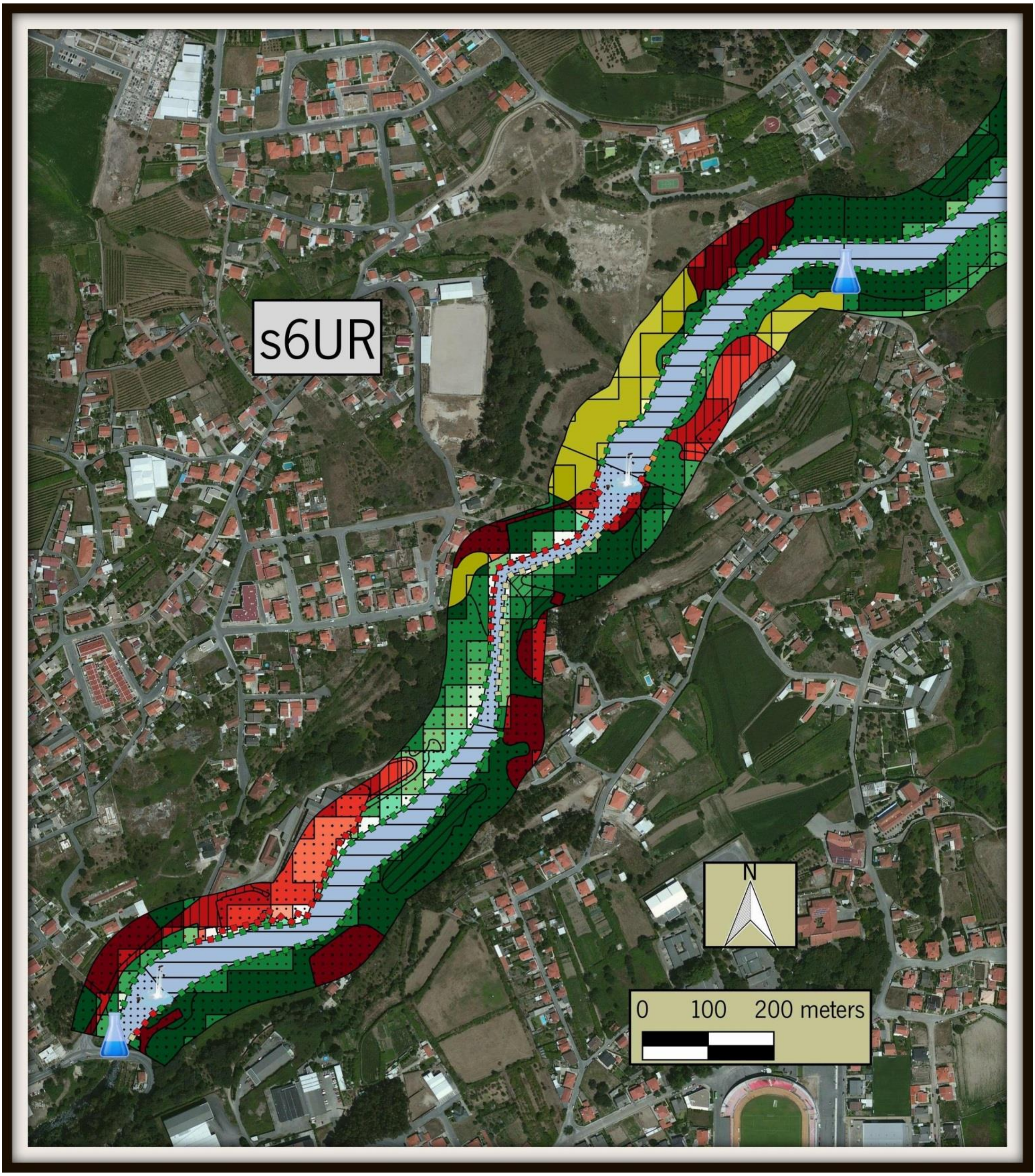
0 100 200 meters


s6UR
N
0 100 200 meters

Appendix 3. River margins classification

Broad margins

River margins without constructions and low slope, which allow river banks to be easy flooded, a good interaction with terrestrial ecosystems, and avoids great impacts on river ecosystems during high floods.

V-shape and deep valleys

Rivers without constructions in the margins but flowing in a deep or natural V-shape valleys, that limits the interaction with terrestrial ecosystems and causes great impacts on river ecosystems during high floods.

Constructed rocky margins

River margins with constructed rocky barriers that limit river channel width and causes great impacts on river ecosystems during high floods, but not totally sealed and allowing a better interaction with terrestrial ecosystems than impervious margins.

Constructed impervious margins

River margins heavily modified by constructed impervious barriers (generally concrete barriers) that limit river channel width and causes great impacts on river ecosystems during high floods.

Appendix 4. Land cover classes and classification framework

Level 1	Level 2	Level 3	Level 4	Level 5
1. Artificial areas	1.1. Buildings	1.1.1. Residential buildings	1.1.1.1. Residential buildings in highly urbanized areas	1.1.1.1.1. Continuous residential buildings in highly urbanized areas
				1.1.1.1.2. Discontinuous residential buildings in highly urbanized areas
			1.1.1.2. Residential buildings in rural areas	1.1.1.2.1. Continuous residential buildings in rural areas
				1.1.1.2.2. Discontinuous residential buildings in rural areas
		1.1.2. Laboral buildings	1.1.2.1. High potential pollutant activities	1.1.2.1.1. Industrial units
				1.1.2.1.2. Wastewater treatment facilities
			1.1.2.2. Moderate potential pollutant activities	1.1.2.2.1. Agricultural facilities
				1.1.2.2.2. Comercial and touristic facilities
		1.1.3. Uninhabited buildings	1.1.3.1. Abandoned buildings	1.1.3.1.1. Abandoned buildings
			1.1.3.2. Buildings with a seasonal use	1.1.3.2.1. Buildings with a seasonal use
	1.2. Artificial surfaces	1.2.1. Highly impervious surfaces	1.2.1.1. Highly impervious surfaces	1.2.1.1.1. Highly impervious surfaces
		1.2.2. Moderately impervious surfaces	1.2.2.1. Dump sites	1.2.2.1.1. Dump sites
			1.2.2.2. Recreational areas	1.2.2.2.1. Sport fields
				1.2.2.2.2. Other recreational areas
			1.2.2.3. Other moderately impervious surfaces	1.2.2.3.1. Other moderately impervious surfaces
2. Agricultural areas	2.1. Horticulture	2.1.1. Arable lands	2.1.1.1. Grain and forage crops	2.1.1.1.1. Grain and forage crops
			2.1.1.2. Vegetable crops	2.1.1.2.1. Vegetable crops
		2.1.2. Permanent crops	2.1.2.1. Orchards and vineyards	2.1.2.1.1. Orchards and vineyards
		2.1.3. Heterogeneous agricultural areas	2.1.3.1. Arable lands with permanent crops	2.1.3.1.1. Arable lands with permanent crops
			2.1.3.2. Complex cultivation patterns	2.1.3.2.1. Complex cultivation patterns
	2.2. Livestock	2.2.1. Livestock	2.2.1.1. Livestock	2.2.1.1.1. Livestock
3. Natural and vegetated areas	3.1. Wooded areas	3.1.1. Dense wooded areas	3.1.1.1. Dense mixture of broad-leaved species	3.1.1.1.1. Dense mixture of broad-leaved species
			3.1.1.2. Dense mixture of broad-leaved with coniferous species	3.1.1.2.1. Dense mixture of broad-leaved with coniferous species
		3.1.2. Sparse wooded areas	3.1.2.1. Sparse mixture of broad-leaved species	3.1.2.1.1. Sparse mixture of broad-leaved species
			3.1.2.2. Sparse mixture of broad-leaved with coniferous species	3.1.2.2.1. Sparse mixture of broad-leaved with coniferous species
	3.2. Shrubs and herbaceous vegetation	3.2.1. Herbaceous vegetation	3.2.1.1. Herbaceous vegetation	3.2.1.1.1. Herbaceous vegetation
		3.2.2. Shrubs	3.2.2.1. Dense shrubs	3.2.2.1.1. Dense shrubs
			3.2.2.2. Sparse shrubs	3.2.2.2.1. Sparse shrubs
		3.2.3. Green urban areas	3.2.3.1. Leisure parks	3.2.3.1.1. Leisure parks
			3.2.3.2. Golf courses	3.2.3.2.1. Golf courses
	3.3. Open spaces with little or no vegetation	3.3.1. Sparse vegetated areas	3.3.1.1. Sparse vegetated areas	3.3.1.1.1. Sparse vegetated areas
		3.3.2. Bare soils	3.3.2.1. Bare rocks	3.3.2.1.1. Bare rocks
			3.3.2.2. Dirt paths	3.3.2.2.1. Dirt paths

1. Artificial areas

Land covers related to human activities such as buildings, paved roads, dump sites or recreational facilities.

1.1. Buildings

Areas covered by buildings regardless the purpose. It includes areas of residential, industrial, commercial and uninhabited buildings.

Areas causing more evident changes on the landscapes than artificialized surfaces due to height constructions, which may differently affect rivers by creating shade and not facilitating surface runoff.

1.1.1. Residential buildings

Areas covered by buildings intended for habitation. Residential buildings at this study area were most villa type so they had less than three floors.

1.1.1.1 Residential buildings in highly urbanized areas

Areas covered by residential buildings inserted or close to highly urbanized areas. Land cover class intends to distinguish residential buildings in highly urbanized areas from residential buildings in rural areas, since population density and social context can significantly affect the amount of pollutant reaching the rivers. Areas assigned with this land cover class indicates that near exists a significant population density and/or some related anthropogenic activities, that despite with a low impact on river hydromorphology as they lay beyond the buffer zone (50 m), they may still to impact the river ecological condition due to a strong human presence and the existing of plumping systems facilitating sewage discharges.

1.1.1.1.1. Continuous residential buildings in highly urbanized areas

Areas covered by residential buildings in highly urbanized areas and by some impervious surfaces associated to them, such as paved surfaces.

1.1.1.1.2. Discontinuous residential buildings in highly urbanized areas

Areas covered by residential buildings in highly urbanized areas and by less impervious surfaces associated to them, such as gardens or dirt soils. Less impervious surfaces does not include areas dedicated to agricultural activities, which were classified apart with agricultural land cover classes.

1.1.1.2. Residential buildings in rural areas

Areas covered by residential buildings inserted or close to rural areas. Land cover class intends to distinguish residential buildings in rural areas from residential buildings in highly urbanized areas. Differ from land cover class 1.1.1.1 by accounting for residential buildings in areas with lower population density, impervious surfaces and plumbing systems.

1.1.1.2.1. Continuous residential buildings in rural areas

Areas covered by residential buildings in rural areas and some impervious surfaces associated to them, such as paved surfaces.

1.1.1.2.2. Discontinuous residential buildings in rural areas

Areas covered by residential buildings in rural areas and less impervious surfaces associated to them, such as gardens or dirt soils. Less impervious surfaces does not include areas dedicated to agricultural activities, which were classified apart with some agricultural land cover classes.

1.1.2. Laboral buildings

Areas covered by buildings for industrial, commercial or agricultural activities.

1.1.2.1. High potential pollutant activities

Areas covered by buildings for laboral activities more prone to generate and discharge pollutants on rivers such as industrial units or wastewater treatment plants.

1.1.2.1.1. Industrial units

Areas covered by buildings for industrial activities. Most industrial activities at this study area were dedicated to textile production and dyeing.

1.1.2.1.2. Wastewater treatment facilities

Areas covered by buildings for wastewater treatment.

1.1.2.2. Moderate potential pollutant activities

Areas covered by buildings for laboral activities less prone to generate and discharge pollutants on rivers relatively to laboral activities with land cover class 1.1.2.1.

1.1.2.2.1. Agricultural facilities

Areas covered by buildings used to support agricultural activities such that to store crops or agricultural equipment.

1.1.2.2.2. Commercial and touristic facilities

Areas covered by buildings for commercial or touristic activities such as fire stations, post offices, small shops, theaters, coffee shops, restaurants or hotels and associated facilities.

1.1.3. Uninhabited buildings

Areas covered by uninhabited buildings, whether abandoned or seasonally used. Land cover class intends to distinguish buildings that although affecting river hydromorphology are not expected to be associated with pollutant discharges.

1.1.3.1. Abandoned buildings

Areas covered by abandoned buildings with no current use.

1.1.3.1.1. Abandoned buildings

Class propagated from the previous level.

1.1.3.2. Buildings with a seasonal use

Areas covered by buildings with a seasonal use. In this study it refers to a summer hotel that was not opened during the sampling period.

1.1.3.2.1. Buildings with a seasonal use

Class propagated from the previous level.

1.2. Artificial surfaces

Lands changed by humans but not involving height constructions like buildings. It includes paved roads, sidewalks, and areas for waste deposition and for recreational activities with impervious surfaces. Differ from the land cover class 1.1 by referring to modified land cover with impervious surfaces but not height constructions, thus with no effect on solar penetration but more prone to facilitate superficial runoff.

1.2.1. Highly impervious surfaces

Areas highly or totally covered by impervious surfaces such as paved roads, sidewalks or parking zones.

1.2.1.1 Highly impervious surfaces

Class propagated from the previous level.

1.2.1.1.1 Highly impervious surfaces

Class propagated from the previous level.

1.2.2. Moderately impervious surfaces

Areas moderately or not totally covered by impervious surfaces such as dirt paths, areas of waste deposition, construction sites, or intended for recreational activities.

1.2.2.1. Dump sites

Areas used for solid wastes deposition without a control system.

1.2.2.1.1 Dump sites

Class propagated from the previous level.

1.2.2.2. Recreational areas

Areas used for recreational activities, moderately or not totally cover by impervious surfaces.

1.2.2.2.1. Sport fields

Areas referring sport fields with dirt soils.

1.2.2.2.2. Other recreational areas

Areas moderately or not totally covered by impervious surfaces used for other recreational activities not related to sport. In this study it was used to define an area referring a leisure area with a pool from a golf course and associated facilities.

1.2.2.3. Other moderately impervious surfaces

Areas moderately or not totally cover by impervious surfaces not intended to waste deposition or recreational activities such as construction sites, abandoned areas or dirt paths in urbanized areas.

1.2.2.3.1. Other moderately impervious surfaces

Class propagated from the previous level.

2. Agricultural areas

Areas used to agricultural activities.

2.1. Horticulture

Areas used to plant cultivation such as fruits, vegetables, nuts, seeds, herbs, flowers, and non-food crops such as grass and ornamental plants. Rainfed and irrigated crops may differently affect runoff but these were not distinguished, since it also depends on the soil properties and management practices.

2.1.1. Arable lands

Agricultural areas regularly ploughed or tilled under a system of crop rotation with annual vegetative cycles (IFAP, 2010).

2.1.1.1. Grain and forage crops

Agricultural areas of grain crops (cereals and legumes) for human consumption, or forage crops whether sown or spontaneous.

2.1.1.1.1 Grain and forage crops

Class propagated from the previous level.

2.1.1.2. Vegetable crops

Agricultural areas of vegetable crops such as cabbage, carrots or lettuces.

2.1.1.2.1. Vegetable crops

Class propagated from the previous level.

2.1.2. Permanent crops

Agricultural areas not regularly ploughed or tilled under a system of crop rotation, with crops providing repeated harvests without being replanted (IFAP, 2010). Arable lands and permanent crops were distinguished by having different plant species and cultivation practices.

2.1.2.1. Orchards and vineyards

Agricultural areas of orchards and vineyards. In this study, areas assigned with this land cover class had a prevalence of vineyards over orchards.

2.1.2.1.1. Orchards and vineyards

Class propagated from the previous level.

2.1.3. Heterogeneous agricultural areas

Agricultural areas with a mixture of arable lands and permanent crops.

2.1.3.1. Arable lands with permanent crops

Agricultural areas dominated by arable lands but including some permanent crops. In this study, most of the areas assigned with this land cover class had a prevalence of arable lands bordered by vineyards.

2.1.3.1.1. Arable lands with permanent crops

Class propagated from the previous level

2.1.3.2. Complex cultivation patterns

Agricultural areas with diverse combinations of arable lands and permanent crops. Areas intended to produce a variety of vegetables and fruit to self-consumption, mostly backyards of habitations.

2.1.3.2.1. Complex cultivation patterns

Class propagated from the previous level.

2.2. Livestock

Areas used to raise domesticated animals, most to food production. In this study, most of the areas assigned with this land cover class were used to raise chicken, but some others also contained horses and sheep.

2.2.1 Livestock

Class propagated from the previous level.

2.2.1.1 Livestock

Class propagated from the previous level.

2.2.1.1.1 Livestock

Class propagated from the previous level.

3. Natural and vegetated areas

Areas covered by natural or planted vegetation. It includes natural areas of trees, shrubs, herbaceous vegetation and bare soils but also arboreal rows in agricultural and urban areas and human land uses covered by planted vegetation, such as golf courses and leisure parks if with no signs of impervious surfaces.

3.1. Wooded areas

Areas covered by forest trees (IFN, 2009, ICNF, 2013b) both natural and planted. Forest trees coverage refers to the canopy coverage based on satellite images and field observations. Land cover intends to distinguish trees from other plant types such as shrubs and herbaceous vegetation since trees can differently affected river ecosystems by having larger root systems and wider canopies.

3.1.1. Dense wooded areas

Areas covered in more than 75% by forest trees. It includes areas with a dense coverage of forest trees (>75%) in forest landscapes or close to human land uses such as roads or habitations, if not in heavily urbanized areas and if the soils beneath the trees canopy are not used for humans activities. It thus refers to areas that even close to human land uses maintain good pristine conditions.

3.1.1.1. Dense mixture of broad-leaved species

Dense wooded areas dominated by broad-leaved species (>75% of the forest trees).

3.1.1.1.1 Dense mixture of broad-leaved species

Class propagated from the previous level.

3.1.1.2. Dense mixture of broad-leaved with coniferous species

Dense wooded areas dominated by broad-leaved species (>50% of the forest trees) but also with coniferous species (15%<x<50% of the forest trees).

3.1.1.2.1. Dense mixture of broad-leaved with coniferous species

Class propagated from the previous level.

3.1.2. Sparse wooded areas

Areas covered in less than 75% by forest trees or in more than 75% if the soils beneath the trees canopy are used for human activities or if in urban and agricultural land uses, such as dense clusters of forest trees in golf courses, leisure parks, and bordering agricultural fields. Land cover intends to distinguish areas with a sparser coverage of forest trees relatively to that assigned to the land cover class 3.1.1, or with a similar coverage of forest trees but with a strong human presence that somehow limits their functionality.

3.1.2.1. Sparse mixture of broad-leaved species

Sparse wooded areas or dense wooded areas in human land uses dominated by broad-leaved species (>75% of the forest trees).

3.1.2.1.1. Sparse mixture of broad-leaved species

Class propagated from the previous level.

3.1.2.2. Sparse mixture of broad-leaved with coniferous species

Sparse wooded areas or dense wooded areas in human land uses dominated by broad-leaved species (>50% of the forest trees) but also with coniferous species (15%<x<50% of the forest trees).

3.1.2.2.1. Sparse mixture of broad-leaved with coniferous species

Class propagated from the previous level.

3.2. Shrubs and herbaceous vegetation

Areas covered by shrubs and herbaceous vegetation. Land cover intends to distinguish shrubs and herbaceous vegetation from trees, since these can differently affect rivers ecological condition. It includes human land uses covered by herbaceous vegetation such as golf courses and leisure parks, if with no signs of impervious surfaces.

3.2.1. Herbaceous vegetation

Areas covered in more than 75% by herbaceous vegetation that is not planted, fertilized or cultivated and may be used for grazing if sporadically and not intensively.

3.2.1.1. Herbaceous vegetation

Class propagated from the previous level.

3.2.1.1.1. Herbaceous vegetation

Class propagated from the previous level.

3.2.2. Shrubs

Areas covered in more than 50% by shrubs.

3.2.2.1. Dense shrubs

Areas covered in more than 75% by shrubs.

3.2.2.1.1. Dense shrubs

Class propagated from the previous level.

3.2.2.2. Sparse shrubs

Areas covered by shrubs in 50 to 75%. It refers to areas with a sparse but homogeneous coverage of shrubs rather than areas covered in less than 75% by isolated but dense clusters of shrubs, since each cluster should be classified with the land cover class 3.2.2.1.1.

3.2.2.2.1. Sparse shrubs

Class propagated from the previous level.

3.2.3. Green urban areas

Areas covered by herbaceous vegetation intended to human activities such as golf courses or leisure parks, if with no signs of impervious surfaces.

3.2.3.1. Leisure parks

Leisure parks covered by herbaceous vegetation, if with no signs of impervious surfaces.

3.2.3.1.1. Leisure parks

Class propagated from the previous level.

3.2.3.2. Golf courses

Golf courses covered by herbaceous vegetation, if with no signs of impervious surfaces.

3.2.3.2.1. Golf courses

Class propagated from the previous level.

3.3. Open spaces with little or no vegetation

Natural areas with little or no vegetation but not intended for human activities, such as forests cuts or bare soils on forest landscapes. Land cover intends to distinguish natural areas without vegetation from natural areas with vegetation, because even shrubs or herbaceous vegetation can influence several ecosystem processes.

3.3.1. Sparse vegetated areas

Areas covered by shrubs and/or herbaceous vegetation in 10 to 50%. In this study, areas assigned with this land cover class were mostly covered by clusters of herbaceous vegetation that account for less than 50% of the total area.

3.3.1.1. Sparse vegetated areas

Class propagated from the previous level.

3.3.1.1.1. Sparse vegetated areas

Class propagated from the previous level.

3.3.2. Bare soils

Natural areas covered in less than 10% by vegetation. It includes bare rocks and dirt paths with little or no human presence.

3.3.2.1. Bare rocks

Areas covered by bare rocks in mountain landscapes.

3.3.2.1.1. Bare rocks

Class propagated from the previous level.

3.3.2.2. Dirt paths

Areas referring dirt paths in natural areas, such as forest paths, which are not paved and poorly affect the connectivity between sites. It also includes dirt paths close to urban centers that poorly affect the connectivity between sites and are used to access naturalized areas. Dirt paths in urbanized areas with poor pristine conditions, greatly disrupting the connectivity between sites or used to agricultural and industrial activities are assigned with the land cover class 1.2.2.3.1.

3.3.2.2.1. Dirt paths

Class propagated from the previous level.

Appendix 5. River flow types classification

Laminar flow

River sections with a laminar flow where pools prevailed over riffles. It may include river sections with different channel width, depth, discharge or current velocity.

Turbulent flow

River sections with a turbulent flow where riffles prevailed over pools. It may include river sections with different channel width, depth, discharge or current velocity.

Appendix 6. River waterfalls classification

Slight

Short differences in elevation at the river bed (<50cm) that increases flow turbulence, but are unlikely to act as a barrier for most aquatic organisms or cause severe changes in flow turbulence and current velocity.

Moderate

Moderate differences in elevation at the river bed (50<x<150cm) resulting from natural or man-made structures that act as a barrier for most aquatic organisms and markedly increases flow turbulence.

Vigorous

High differences in elevation at the river bed (>150cm) resulting from man-made structures that act as a barrier for aquatic organisms and markedly increases flow turbulence.

Appendix 7. Protocols of the habitat characterization methods

FFI RECORD

Basin...Stream name...
Location...
Stretch (metres)............. width (metres)......... altitude...........
date ....record no.................... photo no................. code...................

River margin	right		left
1) Land use pattern of the surrounding area			
a) Lack of anthropization	25		25
b) Co-presence of natural area and anthropic use of territory	20		20
c) Seasonal cultivation and/or permanent cultivation; scarce urbanization	5		5
d) Urbanized area	1		1
2) Vegetation in the primary riparian zone			
a) Co-presence of functional riparian formations	40		40
b) presence of only one or simple series of riparian formations	25		25
c) absence of riparian formations but riparian of anyway functional formations	10		10
d) absence of significant functional formations	1		1
2bis) Vegetation in the secondary riparian zone			
a) Co-presence of functional riparian formations	20		20
b) Presence of only one or simple series of riparian formations	10		10
c) Absence of riparian formations but riparian of anyway functional formations	5		5
d) Absence of significant functional formations	1		1
3) Width of functional formations present in riparian zone			
a) Cumulative width of functional formations greater than 30 m	15		15
b) Cumulative width of functional formations between 10 and 30 m	10		10
c) Cumulative width of functional formations between 2 and 10 m	5		5
d) Absence of functional formations	1		1
4) Continuity of functional formations present in riparian zone			
a) formations zone intact without breaks in vegetation	15		15
b) formations zone with breaks in vegetation	10		10
c) frequent breaks or only continuous and consolidated herbaceous growth or only shrubs with dominance of exotic and infesting vegetation	5		5
d) Soil without or with thin herbaceous vegetation	1		1

5) Water conditions

a) Perennial regime with undisturbed flow and width of wet riverbed > 1/3 of moderate flow riverbed		20	
b) Fluctuations of flow induced with width of wet riverbed < 1/3 of moderate flow riverbed or variation of hydraulic head		10	
c) Frequent flow disturbances or natural non prolonged seasonal droughts or induced constant flow		5	
d) Intense flow disturbances, very frequent or sudden or prolonged droughts induced by anthropic actions		1	

6) Flooding efficiency

a) not diked stretch, flood stage riverbed greater than 3 times the moderate flow riverbed		25	
b) Flood stage riverbed breadth between 2 and 3 times the moderate flow riverbed (or if diked more than three times)		15	
c) Flood stage riverbed breadth between 1 and 2 times the moderate flow riverbed (or if diked more than 2-3 times)		5	
d) Stretches of v-shaped valleys with steep riverbanks and diked stretches with flood stage of the wet riverbed less than 2 times of the moderate flow		1	

7) Riverbed substrate and retention structures of the organic matter

a) Riverbed with boulders and/or old trees trunks embedded (or presence of reed groves or hydrophytes)		25	
b) Boulders and/or branches present with deposit of organic matter (or reed groves or scarce and poorly ranging hydrophytes)		15	
c) Free and mobile retention structures with flooding (or absence of reed groves and hydrophytes)		5	
d) Sandy sediment riverbed or smooth artificial shapes with uniform current		1	

8) Erosion

a) little evident and not important or only in the bends	20		20
b) Only at the straight stretches and/or modest vertical etching	15		15
c) Frequent with cutting of the banks and of roots and/or obvious vertical etching	5		5
d) Very frequent, with undercutting of banks and landslips or presence of artificial interventions	1		1

9) Cross-section

a) Natural riverbed with high morphological diversity		20	
b) Some artificial interventions but with discrete morphological diversity		15	
c) Artificial interventions present or with slight morphological diversity		5	
d) Artificial or almost no morphological diversity		1	

10) Ichthyic suitability

a) Elevated		25	
b) Good or discrete		20	
c) Slightly sufficient		5	
d) Absent or scarce		1	

11) Hydromorphology

a) Clearly distinguished and recurrent hydromorphological elements		20	
b) Clearly distinguished and irregular hydromorphological elements		15	
c) Indistinct or mainly one type of hydromorphological elements		5	
d) Not identifiable hydromorphological elements		1	

12) Plant component in wet riverbed

a) Periphyton scarcely developed and low presence of tolerant riparian vegetation		15	
b) Fair three dimensional periphyton scarcely developed with low presence of tolerant macrophytes		10	
c) Periphyton fair (if significant cover of tolerant cover) from absent to discrete		5	
d) Periphyton thick, and/or relevant presence of tolerant riparian vegetation		1	

13) Detritus

a) Recognizable and fibrous plant fragments		15	
b) Fibrous and pulpy plant fragments		10	
c) Pulpy fragments		5	
d) Anaerobic detritus		1	

14) Macrobenthic community

a) Well-structured and diversified, appropriate to the fluvial type		20	
b) Sufficiently diversified but with altered structure compared to that expected		10	
c) Poorly balanced and diversified with prevalence of taxa tolerant of pollution		5	
d) Absence of structured community, presence of few taxa all relatively tolerant of pollution		1	

Total score		
Functionality level		

HABSCORE (RBP) protocols

a)

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS

STREAM NAME	LOCATION
SITE ID #_ REACH ID __________	STREAM CLASS
UTM N ___________ UTM E ______________	RIVER BASIN
STORET #	AGENCY
INVESTIGATORS	
FORM COMPLETED BY	DATE ________ TIME . AM / REASON FOR SURVEY

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Greater than 70% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	40-70% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	20-40% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 20% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Embeddedness	Gravel, cobble, and boulder particles are 0-25% surrounded by fine sediment. Layering of cobble provides diversity of niche space.	Gravel, cobble, and boulder particles are 25-50% surrounded by fine sediment.	Gravel, cobble, and boulder particles are 50-75% surrounded by fine sediment.	Gravel, cobble, and boulder particles are more than 75% surrounded by fine sediment.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Velocity/Depth Regime	All four velocity/depth regimes present (slow-deep, slow-shallow, fast-deep, fast-shallow). (Slow is < 0.3 m/s, deep is > 0.5 m.)	Only 3 of the 4 regimes present (if fast-shallow is missing, score lower than if missing other regimes).	Only 2 of the 4 habitat regimes present (if fast-shallow or slow-shallow are missing, score low).	Dominated by 1 velocity/ depth regime (usually slow-deep).
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than 5% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 5-30% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 30-50% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 50% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HABITAT ASSESSMENT FIELD DATA SHEET—HIGH GRADIENT STREAMS

	Habitat Parameter	Condition Category: Optimal	Suboptimal	Marginal	Poor
Parameters to be evaluated broader than sampling reach	6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	7. Frequency of Riffles (or bends)	Occurrence of riffles relatively frequent; ratio of distance between riffles divided by width of the stream <7:1 (generally 5 to 7); variety of habitat is key. In streams where riffles are continuous, placement of boulders or other large, natural obstruction is important.	Occurrence of riffles infrequent; distance between riffles divided by the width of the stream is between 7 to 15.	Occasional riffle or bend; bottom contours provide some habitat; distance between riffles divided by the width of the stream is between 15 to 25.	Generally all flat water or shallow riffles; poor habitat; distance between riffles divided by the width of the stream is a ratio of >25.
	SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
	8. Bank Stability (score each bank) Note: determine left or right side by facing downstream.	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
	SCORE ___ LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	SCORE RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
	9. Vegetative Protection (score each bank)	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
	SCORE LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	SCORE ___ RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
	10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
	SCORE LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
	SCORE ___ RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

b)

HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS

STREAM NAME	LOCATION
STATION #_ REACH ID# ________	STREAM CLASS
UTM N____________ UTM E	RIVER BASIN
STORET #	AGENCY
INVESTIGATORS	
FORM COMPLETED BY	DATE ________ TIME _ PM
	REASON FOR SURVEY

Parameters to be evaluated in sampling reach

Habitat Parameter	Condition Category			
	Optimal	Suboptimal	Marginal	Poor
1. Epifaunal Substrate/ Available Cover	Greater than 50% of substrate favorable for epifaunal colonization and fish cover; mix of snags, submerged logs, undercut banks, cobble or other stable habitat and at stage to allow full colonization potential (i.e., logs/snags that are not new fall and not transient).	30-50% mix of stable habitat; well-suited for full colonization potential; adequate habitat for maintenance of populations; presence of additional substrate in the form of newfall, but not yet prepared for colonization (may rate at high end of scale).	10-30% mix of stable habitat; habitat availability less than desirable; substrate frequently disturbed or removed.	Less than 10% stable habitat; lack of habitat is obvious; substrate unstable or lacking.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
2. Pool Substrate Characterization	Mixture of substrate materials, with gravel and firm sand prevalent; root mats and submerged vegetation common.	Mixture of soft sand, mud, or clay; mud may be dominant; some root mats and submerged vegetation present.	All mud or clay or sand bottom; little or no root mat; no submerged vegetation.	Hard-pan clay or bedrock; no root mat or vegetation.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
3. Pool Variability	Even mix of large-shallow, large-deep, small-shallow, small-deep pools present.	Majority of pools large-deep; very few shallow.	Shallow pools much more prevalent than deep pools.	Majority of pools small-shallow or pools absent.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
4. Sediment Deposition	Little or no enlargement of islands or point bars and less than <20% of the bottom affected by sediment deposition.	Some new increase in bar formation, mostly from gravel, sand or fine sediment; 20-50% of the bottom affected; slight deposition in pools.	Moderate deposition of new gravel, sand or fine sediment on old and new bars; 50-80% of the bottom affected; sediment deposits at obstructions, constrictions, and bends; moderate deposition of pools prevalent.	Heavy deposits of fine material, increased bar development; more than 80% of the bottom changing frequently; pools almost absent due to substantial sediment deposition.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
5. Channel Flow Status	Water reaches base of both lower banks, and minimal amount of channel substrate is exposed.	Water fills >75% of the available channel; or <25% of channel substrate is exposed.	Water fills 25-75% of the available channel, and/or riffle substrates are mostly exposed.	Very little water in channel and mostly present as standing pools.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0

HABITAT ASSESSMENT FIELD DATA SHEET—LOW GRADIENT STREAMS

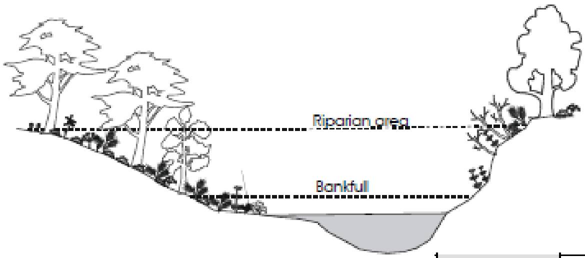
Parameters to be evaluated broader than sampling reach

Habitat Parameter	Condition Category: Optimal	Suboptimal	Marginal	Poor
6. Channel Alteration	Channelization or dredging absent or minimal; stream with normal pattern.	Some channelization present, usually in areas of bridge abutments; evidence of past channelization, i.e., dredging, (greater than past 20 yr) may be present, but recent channelization is not present.	Channelization may be extensive; embankments or shoring structures present on both banks; and 40 to 80% of stream reach channelized and disrupted.	Banks shored with gabion or cement; over 80% of the stream reach channelized and disrupted. Instream habitat greatly altered or removed entirely.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
7. Channel Sinuosity	The bends in the stream increase the stream length 3 to 4 times longer than if it was in a straight line. (Note - channel braiding is considered normal in coastal plains and other low-lying areas. This parameter is not easily rated in these areas.)	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	The bends in the stream increase the stream length 1 to 2 times longer than if it was in a straight line.	Channel straight; waterway has been channelized for a long distance.
SCORE	20 19 18 17 16	15 14 13 12 11	10 9 8 7 6	5 4 3 2 1 0
8. Bank Stability (score each bank)	Banks stable; evidence of erosion or bank failure absent or minimal; little potential for future problems. <5% of bank affected.	Moderately stable; infrequent, small areas of erosion mostly healed over. 5-30% of bank in reach has areas of erosion.	Moderately unstable; 30-60% of bank in reach has areas of erosion; high erosion potential during floods.	Unstable; many eroded areas; "raw" areas frequent along straight sections and bends; obvious bank sloughing; 60-100% of bank has erosional scars.
SCORE (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
9. Vegetative Protection (score each bank) Note: determine left or right side by facing downstream.	More than 90% of the streambank surfaces and immediate riparian zone covered by native vegetation, including trees, understory shrubs, or nonwoody macrophytes; vegetative disruption through grazing or mowing minimal or not evident; almost all plants allowed to grow naturally.	70-90% of the streambank surfaces covered by native vegetation, but one class of plants is not well-represented; disruption evident but not affecting full plant growth potential to any great extent; more than one-half of the potential plant stubble height remaining.	50-70% of the streambank surfaces covered by vegetation; disruption obvious; patches of bare soil or closely cropped vegetation common; less than one-half of the potential plant stubble height remaining.	Less than 50% of the streambank surfaces covered by vegetation; disruption of streambank vegetation is very high; vegetation has been removed to 5 centimeters or less in average stubble height.
SCORE ___ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0
10. Riparian Vegetative Zone Width (score each bank riparian zone)	Width of riparian zone >18 meters; human activities (i.e., parking lots, roadbeds, clear-cuts, lawns, or crops) have not impacted zone.	Width of riparian zone 12-18 meters; human activities have impacted zone only minimally.	Width of riparian zone 6-12 meters; human activities have impacted zone a great deal.	Width of riparian zone <6 meters: little or no riparian vegetation due to human activities.
SCORE __ _ (LB)	Left Bank 10 9	8 7 6	5 4 3	2 1 0
SCORE (RB)	Right Bank 10 9	8 7 6	5 4 3	2 1 0

QBR INDEX

Field data sheet

Riparian habitat quality



Station	
Observant	
Data	

Score of each part cannot be negative or exceed 25

Total riparian cover — Part 1 score

Score		
25	> 80 % of riparian cover (excluding annual plants)	
10	50-80 % of riparian cover	
5	10-50 % of riparian cover	
0	< 10 % of riparian cover	
+ 10	if connectivity between the riparian forest and the woodland is total	
+ 5	if the connectivity is higher than 50%	
- 5	connectivity between 25 and 50%	
-10	connectivity lower than 25%	

Cover structure — Part 2 score

Score		
25	> 75 % of tree cover	
10	50-75 % of tree cover or 25-50 % tree cover but 25 % covered by shrubs	
5	tree cover lower than 50 % but shrub cover at least between 10 and 25 %	
0	less than 10% of either tree or shrub cover	
+ 10	at least 50 % of the channel has helophytes or shrubs	
+ 5	if 25-50 % of the channel has helophytes or shrubs	
+ 5	if trees and shrubs are in the same patches	
- 5	if trees are regularly distributed but shrubland is > 50 %	
- 5	if trees and shrubs are distributed in separate patches, without continuity	
- 10	trees distributed regularly, and shrubland < 50 %	

Cover quality *(the geomorphological type should be first determined*)* — Part 3 score

Score		*Type 1*	*Type 2*	*Type 3*	
25	number of native tree species:	> 1	> 2	> 3	
10	number of native tree species:	1	2	3	
5	number of native tree species:	0	1	1 - 2	
0	absence of native trees	-			
+ 10	if the tree community is continuous along the river and covers at least 75% of the edge riparian area				
+ 5	the tree community is nearly continuous and cover at least 50% of the riparian area				
+ 5	if the riparian community is structured in gallery				
+ 5	when the number of shrub species is:	> 2	> 3	> 4	
- 5	if there are some man-made buildings in the riparian area				
- 5	is there is some isolated species of non-native trees**				
- 10	presence of communities of non-native trees				
- 10	presence of garbage				

Channel alteration — Part 4 score

Score		
25	unmodified river channel	
10	fluvial terraces modified, constraining the river channel	
5	channel modified by discontinuous rigid structures along the margins	
0	totally channelized river	
- 10	river bed with rigid structures (e.g wells)	
- 10	transverse structures into the channel (e.g weirs)	

Final score (sum of level scores)	

Appendix 8. Scoring systems of the habitat characterization methods

Fluvial Functioning Index (FFI)	
Score	River Functioning
300-261	Excellent
260-251	Excellent - very good
250-201	Very good
200-181	Very good - good
180-121	Good
120-101	Good - fair
100-61	Fair
60-51	Fair - poor
50-14	Poor

HABSCORE (RBP)	
Score	River Habitat Quality
200-151	Optimal
150-101	Suboptimal
100-51	Marginal
50-0	Poor

Riparian Forest Quality Index, QBR	
Score	Riparian Habitat Quality
≥95	Riparian habitat in natural conditions
90-75	Some disturbance, good quality
70-55	Disturbance important, fair quality
50-30	Strong alteration, poor quality
≤25	Extreme degradation, bad quality